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Short Communication

What has happened to the females? Population trends in the Aesculapian snake at its northern range limit

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ABSTRACT

Populations at the edge of their main range of distribution are often exposed to suboptimal environments. They therefore exhibit a greater susceptibility towards habitat changes and often clearly differ in their structure and dynamics from populations in the main range. Here we define population parameters of the Aesculapian snake *Zamenis longissimus*, a species endangered in Europe, at its northern range limit based on data obtained in the valley of the River San (Bieszczady Mts., SE Poland) in 2009–2013. We focus on the spatial and temporal sex ratio patterns by comparing data obtained in 2009–2013 with published records from 1990 to 1998 and five other northern populations (1 from the main range, 1 at the northern edge of its contiguous range, and 3 entirely isolated ones). Using the capture-mark-recapture (CMR) method, we estimated the population size to be about 230 snakes. Our data show that among adults the percentage of females (17.74%) in the San valley population was significantly lower than in those other five populations and the same population studied two decades earlier. Because of the male-biased sex ratio, we estimated the effective population size to be about 74 snakes. This disproportion between the sexes in adults may be interpreted as an early stage of the population extinction process at the northern range limit of this species' distribution. It is probably due to the limited availability of egg-laying sites, compelling females to undertake longer movements, which may heighten the risk of mortality.

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1. Introduction

One of the key questions of conservation biology is what traits render a species prone to extinction (Foufopoulos and Ives, 1999). Many extinctions can be explained by habitat loss, small populations, occurrence at the main range limits, isolation, and specialized habitat requirements (Webb et al., 2002). However, the processes causing populations to dwindle, especially

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at species' range limits, are poorly understood; this also applies to snakes, vertebrates that are in worldwide decline (Dodd, 1993; Gibbons et al., 2000; Reading et al., 2010). Populations at the limits of their ranges are additionally exposed to sub-optimal environmental conditions, which makes them particularly vulnerable to changes in the environment (Kurek et al., 2018; Reinert, 1993). Because of their dependence on warmth and in the context of climate change, reptiles can make an important contribution to our understanding of the dynamics of peripheral populations. At their northern distribution limits, reptiles exhibit a strong affinity for anthropogenic habitats (Martino et al., 2012; Kurek et al., 2018), which may influence their population size and reproductive success, and consequently, their viability (Hagman et al., 2012).

Population decline is usually discussed in terms of a drop in population size. But the viability of populations is also strongly influenced by their sex ratio (Reed et al., 2003). In species with genetic sex determination, as in all snakes (Bull, 1980), this is assumed to be set at 1:1 at birth (Fisher, 1999; Shine and Bull, 1977). But the sex ratio as determined by genetic inheritance may not necessarily be tantamount to the sex ratio of juvenile and adult snakes, since sex-specific mortality may occur in both the prenatal and postnatal stages (Burger and Zappalorti, 1988). Such possible deviations in the sex ratio of snake populations, when analysed in the context of age (Lind et al., 2005), may provide crucial insight into their dynamics, and further, into the risk of their extinction. Thus, the sex ratio in snake populations should be considered in different life stages: (1) at fertilization, (2) at birth, and (3) at the time of reaching sexual maturity (Burger and Zappalorti, 1988). The best way of determining the sex ratio at different life stages in the field (without keeping pregnant females in captivity) is to place them in juvenile, subadult and adult age classes (Lind et al., 2005; Webb and Shine 1998). This is due, e.g. to the differential activities of snakes during growth, which leads to differences in mortality in different age/size classes and between the sexes (Seigel et al., 1987). The costs of reproduction and associated changes in movement rate in different age classes may also affect the sex ratio in adults (Hyslop et al., 2012; Jayne and Bennett, 1990; Shine, 1980; Madsen, 1987; Seigel et al., 1987; Bonnet et al., 1999; Shine and Bonnet, 2009). To date, however, the association between sex ratio and population dynamics in snakes remains unexplored.

