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About diet of *Vipera ursinii* (Bonaparte, 1835) in a central Apennine grassland landscape: what do they eat when Orthoptera are not present?

Sobre la dieta de *Vipera ursinii* (Bonaparte, 1835) en un paisaje de pastizales de los Apeninos centrales: ¿qué comen cuando no hay ortópteros?

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Abstract

Vipera ursinii has a very short period of activity and has a fragmented population; it lives in similar habitats but at different altitudes. In view of its Italian distribution, this study dealt with mountain populations that frequent on primary pastures above the forest line, from 1600 to 1850 m a.s.l. At these altitudes and latitudes, the snake is known as a large consumer of meadow orthoptera. The aim of the research is to identify and analyze the diet of *Vipera ursinii* in a period when orthoptera is not available. The present work was carried out in the Apennines (central Italy), the subjects studied were juveniles and adults. The diet of this snake has never been studied in the subject areas, therefore the research results provides new insight. Regurgitated material and fecal droppings obtained by non-invasive ventral palpation were analyzed.

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The main results indicate the presence in the diet of new taxa such as ants, slug and caterpillar. This new knowledge may give a new perspective on this taxon that is potentially vulnerable to environmental changes due to environmental thermal alteration phenomena.

Keywords: *Vipera ursinii*, predation strategy, feeding habits, niche breadth.

Resumen

Vipera ursinii tiene un período de actividad muy corto y una población fragmentada; vive en hábitats similares, pero a diferentes altitudes. Dada su distribución en Italia, este estudio se centró en poblaciones de montaña que frecuentan pastos primarios por encima del límite forestal, entre 1600 y 1850 m s.n.m. En estas altitudes y latitudes, la serpiente es conocida por ser un gran consumidor de ortópteros de pradera. El objetivo de la investigación es identificar y analizar la dieta de la *Vipera ursinii* en un período en el que no dispone de ortópteros. El presente trabajo se llevó a cabo en los Apeninos (Italia central), y los sujetos estudiados fueron juveniles y adultos. La dieta de esta serpiente nunca se había estudiado en las áreas mencionadas, por lo que los resultados de la investigación aportan nuevos conocimientos. Se analizó material regurgitado y excrementos obtenidos mediante palpación ventral no invasiva. Los principales resultados indican la presencia en la dieta de nuevos taxones, como hormigas, babosas y orugas. Este nuevo conocimiento podría brindar una nueva perspectiva sobre este taxón, potencialmente vulnerable a los cambios ambientales debido a fenómenos de alteración térmica ambiental.

Palabras clave: *Vipera ursinii*, estrategia de depredación, hábitos alimentarios, amplitud de nicho.

INTRODUCTION

Current knowledge about meadow viper (*Vipera ursinii*) is extensive, with numerous studies reporting data on various ecological aspects, but only a few of them date back to recent times, despite the climate changes we observe (Ozol, 1941; Krassawzeff, 1943; Terentiev and Chernov, 1949; Dreux and Saint Girons, 1951; Kramer, 1961; Makeyev, 1964; Naulleau, 1973; Kofron, 1978; Saint Girons, 1975, 1978, 1979; Baron, 1980; Saint Girons and Naulleau, 1981; Naulleau, 1984; Baron, 1989; Nilson, Andrèn, Jorge, 1993; Hilpold, Seeber, Fontana, Niedrist, Rief, 2018).

Studies on another species with similar ecological conditions, *V. graeca* (Mizsei , Boros, Lovas-Kiss, Szepesváry, Szabolcs, et al., 2019); show that *V. graeca* feeds on crickets and grasshoppers and that vipers limit their feeding to periods of high Orthoptera abundance. This remains confirmed provided that vipers have to reach a certain body size to catch large prey. Studies on the feeding behaviour of these vipers conducted in different habitat by Baron (1989) on French alpine populations and by Kotenko (1989) in various areas of Ukrainian plain confirm a heterogeneous but substantially similar diet composed mainly of *Orthoptera*, with occasional additions of lizards and small mammals. From an evolutionary point of view, the French populations are very similar to the Italian ones and recent molecular studies demonstrate that they belong to the same subspecies of *Vipera ursinii ursinii* (Ferchaud, Ursenbacher, Cheylan, Luiselli, Jelić, 2012; Mizsei, Jablonski, Roussos, Dimaki, Ioannidis et al., 2017; Mizsei Zinenko, Sillero, Ferri, Roussos, 2018). Phylogeographically speaking, the *Vipera ursinii* complex is influenced by Quaternary glacial-interglacial cycles, with the Balkan Peninsula appearing to have been the geographical centre of diversification around 2.1 million years ago (Ferchaud et al., 2012). The ancestral lineage giving rise to present-day *V. u. ursinii* and Croatian *V. u. macrops* populations (Méhely, 1911) that migrated northward from Greece to Croatia and Slovenia, while the lineage leading to *V. u. moldavica* (Nilson et al., 1993) and *V. u. rakosiensis* (Méhely, 1893) emerged between Greece and Montenegro before moving north-eastward to the steppes of Hungary, Romania, and Moldova (Ferchaud et al., 2012; Zinenko, Stümpel, Mazanaeva, Bakiev, Shiryaev et al., 2015). Subsequently, *V. u. ursinii* colonized the Alpine mountains (extinct population) before spreading southward to Apennines (Ferchaud et al., 2012).

