Online ISSN: 2588-3526
Journal of Wildlife and Biodiversity
Volume 9(1): 202-208 (2025) (http://www.wildlife-biodiversity.com/)
Short communication

New data on the reproduction and possible prey objects of *Elaphe dione* (Pallas, 1773) (Reptilia: Colubridae) in specially protected areas of the Samara Region

Anastasia A. Klenina, Victoria A. Vekhnik*

Samara Federal Research Scientific Center RAS, Institute of Ecology of the Volga River Basin of RAS, Togliatti, Russia
Email: ivavika@rambler.ru*
Received: 05 October 2024 / Revised: 01 November 2024 / Accepted: 02 November 2024/ Published online: 02 November 2024.
How to cite: Klenina, A.A., Vekhnik, V.A. (2024). New data on the reproduction and possible prey objects of *Elaphe dione* (Pallas, 1773) (Reptilia: Colubridae) in specially protected areas of the Samara Region, Journal of Wildlife and Biodiversity, 9(1), 202-208. DOI: https://doi.org/10.5281/zenodo.

Abstract.
The new data on the reproduction and probable prey objects of *Elaphe dione* are summarized. Body size and body mass of pregnant females, the timing of egg laying, the number and parameters of the eggs, and the parameters of the newborn snakes are given. High energy expenditures and RCM were found in females. Based on the results of the census of mouse-like rodents as the main food items of the snakes, the first data on the potential composition of the prey objects of pregnant *E. dione* in the Zhiguli Nature Reserve was obtained.
Keywords: Colubridae, *Elaphe dione*, reproduction, specially protected natural areas

Introduction

The steppe ratsnake *Elaphe dione* (Pallas, 1773) is a representative of the Colubridae family, widespread in the Palearctic fauna (Ananjeva et al., 2004). The species is included in the IUCN list (The IUCN, 2017) with the category “Least Concern” and in Appendix II of the Bern Convention, “strictly protected species of fauna” (ETS No. 104, 1979). In the Samara Region, it lives on the northern border of the range, where it is listed in the regional Red Book (2019) with the status 3 "rare species". It is found on the Samarskaya Luka peninsula, formed by the bend of the Volga River, within the boundaries of specially protected natural areas (hereinafter referred to as PAs) National Park "Samarskaya Luka" and I.I. Sprygin Zhiguli State Nature Biosphere Reserve (the Middle Volga Region, Russia). Due to its low population size, secretive lifestyle, and inaccessibility of certain habitats, *E. dione* remains one of the least studied species of colubrids throughout its range, especially in its northern part. For the

Samarskaya Luka peninsula, a number of authors provide some information on the distribution, abundance, phenology, morphology, and diet of the species (e.g., Shaposhnikov &, Zhukov, 1988; Bakiev et al., 2009; “Chronicle of nature”, 2024). Data on the diet and reproduction of *E. dione* in the north of its distribution range have been published for a number of localities in the Samarskaya Luka peninsula and do not cover the Zhiguli Reserve (Klenina, 2013; Klenina, 2015). It was not possible to find any works considering the relationships of the species in the predator-prey system, whereas such relationships have been studied for other snake species (e.g., Luiselli et al., 1997; Madsen et al., 2006) and have fundamental importance in many areas of ecology and nature conservation (Yoshida et al., 2007; Radovics et al., 2023). Also, the available publications considering *E. dione* in the studied region do not pay enough attention to one of the most important characteristics of the life cycle of reptiles – relative clutch mass (RCM), which reflects an indirect assessment of the reproductive efforts of squamates (Vitt & Price, 1982; Seigel & Fitch, 1984; Suárez-Varón et al., 2019). This work aimed to obtain new data on the reproduction of *E. dione* in the PAs of the Middle Volga Region and to study the prey objects of the snakes as a possible way to compensate for their high cost of reproduction.