Populations of the Aesculapian snake *Zamenis longissimus* at its northern range limit in central Europe can be characterized as small, isolated and critically endangered (Edgar and Bird, 2006). According to Reed and Shine (2002) description of snake species disproportionately sensitive to habitat disturbance, this European oviparous snake has a relatively large body size, a sedentary life style, a low reproductive investment, a tendency to form aggregations, and – in suboptimal conditions – a late onset of the breeding season and oviparity (Kurek et al., 2018). Detailed and reliable demographic data are assumed to provide a basis for understanding the life history and ecology of a species (Stanford and King, 2004) and to predict their declines before they occur (Reed and Shine, 2002). Hence, the main aim of the present study was to define the size, age structure and sex ratio of the Aesculapian snake population in Poland. We also compared the sex ratio of adults with data obtained in the same population two decades earlier and with five other northern European populations (three isolated ones, one at the northern edge of its contiguous range and one in the centre of this range).

2. Methods

The study area (ca 25 km²) was situated in the valley of the River San in the Bieszczady Mountains (SE Poland), where the most numerous populations of the Aesculapian snake in Poland occur (for a detailed description, see Kurek et al., 2017, 2018, Fig. 1a, b). Capture-mark-recapture (CMR) studies were conducted from 2009 to 2013. Snakes were captured by hand in 20 monitoring localities and on transects between them; in 17 of these localities artificial reproduction mounds were established at 250 m intervals on average (detailed description in Kurek et al., 2017, 2018). The survey area was monitored every two weeks or so from April to September (39 visits in 2009–2013; 6, 7, 9, 9 and 8 visits in the successive years) by 2–6 persons, i.e. when the snakes were active and in similar weather conditions (detailed description in Kurek et al., 2017, 2018). The average area of the monitored localities was about 3 ha: we searched for the snakes along ecotones, in their potential hiding places, and on breeding mounds. The date and geographical coordinates of each snake encounter were recorded. The animals were individually marked by ventral scale clipping (Brown and Parker, 1976). The snout-vent length (SVL) and tail length (TL) of each specimen were recorded. To determine the sex of individuals we used a non-invasive method, i.e. we examined the tail shape: males have a characteristic thickening of the body near the cloaca, well visible in individuals with a total length of over 60 cm.

Based on total body length, each individual was assigned to one of three age classes: juvenile, subadult and adult. The Aesculapian snake reaches sexual maturity on reaching a total body length of 90 cm (age 4 + years; Heimes and Waitzmann, 1993); such individuals were considered to be adults. Individuals from 40 to 89 cm long (age 2–4) and less than 40 cm long (age 1–2) were considered to be subadults and juveniles respectively.

Male snakes have longer tails relative to the body length ($RTL = TL/SVL$; King, 1989) than females, a feature visible even in hatchlings, which is helpful in determining the sex of juveniles or unsexed individuals (Madsen and Shine, 1992). We tested these differences between sexes using one-way ANOVA. To calculate the sex ratio between the different life stages, the sex of 22 unsexed individuals in total (mostly juveniles and subadults) was determined using RTL in Discriminant Analysis (DA). The discriminant function was obtained using 111 RTL values (19 females and 92 males).

Based on the home range of this species (3 ha; Lelièvre et al., 2011, 2012, 2013; Naulleau and Bonnet, 1995), we grouped the localities of snake observations into clusters <1 km apart. In this way we distinguished seven areas (labelled A to G; Fig. 1B) in order to illustrate the spatial distribution of sites occupied by this species and the abundance of males, females and new-born snakes, including individuals whose sex was determined using DA.