Generally speaking, populations of the *V. ursinii* complex tend to be associated with the forest edge and its conservation. Therefore, critical habitat limitations can be detected because grasslands face increasing forestation due to the abandonment of traditional pastoral practices and upward migration of the forest line driven by climate warming (Lyet, Thuiller, Cheylan and Besnard, 2013; Ferrarini, Alatalo and Gustin, 2017). However, important work by Agrimi and Luiselli (1992) and Cerasoli, Besnard, Marchand, D'Alessandro, Iannella et al. (2021) indicates some niche overlap between these populations, where the diet of *V. ursinii* shows some heterogeneity, although it mainly preys on orthopterans, lizards and micromammals. The maximum size of *V. ursinii* is around 50 cm (Baron, 1992), with the average size reported for Apennine populations being around 35 cm (Luiselli, Filippi and Di Lena, 2007).

Due to these relatively small biometric values, vertebrates such as lizards, micro-mammals, and lark birds are less frequently preyed upon and such occurrences are attributable to individuals of considerable size that constitutes a small part of the population.

Knowledge of trophic habits, including trophic resources, is a critical factor for population conservation (Soberón and Nakamura, 2009), particularly for *V. ursinii* and its distribution. Understanding dietary habits is important for studies of trophic niches and ecological relationships (Oliveira, Gottschalk, Loebmann, Santos, Miranda, 2015) as prey diversity and the amount of food items used by each species are directly related to differentiation in habitat use (Carvalho, Freitas, Faria, Batista, Batista, 2008). Baron (1989) found in French populations that the feeding period of this reptile spans approximately six months for adult males and five months for females (late June to late September). This suggests that adults may prey on micro-mammals and lizards soon after emerging from hibernation, while juveniles likely feed on other invertebrate prey until they are older. The cycle of primary grassland orthopterans in the sub-adult and adult stages occurs between late June and the arrival of cold temperatures in September (Baron, 1992). Studies that aim to represent the feeding habits of small snakes are also obtained from faecal remains. The nervous character of *V. ursinii* favours its spontaneous defecation when handled. By mid-September, orthopterans are already scarce, and observations during this period may yield important insights into alternative prey availability. The Appennine populations of meadow viper occurs in isolated areas dominated by subalpine grasslands (Luiselli, 2004; Lyet, Thuiller, Cheylan and Besnard, 2013) and in our opinion rare and cryptic species should be studied more extensively than others, as has been observed for various European bats (Rebelo, Tarros and Jones, 2010), viperids (Mizsei, Budai, Móré, Rák, Radovics et al., 2023) and, beetles (Bosso, Rebelo, Garrona and Russo, 2013). Our study focused on an upland population, like that studied by Agrimi and Luiselli (1992), but located in a more northerly region within the same grassland landscape. The aim of our research is to explore the diet of *Vipera ursinii* covering the period of season when orthoptera ceased to be available. We assume the species follows a larger seasonal and monthly variation of diet and we believe that the results obtained with this research can increase knowledge about new and unexpected taxa in the meadow viper diet. Last but not least, we believe we can detect a marked change during the active season.

MATERIALS AND METHODS

Study area

The study area is located within the Monti Sibillini National Park; specifically on Mt. Vettore (42°48’24” N, 13°16’1” E, coordinate system UTM-WGS94, 2476 m a.s.l.) and Mt. Rotondo (42°57’27” N, 13°12’21” E, coordinate system UTM-WGS84, 2012 m a.s.l.) (Fig. 1). The elevation ranges of our study is 1,600 to 1,850 m a.s.l. and it is situated above the upper limit of the beech forest (Pedrotti, 1969). This areas, of about 25 hectares (each), is characterized by limestone bedrock geology, with rocky outcrops and scree among the vegetation. The vegetation comprises a rich mosaic of herbaceous species, share stretches of habitat with typical upland grassland species (Brusaferro, Iesari, Tardella and Catorci, 2019), including plant communities such as *Thlaspietea rotundifolii* Br. Bl. 19848, *Drypidatea spinosae* Quézel 1964, *Asplenietea trichomanis* (Br.-Bl. in Meier et Br.-Bl. 1934) praterie di *Nardetea strictae* Rivas Goday et Borja Carbonell in Rivas Goday et Mayor López 1966, *Festuco hystricis-Ononidetea striatae* Rivas- Mart. et al. 2002, *Festuco-Brometea* Br.- Bl. et Tx. ex Soó 1947, and *Elyno-Seslerietea* Br.-Bl. 1948. In general, the habitat is dominated by herbaceous vegetation, with *Juniperus nana* and *J. communis* present. Considering the prevalence of grassland species and the absence of trees combined with elevation, the primary grasslands are predominant in the study area (Fig. 2).

