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Short Communication

Analysis of mammal mesopredator scat samples indicates significant predation on the endangered Hungarian meadow viper (*Vipera ursinii rakosiensis*)

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Animal populations are regulated by multiple factors, e.g. resource availability, diseases and predators. However, these factors can be significantly influenced by anthropogenic effects (e.g. biological invasions, mesopredator release) and either subserve populations or push them towards local extinction. The Hungarian meadow viper *Vipera ursinii rakosiensis* is an endangered reptile, with only a few surviving populations following recent alterations and fragmentation of their habitats. In Hungary, all documented populations are located within nature conservation areas. Significant practical conservation efforts have been made, including habitat reconstruction and development, as well as ex situ breeding and reintroductions. Nevertheless, the estimated overall population size of Hungarian meadow viper remains very low, and the impact of conservation interventions is practically immeasurable according to low densities. It is hypothesized that predation is the main factor interfering with conservation efforts of the vipers. Here, we analysed the diet of two mammalian predators (European badger and red fox) in a Hungarian meadow viper habitat. We found a high prevalence of viper remains in the processed faecal samples, indicating that a high number of individuals vipers are subject to predation even within a short period of time. We conclude that effective predator control might be necessary to increase the impact of standard conservation measures (e.g. habitat development, ex situ breeding and reintroduction) that target the restoration of Hungarian meadow viper populations.

Keywords: conservation, predation pressure, predator control, reptiles, snakes, wildlife management

Introduction

Meadow vipers (*Vipera ursinii* complex) are grassland specialist reptiles preying mostly on grasshoppers (Baron 1992, Filippi and Luiselli 2004, Mizsei et al. 2019). Their global



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distribution follows the Steppe biome with some habitats located in extra-zonal grasslands in Asia and Europe (Nilson and Andr en 2001). In Europe, meadow vipers are present in the Western Alps and Apennines *Vipera ursinii ursinii*, in the Dinaric Alps *Vipera ursinii macrops*, in the Hellenides mountain ranges of the Balkan Peninsula *Vipera graeca* and in the Pannonian *Vipera ursinii rakosiensis* and Bessarabian *Vipera ursinii moldavica* lowlands in Hungary and Romania, respectively (Mizsei et al. 2018). Lowland populations are threatened by intense habitat alteration, fragmentation and degradation. Nearly all former habitats of meadow vipers have suffered complete destruction, and all former populations of the animals that were situated in Austria and Bulgaria are now extinct, with only a few populations remaining in Hungary and Romania (Kre s ak et al. 2003).

The Hungarian meadow viper *Vipera ursinii rakosiensis* is one of the most threatened reptiles of Europe, listed as Endangered by the IUCN Red List (European Reptile and Amphibian Specialist Group 1996), the subspecies is listed in annex II and IV of the EU Habitat Directive and it is strictly protected in Hungary. A hundred years ago, Hungarian meadow vipers were common and abundant on grasslands and marshlands across the Vienna Basin (east Austria and western Hungary), Turj nvid ek and Kiskuns g (central Hungary) and Transylvanian plains (Romania). While historical density data is not available, several reports mention high densities in the past (Boulenger 1913, L nyi 1957, Dely and Janisch 1959, Janisch 1979, Kors s 2005). Moreover, it is the most common subspecies in natural history collections (87% of 1356 specimens across the five largest reptile collections in Europe), but by the end of the 20th century, only a few populations remained.

In the last two decades, huge conservation efforts and significant improvements have been implemented to enhance the long-term survival of Hungarian meadow vipers in Hungary. Since 2004 two consecutive LIFE projects have been carried out, mainly focusing on habitat reconstructions, ‘viper friendly’ grassland management, ex situ breeding, reintroductions to reconstructed habitats and restocking declining populations (P chy et al. 2015). According to reports of former herp collectors, in the 1970s it was possible to collect 30–40 vipers on an average day (T. P chy pers. comm.). By contrast, currently less than one individual on average is detected during a full-day search. Accordingly, despite intensified survey efforts and conservation interventions, the density of Hungarian meadow vipers is currently considered to be much lower than in historical times (P chy et al. 2015, Mizsei et al. 2020).

