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Population and ecological characteristics of the dice snake, *Natrix tessellata* (Laurenti, 1768), in lower portions of the Vrbanja River (Republic of Srpska, Bosnia and Herzegovina)

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Abstract: Despite their comparative richness and accessibility in the Republic of Srpska and in Bosnia and Herzegovina in general, population studies of reptiles have not been performed in Srpska until recently. For example, one of the most common snake species in this area is the dice snake; nevertheless, previous studies have only reported its distribution. The aim of the present study was to analyze characteristics of the dice snake population along the Vrbanja River. Animals were processed during 2011 throughout their activity period. In total, 199 individuals of all ages were collected. We observed substantial differences in numbers of animals captured in different habitat types classified according to the level of anthropogenic influence. Unexpectedly, the largest number of snakes was captured in the zone with the highest anthropogenic influence, while the smallest number was observed in the zone with no anthropogenic pressures. The above is probably connected with the observed greater number of their most common prey, as well as the absence of raptors in areas with human impact. In the surveyed area, dice snakes feed predominantly on cyprinid fishes, and their reproductive traits are in accord with literature data.

Key words: *Natrix tessellata,* Vrbanja River, diet, fecundity, seasonal activity, sex ratio, habitat type

Different aspects of dice snake (*Natrix tessellata*) population ecology have been investigated in western and central Europe (Italy: Filippi et al., 1996; Capula and Luiselli, 1997; Luiselli and Zimmermann, 1997; Luiselli et al., 2007; Capula et al., 2011; Germany: Gruschwitz, 1978; Lenz and Gruschwitz, 1993a; Lenz and Gruschwitz, 1993b; Switzerland: Metzger et al., 2009; Conelli et al., 2011; Mebert et al., 2011; Austria: Zimmermann and Fachbach, 1996; Duda et al., 2007). In the Balkan Peninsula, the first comprehensive study of the dice snake took place in 2008, in the Prespa Lake region (North Macedonia): morphometry, population structure, and ecology were analyzed (Sterijovski et al., 2011; Ajtić et al., 2013). Information concerning some aspects of 2 *Natrix* species' diet in the Balkans is also available (Janev Hutinec and Mebert, 2011; Šukalo et al., 2014).

Although the dice snake is widely distributed, often abundant, and usually regarded as a common representative of freshwater habitats in Bosnia and Herzegovina (Tomasini, 1889, 1890; Werner, 1898, 1904; Bolkay, 1919, 1929; Radovanović, 1951; Jelić and Lelo, 2011), there are still no population studies which would provide quantitative data for this and other snake species. Here we present the preliminary population characteristics of *Natrix tessellata* in the Republic of Srpska, Bosnia and Herzegovina. This paper represents an integral element of dice snake population studies which have been initiated in the central and western Balkans. The information collected along the Vrbanja River should provide data for comparative analyses of populations from different areas and various ecosystems. Based on such an approach, we should gain insight into crucial characteristics of different habitats, i.e. in the future, we expect to have collected enough data to assess their carrying capacities. Also, because it is often a top predator in aquatic ecosystems, the dice snake can be significant as an indicator species in assessing the state of an environment (e.g., Chin et al., 2013). These ideas remain for the continuation of our studies.

The dice snake, *Natrix tessellata*, is an oviparous snake with an average total body length between 100 cm and 120 cm (Arnold and Burton, 1978). Its populations can reach high densities, probably higher than any other Eurasian snake species (Gruschwitz et al., 1999). According to its lifestyle and preferred habitats, it is regarded as a

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riverine species, commonly found in fast-flowing waters (Radovanović, 1951) or along suitable lakeshores (Ajtić et al., 2013). The range of the dice snake expands from central and southern Europe to western and central Asia and the Near East as far as Egypt (Gruschwitz et al., 1999). Sexual dimorphism in size is female-biased (Filippi et al., 1996; Mebert, 2011; Ajtić et al., 2013). Males become sexually mature at approximately 2.5 years and 46–52 cm snout–vent length (SVL), while females mature at about 3.5 years and 54–60 cm SVL (Carlsson et al., 2011). The dice snake feeds predominantly on fish, but it can prey upon amphibians and small mammals as well (Bilcke et al., 2007; Luiselli et al., 2007; Metzger et al., 2009; Šukalo et al., 2014). Its seasonal activity in Europe starts in early April (when it emerges from hibernation) and lasts until the end of October or beginning of November (Ghira et al., 2009). Mating usually takes place during April or May (Radovanović and Martino, 1950; Luiselli and Zimmermann, 1997). The young hatch in August–September (Herczeg et al., 2005). Clutch size varies from 4 to 29 eggs (Luiselli and Rugiero, 2005).

