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A preliminary study on population characteristics and ecology of the critically endangered meadow viper *Vipera ursinii* in the Romanian Danube Delta

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Abstract: The present paper discusses preliminary data on population characteristics and ecology of the meadow viper *Vipera ursinii* in the Romanian Danube Delta. Using line transects and the Distance software, the size of the studied population was estimated at 321 (95% confidence interval: 166–618) individuals on a 62 ha area. The sex-ratio of the population was close to 1:1 and juvenile specimens were well represented. Half of the captured adult females were gravid, possibly indicating a more than annual reproductive cycle. Analysis of sexual dimorphism of 12 morphometric traits indicated significant differences only in tail length and height. No significant relationships were detected between the size, sex, age or reproductive status and the thermal ecology of the vipers. Microhabitat selection and activity patterns varied with age, sex and reproductive status and are probably linked to an onthogenetic shift in feeding ecology and to behavioral differences between reproductive and non-reproductive females.

Key words: snakes; population size; sexual dimorphism; reproduction; microhabitat selection

Introduction

Snakes exhibit many of the biological characteristics which increase an organism's proneness to extinction (e.g., Seigel et al. 1987). This, together with the ongoing pressures of habitat loss and intentional human persecution, rendered European snakes, especially venomous species, particularly vulnerable (e.g., Santos et al. 2006; Ursenbacher et al. 2009). The meadow viper *Vipera ursinii* (Bonaparte, 1835) has a wide but fragmented range, covering parts of Italy, France, Hungary, Romania and the Balkan Peninsula and is considered extinct from Bulgaria, the Republic of Moldova and Austria (Nilson & Andrén 2001; Edgar & Bird 2006). *Vipera ursinii* is considered to be the most endangered viper in Europe (Edgar & Bird 2006), being labeled as vulnerable in the IUCN Red List, included in Annex II of the European Habitat Directive and listed in CITES Appendix I. Consequently, the species has been the subject of several ecological studies throughout its range (e.g., France – Lyet et al. 2009; Italy – Luiselli et al. 2006; Hungary – Ujvari et al. 2000; Montenegro – Tomović et al. 2004).

In Romania, *V. ursinii* reaches its easternmost range limit and is currently known from three distinct and isolated lowland regions: north-eastern Romanian Moldavia (east of Carpathian Mountains), Transylvania (within the Carpathian Mountain basin) and the Danube Delta (for review see Krecsak & Zamfirescu 2008). At a national level, the species is listed as critically endangered in the Red Data Book of Romanian Vertebrates (Iftime 2005). Whilst several ecological studies have been conducted on the two populations from Romanian Moldavia (Krecsak & Zamfirescu 2001; Krecsak et al. 2003; Zamfirescu & Krecsak 2002; Zamfirescu et al. 2007, 2008, 2009) and some ecological data were given about the Transylvanian population (Ghira 2007), the deltaic meadow viper populations are known only from very scarce and purely speculative data (Török 2002), with the exception of one population studied within a wider herpetofaunal survey of the Perişor-Periteaşca area, for which population size and density were estimated (Kotenko & Oţel 1997). This paper aims to present preliminary data on population size and structure, density, microhabitat selection and morphology of *V. ursinii* from the Romanian Danube Delta.

Material and methods

Our study was carried out in the Danube Delta Biosphere Reserve, on the marine levee of Sărăturile, near the village of Sfântu Gheorghe (Tulcea County, Romania), in a 62 ha site located about 1 km from the Black Sea (44°54′

Table 1. Descriptive statistics of body mass, morphometric characters and substratum temperatures for adult and immature male and female meadow vipers: mean ± standard deviation (min.–max.).