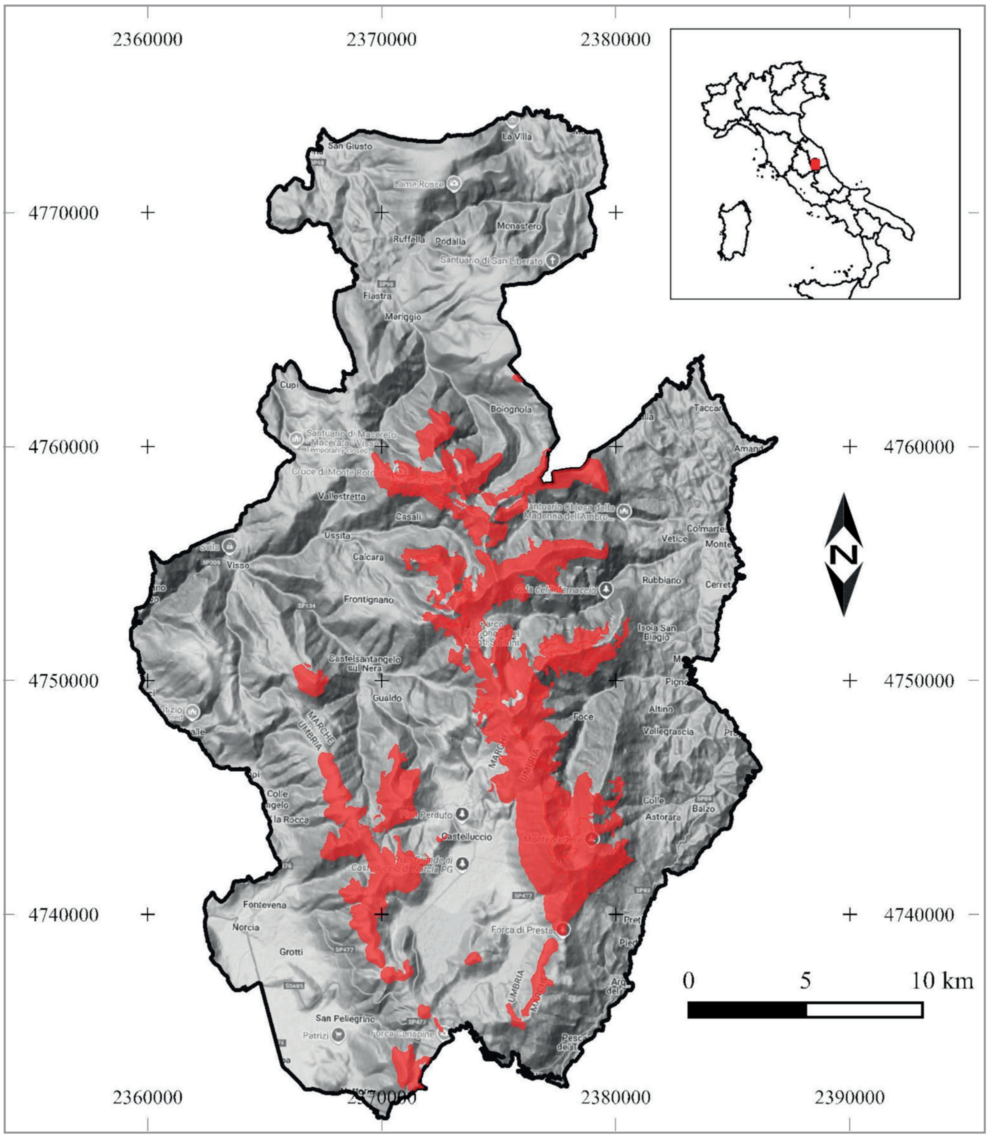

Figure 1. Location of the study area (indicated with a red rectangle in the map of Italy) and distribution range of *Vipera ursinii* (red areas) in the Monti Sibillini National Park.

The temperate climate of the study area is characterized by cold winters and summer droughts of varying intensities depending on altitude and soil conformation (Rivas-Martínez and Rivas-Saenz, 2016). The average annual temperature ranges from 3.5°C to 7.0°C, with average annual precipitation ranging from 1,300 to 1,600 mm. Two seasonal peaks of precipitation occur in late spring and autumn, with reduced precipitation in summer. The rural landscape has historically been characterized by extensive animal grazing, but since recent decades grazing cessation and abandonment of mountain agriculture have particularly increased. Dynamic processes of abandonment and spontaneous vegetation recovery are underway in several areas of the Monti Sibillini National Park. Malatesta, Tardella and Tavolini (2019) observed a decrease in areas covered by open grassland turf communities and an increase in pastures with dense, closed turf dominated by *Brachypodium genuense*. Abandonment significantly impacted the habitat of *V. ursinii*, which frequents south-facing slopes undergoing dynamic changes in grassland mosaic, resulting in high patch turnover and landscape structure homogenization, especially on south-facing slopes. The effect of grassland use (abandonment of grazing) needs to be further studied, in order to understand and predict is effect in longer term. Due to its high narrow niche and fragmented distribution, *V. ursinii* is considered "vulnerable" on the IUCN Red List, according to criterion B2ab (ii, iii, iv, v), referring to range fragmentation, continuous decline in population size, and habitat quality decline across Europe and the EU-27 (Halpern and Bowles, 2024). The conservation perspective and challenges are discussed by Console, Iannella, Cerasoli, Luiselli, Jelic, et al. (2020), summarizing different historical knowledge models and varying levels of protection depending on the country and subspecies.

Data collection

To collect data, we randomly selected two sites where *Vipera ursinii* was very common. Within these sites we used in 2011 the hand capturing method (Sutherland, 2006), we used 40 transects for a total of 12 km-long walking routes. Each fieldwork sessions lasted five hours with two operators walking about three meters apart. Our research took place during two periods which correspond two seasonal phases: (series 1 - spring) vipers collected between May 15th and June 15th and (series 2 - autumn) vipers examined between September 1st to 31st. For each period, ten monitoring replications were carried out and for each replication the 300 meter-long transect starting point was always randomly chosen; each transect was covered at the same altitude. The data collected in the two sites were clustered as a single sample (Sutherland, 2006). The vipers were divided into two categories according to size (Agrimi and Luiselli, 1992): (1) vipers shorter than 28 cm in total length and (2) vipers longer than 28 cm in total length.