The Vrbanja River watershed lays in the northern part of Bosnia and Herzegovina, i.e. in the northwestern part of the Republic of Srpska. It is situated between 44°47′39″–44°20′44″ N and 17°12′33″–17°14′42″ E; its total area is 791.33 km^2 (Rajčević and Crnogorac, 2011). The climate of this area is moderate continental; average annual temperature is 10.6 °C, and average annual rainfall is 1.135 mm.

Field surveys were performed from the end of May to the end of September 2011 along the 2400 m of the Vrbanja River's course. We collected, measured, and permanently marked 199 dice snakes of all age classes. In total, 41 days were spent in the field; 1–3 people participated in the search. The snakes were captured by hand between 8:00 and 17:30, and kept in canvas bags until processing. After processing and marking, the animals were returned to the exact sites of capture. The snakes were assigned to one of the age categories based on literature data: we regarded females larger than 55 cm SVL and males larger than 48 cm SVL to be adults (Luiselli and Rugiero, 2005); individuals smaller than 30 cm SVL were considered juvenile, and those in between the given values were grouped as subadults. Sex was determined according to tail morphology (Feriche et al., 1993); when necessary, the hemipenes were everted for confirmation of sex. For body length measurements, we used tape meter (accuracy 1 mm); body weight was measured with a digital scale (precision 1 g). In order to enable future monitoring of population trends, all the animals processed during this research were marked with the standard method of clipping the ventral scales (Bonnet et al., 2002, Dorcas and Willson, 2009). None of the snakes was hurt or displaced.

Considering the types of activity of dice snakes, the survey period was divided into 3 parts, i.e. seasons: 1) "the end of spring" – May and June (for females this coincides with the pregestation phase); 2) "midsummer" – July (gestation); 3) "the beginning of autumn" – August and September (postgestation). For every snake, we recorded the type of habitat it was found in. We distinguished three habitat types, which were coded as follows: **vege** = banks of the Vrbanja watercourse overgrown with vegetation, away from human settlements; **anthr** = banks of the watercourse adjacent to human settlements (under direct anthropogenic influences); **voda** = the middle of the stream, i.e. the free, open portion of the watercourse.

Based on habitat characteristics and the level of anthropogenic influence, the surveyed area was divided into 4 zones, each 600 m long. The respective parts of the river course are as follows: **zone 1** = part with sporadic/weak anthropogenic influence (a); **zone 2** = part with intense anthropogenic influence (b); **zone 3** = part without immediate anthropogenic influence (c); **zone 4** = part with anthropogenic influence evident along one and absent on the other bank (d) (see Figure 1).

For the basic analyses, e.g., of body dimensions and clutch size range, we used descriptive statistics. Chi-square tests were performed for comparison of sex ratios in various seasons and zones. To evaluate the preference of snakes for various habitats during our study seasons, we implemented correspondent analysis.

We caught and processed a total of 199 dice snakes: 66 adults (33.2%), 88 subadults (44.2%), and 45 juveniles (22.6%). Of these, there were 126 females (63.3%) and 73 males (36.7%) of all age classes. Among juveniles, sex ratio was balanced (22 ♀ : 23 ♂): $\chi^2 = 0.02$, df = 1, n.s.; among subadults, it was slightly female-biased (52 ♀ : 36 ♂): $\chi^2 = 2.91$, df = 1, n.s. Interestingly, in the category of adults there were almost 4 times more females than males (52 ♀ : 14 ♂): $\chi^2 = 21.88$, df = 1, $P < 0.001$.

Total length of the largest adult female was 93.6 cm (SVL = 75.7 cm), while the largest male had a total length of 71.0 cm (SVL = 54.3 cm).

We had 20 recaptures; i.e. the total number of animals we encountered during the research period was 219. This was the number we used for the analysis of seasonal and zonal presence and activities.

By spontaneous, defensive regurgitation, 15 snakes provided intact prey (7.54% of the total number); we determined the species of these prey items. The most common prey was the large-spot barbel (*Barbus balcanicus*): 53.3% of the identified prey. The next in frequency were chub (*Squalius cephalus*) and spirlin/schneider (*Alburnoides bipunctatus*) (13.3% each), while bleak (*Alburnus alburnus*), European bitterling (*Rhodeus amarus*), and spined loach (*Cobitis elongatoides*) each

Figure 1. Portions of the Vrbanja River watercourse under various anthropogenic influences or without them: **a** = part with sporadic/weak anthropogenic influence; **b** = part with intense anthropogenic influence; **c** = part without direct anthropogenic influence; **d** = part with anthropogenic influence evident along one and absent on the other bank. (Photo: G. Šukalo).

represented 6.7% of the identified prey. We analysed feeding frequencies in different seasons. The largest number of snakes with prey (intact, half-digested, or fully digested, detectable by abdominal palpation, in stomach or intestine) was found during the "beginning of autumn" season (78.5%) and the smallest at the "end of spring" (48.4%). The highest percentage of snakes with ingested prey was found in adult females: 78.8%. Substantial numbers of satiated individuals were also found among subadult males and females (75%), while the smallest frequency of prey was found in juvenile males (21.7%).