	Males		Females	
	Adults ($n = 6$)	Immature ($n = 3$)	Adults ($n = 6$)	Immature ($n = 2$)
BM (g)	44.68 ± 10.17 (32.5–56.4)	12.06 ± 1.3 (10.6–13.1)	74.8 ± 32.21 (38.3–127.2)	13.55 ± 3.04 (11.7–15.7)
LTot (cm)	45.66 ± 3.27 (40.7–49.4)	28.2 ± 0.5 (27.7–28.85)	46.08 ± 5.55 (37.2–53)	28.9 ± 0.70 (28.4–29.4)
SVL (cm)	39.56 ± 2.96 (35.5–43)	24.66 ± 0.73 (24.1–25.5)	41.95 ± 4.61 (35–48)	25.85 ± 0.49 (25.5–26.2)
TL (cm)	6.1 ± 0.48 (5.2–6.5)	3.53 ± 0.16 (3.35–3.6)	4.13 ± 1.03 (2.2–5)	3.05 ± 0.21 (2.9–3.2)
HL (mm)	17.15 ± 2.05 (15.1–20)	13.33 ± 0.49 (13–13.9)	18.16 ± 1.77 (16.2–20.9)	12.85 ± 0.91 (12.2–13.5)
HW (mm)	14.48 ± 0.45 (14–15.2)	11.13 ± 0.7 (10.5–11.9)	15.98 ± 1.95 (13.9–18.1)	11.15 ± 0.91 (10.5–11.8)
HH (mm)	7.08 ± 1.31 (5.4–8.9)	4.73 ± 0.64 (4–5.2)	6.75 ± 0.94 (5.1–8)	5.7 ± 1.13 (4.9–6.5)
BW (mm)	14.83 ± 1.42 (13–16.5)	9.33 ± 1.15 (8–10)	16.96 ± 2.3 (13.9–20)	9.75 ± 0.35 (9.5–10)
BH (mm)	15.48 ± 0.96 (14.1–17.1)	8.86 ± 1.26 (7.5–10)	17.06 ± 1.79 (14.8–19)	10.5 ± 0 (10.5–10.5)
NW (mm)	7.53 ± 1.25 (6.2–9.5)	5.23 ± 0.58 (4.8–5.9)	7.38 ± 0.83 (6.1–8.5)	5.75 ± 0.35 (5.5–6)
NH (mm)	9.05 ± 1.22 (6.9–10)	5.63 ± 0.55 (5–6)	9.25 ± 1.29 (7.5–11)	6.75 ± 0.35 (6.5–7)
TW (mm)	6.1 ±1 (5–7.3)	3.13 ± 0.05 (3.1–3.2)	5.06 ± 0.8 (4–6.1)	3.8 ± 0.98 (3.1–4.5)
TH (mm)	6.8 ± 0.7 (6–7.9)	4.06 ± 0.95 (3.1–5)	5.56 ± 1.07 (3.9–7.1)	4.25 ± 0.49 (3.9–4.6)
T (℃)	28.68 ± 3.47 (24.8–34.1)	29.2±0.79 (28.3–29.8)	29.11 ± 4.08 (24–33.7)	31.25 ± 2.47 (29.5–33)

Explanations: For abbreviations see Material and methods.

N, 29°35′ E; ca. 0 m a.s.l.). Sandy soils with characteristic xerophilous vegetation (most common plant species: *Juncus littoralis, Festuca valesiaca, Cynodon dactylon, Bromus tectorum, Elymus elongatus, Salicornia europaea, Suaeda maritima* and *Eleagnus angustifolia*) prevail. The climate of the area is semi-arid, with mean annual temperatures and precipitations of 11.4℃ and 403.6 mm, respectively (Gâştescu & Ştiucă 2006).

Field research was conducted daily between 12th and 16th July 2008 and between 07:00 and 15:00 h. Snakes were captured by hand or with a herpetological hook along line transects. Vipers which exceeded 30 cm (males) or 35 cm (females) in snout-vent length (SVL) were considered adults according to the pattern observed in the Moldavian meadow vipers (Strugariu & Zamfirescu, unpublished data). All snakes were sexed, weighed (body mass: BM) with a digital scale to the nearest 0.1 g, measured for 12 metric characters with a string (to the nearest 0.1 cm: total length – Ltot; snout-vent length – SVL; tail length – TL) or with digital calipers (to the nearest 0.1 mm: head length – HL; head width – HW; head height – HH; body width at midbody – BW; body height at midbody – BH; neck width – NW; neck height – NH, tail width at the base of the tail – TW; tail height at the base of the tail – TL), individually marked by a unique combination of ventral scale clippings, photographed, and released at the exact place of capture. The reproductive status (gravid or non-gravid) of adult females was checked by gentle palpation of the ventral side. Also, for each captured snake, we recorded the geographical coordinates with a handheld GPS and the following environmental variables: substratum temperature (to the nearest 0.1℃), vegetation type (1 – Ass. *Holoschoeno-Calamagrostetum epigeios*; 2 – Ass. *Juncetum littoralis*), time of capture (expressed in hourly intervals: 07–08H; 08–09H; 09–10H; 10–11H), nebulosity (sunny, cloudy or partially cloudy), microhabitat exposure (S Exposure; S-W Exposure; E Exposure, W Exposure) and elevation of the microhabitat ("low area" if a snake was found at the base of a sand dune or "high area" if it was found on top of one).