Figure 2. Habitat of the Ursini's viper (A) and a individual feeding on an orthopteran of the *Decticus* genus (B).

The measurement was made with an aluminum millimeter stick resting on the ground. Subjects held between two hands were placed on the measuring surface and slowly distracted to reach the maximum head-tail length. Sex was determined for all vipers examined.

Analysis of gastrointestinal contents.— The stomach analytical method (Kjaegaard, 1981, Capula and Luiselli, 1990; Glaudas, Glennon, Martins, Luiselli, Fearn, 2019; Filippi and Luiselli, 2002; Filippi, Rugiero, Capula, Capizzi and Luiselli, 2005) was applied to all 69 vipers collected (33 males and 36 female); this method involves palpation to let them regurgitate their prey. When this method provided sample, no further sample collection were performed, and the vipers were immediately released. Unfortunately, this method does not always allow a complete examination of all stomach contents; to overcome this problem we used a method which require washing the final section of the intestine. To wash out the intestine, we gently flushed the cloaca with a saline solution, like a medical enema (De la Navarre, 2006). We used sterilized equipment for each sample, including a syringe, a small rubber probe, saline solution, Petri dishes, and collection tubes. The probe helped ensure the saline solution flowed smoothly, carrying any waste material out. For larger vipers, we used two tubes, one to add the saline and the other to remove it. After completing the sample collection, we released the subjects immediately to reduce stress. Two handlers carefully held the snakes to make sure they were comfortable throughout the process.

Data analysis.— The data collected in the field were entered into a spreadsheet database with sequential numbering. Each snake handled is therefore one of the numbers. These numbers corresponded to the gastro-intestinal sample collected.

The food data were expressed in frequency of presence or recurrences (Rolando, 1986; Prigioni, 1991; Borgo, 2015) defined as follows:

$$F = n / N$$

where n is the number of times the same food component appears on the total stomach and intestinal samples examined (N); the prey consumed, therefore, has the same importance and it does not depend on size. This method tends to overestimate the importance of small categories more often ingested and to underestimate the prey larger in size that appear less frequently. The samples were attributed to nine food categories: Orthoptera, Formicidae, lepidopteran larvae, Stylommatophora, Opilionidae, beetle larvae, Araneidae, Microtidae and Sauria.

The trophic niche breadth was calculated using the Levin's normalized index (L) (Feinsinger, Speers and Polle, 1981) and the Simpson's (1949) diversity index (B); for trophic niche overlapping Pianka's (1974) index (O_{jk}) was used. Pearson's χ-squared statistical test (Pearson, 1900) was applied to verify the differences between the food categories and viper size classes, food categories/seasonal differences and food categories/sample method. Since the data on occurrence frequencies did not have a normal distribution (Shapiro-Wilk Normality test; p-value$<$ 0.05), the nonparametric Wilcoxon

Mann-Whitney U test was applied to verify the statistical differences among the overall viper seasonal diet, sex and size. We performed the statistical analysis using R, version 3.4.1 (R Core Team, 2017).

Ethical statements

The authors are committed to upholding the integrity of the scientific documentation. They follow the guidelines of the Committee on Publication Ethics (COPE) with the aim of good conduct. Maintaining the integrity of the research was ensured by ethical and proper conduct. The methodologies used for this research are established and accepted by the scientific community for this type of study. The authors have the authorization to handle the species under study. Prot. 0039999/PNM 3-7-2013 DIV II internal reference n. 19129-22513-25598/2013 registered application 3 May 2013 n. 35027.

RESULTS

A total of 69 (33 males and 36 females) *V. ursinii* were collected in two seasonal phased: (series 1 – spring) vipers collected between May 15th and June 15th and (series 2 – autumn) vipers examined between September 1st to 31st. Data analysis on preyed *taxa* was carried out on 52 viper individuals, since for 17 of them (24.6%) gastrointestinal observations did not provide any samples; these belonged to both size, sex and season categories. Observations of fecal remains were obtained from 19 vipers (27.5%) belonging to both size categories (size 1 = 12 adders; size 2 = seven adders) and both sexes (11 males; eight females, Table 1).

Table 1. Samples examined by size, season categories and sex.

Size	Sex	Spring	Summer	Total
Shorter than 28 cm	Male	6	7	13
	Female	6	7	13
	Total	**12**	**14**	**26**
Longer than 28 cm	Male	3	10	13
	Female	5	8	13
	Total	**8**	**18**	**26**

Composition of diet

The total of 67 ingested food items shows a high degree of invertebrate in the diet (85.1% of identified prey), particularly Orthoptera (29.9%), Oplyonidae (14.9%) and Arachnida (11.9%); these preys represent over 50% of *Vipera ursinii* Arthropod diet. Vertebrate remains was 14.9% of the total number of preys: these consisted of 9.0% Sauria (Reptilia) and 6.0% Microtidae (Mammalia, Rodentia) (Table 2).

Table 2. Seasonal list of prey obtained from gastrointestinal tract of all *Vipera ursinii* captured in the Apennines Sibillini mountains. Spring, vipers collected between May 15th and June 15th; autumn, vipers collected between September 1st to 31st; %Sp, frequency of species collected in spring; %Su, frequency of species collected in autumn; %n frequency of total collected species.