In contrast to the population trend of Hungarian meadow vipers, the density of medium-sized predators has increased significantly in the last decades across the entire temperate climate zone (M rton et al. 2016, Krofel et al. 2017). Extinction or decline of apex predator populations caused significant changes in trophic networks, increasing the density of medium-sized predators, as explained by the mesopredator release hypothesis (Soul  et al. 1988). Furthermore,

some mesopredator populations expanded significantly, as a result of vaccination campaigns against rabies (Freuling et al. 2013). Currently, there is limited knowledge on the effect of native mammalian predators on reptile populations (Graitson et al. 2018). However, the available studies show a catastrophic impact of wild boar *Sus scrofa* on adder *Vipera berus* populations and of American badgers *Taxidea taxus* on Agassiz’s desert tortoise *Gopherus agassizii* populations (Emblidge et al. 2015). It was hypothesised that the past abundance of potential viper predators was considerably lower in Hungary (Reason et al. 1993, Heltai et al. 2001), which likely resulted in a low predation pressure on local viper populations. Consequently, the current increase of mesopredator population sizes was proposed to explain the low impact of conservation efforts on viper densities.

Increased predation by mesopredators was hypothesized to be an obstacle in reaching a more favourable conservation status in Hungarian meadow vipers. Nevertheless, to date only a handful, mostly unpublished observations are known on vipers being preyed upon by mesopredators. Most of these were made by biologists, rangers or stakeholders in Hungarian meadow viper habitats. However, no systematic evaluation had been conducted so far to quantify the predation pressure on Hungarian meadow vipers by mesopredators. Therefore, we explore the predation pressure on Hungarian meadow vipers by potential mammalian mesopredators through the analysis of faecal samples. We aimed to answer the following questions: 1) is the Hungarian meadow viper preyed upon by red foxes and/or European badgers? 2) Can predation by red foxes and/or European badgers be considered a significant threat to Hungarian meadow viper populations? We conducted this study to provide basic information for future conservation actions of a LIFE Nature and Biodiversity project application, which is now carried out within the framework of the LIFE HUNVIPHAB ‘Viability improvement of Hungarian meadow viper populations and habitats in the Pannonian region’ LIFE18 NAT/HU/000799 project.

Material and methods

We collected European badger *Meles meles* and red fox *Vulpes vulpes* faecal droppings between June 2018 and April 2019 biweekly at the second largest unfragmented viper habitat in Hungary, at B csa-Bugac homokpuszta HUKN20024 SCI in the Kiskuns g National Park. Sampling was designed to cover all documented viper habitat patches (exact locations are not given due to the strict rules on not providing spatially explicit data related to the occurrence of Hungarian meadow viper to avoid illegal collection for the pet trade). At the beginning of the field data collection period (June 2018) four days were spent searching for European badger latrines and red fox faeces deposition localities by random walking covering 3000 ha of grassland and forest edges. Both of the targeted mesopredator species are known to deposit their faecal droppings at specific sites within their territory. European badgers

allocate few fix sites (badger's latrines), while red foxes use the edge of their territory. Latrines/faeces deposition localities were recorded by GPS and were regularly surveyed during the entire field data collection period. All detected faecal droppings were collected and all data gained from these were included in subsequent analyses.

Faecal samples were kept in dry (in silica gel) and cold (2–5°C) conditions until processing. We have processed the faecal samples following the protocols described by Jędrzejewska and Jędrzejewski (1998): 1) faecal samples were weighed on a balance, 2) food remains were separated and sorted, 3) diet components were identified and grouped, at order-level in the case of most taxonomic groups, and at species-level in case of reptiles and 4) weighed (0.01 ± 0.005 g precision). Diet component grouping was based on the preliminary examination of faecal samples and according to the questions of the study as 1) plants (leaves, remains of fleshy fruits and seeds), 2) invertebrates (earthworm bristles, remains of chitinous exoskeleton of arthropods), 3) non-reptile vertebrates (feathers of birds, hair or bones of mammals), 4) Hungarian meadow viper (body parts, bones and keratinous scales), 5) other reptiles (body parts, bones and keratinous scales of reptiles excluding the Hungarian meadow viper). Identification of snake dorsal scales was done based on the presence or absence of keel: absent in *Coronella austriaca*, present in *Natrix natrix* and *Vipera ursinii*. Keeled dorsal scales and ventral scales were determined to species level using comparative material (shed skins and full carcasses). Bones (vertebrae) were identified based on the key by Venczel (2000). The diet composition of the predators was characterised by calculating the relative frequency of occurrence (%O or RFO) and the percentage of biomass consumed (%B). %O highlights the presence of rare or small and soft-bodied prey which can be found in small quantities. %B is an estimation of the alive prey biomass consumed based on the dry weight of prey item/group/diet component multiplied by a conversion or correction factors (× 14 plants, × 5 arthropods, × 18 reptiles and × 23 mammals) because only limited remains of large-bodied prey go through the digestive system of predators (Jędrzejewska and Jędrzejewski 1998). %O and %B were calculated on a monthly basis throughout the temporary range of sampling in the R statistical environment (<www.r-project.org>).