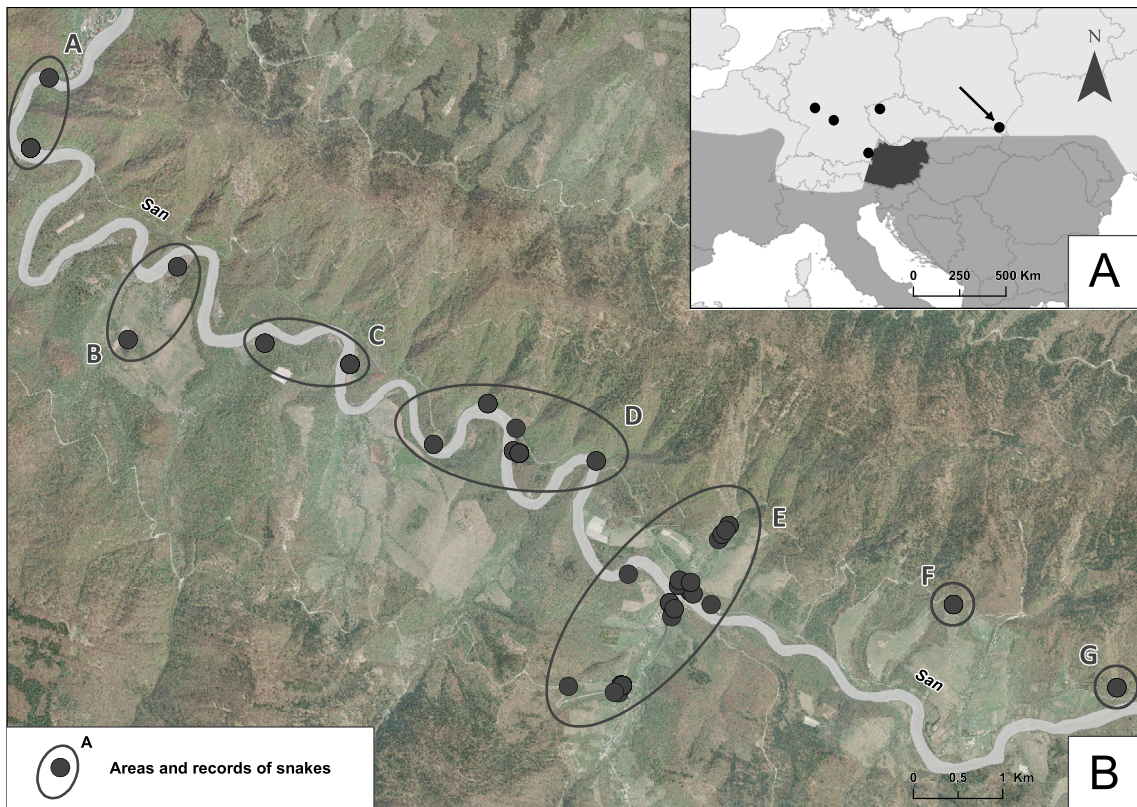


Fig. 1. (A) Geographical range of the Aesculapian snake in central Europe; isolated populations are shown by black dots, the population in Austria by the large black patch (based on Musilová et al., 2007). The location of the study area in the Bieszczady Mts. (SE Poland) is indicated by the arrow; (B) Spatial distribution of sites occupied by the Aesculapian snake in the study area (San valley, Bieszczady Mts.) with the 7 areas highlighted (labelled consecutively from A to G), 2009–2013.

The sex ratio of adults was compared to the sex ratio obtained in the same population in 1990–1998, to three other isolated northern populations (Germany – Odenwald, Rheingau-Taunus, and the Czech Republic – Karlovy Vary), to one at the northern edge of its contiguous range in Germany (the Danube valley near Passau) and to one in the centre of this range in Austria (Table 3). We used data from the age structures in the length classes to compare the sex ratio of the adults in each population. We excluded our research data for 2009 and 2013 from this comparison because in 2009 we only started the season in June, and in 2013 we only monitored localities with artificial egg laying sites.

The differences in the sex ratios between populations and in the sex ratios between the age classes were tested using the two-proportions test in Statistica 13 Software.

Population size was determined using the model of Otis et al. (1978) on the basis of the encounter history of all captured, marked and recaptured individuals in 2009–2013 ($N = 137$). The number of females and males was estimated using the same method, but only the encounter histories of adult snakes were used ($N = 21$ for females, $N = 89$ for males). CMR analyses were performed using the MARK Program, version 9.0 (Lucas, 2019). Two field visits were usually needed to cover the whole study area, which gives a total of 22 encounter occasions (3, 4, 5, 5 and 5 in the successive years); this corresponds to the number of months during which field work was carried out.

The effective population size (N_e) was estimated for an unequal sex ratio using the equation from Frankham (1995): $N_e = 4N_mN_f/(N_m + N_f)$ where N_m is the number of males and N_f is the number of females, both estimated using CMR.