Food items	Spring	%Sp	Summer	%Su	Total	%n
Orthoptera	0	0.0	20	50.0	20	29.9
Formicidae	3	11.1	1	2.5	4	6.0
butterfly larvae	4	14.8	1	2.5	5	7.5
Stylommatophora	5	18.5	1	2.5	6	9.0
Oplyonidae	6	22.2	4	10.0	10	14.9
beetle larvae	1	3.7	1	2.5	2	3.0
Arachnida	4	14.8	4	10.0	8	11.0
Microtidae	1	3.7	3	7.5	4	6.0
Saura	2	7.4	4	10.0	6	9.0
undetermined	1	3.7	1	2.5	2	3.0
Total	27	100	40	100	67	100

Sex of individuals did not show significant differences in the composition of the diet: it appears that males slightly prefer an insect diet, while females seem to have a greater predilection towards arachnids and vertebrates (Fig. 3). We tested this difference using bootstrap confidence intervals that does not highlight significant differences in the composition of the diet (observed mean difference = 0.1; 95% confidence interval -2.2 – 2.6). Both sexes have very similar values of Levin's niche breadth (L_m = 0.54; L_f = 0.7), similar value of Simpson's index (B_m = 5.3; B_f = 6.7) and values close to one for the Pianka's index (O_{mf} = 0.9) (Table 3).

Table 3 – Niche breadth by sex.

	Size	Levin's index	Simpson's index	Pianka's index
Niche breadth	Male	0.5	5.3	0.9
	Female	0.7	6.7	

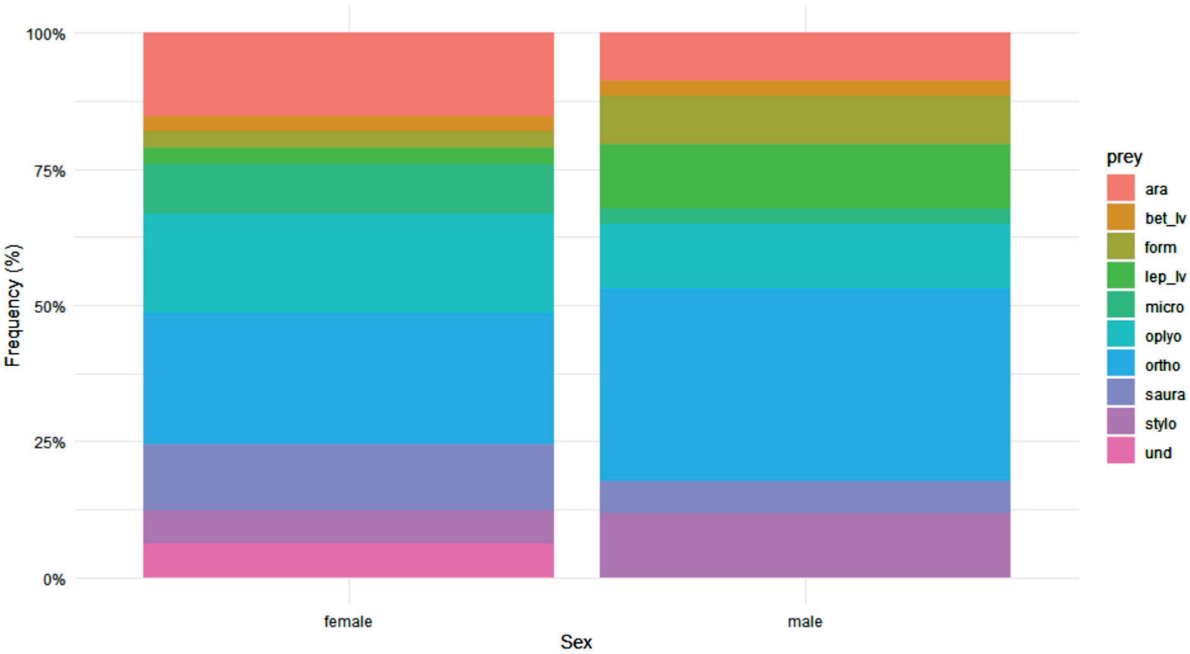

Figure 3. Frequency of the preyed data of *Vipera ursinii* for both sexes. ortho= *Orthoptera*; form= Formicidae; lep_lv= butterfly larvae; stylo= *Stylommatophora*; oplyo= *Oplyonidae*; bet_lv= beetle larvae; arach= *Arachnidae*; micro= *Microtidae*; sauria= sauria; und= undetermined.

Composition of diet based on viper size

Vipers shorter than 28 cm have a broader dietary niche than the larger ones (Fig. 4). Small vipers show a very high degree of invertebrates in their diet (90.0% of the identified prey), particularly clams (26.3%), Arachnida (21.1%) and Formicidae (15.8%); in these vipers' vertebrate are quite rare, amounting to 7.5% of the total number of prey and are predominantly represented by Sauria (5%). Vipers over 28 cm long have a more balanced diet, with invertebrate that are always the main component of their prey (70.4%), and vertebrates represent almost a quarter of the overall diet.

The two size classes show different Levin's value (L_{short}= 0.7; L_{long}= 0.4) and this indicates that vipers smaller than 28cm have a much broader trophic niche; the Pianka's index (O_{sl}= 0.7) also highlights a slight separation in the trophic niche between these two size classes. The χ^2 test highlighted significant differences in the composition of the invertebrate diet regarding Stylommatophora (χ^2=4.2981; p-value=0.038*) and Arachnida (χ^2=6.6509; p-value=0.009**); these data therefore indicate that only small vipers feed on these taxa which represent 35.0% of the overall diet and the 38.9% of the invertebrate diet.