A rough estimation of the population size and density was made through line transect surveys and Distance 5.0 software (Thomas et al. 2005), a method frequently used when studying species which occur in homogenous landscapes (e.g., Luiselli 2006). Patterns of daily activity and microhabitat selection were investigated using multiple correspondence analyses (MCA), for which vipers were categorized according to age (adult or immature), sex and reproductive status (only for females : gravid or non-gravid). All the previously mentioned environmental variables and variable states, with the exception of substratum temperature were used in MCA. All parametric assumptions were verified prior to using parametric tests. All statistical analyses were performed with XLSTAT-Pro software.

Results

Seventeen vipers were captured during the study pe-

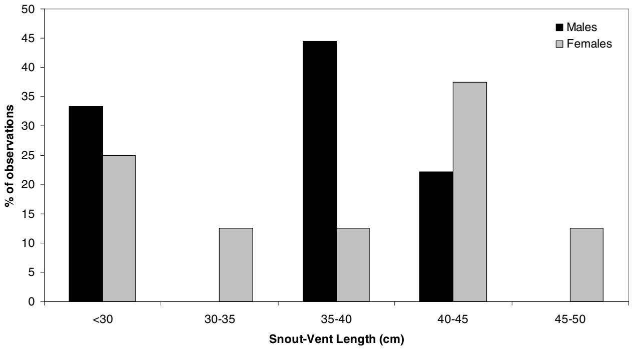

Fig. 1. Observed size structure of the *Vipera ursinii* population.

riod. Population size and density were estimated at 321 (95% confidence interval: 166–618) individuals and 5.21 (95% confidence interval: 2.7–10.05) ind./ha. The observed male to female ratio was 1.125 : 1, which does not differ significantly from the expected 1 : 1 (χ^2 test: $\chi^2_{\text{Yattes}(1)} = 0.238$, $P = 0.625$). Half ($n = 3$) of the captured adult females were gravid. The observed size structure of the viper population is given in Fig. 1.

Descriptive statistics of body mass, substratum temperatures and the twelve morphometric characters are given in Table 1. There were significant differences between the body mass of adult males, adult gravid and adult non-gravid females (Single-Factor ANOVA: $F_{(2,9)} = 13.102$; $P < 0.01$.). Tukey HSD post-hoc test revealed that gravid females were significantly heavier than non-gravid ones ($P < 0.001$) and adult males ($P < 0.01$). No significant differences were found between the body mass of non-gravid females and adult males ($P = 0.859$). Student's t test revealed that adult males were superior to females in tail length ($t_{(10)} = 4.208$, $P < 0.01$) and tail height ($t_{(10)} = 2.354$, $P < 0.05$) and that there are no significant differences between any of the other recorded characters ($P > 0.05$ for all comparisons). Analysis of covariance (ANCOVA) with substratum temperature as dependent variable, SVL as quantitative variable, and sex and age as qualitative variables showed that the substratum temperature is not significantly related to the age, sex or size of the individuals ($F_{(6,10)} = 1.608$, $P = 0.242$).

MCA showed that, during the survey period (mid-July) immature specimens displayed similar patterns regardless of sex but were quite different from adult vipers. MCA revealed a relevant separation between the gravid female vipers, which were active in the early morning, in low areas and in microhabitats with vegetation type 2 while non-gravid females were more active during later hours and only in vegetation type 1 (Fig. 2).

