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FEMALE REPRODUCTIVE CHARACTERISTICS OF THE HORVATH'S ROCK LIZARD *(IBEROLACERTA HORVATHI)* FROM SLOVENIA

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Abstract - In this paper, we present data on the female reproductive traits of the Horvath's rock lizard from Slovenia. The clutch, egg and hatchling characteristics were investigated based on clutches laid in laboratory conditions by pregnant females collected from a natural population. A female lays one clutch of eggs annually with an average number of three (range 1-5) eggs. We found a significant positive correlation between female size and egg width and volume. The mean egg length and volume in a clutch decreased significantly with clutch size independently of female size. The incubation period averaged 44 days. Significant positive correlations were found between the hatchling total length and mass, and egg mass. There was a significant negative relationship between the egg mass and incubation duration. The life-history strategy of the Horvath's rock lizard appeared to be shaped by several factors, such as the constraints of a high-altitude environment, flattened body morphology and possibly phylogenetic background.

Key words: Life history, lacertid lizard, trade-off, clutch size

INTRODUCTION

Life history traits such as age of maturity, fecundity, reproductive life span, are related to the timing and amount of reproduction, survival, and the survival of offspring, thus contributing directly to an organism's fitness (Roff, 2002). For an organism, the achievement of an optimal life-history strategy within the framework of environmental constraints presents particular challenges. Consequently, many ectotherms, such as reptiles, show variation in growth trajectories, size-specific mortality rates and size-fecundity relationships as plastic responses to suboptimal thermal conditions in cold environments (Arendt, 2011).

Up to now, there have been several studies of the life history traits of high altitude lacertid lizards with different phylogenetic histories (e.g. Castilla et al., 1989; Arakelyan, 2002; Arribas and Galán, 2005; Ljubisavljević et al., 2007). Among them, maybe the most prominent adaptations to harsh climatic conditions appeared in high-altitude species of the *Iberolacerta* genus, which is also evolutionarily distinct among the lacertids (see Arnold et al., 2007). They show a very low fertility, delayed sexual maturation and prolonged egg retention which is interpreted as an advanced stage in the tendency towards viviparity (Arribas, 2004; Arribas and Galán, 2005).

The Horvath's rock lizard (*Iberolacerta horvathi*) is separated from the rest of the representatives of the genus on the Iberian Peninsula by a large disjunction of about 1100 km (Arnold et al., 2007). Like other congenerics, it is a relict species, most likely without significant range expansions since its origin (Carranza et al., 2004). It is patchily distributed in the Alpine-north Dinaric mountain areas in western Croatia, western and southern Slovenia, northeastern Italy and southern Austria, while its presence in Germany is thought to be the result of human introduction (Bischoff, 1984; De Luca, 1989; Lapini et al., 2004; Arnold et al., 2007). Although it is believed that *I. horvathi* is restricted to rocky habitats with poor pioneer vegetation at higher altitudes between 600-1900 m (De Luca, 1989), new findings pointed to occurrence of this species at much lower locations of 200-370 m in Slovenia on rocks in shady and moist conditions (Žagar et al., 2007; Žagar, 2008). The Horvath's rock lizard is a diurnal, heliothermic, insectivorous, oviparous, and a distinctly flattened small lacertid species (Arnold and Burton, 1978). Although a few studies have focused on the ecology of the species (De Luca, 1992; Lapini et al., 1993), information regarding clutch and hatchling characteristics is missing.

In this paper, we provide data about the female reproductive characteristics of the Horvath's rock lizard from Slovenia obtained through pregnant females from a natural population. We report data on clutch and hatchling characteristics and their relationships and incubation duration under controlled, laboratory conditions. Thus, we provide more complete information regarding the life history of this environmentally constrained species.

MATERIALS AND METHODS

Study population and laboratory procedure

Analysis of the female reproductive traits and laboratory hatching study were carried out on a sample from the population of *I. horvathi* from the Julian Alps in western Slovenia and the Dinarides in southern Slovenia. Pregnant females were collected from tree localities in the Alpine region: Kluže (46°22' N, 13°36' E, 1033 m a.s.l.), Strmec (46°25' N, 13°35' E, 1050 m), Predel (46°25' N, 13°34' E, 1173 m) and two localities in the Dinaric region: Male Bele Stene (45°41' N, 14°41' E, 1010 m), Taborska Stena (45°34' N, 14°43'E, 960 m). These populations inhabit calcareous rocks, concrete road margins and old stonewalls partially covered with petroliferous vegetation, often surrounded by beech forests (*Anemono-trifoliae-Fagetum in* the Alpine region and *Omphalodo-Fagetum* in the Dinaric region (Čarni et al., 2002). The climate is moderate continental, modified by mountain climatic conditions. Mean annual temperatures are between 6-8°C, while annual amount of precipitation vary between 1600-2000 mm in the Dinaric region and 2600-3200 mm in the Alpine region (Ogrin and Plut, 2009).