The data on female fecundity were obtained by abdominal palpation, i.e. assessment of the number of growing follicles/eggs through the body wall. In the second half of June and first half of July, we caught and palpated 17 adult females. Seven were gravid (41.2%). Importantly, one of the gravid females was assigned to the subadult category based on previous data concerning threshold adult trunk length. The average number of growing follicles/eggs was 8.7 ± 3.73, with a range of 5–16. The highest number of eggs was found in the largest of the gravid females (70.5 cm SVL). Number of eggs was highly correlated to trunk length (log-transformed SVL and numbers of eggs: r = 0.80). During the second half of July, we could not record the presence of growing follicles/eggs in the processed snakes, which implies that the snakes had laid eggs before the second half of July.

Correspondent analysis of frequencies of males and females of all 3 age categories, with habitat type, season, and zone as factors, revealed that adult (FA) and subadult females (FSA) were usually found in zones 1 and 3, in habitat covered with vegetation ("vege"), during summer (gestational period for females, GEST). On the other hand, adult (MA) and subadult males (MSA) were most frequently found in zone 2, in water ("voda"), in early autumn (postgestational period for females, POSTG). Juvenile individuals of both sexes (MJ, FJ) were associated only with zone 4 and, to some extent, with anthropogenic-influenced habitat ("anthr") (Figure 2).

Analysis of seasonal activity (all 219 encounters considered) showed that the largest number of snakes (110, 50.23%) were caught during "midsummer" (July),

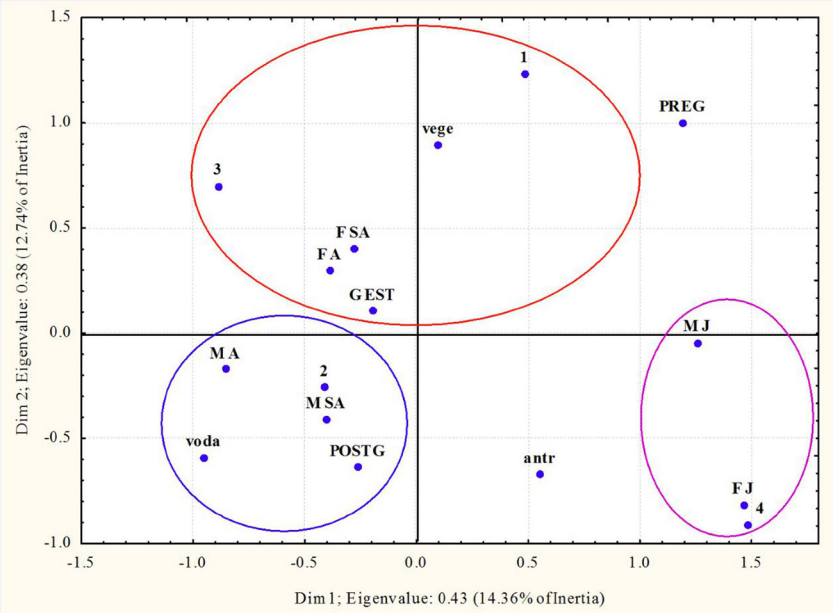

Figure 2. Correspondent analysis of frequencies of males and females in all 3 age categories, with habitat type, season, and zone as factors.

while the smallest (32 individuals, 14.61%) were found in "late spring" (May and June).

The largest number of snakes (124, 56.6% of the total number) were caught in zone 2 (part of the river stream under intense anthropogenic influence). Of these, 77 snakes were caught in "midsummer" (70% of the 110 caught in this season). The smallest number of snakes (25, 11.4%) were caught in zone 3 (area with no anthropogenic influence): in this zone, only 3 individuals were caught in "late spring".

In all seasons, we caught 2 times more females than males. Again, to check if sex ratios (all age classes) in 3 seasons departed from 50 : 50, we performed chi-square tests. In "late spring" the difference was not significant ($\chi^2 = 3.13$), but in "midsummer" and "early autumn" the ratios of females among the encountered snakes were significantly larger compared to males: $\chi^2 = 7.13$, $P < 0.01$, and $\chi^2 = 8.12$, $P < 0.005$, respectively.