3. Results

During our study (between 2009 and 2013), 137 snakes were captured and marked (65.0% males (89 individuals), 15.3% females (21) and 19.7% unsexed (27)), 12 of which were recaptured (8.8%). The mean number of individuals in the population as estimated using the Otis et al. (1978) model was 227.77 (SD = 85.12, Median = 202.85, 95% CI of mean: 141.38–402.89). The mean number of males was 95.29 (SD = 8.39, Median = 92.75, 95% CI of mean: 89.00–108.53) and the mean number of females was 22.91 (SD = 4.38, Median = 21.67, 95% CI of mean: 21.00–27.78). The effective population size was 74.1.

The mean RTL in males was 0.24 (SD = 0.03, 95%CI: 0.232–0.243), while the mean RTL in females was 0.20 (SD = 0.04, 95% CI: 0.183–0.208). The RTL differed significantly between males and females (one-way ANOVA; $F = 36.94$, $p < 0.0001$). The

correct classification of cases in discriminant analysis using RTL achieved 82.14% (83.7% for males and 73.7% for females). Most of the 27 unsexed snakes were juveniles and subadults (20 individuals); only two of were adults, five (new-born snakes) weren't measured. Among the 22 unsexed snakes, 13 were classified as males and 9 as females. After RTL classification, the proportion of sexes for all snakes (with and without sex determination) in the <90 cm length class (N = 43, individuals before reaching sexual maturity) was 69% males and 31% females; it did not differ significantly from Fisher's theoretical sex ratio at birth (the difference between the two-proportions test; $p = 0.0680$).

A small number of females and new-born snakes were recorded in each year of the study (Table 1). The same trend emerged in all the Aesculapian snake's localities: freshly-hatched snakes were found at only three sites/plots (Table 2).

The percentage of adult females (total length >90 cm, 17.74%) in the San valley population studied in 2010–2012 was significantly lower than in other populations and in the same population studied earlier (1990–1998 – Table 3).

4. Discussion

We have shown that the sex ratio in the Polish population of the Aesculapian snake clearly deviated from 1:1. The ratio of 4:1 that we established is one of the strongest, adult male-biased ratios compared to other snake species (Madsen, 1987; Parker and Plummer, 1987; Madsen and Shine, 1992; Blouin-Demers et al., 2002; Lind et al., 2005), in which the male-to-female ratio does not exceed 3:1. The proportion of females in the Bieszczady Mts. has declined in comparison with the data obtained from the same population two decades ago. The adult sex ratio in all isolated populations of the Aesculapian snake at the northern limit of its distribution was found to be male-biased; this did not apply to the Austrian population, in the centre of its distribution range, where the ratio was almost 1:1 (Table 3). Those populations harboured no less than 30% of females, unlike the Polish population investigated in 2010–2012, which contained a mere 18% of females; this is the smallest percentage of female Aesculapian snakes yet recorded. One factor causing this state of affairs might be the lower detection rate of females in field studies. However, we tried to minimize this factor through regular field visits and the use of artificial breeding sites (mounds). Even though the research efforts with regard to different populations are not fully comparable – only the Czech population was studied in a similar way (Musilová, 2011) – we wanted to draw attention to the fact that there were more females in each of those other populations than in our population.

Population size is the one of major determinants of extinction risk (Reed et al., 2003): our study was thus the first to demonstrate this parameter for the Aesculapian snake based on the systematic use of the capture-mark-recapture method. The CMR model revealed a low number of females in this population, and also that the effective population size was low. In the Allee effect, a small population may have a diminished ability to survive and exhibit deviations in the sex ratio (Stephens et al., 1999). Not only the small population size, but above all the small number of females found in each year of the study and the fact that hatched juveniles were found in only three of the seven localities (plots) endorse our conjecture that this population is in poor condition.

The stronger environmental pressure on females in peripheral populations of the Aesculapian snake may be intensified by the high costs of reproduction because of the limited availability of suitable reproductive habitats (Kurek et al., 2018). A greater disproportion in the sex ratio between subadults (individuals yet to reach sexual maturity) and adults may be a consequence of a naturally higher mortality rate among adult females (Seigel et al., 1987; Bonnet et al., 1999). Oviparous females have to find oviposition sites that provide safety for their clutch, as well as suitable thermal and hydric conditions throughout incubation (Shine and Bonnet, 2009). At the northern range limit, artificial egg-laying sites may be the most important factor enabling oviparous snake species to survive, a suggestion made in Sweden for grass snakes *N. natrix* (Hagman et al., 2012; Löwenborg et al., 2012). This also applies to the Aesculapian snake in the Bieszczady Mts., since access to optimal egg-laying sites is restricted to just a few areas in that region (Najbar, 2004). Such a dependence was also highlighted by the increase in the numbers of females and juveniles in each study year, probably as a result of the artificial egg-laying sites built in 2009 and extended in later years (Kurek et al., 2017). Habitat changes in this region have severely restricted access to suitable microhabitats (Kurek et al., 2018), so female Aesculapian snakes are probably forced to move longer distances to find appropriate breeding sites, thus exposing them to a greater risk of mortality. This stands in contrast to the findings of Lelièvre