Sixteen pregnant females were captured from the first to the third week of June in 2010 and transported to the laboratory (located in Koper, Slovenia). The lizards were collected under permits provided by the Ministry of the Environment and Spatial Planning – Slovenian Environment Agency, Republic of Slovenia (no. 35601-12/2010-4). They were housed in individual terraria under the same conditions, with exposure to natural and additional artificial light that created a thermal gradient for 12 h a day from sunrise to sunset. Food consisted of mealworms and insects and water was provided *ad libitum*. The females were inspected daily. Following oviposition, they were measured for snout-vent length (SVL) and weighed. Some of them were autopsied to check for the presence of vitellogenic follicles, while others were released at the point of capture. Immediately after oviposition, the eggs were dug up and carefully removed from the terrarium, weighed and measured (maximum length and width). A digital caliper (0.01 mm precision) was used for 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: $V=4/3\pi a^2 b$, **a** and **b** being half of the width and length of the egg, respectively (see e.g. Amat et al., 2000). 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.

The eggs were marked and placed in plastic boxes filled with moistened vermiculite for incubation in

Table 1. Summary of statistics for measurements of pregnant females, clutch, egg and hatchling size of *I. horvathi*. For egg and hatchling attributes, the average values for each clutch were used.

measurement	mean ± SE	range	N
Female SVL (mm)	55.06 ± 0.88	49.70 – 62.06	16
Clutch size	3.31 ± 0.25	1 – 5	16
Post-oviposition female body mass (g)	3.022 ± 0.202	1.972 – 4.721	16
Clutch mass (g)	1.304 ± 0.112	0.450 – 2.217	16
Relative clutch mass (RCM)	0.435 ± 0.028	0.155 – 0.632	16
Egg mass (g)	0.395 ± 0.012	0.332 – 0.461	16
Egg length (mm)	13.63 ± 0.22	12.29 – 15.65	16
Egg width (mm)	7.48 ± 0.06	7.17 – 8.11	16
Egg volume (mm^3)	398.74 ± 8.96	347.34 – 480.83	16
Hatchling total length (mm)	62.98 ± 2.01	52.00 – 70.00	8
Hatchling SVL (mm)	24.83 ± 0.42	22.93 – 26.68	8
Hatchling mass (g)	0.406 ± 0.017	0.350 – 0.477	8

Table 2. Summary statistics of linear regression of egg and hatchling characteristics (based on average values for each clutch) on female SVL of *I. horvathi*.

Dependent variable	*r*	*F*	d.f.	*P*
Clutch size	0.38	2.32	1,14	0.15
Clutch mass	0.50	4.59	1,14	0.05
RCM	-0.05	0.03	1,14	0.87
Egg mass	0.40	2.58	1,14	0.13
Egg length	-0.03	0.01	1,14	0.92
Egg width	0.77	20.05	1,14	0.0005
Egg volume	0.53	5.33	1,14	0.04
Hatchling total length	0.05	0.02	1,6	0.90
Hatchling SVL	0.08	0.04	1,6	0.85
Hatchling mass	0.01	0.0007	1,6	0.98

the laboratory. The room temperature was controlled within an interval of 28-30°C during the incubation period. The eggs were inspected daily to verify their viability. The moisture of the substrate was checked daily and, if necessary, distilled water was mixed evenly into the substrates to compensate for water absorbed by the eggs and for loses due to evaporation, so that the water potential of the substrate was maintained constant. Immediately after hatching, the hatchlings were weighed (to the nearest 0.01 g) and measured (SVL and total length (L) to 0.01 mm). The duration of incubation was defined as the elapsed time from egg laying to hatchling emergence.

