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Female reproductive characteristics of the Balkan wall lizard (*Podarcis taurica*) in the northwestern periphery of its range

Communication

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Abstract: We present data on the female reproductive traits of the Balkan wall lizard in the Deliblato Sand, a large continental sandland in the Pannonian area in the northwestern periphery of the species range. The clutch and egg characteristics of the population were investigated on the basis of clutches laid in laboratory conditions by gravid females captured in one locality. Balkan wall lizards produced at least two clutches in a breeding season. Individual females laid clutches of commonly two (range 1 – 4) eggs. The female body size had no effect on clutch and egg size. There was no trade-off between egg size and clutch size.

Keywords: *Life history • Lacertid lizard • Clutch size • Egg size • Peripheral population*

1. Introduction

Lizards show complex relationships between the maternal body size, clutch or litter size, size of eggs and hatchlings which can exhibit great variability within and among populations, as well as among different species [1,2]. The population level analyses of lizards' reproductive characteristics may play an important role in understanding the evolution of life-history traits, especially as methods for incorporating population-level phylogenetic information are developed [3]. Although the need for enlarging and improving the dataset on life history variation through detailed studies of single species has been emphasised by many authors [e.g., 1,2], even the most recent studies on theoretical models of life history patterns indicated that our understanding of one of the fundamental trade-offs in life history evolution (*i.e.*, division of resources into offspring size and number) remains surprisingly incomplete [4].

The Balkan wall lizard (*Podarcis taurica*, Pallas 1814) is a small, diurnal, heliothermic and actively foraging ground dwelling lizard, that lives in open, sunny, flat, dry areas with low vegetation usually below 700 – 800 m elevation [5,6]. It is distributed throughout Hungary, the largest part of the Balkan Peninsula, Crimea and a few locations on the coasts of NW Asia Minor, displaying notable geographical variations in colour, pattern and size [7] (Figure 1).

Although reproductive biology of *P. taurica* has been widely researched [8-11], none of the studies concern populations in the north-western part of the range (see Figure 1). In fact, all aspects of biology in Serbian populations where the species reaches western and north-western limits of its distribution remain unknown.

The main aim of our study was to provide basic data about the female reproductive characteristics of the Balkan wall lizard population at the northwestern periphery of the species range, obtained through gravid

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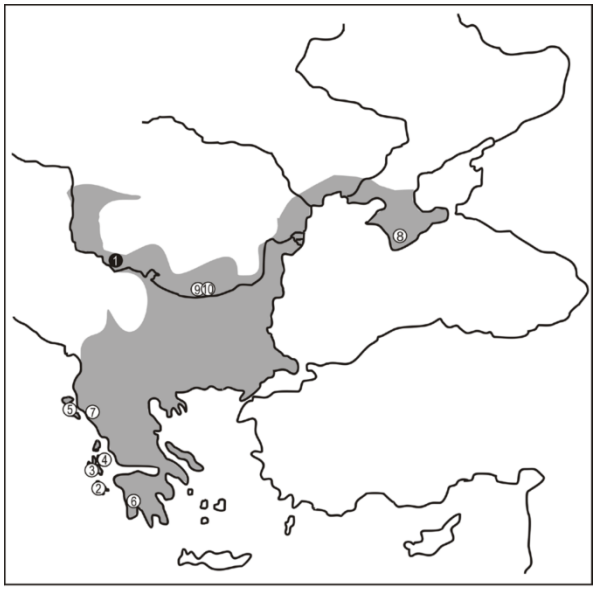

Figure 1. General distribution and localities of the populations of *P. taurica* where reproductive characteristics were analysed in this (closed circle) and previous studies (open circles). Numbers correspond to those in Table 3.

females from the natural population that laid eggs under controlled, laboratory conditions. We report data on clutch size, egg mass and size, and analyze these variables in relation to maternal size.

2. Experimental Procedures

2.1 Study population

The study was carried out on a population of *P. taurica* from a Deliblato Sand location (UTM EQ27, 88 m a.s.l.) in the extreme southeastern part of the Pannonian depression between the Danube and the Carpathian Mountains in northeastern Serbia.

The Deliblato Sand, the dune character area of formerly moving sands, has semi-arid continental climate with great diurnal and seasonal fluctuations in temperature and quantity of rainfall during the year. The average annual temperature at the studied location is 11.4°C (January -2.5°C, July 27.8°C). The mean annual precipitation is 639 mm and the period of drought lasts from July to October (for a more detailed description of the area see reference [12]).

Here *P. taurica* occupies open areas of the sand dunes with grassy xerophyle vegetation of sandy and steppe habitats or is found in open areas where the vegetation has been removed such as abandoned sand extraction sites.