The highest number of snakes were caught in the part of habitat defined as "vege": 94 (42.9%), while the lowest number were found in water (51, 23.3%). In the part under anthropogenic influences, we found 74 snakes (33.8%). The majority of recaptures (13 of 20) occurred during September, again in zone 2.

During our field surveys, we found 11 dead snakes. Of these, 9 were obviously killed (81.8%), and these were all adult females. All were killed with blunt objects, most probably by local people. We also found 12 snakes with numerous scars on their bodies, and an additional 18 were missing their tails. We did not observe predation by animals, although we recorded potential predators grey heron (*Ardea cinerea*) and little egret (*Egretta garzetta*).

Our field surveys suggest that *Natrix tessellata* is the dominant snake species in the lower portions of the Vrbanja River course: during 5 months of frequent patrolling of the selected part of the river course, we observed only one individual of the other semiaquatic snake, *Natrix natrix*. The population density of dice snakes is relatively high (82.9 individuals per km of the surveyed river stretch). Of 199 captured and marked snakes, we managed to recapture only 20 individuals (approximately 10%). Such a small proportion of recaptured animals usually indicates a fairly large population.

Sex ratio in the dice snake population in the surveyed area is balanced among juveniles, but is significantly female-biased among adults (3.7 : 1). In other parts of the species' distribution range, higher proportions of females were also recorded (Gruschwitz, 1986; Velenský et al., 2011; Ajtić et al., 2013). The same stands for other Natricinae species (Gibbons and Dorcas, 2004; Parker and Plummer, 1987). Unequal sex ratio in reptiles can result from biases in sampling procedure or from some biotic factor: unbalanced sex ratio at hatching, differential mortality between the sexes, sex differences in emigration and immigration, or from sex differences in reaching maturity (Gibbons, 1990). Given the 1 : 1 sex ratio in juvenile category, we assume that one or several other possible factors determined the observed sex ratio among adults. Based on the data gathered to date, it was not possible to determine if differential mortality or sex-

specific migrations could have led to higher numbers of females. However, in juvenile males we found the smallest frequency of prey (21.7%). This might be the cause of their higher mortality and could lead to an unbalanced sex ratio in subadult and adult categories. Another possible cause of female prevalence in our sample of adults could be their larger body size: large females are easier to spot and capture (Luiselli et al., 2011). Furthermore, their thermal demands are different from those of males, i.e. females need to spend more time basking (Simonov, 2009; Capula et al., 2011). These assumptions are supported by the fact that the 9 animals killed by people (81.7% of all dead) were adult females. The significantly smaller number of males captured during the present study may also be related to lower terrain accessibility during the mating season due to spring floods (Velenský et al., 2011); during this period, in the majority of snake species, males are usually the sex easier to catch (Parker and Plummer, 1987). Kärvemo et al. (2011) also reported seasonal differences in *N. tessellata* sex ratios: the majority of males (63%) were captured during the spring mating period, while females were more often caught during periods of feeding and vitellogenesis (68%). One of the causes of low number of males in our sample could be strong Vrbanja flooding in spring 2010. On that occasion, a large number of snakes (approximately 200 individuals) were removed from the locality by the local utility company. It is possible that most of these animals were males, because in that period males would have been actively searching for females (Shine and Bonnet, 2009). This could have led to the female-biased sex ratio in 2011. After mating, females need excess food intake to increase fat reserves, because fat is the main factor for the onset of vitellogenesis (Plummer, 1983; Bonnet et al., 1999).

In the age pyramid of our dice snake population, juveniles constituted 22.6% of all captured animals. A similar proportion (23%) of juvenile dice snakes was found by Ahmadzadeh et al. (2011) in an Iranian population. In contrast, in the population from Prespa Lake (Ajtić et al., 2013), juveniles constituted merely 8%. The ratio of adult vs. subadult and juvenile snakes in the Vrbanja sample was 1 : 2 ($\chi^2 = 30.6$, df = 1, $P < 0.000$). Here we need to emphasize the possibility that some "subadult" dice snakes might have been sexually mature, because there are data for cases of earlier maturation, i.e. maturity at SVL smaller than those previously reported (36 cm for males and 35 cm for females) (Ahmadzadeh et al., 2011). However, further studies are necessary to adequately address this question.