Results

We detected six European badger latrines, from which the droppings were regularly collected across the whole study season. Red fox droppings were only found during two months (June and August). In total, 69 European badger and 11 red fox faecal droppings were collected and included in further analyses (Table 1).

Remains of *Vipera ursinii rakosiensis* were found in European badger droppings in almost every month, including winter, with 33.3% of detected European badger droppings including viper remains (n=23, Fig. 1). Viper scales were found in 90.1% of red fox droppings (n=10), which most likely originated from at least three different red fox individuals. Based on the size of scale and vertebrae remains, potentially 86.7% of remains belongs to adult (88.5% in European badger and 11.5% in red fox samples) and 13.3% of remains belongs to subadult or juvenile (75% in European badger and 25% in red fox samples) vipers. These results provide clear evidence for a significant predation pressure on Hungarian meadow viper by both mammalian mesopredators inspected here. Assuming that viper remains found in faecal droppings could have belonged to different viper individuals, our results suggest that at least 34 Hungarian meadow vipers have been subject to predation by European badgers and red foxes at the study site, considering only the droppings analysed here.

The temporal pattern of observed diet composition of European badgers varied across the study season: arthropods were present in faecal droppings during summer and early autumn and were absent in colder months (Fig. 2). The main arthropod preys found in European badger faecal droppings were beetles *Polyphylla fullo* (including larvae), *Copris lunaris* and Orthopterans not determined to lower taxonomic level. No viper remains were detected in European badger faecal droppings collected in June 2018, but during all the other months Hungarian meadow viper remains were frequent in European badger droppings. Other reptile species (*Podarcis tauricus*, *Lacerta agilis*, *Lacerta viridis*, *Coronella austriaca*) were preyed upon in late summer and during the winter hibernation period along with Hungarian meadow vipers. According to our calculations, vipers represented 3.8% of the fresh weight of biomass consumed by the European badger during the sampling period. We were unable to assess the

Table 1. Number of collected faecal droppings during the study period.

	June 2018	July 2018	August 2018	September 2018	October 2018	November 2018	December 2018	January 2019	February 2019	Total
Red fox										
No. of detected faecal droppings	9	0	2	0	0	–	0	–	0	11
No. of faecal droppings including viper remains	8	0	2	0	0	–	0	–	0	10
European badger										
No. of detected faecal droppings	1	7	34	9	10	–	3	–	5	69
No. of faecal droppings including viper remains	0	3	14	1	3	–	1	–	1	23



Figure 1. Healed injury on a living Hungarian meadow viper (A), viper preyed and vomited back by red fox (B), viper dorsal and ventral scales in European badger faeces as found in the field (C), viper scales and bones in European badger faeces (D–E), viper and *Polyphylla fullo* remains in European badger faeces (F).

seasonal change in the diet of red foxes due to the limited sample size, however, faecal samples showed a very high prevalence of reptiles in the diet of red foxes.

Discussion

The Hungarian meadow viper is a frequent prey item of European badgers and red foxes, as highlighted by a high prevalence of vipers in the analysed faecal droppings of both predators. Our results support the assumption that common mammalian mesopredators regularly prey upon Hungarian meadow vipers, despite its rarity and elusive nature. Populations become threatened with extinction when mortality is no longer balanced by recruitment, for example, due to a decrease in habitat quality or an increase in predation pressure (Peterson 1994). The negative impact of the increase in predation pressure by non-native and invasive mammalian

predators is well documented (Hilton and Cuthbert 2010), but native mammal species could also drive devastating declines in prey populations (Kurz et al. 2011, Darst et al. 2013, Dinkins et al. 2016). Predation control and management of predation risk could significantly contribute to the recovery of at-risk species (Darst et al. 2013, Kirkwood et al. 2014, Nafus et al. 2017).