2.2 Data collection

Clutch characteristics were investigated on the basis of clutches laid by females captured at the Deliblato Sands location in 2008. All gravid females (N=16) found to have oviductal eggs (by ventral palpation) were collected by hand and housed in terraria on the same day (20 June 2008). The lizards were collected under permits provided by the Ministry of Environmental Protection, Republic of Serbia (no. 335-00-113/2008-03). The laboratory work was approved by the Ethical Committee of the Institute for Biological Research "Siniša Stanković" (no. 16/08). The specimens used for this study were also included in our other ongoing studies (such as analyses of variability in skeletal morphology in Lacertidae) that required sacrificing the animals.

The gravid females were kept in individual terraria under the same conditions in the laboratory with exposure to natural and additional artificial light that created a thermal gradient for 12 hours a day from sunrise to sunset. Food consisting of mealworms and insects as well as water were provided *ad libitum*. The females were inspected daily. Following oviposition, they were measured for snout-vent length (SVL), weighed and autopsied to check for the presence of vitellogenic follicles.

The number of enlarged vitellogenic follicles (diameter >3 mm) in females, was considered as their next clutch of the season. This was done according to a standard procedure used in similar studies [e.g., 13,14] and provides a reliable estimate of clutch size.

Immediately after oviposition, the eggs were dug up and carefully removed from the terrarium, weighed and measured (maximum length and width). A digital calliper (0.01 mm precision) was used for the linear measurements, while mass measurements were taken with an electronic balance (accuracy 0.001 g). Egg volumes were obtained by approximating the volume of the ellipsoid: $V=4/3\pi a^2 b$, with a and b being half of the width and length of the egg, respectively [see e.g. 13]. Clutch mass was calculated as the total mass of eggs in a clutch. In all cases, each clutch was unequivocally assigned to an individual female, allowing us to calculate the relative clutch mass (RCM) as the ratio of clutch mass to post-oviposition body mass.

2.3 Statistical procedures

Descriptive statistics (mean, standard error and range) for all traits were calculated. Throughout this study, values were presented as mean ± SE. For subsequent analyses all variables were log-transformed, to ensure data normality and to generate homogeneous variances [15]. Linear regression and correlation analyses were

measurement	mean ± SE	range	N
Female SVL (mm)	52.71 ± 0.71	46.83 – 58.13	16
Clutch size	2.25 ± 0.19	1 – 4	16
Post-oviposition female body mass (g)	2.854 ± 0.109	2.240 – 3.888	16
Clutch mass (g)	0.916 ± 0.060	0.495 – 1.373	16
Relative clutch mass (RCM)	0.328 ± 0.020	0.165 – 0.486	16
Egg mass (g)	0.424 ± 0.020	0.326 – 0.638	16
Egg length (mm)	14.01 ± 0.31	11.99 – 17.62	16
Egg width (mm)	7.41 ± 0.08	6.90 – 8.05	16
Egg volume (mm^3)	406.08 ± 15.70	348.71 – 556.99	16

Table 1. Summary of statistics for measurements of reproductive females, clutch and egg size of *P. taurica*. For egg attributes the average values for each clutch were used.

used to study the interrelationships among various reproductive traits. In order to test whether the oviposition date was associated with female body size and whether the time that females spent in captivity influenced the results obtained on egg attributes, we regressed female size and all reproductive variables against the duration of retention in captivity prior to oviposition. Variation in the clutch size between the successive clutches (laid eggs and enlarged vitellogenic follicles) of an individual was analysed by paired sample t- test. We used unpaired t-test to determine whether clutch size (those of 2 and 3 eggs) was associated with differences in egg characteristics. The analyses were carried out using the computer package Statistica® (STATISTICA for Windows. StatSoft, Inc., Tulsa, OK), considering $P<0.05$ as the level for significance.

3. Results and Discussion

3.1 Female characteristics and oviposition

Gravid females had an average SVL of 52.7±0.7 mm (Table 1). Oviposition occurred between 25 June and 5 July (5-15 days after capture), but any given clutch was always laid within a single day (and generally, within a few hours). There was no statistically significant correlation between female size and duration in captivity prior to oviposition, although the trend was observable ($r=-0.50$, $P=0.06$). Nevertheless, we assumed that female condition did not affect the oviposition date. The presence of enlarged vitellogenic follicles in eight females after oviposition suggested that at least a proportion of females of the Balkan wall lizard from Deliblato sand locality was able to lay at least two clutches per season. The production of multiple (two or even three) clutches per season was also documented in other populations of the Balkan wall lizard [9,11,16]. Production of single or multiple clutches within the same season is primarily related to female body size and to the timing of oviposition (reviewed in [2]).

3.2 Clutch and egg characteristics

The number of eggs in a laid clutch varied from 1 to 4, with the most (56%) of the clutches containing 2 eggs. We recorded two clutches of one egg, nine clutches of two eggs, four clutches of three eggs, and one clutch of four eggs. The eggs had a mean length of 14.0±0.3 mm, a mean width of 7.4±0.1 mm, and a mean mass of 0.42±0.02 g. The relative clutch mass ranged from 0.17 to 0.49, with the mean value of 0.33±0.02 (Table 1). A paired t- test on limited sample of females (N=8) that possessed enlarged vitellogenic follicles showed that clutch size did not differ significantly between successive clutches (paired t test: df=7, t=-0.27, P=0.80).