Statistical analyses

Descriptive statistics (mean, standard error, range) for all traits were calculated. For subsequent analyses, all variables were log-transformed to ensure data normality and to generate homogeneous variances (Sokal and Rohlf, 1981). Linear regression analyses were used to study the interrelationships among var-

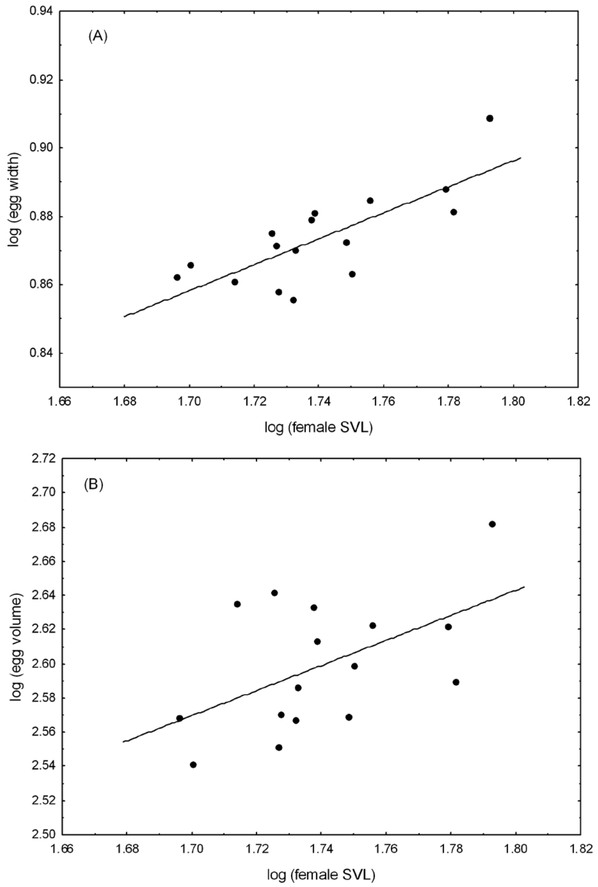

Fig. 1. Relationship between female size and egg width (a) and egg volume (b) in *I. horvathi.*

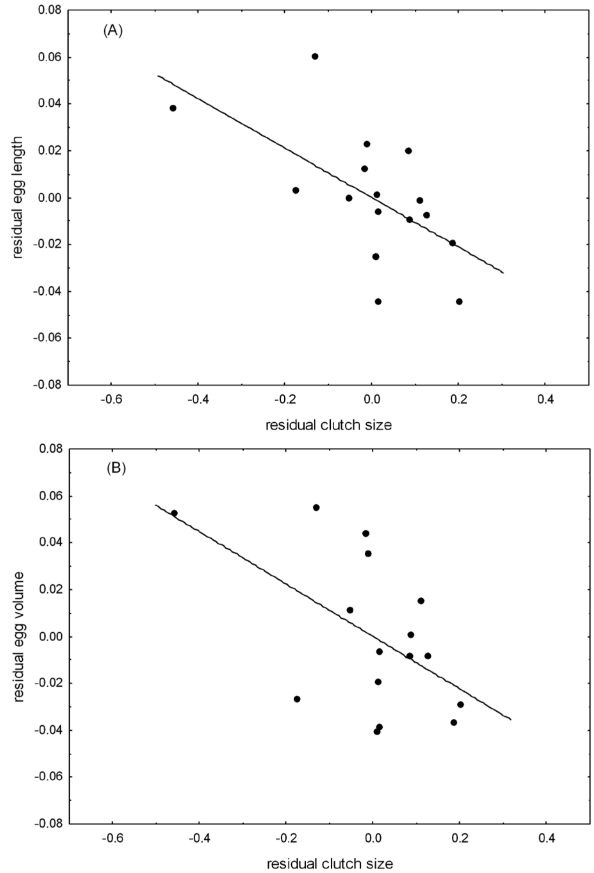

Fig. 2. Relationship between residual clutch size and egg mass (a) and egg length (b) in *I. horvathi.*

ious reproductive traits. The effects of maternal SVL were removed by calculating residual scores from the separate regressions of egg and clutch characteristics on female SVL. The analyses were carried out using the computer package Statistica® (STATISTICA for Windows. StatSoft, Inc., Tulsa, OK).

RESULTS

Oviposition and clutch frequency

Oviposition in laboratory conditions took place from the end of June to the second week of July 2010. The smallest pregnant female in our sample was about 50 mm in SVL. The examined post-gravid females did not have enlarged vitellogenic follicles, suggesting the production of one clutch per season.