Limitations of our study are the narrow spatial and temporal extent of the sampling, as well as the inclusion of only two predator species. We were only able to study a single large habitat of Hungarian meadow vipers, thus there is a lack of spatial replication of the sampling. Hence, our results should be interpreted and generalised with caution. Samples analysed here covered a nine-month long period and we were able to assess the seasonal variation in the diet of European badgers. However, red fox scat samples were very limited, hence our results regarding the diet of red foxes represent only a snapshot, limiting the ability to infer seasonal patterns,

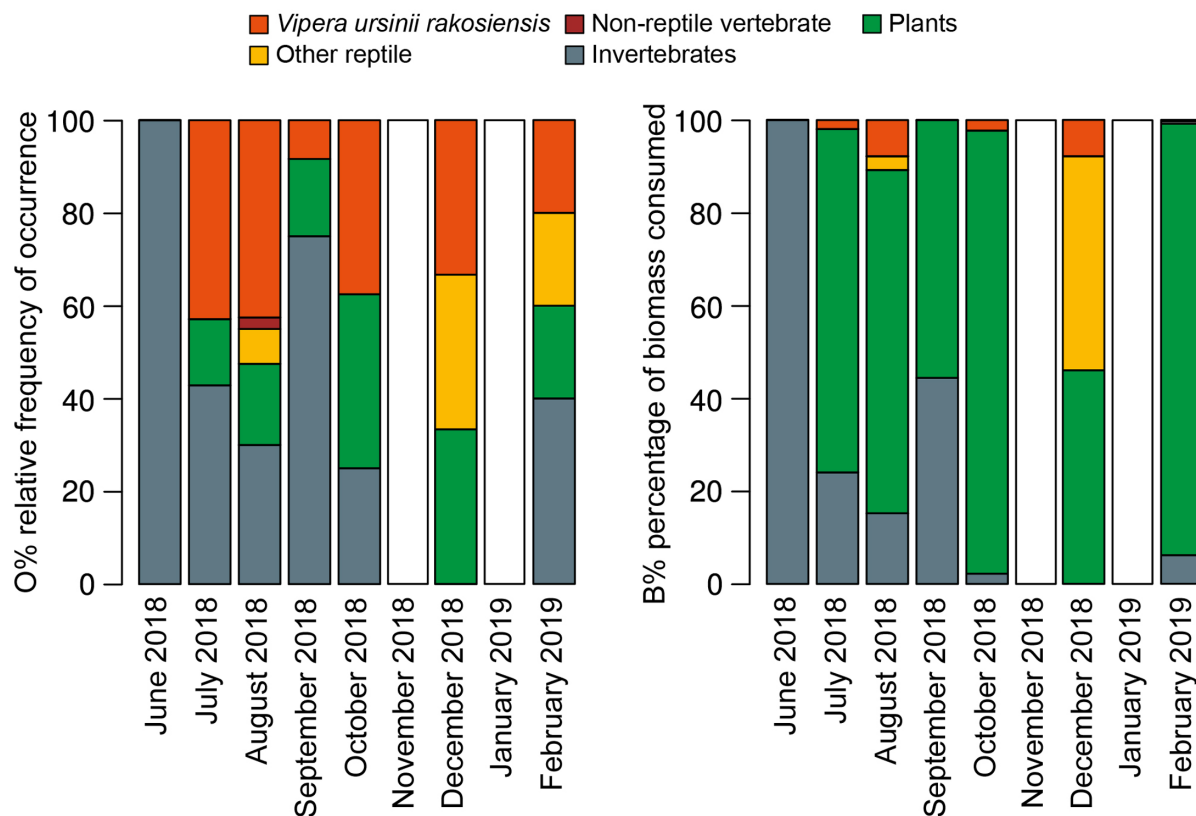


Figure 2. Monthly variation in diet composition of European badgers. White bars indicate missing data due to missed samplings.

but were enough to confirm the presence of vipers in their diet. Moreover, we had to terminate sampling sooner than planned, because based on our preliminary results the decision was made to enhance predator control at the study site. Lastly, in the present work, we only focused on mammalian mesopredators. However, predation by avian raptors could be significant as well, since avian predators are known to be important regulators of reptile populations (Selås 2001), thus our study did not cover all possible predators of Hungarian meadow vipers.