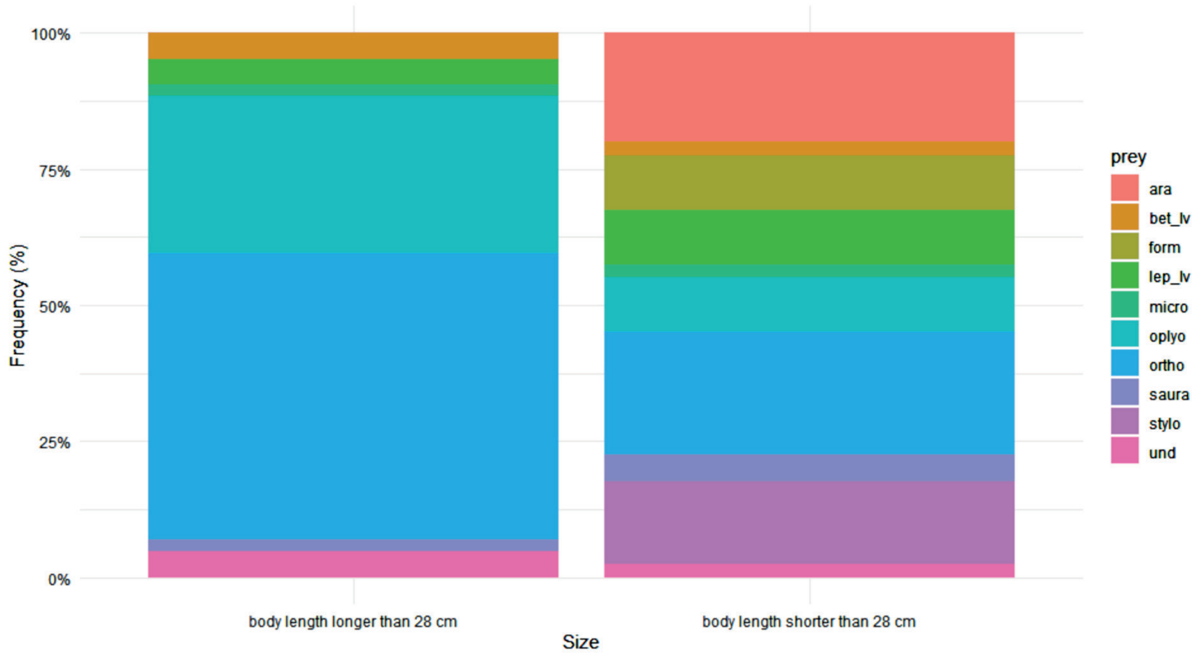

Figure 4. Frequency of the preyed data of *Vipera ursinii* for both sizes. ortho= *Orthoptera*; form= *Formicidae*; lep_lv= butterfly larvae; stylo= *Stylommatophora*; oplyo= *Oplyonidae*; bet_lv= beetle larvae; arach= *Arachnidae*; micro= *Microtidae*; *sauria*= sauria; und= undetermined.

The differences in the composition of the trophic niche between these two size classes are also evident in the seasonal examined periods: spring and summer (Fig. 5). During the spring, vipers have differing niche breadth ($L_s = 0.6$; $L_l = 0.3$) and values close to zero for the Pianka's index ($O_{sl} = 0.3$); small vipers feed mainly on formicides (W = 60; p-value = 0.15), mollusks (W = 68; p-value = 0.04*) and arachnids (W = 64; p-value = 0.08) which overall represent almost 50% of the diet while in the large ones are the opilionids the 50% of the diet. During the summer the non-parametric Mann-Whitney test (W = 171; p-value = 0.03*) highlight significant differences in the composition of the diet between the two size classes, they have similar values of niche breadth ($L_s = 0.4$; $L_l = 0.3$) and values close to one for the Pianka's index ($O_{sl} = 0.9$). But small vipers eat not only orthoptera but also formicides (W=135; p-value=0.28), lepidopteran (W = 135; p-value = 0.28) and beetle (W = 135; p-value = 0.28) larvae, and especially spiders (W = 162; p-value = 0.02*) which overall represent almost 20% of the diet.