The European dice snake's diet mostly comprises fish (Filippi et al., 1996; Gruschwitz et al., 1999; Luiselli et al., 2007; Šukalo et al., 2014). In central Italy, the dice snake mostly consumes cyprinid fishes, and occasionally feeds on amphibians: green frogs and common toad tadpoles (Luiselli and Rugiero, 1991; Zimmermann and Fachbach, 1996). However, the percentage of fish in the dice snake diet can vary substantially (Weiperth et al., 2014). In the lower portions of the Vrbanja River *N. tessellata* is exclusively piscivorous (100% fish in its diet), and its predominant prey are cyprinid species: *Barbus balcanicus*, *Squalius cephalus*, and *Alburnoides bipunctatus*, which is in accord with findings from other regions (Zimmermann and Fachbach, 1996; Filippi et al., 1996; Gruschwitz et al., 1999; Luiselli et al., 2007; Janev Hutinec and Mebert, 2011).

Some data on the Italian dice snake's fecundity were provided by Luiselli and Rugiero (2005). Dice snakes lay between 4 and 29 eggs (15 on average). Ajtić et al. (2013) recorded a range between 4 and 15 eggs (9 on average) in a population from Prespa Lake. Larger females tend to produce more eggs (Whittier and Crews, 1990; Capula et al., 2011). In the lower region of the Vrbanja River, abdominal palpation revealed between 5 and 16 eggs (9 on average) in the dice snake. We also found positive correlation between female body size (SVL) and clutch size. Clutch size in the population from the Vrbanja River corresponds to that found in Prespa Lake, but is much smaller than the size of clutches in Italy (Luiselli and Rugiero, 2005). Apparently contradictorily, female dice snakes from Prespa Lake (83.8 ± 9.1 cm SVL: Ajtić et al., 2013) are larger than these along the Vrbanja River (63.1 ± 5.8 cm SVL) and snakes in Italy (70.2 ± 10 cm SVL: Luiselli and Rugiero, 2005). The egg-laying period in our study population (June and July) is in accord with previously published data (Velenský et al., 2011). However, the relatively low percentages of gravid dice snakes in our sample suggest a biannual reproductive cycle. This was also seen in populations from Austria and North Macedonia providing conditions (Luiselli and Zimmermann, 1997; Ajtić et al., 2013). This assumption needs to be verified in the future.

The percentage of gravid females varies in relation to annual variations in prey availability (Miller et al., 2011). We speculate that the low percentage of gravid females in 2011 could have resulted from the summer floods of 2010: floods probably delayed the onset of the feeding period due to bad water quality (e.g., increased turbidity) (Velenský et al., 2011). Shortage of food could have led to insufficient fat reserves in many females, preventing their vitellogenesis in 2011 (Capula et al., 1995).

Correspondent analysis of frequencies of males and females of all 3 age classes associated with habitat type, season, and zone as factors showed that adult and subadult females were usually found in the habitat covered with vegetation during summer (gestation period). This result can be related to deeper water in the given zones, populated by larger prey items (Velenský et al., 2011), or a better visual protection against birds of prey, while still for thermoregulation in the vegetation. Previously, ecological differences between the sexes were documented in the

file snake, *Acrochordus arafurae*: adult males are smaller and they feed on small fish in shallow waters, while adult females are much larger and tend to eat larger fish in deeper waters (Houston and Shine, 1993). Nevertheless, further detailed research is necessary to confirm sex differences in qualitative and quantitative composition of diet and different positions in the spatial niche. On the other hand, the largest proportion of adult and subadult males in the open water at the beginning of autumn could be explained by their search for smaller fish in shallow water (Velenský et al., 2011) in preparation for hibernation. The association of juveniles with the zone under anthropogenic influence could be related to numerous embankments along this river portion: it is possible that females lay their eggs along this part of the river in suitable thermal and hydric conditions. Juveniles were mostly captured in shallows, probably because water is warmer and fish fry shoals there (Velenský et al., 2011).

During the present study, we captured large numbers of snakes near human settlements and beaches. A possible explanation for this phenomenon should be sought in the higher presence of fish in these river portions resulting from underdeveloped sewage system along the Vrbanja River. In the sections covered during this study, in places where sewage discharges directly into the river, *Barbus balcanicus*—the preferred dice snake prey—gathers in masses. The main factor for high density of piscivorous snakes is the abundance of their prey (Chim and Diong, 2013). Numerous studies have shown that certain infrastructural objects are suitable for dice snakes as hiding places; in some instances, they even improved the suitability of habitats (Conelli et al., 2011; Kammel and Mebert, 2011; Žagar et al., 2011). On the other hand, in close proximity to human settlements, potential bird predators were not observed, while outside the areas under anthropogenic pressure numerous herons were encountered (G.Š., personal observation). In such places, the numbers of snakes were very low, or they were completely absent.

The data we presented here provide the first population and ecological research of the dice snake in Bosnia and Herzegovina.

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