The temporal pattern of feeding habits of European badgers reveal that vipers may be preyed upon throughout the entire year, and it is not limited to the active period of snakes. Viper remains found in European badger droppings collected during winter months, suggest that European badgers dig up hibernating reptiles. This behaviour could be a significant threat as Meadow vipers that are known to overwinter in small groups, suggesting that multiple individuals can be killed by single European badgers. Hibernaculum availability is a recognized limiting factor in habitat occupancy (Graves and Duvall 1995), and digging by European badgers can physically destroy important hibernacula. Preying on hibernating reptiles is also supported by our observation of remains of at least 19 Balkan wall lizard *Podarcis tauricus* individuals in a single European badger faeces in December, collected within the framework of the present study. The absence of other snake species in the examined samples can

be explained by the relative rarity of other snakes present at the study site (*Natrix natrix*, *Coronella austriaca*) compared to the Hungarian meadow viper, but also by the anti-predatory behaviour of *N. natrix* (Gregory et al. 2007).

The results of our study suggest that predation by mammalian mesopredators could be one of the most important current threatening factors for the studied Hungarian meadow viper populations. According to the data presented in the standard data form (SDF) of the studied Natura 2000 site, the overall size of the local Hungarian meadow viper population is estimated to be 70–110 individuals (<<https://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=HUKN20024>>), but see below. Fatalities caused by mammalian mesopredators (at least 34 viper individuals in our samples within a nine month period), indicates that predation likely affects a very significant portion of the local viper population. The number of viper individuals preyed upon during our sampling is still just an estimate and represents the minimum number of individuals killed. Furthermore, it is possible that not all mammalian mesopredator territories were identified and sampled within the study area. We hypothesize that a considerably higher number of viper individuals had been preyed during the study period than 34 individuals.

Hungarian meadow vipers have other predators besides mammalian mesopredators. Consequently, based on the recorded fatalities, it is likely that the size of the local viper population exceeds considerably the population size formerly

estimated. Estimation of the population size of elusive reptiles is often challenging, because of their low detectability and restricted activity (Durso et al. 2011, Erb et al. 2015). Newly collected data suggest that the population size of Hungarian meadow vipers is at least two orders of magnitude larger than previous estimates, as indicated by the SDF (Móré et al. unpubl.). Otherwise – if truly 31–48% of the viper population was subject to predation within nine months – the local viper population would likely face an instant, predation-driven extinction. Problems caused by habitat loss and degradation have already been eliminated at important Hungarian meadow viper habitats. Consequently, we suggest that reduction of predation pressure could now help to achieve significantly increase the size of the local viper populations.

Due to the lack of adequate data it is not possible to estimate demographic trends of Hungarian meadow viper populations inhabiting the study site or the impact of European badger and red fox predation. As a proxy, the increased time demand of viper detection (i.e. the average amount of search time necessary for detecting one viper, unpublished data) may indicate a decline in population size despite ‘viper friendly’ habitat management and other conservation actions. To enhance population growth and to contribute to the success of future reintroductions of Hungarian meadow vipers to reconstructed habitats, effective predation control is highly recommended. European badgers and red foxes are not protected by law and can be legally hunted throughout the year in Hungary. Both species can be effectively trapped or excluded by fencing (Kirkwood et al. 2014). A controlled study with a full block design should be carried out to test the effect of predation control on the demography of Hungarian meadow viper populations. Moreover conducting a population viability analysis is highly recommended.

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Author contributions

Attila Móré: Conceptualization (equal); Data curation (equal); Investigation (equal); Methodology (equal). **Edvárd Mizsei:** Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (equal); Methodology (equal). **Csaba Vadász:** Conceptualization (equal); Methodology (equal). **Béla Tóthmérész:** Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal). **Miklós Heltai:** Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.bcc2fqzdx>> (Móré et al. 2022).

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