Discussion

The estimated size of the studied population is similar to the estimations made for one (from "Dealul lui Dumnezeu") of the two studied meadow viper populations from Romanian Moldavia but lower than the other one (from "Valea lui David"; Zamfirescu et al. 2008). However, the density estimated for the population described here is much lower than that described from

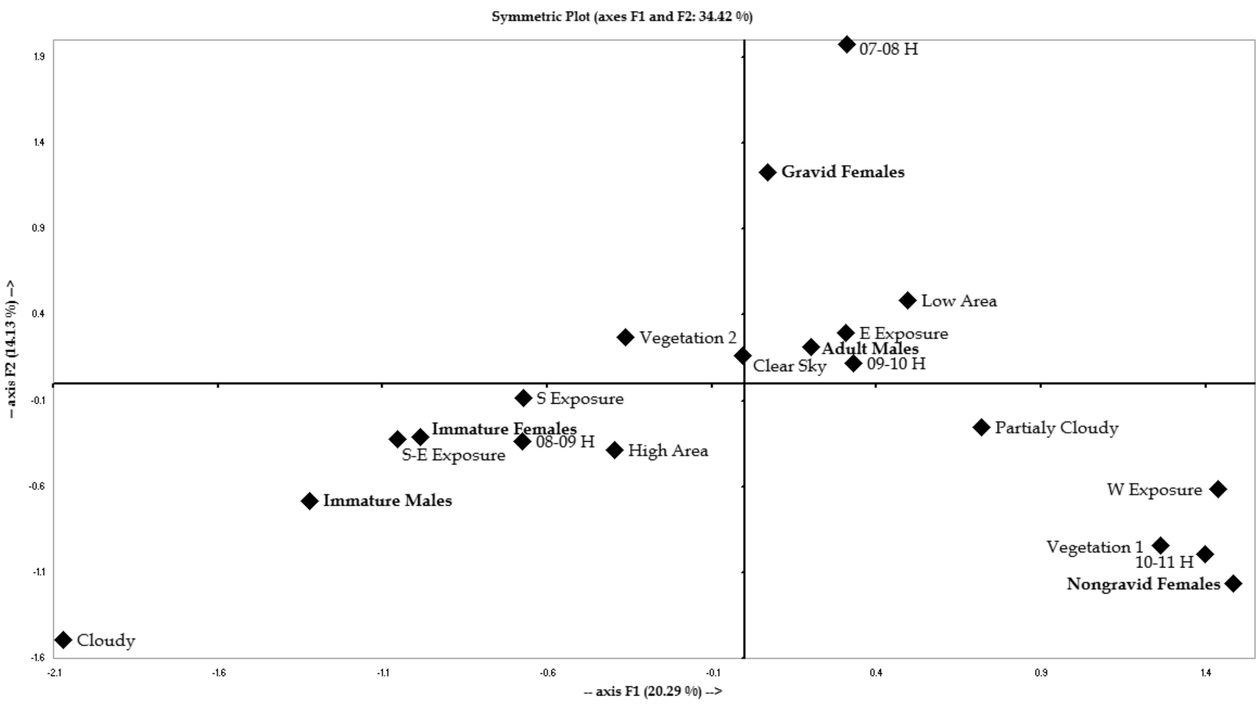

Fig. 2. Associations of microhabitat variable and *V. ursinii* observations in the space described by the first two correspondent axes of multiple correspondence analysis.

Romanian Moldavia (Zamfirescu et al. 2008). Kotenko & Oţel (1997) gave a rough estimation of 2,200 meadow viper specimens and an average density of 10 specimens/ha for the Perişor-Periteasca population, values which greatly exceed our estimations for our study area. Török (2002) estimated the population size of *V. ursinii* in our study site at ca. 800 individuals. However, given our results and the fact that Török (2002) mentioned no method and sample, we consider the estimation's validity to be highly unlikely. The observed size structure of the studied population, through the high frequency of immature specimens, may indicate a breeding (persistent) population, as the apparent absence of juvenile and subadult snakes signals a rapidly decreasing population (e.g., Nilson & Andrén 2001; Halpern & Péchy 2002).

The relatively small number of females with a SVL between 30 and 40 cm could either indicate a higher mortality rate for this age/size group or a sampling bias due to lower detection rates. The fact that only half of the captured females were gravid during midsummer could indicate that the deltaic meadow vipers follow a biennial reproductive cycle, the pattern most frequently reported in European vipers (e.g., Mallow et al. 2003). However, long term capture-mark-recapture data are necessary in order to test this supposition. A biennial female reproductive cycle has also been observed in mountain *V. ursinii* populations (France – Baron 1997; Italy – Luiselli 1990) while lowland *Vipera renardi* (Christoph, 1861) populations from the Crimea (Ukraine) and other Ukrainean lowland areas appear to present an annual reproductive cycle (Kukushkin 2004; Kotenko & Kukushkin 2008). So far, no precise data exist with regard to the reproductive cycle of other Romanian *V. ursinii* populations. The minimum recorded total length of a gravid female in the studied population was 47.6 cm, which exceeds the value given from mountain *V. ursinii* populations from Montenegro (34.5 cm – Tomović et al. 2004) and France (31.5 cm – Baron 1997). The heaviest gravid female in our study (127.2 g) greatly exceeded the values from Montenegro (82 g – Tomović et al. 2004) and France (84.5 g – Baron 1992). The heaviest adult male in our study weighed 56 g, being similar to the maximum value from France (58 g – Baron 1992) but lighter than that from Montenegro (72 g – Tomović et al. 2004).