Table 1

Numbers of male, female and unsexed Aesculapian snakes in different length classes in the San valley between 2009 and 2013 following discriminant analysis using RTL.

Age/sex class	Years				
	2009	2010	2011	2012	2013
Unsexed juveniles	0	0	0	0	5
Male juveniles	1	0	2	0	1
Female juveniles	0	0	2	1	3
Male subadults	1	5	9	9	3
Female subadults	0	1	4	2	0
Male adults	11	10	19	22	9
Female adults	0	3	3	5	6
Total	13	19	39	39	27

Table 2

Male, female and unsexed Aesculapian snakes (n = 137) in different length classes in 7 areas (A-G) in the San valley. M-male, F-female, U-unsexed, length classes: <40 cm – juvenile, 41–90 cm – subadult, >90 cm – adult (2009–2013).

Plot	Total		Juveniles			Subadults		Adults	
	M	F	U	M	F	M	F	M	F
A	11	6	0	0	2	1	1	10	3
B	2	2	0	0	0	1	0	1	2
C	13	2	0	0	0	7	1	6	1
D	24	6	3	1	2	3	0	20	5
E	43	10	2	3	2	14	4	26	3
F	6	2	0	0	0	1	0	5	2
G	3	2	0	0	0	0	1	3	1

Table 3

Numbers of Aesculapian snakes in regions of occurrence in different years divided into adults, subadults, males and females, percentages of females and subadults. The differences between the sex ratio in the San valley population in 2010–2012 vs. populations from other regions were tested using the two-proportions test

N – number of individuals, %F – percentage of females longer than 90 cm; N, %N – number, percentage of individuals shorter than 90 cm.

Region	Years	N	Adults				Juveniles and subadults		p	Source
			Total	Males	Females	%F	N	%N		
Odenwald	1998	113	85	60	25	29.41	28	24.78	0.0478	Gomille (2002)
Odenwald	1987–88	190	144	93	51	35.42	46	24.21	0.0041	Waitzmann (1993); Heimes and Waitzmann (1993)
Danube valley near Passau	1987–88	133	117	77	40	34.19	16	12.03	0.0070	Waitzmann (1993); Heimes and Waitzmann (1993); Böhme, 1993
Rheingau-Taunus	1988	215	125	80	45	36.00	90	41.86	0.0031	Heimes and Waitzmann (1993)
Karlovy Vary	2005–07	361	273	159	114	41.76	88	24.38	0.0002	Musilová (2011)
Austria	1990–96	589	367	202	165	44.96	222	37.69	<0.0001	Kammel (2009)
San valley	1990–98	104	68	43	25	36.76	36	34.62	0.0021	Najbar (2000)
San valley	2010–12	97	62	51	11	17.74	35	36.08	–	This study

et al. (2012) in France (centre of species distribution range): during the reproductive period there, females moved less frequently and for shorter distances than Aesculapian males.

In conclusion, strong environmental pressure seems to act asymmetrically on both sexes, leading not only to a drop in population size but also to a change in its structure. Such alterations in the sex ratio may accelerate population decline by exacerbating the spatial division of the population and limiting the occurrence of females to sites where oviposition is possible. This brings up the important question about the future of the northernmost Aesculapian snake population, that is, whether the low number of females may be the next step towards the extinction of this species at its northern range limit: we would answer it in the affirmative. The small Polish population, unlike the relict German and Czech populations, has become completely isolated only quite recently (Kurek et al., 2018); in addition, there are fewer and fewer females as a consequence of lost nesting habitats. This problem could also apply to other snake species and therefore requires further study.

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