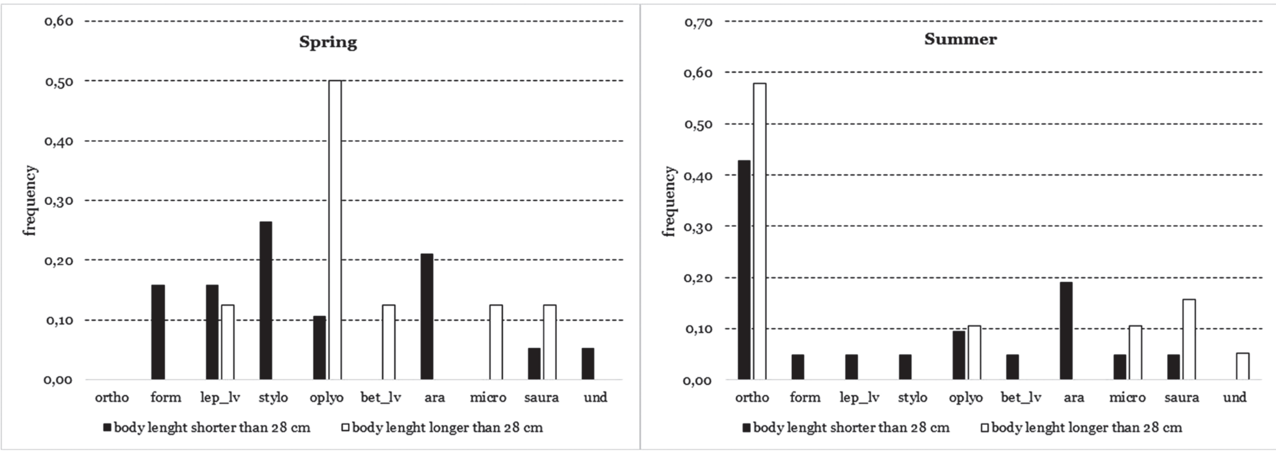

Figure 5. Frequency of the preyed data of *Vipera ursinii* for both size during spring and summer: ortho= Orthoptera; form= Formicidae; lep_lv= butterfly larvae; stylo= Stylommatophora; oplyo= *Oplyonidae*; bet_lv= beetle larvae; ara= *Araneidae*; micro= *Microtidae*; *sauria*= sauria; und= undetermined.

Trophic niche

By means of the data obtained in the respective periods, it was possible to obtain and draw a trophic niche. This appeared to be broad in the spring rather than the summer season. Small adders have a larger feeding niche than larger ones (Fig. 6).

The diet of Sibillini *V. ursinii* population includes nine food items (of which two are vertebrates), some of these taxa being confirmed by other authors in their research but new preys have been observed for this viper: caterpillars, ants and mollusks. In effect, we found in the intestinal and gastric tracts leavings of ants, slugs mineralized organs and other remains attributable to lepidopteran larvae (Table 2). No birds were found, and this does not mean that these populations do not feed on this taxon, but rather that it is not a common element of their diet.

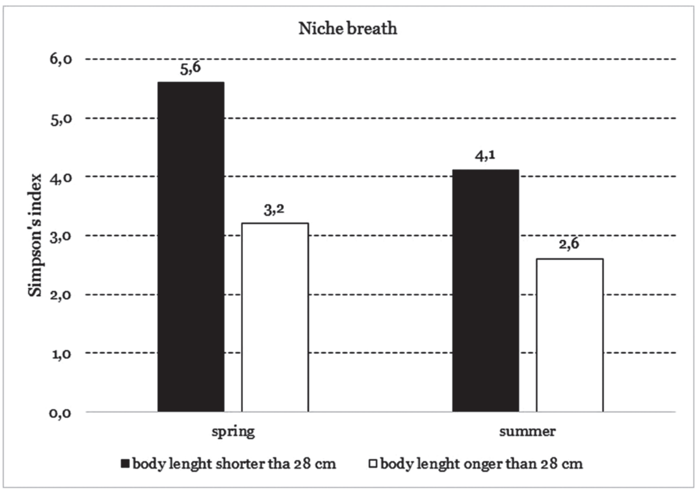

Figure 6. Food niche of *V. ursinii* calculated using a Simpson's index (1949) in spring and summer season.

The total absence of Orthoptera in the spring period (Fig. 5) is clearly the reason why this viper is forced to feed on unconventional prey and the type of prey captured is closely correlated with the size of the viper itself. Small vipers are forced to expand their trophic niche, increasing the diversity of the taxa captured but focusing on small prey which provide a lower energy supply than larger ones (orthopterans and vertebrates) but which are more easily catchable. This is confirmed by the fact that the females of small vipers, being larger than their respective males, are the only ones that have a vertebrate component in their diet (Fig. 7).

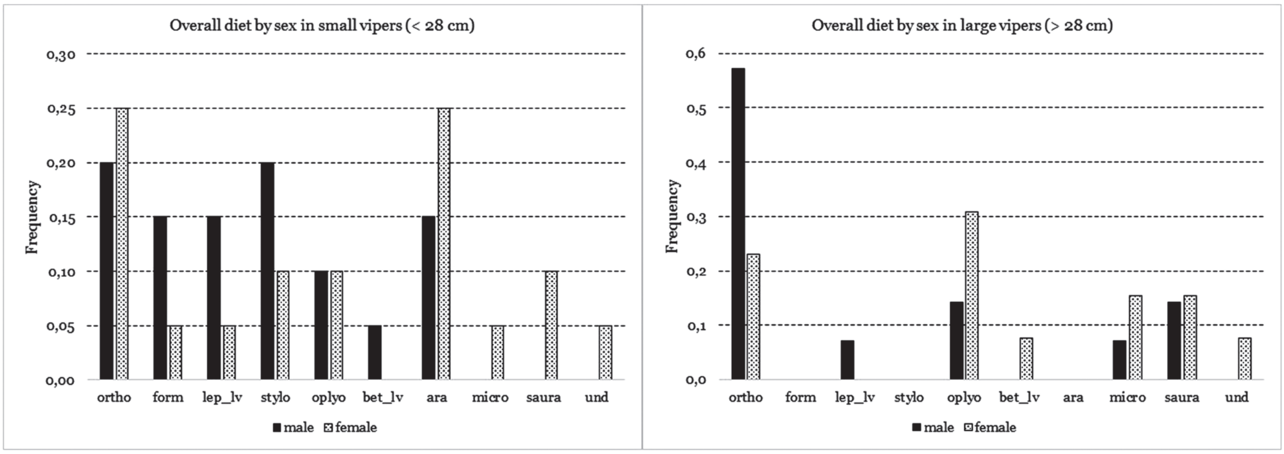

Figure 7. Frequency of the preyed data of *Vipera ursinii* for both sexes. ortho= *Orthoptera*; form= *Formicidae*; lep_lv= butterfly larvae; stylo= *Stylommatophora*; oplyo= *Oplyonidae*; bet_lv= beetle larvae; ara= *Araneidae*; micro= *Microtidae*; *sauria*= sauria; und= undetermined.