In the lizards that produce multiple clutches per activity season, females switch from producing more but smaller eggs in the first clutch to fewer but larger eggs in the subsequent clutches, which presumably reflects differences in the proximate source of the energy allocated to different clutches [e.g., 17,18]. Due to the small sample size it was not possible to determine whether clutch size varied within the season in the analysed population.

No significant relationship was found between the SVL and clutch and egg characteristics (for values of estimated coefficients see Table 2). However, the positive relationships between female size and clutch size and mass were of borderline significance (P=0.07

Dependent variable	r	F	d.f.	P
Clutch size	0.46	3.78	1,14	0.07
Clutch mass	0.48	4.08	1,14	0.06
RCM	0.20	0.56	1,14	0.47
Egg mass	-0.30	1.41	1,14	0.26
Egg length	-0.32	1.56	1,14	0.23
Egg width	-0.26	1.04	1,14	0.32
Egg volume	-0.35	1.93	1,14	0.19

Table 2. Summary statistics of linear regression of egg characteristics (based on average values for each clutch) on female SVL of *P. taurica*.

Population	SVL (mm)			Clutch size			Reference
	mean ± SE	range	N	mean ± SE	range	N	
Deliblato Sand (Serbia)	52.7 ± 0.7	46.8 - 58.1	16	2.2 ± 0.2	1 - 4	16	this study
Zakynthos (Greece)	63.7 ± 0.8	53.5 - 72.5	38	3.6 ± 0.3	2 - 10	33	[9]
Kefalonia (Greece)	64.8 ± 1.4	54.0 - 73.7	32	3.6 ± 0.2	2 - 7	25	[9]
Ithaki (Greece)	62.4 ± 1.9	54.3 - 66.0	16	3.8 ± 0.3	2 - 6	11	[9]
Kerkyra (Greece)	62.6 ± 0.8	56.2 - 69.5	31	4.3 ± 0.2	2 - 8	28	[9]
W. Peloponnese (Greece)	66.7 ± 1.1	53.5 - 79.5	15	5.8 ± 1.0	4 - 10	6	[9]
Epirus (Greece)	62.6 ± 1.6	53.3 - 69.5	12	4.6 ± 0.5	3 - 6	5	[9]
Crimea (Ukraine)	-	52.5 - 65.5	17	2.9 ± 0.5	2 - 4	17	[11]
Lunca Jiului (S. Romania)	54.1	52.5 - 66.6	-	(3-4)*	2 - 6	77	[8]
Nisipurile Obedeanu (S. Romania)	54.7	53.2 - 65.7	-				

Table 3. The clutch size and size of reproductive females (SVL) of different populations of *P. taurica*. * only range is available, - data are not available.

and 0.06 respectively), suggesting that there was some tendency of increasing clutch size and mass with female size which appeared not to be statistically significant perhaps because of the small sample size. The rest of the variables did not show significant effects (all P>0.19, see Table 2).

Also, we regressed all egg and clutch characteristics against the duration in captivity. All regressions were non-significant (P>0.14 for all variables), suggesting that females held longer in captivity prior to producing their clutch did not modify attributes of those clutches.

Within lizard species, clutch size usually increases with female size unless clutch size is small [19-24; but see 17,25]. A significant body size-clutch size correlation exists in southern *P. taurica* populations [9,10]. There is a suggestion of a similar relationship in the studied population, however for egg dimensions we found little evidence for strong association with female size.

Differences in egg characteristics among different-sized clutches were tested only between two-egg and three-egg clutches, due to the small samples of other clutch-sizes. Eggs from two-egg clutches were not significantly larger (egg length, width and volume, t test; t=0.20–0.38; df=11 and P>0.71 in all cases), or heavier (t=1.27; df=11, P=0.23) than eggs from three-egg clutches. Thus, a trade off between the egg mass and/or size and clutch size (usually arises as a consequence of space constraints or limited food resources), although widespread in the lacertid lizards species [e.g. 26,27], was not detected in this as in other studies on the Balkan wall lizard [9,11,28].

To make a comparison, we presented clutch size and female size of the Deliblato Sand population of *P. taurica* with the data available for other studied populations of the same species (Table 3). Although statistical significance of differences in these traits between the Deliblato Sand and other presented populations could not be determined, it appears that the Balkan wall lizard in Deliblato Sand exhibited smaller female body size and reproductive output than those reported in previous studies on the same species. However, "reproductive strategy" concerning relationships between reproductive characteristics seems not to be different from that in more core areas, or in southern peripheral populations.

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