Material and methods

The studies were conducted from April to October in the protected areas of the Samara Region: in 2019 in the National park "Samarskaya Luka" (near the Perevoloki Village, Syzransky District, average coordinates 53°14'N 49°11'E) and in 2022 in I.I. Sprygin Zhiguli State Nature Biosphere Reserve (near the village of Bakhilova Polyana, Stavropolskiy District, average coordinates 53°25'N 49°41'E).

Pregnant females (n = 8) were captured during the periods of maximum daily activity (Bakiev et al., 2009). The sex of adult specimens was determined visually by the shape of the tail. The body mass of females and the eggs they laid was measured by the portable electronic scale AND HL-400 (400 g / 0.1 g; A&D, Japan). The relative clutch mass, hereinafter RCM, was determined based on the loss of female mass during birthing, divided by her mass after it (Shine, 1980). For ease of perception, this indicator was expressed as a percentage.

Pregnancy was determined visually by enlarged oviducts, which, when filled with large eggs, stretch greatly and occupy almost the entire body cavity (Gurtovoy et al., 1978). Pregnant females (n = 8) were temporarily removed from the wild and kept in laboratory conditions until egg-laying. The length and diameter of the eggs were measured using an electronic caliper, "ShTT-1-150 0.01 Etalon"(Etalon, China). If the eggs in the clutch were glued

together, the number of measurements of the two parameters could change. The newborn snakes were measured and weighed on the day of hatching.

Possible prey objects of the snakes, mouse-like rodents, were caught in the places of snake captures with standard metal live traps with a drop door measuring 120 × 65 × 65 mm, located in two lines of 25 traps at a distance of 5 m. Checks were conducted twice a day. The census was carried out in the first and second half of June 2022 for 5 days. Species, sex, and age were determined visually. During each round, rodents were marked with a solution of brilliant green dye. The primary data were processed using statistical methods with the calculation of the arithmetic mean (M), its error (m), and standard deviation (sd). Preliminary data processing and analysis were performed in Microsoft Office Excel 2010 and Statistica 8.0.

The work with animals was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Directive 2010/63/EU). After the necessary measurements, all snakes were returned to the wild.

Results and discussion

Morphological parameters

The length *L. corp.* of pregnant *E. dione* females (n = 8) caught in the PAs of the Samara Region ranged from 730 to 960 mm (821.9 ± 25.84 / 73.09). Before laying eggs, their body mass varied from 224.7 g to 420.0 g (314.7 ± 24.77 / 70.07). The obtained figures are consistent with the data published earlier for the species (Klenina, 2015). Thus, *L. corp*. of the smallest female caught pregnant in the region was 725 mm; the largest pregnant female reached 970 mm in length (810.6 ± 16.08, n = 17). The weight of females before parturition ranged from 229.1 g to 451.6 g (303.2 ± 18.05, n = 12) (Klenina, 2015). The number of eggs laid in eight clutches ranged from 6 to 14 (9.1 ± 1.08 / 3.04). The length of fertilized eggs ranged from 35 to 57 mm (n = 56; 43.1 ± 0.69 / 5.16), the diameter – from 20 to 27 mm (n = 58; 23.2 ± 0.22 / 1.67). The length *L. corp*. of the snakes hatched from eggs (n = 70) ranged from 170 to 238 mm (212.6 ± 1.88 / 15.74), the tail length *L. cd*. – from 34 to 57 mm (46.4 ± 0.53 / 5.27), weight – from 4.1 to 11.4 g (8.5 ± 0.16 / 1.32). The values revealed are fully consistent with the limits published for the species in the Samara Region (Klenina, 2015).

RCM in percent varied from 68 to 96% (86.2 ± 3.92 / 11.08). No statistically significant correlation was found between this indicator and the length of the female, as well as between it and the weight of the female, which may be due to the insufficient sample size of the snakes (n = 8). This dependence was confirmed for another species of the Colubridae family, the

smooth snake *Coronella austriaca* Laurenti, 1768, during long-term observations in the Italian Alps on a sample of 28 individuals (Luiselli et al., 1996).