Clutch characteristics

Pregnant females had an average SVL of 55.1 ± 0.9 mm, and a clutch size of 1 to 5 eggs (mean 3.3 ± 0.3, Table 1). The laid eggs had a mean length of 13.6 ± 0.2 mm, a mean width of 7.5 ± 0.1 mm, and a mean mass of 0.40 ± 0.01 g. The relative clutch mass ranged from 0.16 to 0.63, with the mean value of 0.44 ± 0.03 (Table 1). Clutch size was not significantly correlated with female SVL (r = 0.38, P = 0.15), while the positive relationship between female SVL and clutch mass was of *borderline significance (*r = 0.38, P = 0.05), suggesting that there was some tendency of increasing clutch size and mass with female size. Egg width and volume increased significantly with female SVL (r = 0.77, P < 0.001 and r = 0.53, P = 0.04, for egg width and egg volume respectively (Fig. 1). The rest of the variables did not show significant effects (for values of estimated coefficients see Table 2).

Residual egg length and volume decreased with increasing residual clutch size (r = -0.61, $F_{1,14}$ = 8.43, P < 0.01 and r = -0.55, $F_{1,14}$ = 5.94, P = 0.03, for egg length and egg volume, respectively). Hence, larger clutches were composed of shorter eggs of less volume and this relation was independent of female SVL (Fig. 2). No significant correlation between egg width and clutch size was found when removing the effect of female body size (r = -0.02, $F_{1,14}$ = 0.004, P = 0.95).

Incubation period and hatchling characteristics

Clutches deposited in the laboratory hatched in the second half of August 2010. Eggs required between 40 and 47 days to hatch (mean 43.69 ± 0.82 days). There was no relationship between the SVL of females and their offspring mass and size characteristics (for values of estimated coefficients see Table 2). The hatchling mass and size showed significant positive correlation to egg mass (hatchling mass: r =0.87, hatchling SVL: r = 0.74, hatchling total length: r = 0.76, P < 0.05 in all cases). There was a significant negative correlation between the egg mass and incubation duration (r = -0.82, $P < 0.05$).

DISCUSSION

The Horvath's rock lizard exhibits several life-history characteristics common for small high-altitude lacertids that appear to be linked to the unfavorable thermal regimes and short annual activity period in the mountains (Arribas and Galán, 2005; Ljubisavljević et al., 2007). For example, similarly to Balkan endemic mountain *Dinarolacerta* and other representatives of *Iberolacerta* genus, the Horvath's rock lizard produces one small clutch annually. In addition, a significant negative relationship between the incubation duration and egg mass (which is translated directly into hatchling size and mass) has also been found in some lacertids living in harsh conditions (Marco and Pérez-Mellado, 1998; Ljubisavljević et al., 2007). This pointed out the benefits associated with an increment of egg and juvenile size due to the survival advantage of larger and earlier-hatched offspring over smaller and later-hatched. However, other high altitude rock lizards are characterized by prolonged egg retention inside the female body and consequently short incubation period (Arribas and Galán, 2005; Ljubisavljević et al., 2007), while such a tendency was not observed in *I. horvathi* (De Luca, 1992; this study). Actually, in this species the incubation period appeared to be longer than in the majority of small Balkan lacertids from low altitudes (Ljubisavljević et al., unpublished data). This pattern could be related to the fact that the studied population inhabits an intermediate altitude relative to the Pyrenean species of *Iberolacerta* (e.g. Arribas, 2004; Arribas and Galán, 2005), but may also relate to phylogenetic factors and/or particular environmental characteristics of the habitats of the Horvath's rock lizard.

In the *I. horvathi,* egg shape is likely to reflect some morphological constraints of maternal body shape. A trade-off between egg and clutch size is apparent in the Horvath's rock lizard. At a given female size, larger clutches are composed of shorter eggs than are smaller clutches. This relationship between clutch size and egg shape indicates that reproductive allocation is limited by body volume (Castilla and Bauwens, 2000). Furthermore, we found that larger females laid shorter but wider eggs than smaller females, indicating morphological constraints of the pelvic canal width (Congdon and Gibbons, 1987; Sinervo and Licht, 1991).

Abdominal volume is mainly determined by body and pelvic width (Goodman, 2006). It was shown that body flattening is negatively correlated with abdominal volume, so that flatter species, such as *I. horvathi,* have reduced abdominal volumes (Goodman et al., 2009). Therefore, flat species may exhibit reduction in reproductive output (RCM), or alternatively, be "more full" of eggs. RCM in *I. horvathi* appeared to be equivalent or greater to that of more cylindrical Balkan lacertids (e.g. Bejaković et al., 1996; Ljubisavljević et al., 2010). Therefore, by producing less elongated but wider eggs, larger and wider females "pack" eggs more efficiently by being "more full" of eggs, allowing their body cavity to bulge more.

To sum up, the life-history strategy of the Horvath's rock lizard appeared to be shaped by several factors such as the constraints of a high-altitude environment, flattened body morphology and possibly phylogenetic factors.

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