Sexual size dimorphism (SSD) has not been adequately studied in *V. ursinii*. In the studied population, females were slightly, but not significantly longer (total length and SVL) than males. Tomović et al. (2004) gives higher values for length in males than in females in a population from Montenegro; Luiselli et al. (2006) reported that in Italian populations, females are significantly longer than males while Zamfirescu et al. (2008) also reported no significant differences between the lengths of male and female *V. ursinii*. It is widely believed that the occurrence of male-to-male combat and the fecundity advantage of larger size in females play a strong influence on SSD (e.g., Shine 1994). The intra-specific variation in SSD which is apparently present in *V. ursinii* could make this species a useful model organism in future detailed testing of evolutionary hypotheses concerning the presence of this trait in viviparous snakes. The males in our sample had significantly longer and higher tails than females, a commonly encountered sexual dimorphic trait in snakes, usually linked to male reproductive success (Shine et al. 1999).

Substratum temperatures are usually strongly correlated with cloacal temperatures in snakes (e.g., Ujvari & Korsos 1997; Tomović et al. 2004). Thus, even in the absence of data on cloacal temperatures (such as this case), substratum temperatures could be used to investigate potential ontogenetic and sexual differences in thermal ecology. Our analysis, however, indicated no such differences during the study period. Nevertheless, this may result from the small size of the studied subsamples and short period of investigations (mid-July only). Comparisons between the body and substratum temperatures of reproductive versus non-reproductive female *V. ursinii* in Montenegro also showed no significant differences (Tomović et al. 2004).

Patterns of activity and microhabitat selection in reptiles vary according to different environmental (e.g., temperature, humidity, light) and individual biological (e.g., sex, age, reproductive status) factors (e.g., Brito 2003; Crnobrnja-Isailović et al. 2007; Simonov 2009). Our analysis has shown that immature specimens were clearly separated from adults in MCA but no inter-sex differences were revealed within the immature group. The ontogenetic differences in microhabitat use and activity patterns in reptiles are often explained through a dissimilar thermal preference between age groups (e.g., Carretero et al. 2006; Simonov 2009). However, due to the non-significant ontogenetic differences in thermal ecology shown above, we consider that an ontogenetic difference in feeding habits is the most probable responsible factor. An ontogenetic shift in feeding ecology is frequent in several snake species, with immature specimens feeding on smaller-sized prey of different taxa and ecological groups (e.g., Luiselli et al. 2000; Valdujo et al. 2002). Thus, although there are no available data on the feeding ecology of *V. ursinii* in the Danube, it is highly probable that the previously mentioned feeding characteristics also apply to this population and that the immature vipers select the most suitable microhabitats for acquiring their specific prey. The fact that gravid and non-gravid females were associated with distinct vegetation types could be explained by the main summer activities of these categories. Gravid female vipers spend most of their time actively basking in more open areas and feed only opportunistically (e.g., Luiselli & Agrimi 1991; Bonnet et al. 2001; Crnobrnja-Isailović et al. 2007) while non-gravid females forage actively to accumulate sufficient reserves for reproduction in the forthcoming year (e.g., Bonnet et al. 2001). Thus, vegetation type 2 (Ass. *Juncetum littoralis*), which is sparser (40–100 % vegetation cover – unpublished data) seems to offer more favorable conditions for basking gravid females, whereas vegetation type 1 (Ass. *Holoschoeno-Calamagrostetum epigeios*) which is much denser (90–

100% vegetation cover – unpublished data) is most probably a more favorable foraging habitat for adult vipers. However, future detailed studies on the feeding ecology of *V. ursinii* in this population and on prey abundance across the different microhabitats are needed in order to verify these assumptions.

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