Previously published materials indicate that in the patterned snake in the Samara Region, the relative weight loss of females can reach 131% (Klenina, 2015). The primary data on *E. dione* were compared with the RCM values of other colubrids inhabiting the study area: the grass snake *Natrix natrix* (Linnaeus, 1758), the dice snake *Natrix tessellata* (Laurenti, 1768), and the smooth snake *C. austriaca* (Table 1).

Table 1. RCM in snakes of the Colubridae family in the Samara Region (Russia) (Klenina, 2015; original data 2019, 2022)

Species	RCM, %		
	n	*Min-max*	*M±m / sd*
E. dione	20	66 – 131	86.4 ± 3.56 / 15.94
N. tessellata	15	54 – 106	84.4 ± 4.03 / 15.61
N. natrix	8	39 – 99	67.7 ± 6.07 / 17.17
C. austriaca	12	33 – 83	61.6 ± 4.43 / 15.34

Table 1 shows that the highest average, minimum, and maximum RCM values are observed in *E. dione* and *N. tessellata*. Both species are oviparous and inhabit the Samara Region at the northern limit of their range (up to 53° N) (Bakiev et al., 2009). The oviparous *N. natrix*, whose distributional range reaches 67° N on the Scandinavian Peninsula (Ananjeva et al., 2004), has a lower RCM and is closer to the ovoviviparous *C. austriaca*. The lowest RCM values of *C. austriaca* are explained by the fact that viviparous species can reduce the risk of mortality by reducing RCM, since they have a longer reproductive season compared to oviparous species (Seigel & Fitch, 1984). Table 1 shows that female *E. dione* spends a lot of energy on reproducing in the northern range. It should also be noted that among the oviparous snakes of the Colubridae family inhabiting the Samara Region, *E. dione* has the shortest "weight gain period" after giving birth. Thus, females caught in 2019 laid eggs from July 6 to July 24, and in 2022 from July 16 to August 1. According to the previously published data, egg laying in this species in the region can be extended until August 15 (Klenina, 2015). *N. natrix* and *N. tessellata* laid eggs from June 29 to July 8 and from June 26 to July 16, respectively (Klenina, 2015). In other words, female colubrids that have laid eggs have the opportunity to start feeding and replenishing energy reserves earlier than female steppe rat snakes.

Significant energy expenditures on reproduction mean exhaustion of the female that has given birth and can lead to her death (Luiselli et al., 1997). The obtained data on the highest RCM among colubrids and a short period of weight gain in *E. dione* indicate possible fatal postpartum risks for females in northern populations. In this regard, it seems especially important to obtain further data on the features of replenishment of expended energy and the specifics of the diet of pregnant females, as well as the snakes in other physiological states of snakes in the studied PAs.

Probable prey objects of the snakes

It is known that the main prey object of *E. dione* in the Samarskaya Luka is rodents (Shaposhnikov & Zhukov, 1988; Bakiev et al., 2009; Klenina, 2015). As a result of the rodent census conducted in the Zhiguli Reserve in June 2022 in the snake habitat area, three species were encountered: *Apodemus flavicollis* (Melchior, 1834), *Apodemus uralensis* (Pallas, 1811) and *Clethrionomys glareolus* (Schreber, 1780) (Fig. 1).