DISCUSSION

The main novelty of this study is that it provides evidence that this reptile has a wider trophic niche than previously thought. The trophic niche thus appears to be larger in the orthopteran-free season in young vipers. In addition to body size, several aspects of prey have been found. Each new predated taxon has low constants in its ability to escape predators. Each new prey does not know how to fly or jump and all are grassland frequenters. Orthopterans, therefore, provide both small vipers (especially males) and larger ones the right energy ratio between the energy spent in the capture effort and that acquired through feeding. In our opinion, this is the reason why Orthoptera are the fundamental component (50% in summer season) of the *Vipera ursinii*'s diet; during summer, when this taxon is very common in grassland, the diversity of the food niche is lower, and the reduction is more marked in small vipers compared to larger ones (Fig. 6).

Opilionids are already reported as prey of *V. ursinii* (Baron, 1980; Baron, 1992; Agrimi and Luiselli, 1992); in Sibillini population seems to be particularly welcome and frequent. We hypothesize that it is due to a set of ecological factors such as their mobility and high abundance in meadows and vegetation. Young vipers, on the other hand, behave like food generalist; they do not select a particular type of food. In summer, even if orthopterans represent an important food component, the 60% of their diet is represented by other invertebrates and vertebrates; during spring the orthopterans food component of young vipers is replaced by lepidopteran larvae, mollusks and ants. The observations of the results tend to show a more extensive diet segmentation in young individuals. The unique ecology regarding the distribution and habitat of *Vipera ursinii* makes bibliographic comparisons with other species questionable and debatable. However, this is in accordance with the well-known peculiarity that snakes undergo ontogenetic diet change. Well-known studies by Saint Girons in the 1980s showed how juveniles consume lizards and adult small mammals. In some cases, and for some species, such as *Vipera ammodytes*, lizards appear to be 100% of the diet in juveniles with decreasing values in more mature specimens (Luiselli, 1996).

According to Baron (1989) and Agrimi and Luiselli (1992) the feeding period is limited to a few months, but our findings seem to indicate that the feeding period start earlier among the Sibillini populations (at the beginning of May) than what was found in Italy by Agrimi and Luiselli (1992) in the *V. ursinii* population of Monte Gran Sasso and Monte Velino. Obviously, the beginning of the feeding period coincides with the end of the wintering and therefore the altitude where these vipers live play a fundamental role to regulate their biological cycle and the beginning of the feeding phase. Thus, the assumptions seem matching well with a different trophic niche, not so common at higher altitudes. The *ursinii* population of the Sibillini mountains live at lower altitudes and latitudes compared to other populations studied (Baron, 1980; Agrimi and Luiselli, 1992) and therefore it is probable that the end of the wintering will be earlier.

We believe that methodological advances derived from the creation of a database of prey traits from the measurements obtained, using reference material collected in the field, can be utilised. The aim is to minimise the possibility of bias in discussion derived from literature data. We used a moderately invasive method to collect gastric samples while faecal samples did not confer handling stress. Palpation-induced regurgitation never caused mortality in snakes.

A modest criticism concerns the application of statistical models to observe the effects of prey availability and prey traits on prey selection and its ecologically plausible relationships.

Last but not least are the new perspectives that these data suggest. To improve the degree of taxa determination, it would be interesting to repeat the research in the same areas and periods with molecular methods or by involving other expertise among the authors. This would provide a solid foundation to be passed on to other populations of the same complex. However, the use of genetic analyses should always be flanked in the interpretation of data by morphological aspects. it is likely that each sample collected will have to undergo a series of preparatory observations in the future to confirm or otherwise the quality of the data. Following these data, it is possible to observe even better the richness of prey and thus of ecological interactions between them. We believe that following these studies highlighting new taxa as prey for highly selective animals, it is important to study the individual relationships between the new groups and predators. This research could be repeated by adding rich-studies on the microfauna present in the vicinity of the vipers collected during the study. this would make it possible to determine how many other animals are present at the same time. all in all, we would have no chance of excluding hidden prey.

In conclusion, this research confirms the dietary spectrum of *Vipera ursinii* but also documented new taxa for this snake: ants, mollusk and caterpillars. From the richness of groups preyed on in the spring season it is presumable to assume that the trophic spectrum is even broader than what has been published. Young vipers have invertebrates at their disposal which are present from spring until the end of summer. This is confirmed by the size of the niche in opilionids, slugs and ants that are actually present regardless of the season. It is likely that if this research was carried out with the help of molecular tools to support the determination of taxa, it could give faster results and with less specialized personnel in determining prey groups. This is particularly valid especially for samples coming from the intestinal tracts. Male *V. ursinii* vipers appear to have less selection in their prey and consume prey from different families even when Orthoptera appear. Generally young specimens that have a more complex diet than adults, the latter occasionally dedicating themselves to predation on small vertebrates which offer a notable energy and protein supply. Considering the observations made and the evidence emerged, it is important that habitat management takes into account the trophic importance of each individual organism that could potentially be preyed upon by this viper.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization G.A.; methodology and validation, N.P.; formal analysis, A.B.; investigation, G.A. and N.P.; data curation, G.A.; writing-original draft preparation, G.A.; writing-review and editing, C.S.; supervision, C.S.

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