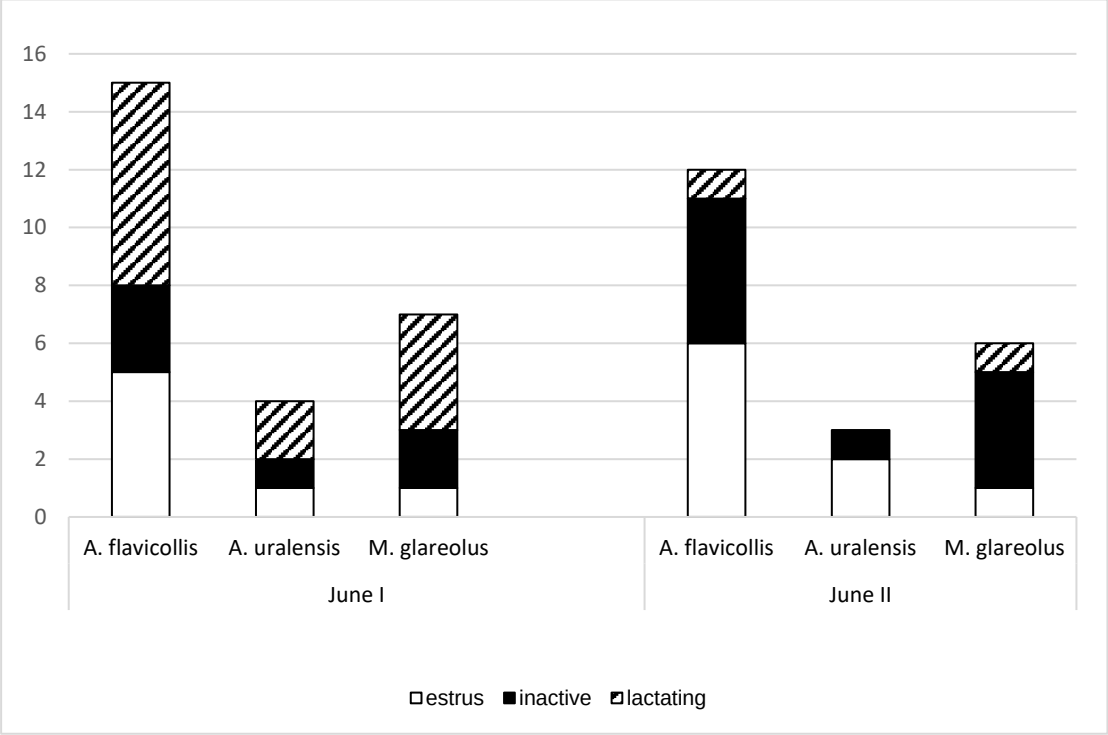

Figure 1. The number of female mouse-like rodents in different reproductive states in June 2022 in the territory of the I.I. Sprygin Zhiguli State Nature Biosphere Reserve (The Middle Volga Region, Russia)

Figure 1 shows that in the first half of the month, lactating females predominate among the rodents, spending most of their time in the nest while feeding their juveniles. At this time,

pregnant *E. dione* females are no longer able to move actively due to the embryos growing inside the oviducts, but they can still feed (at a later stage, the embryos block the esophagus, and the pregnant snake is unable to swallow prey). We believe that female rodents with offspring become easy prey for the snake, which, after finding of the nest, will eat all its inhabitants or, in the case of later stages of pregnancy, only small juveniles. It can also be assumed that the snakes, exhausted after laying eggs, also feed mainly on young, inexperienced rodents in early July – mid-August, which leave the nests by this time.

Thus, the data on the reproduction of *E. dione* in the Zhiguli Reserve and the National Park "Samarskaya Luka" obtained in 2019 and 2022 are consistent with the data collected by A.A. Klenina in the Samara Region from 2009 to 2015. Information on the length of *L. corp*. of pregnant females, their weight, the timing of their egg laying, their number, length, and width, as well as the parameters of hatching young, are consistent with and complement the published limits (Klenina, 2015). Information on high RCM values in the species at the northern limit of distribution and on possible fatal risks for females exhausted after egglaying requires further study. Information on the composition of the prey of different physiological groups of *E. dione*, especially females at different stages of pregnancy and after it, point to the possible role of the reproduction timing of rodents, especially the appearance of juveniles, in the compensation of energetic demands of the snakes during reproduction and indicates the need for further study of the characteristics of reproduction of *E. dione* in protected areas in the northern part of the range.

Acknowledgment

The authors thank the Anonymous Reviewer and the Editor for the valuable comments and quick response. The work was conducted within the framework of the Russian Scientific Foundation, project No. 24-24-00350 “Regulation of reproduction in terrestrial vertebrates in communities with seasonal change of foods”.

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