Journal of Zoological Research
https://ojs.bilpublishing.com/index.php/jzr

ARTICLE

Reproductive Characteristics of the Kulzer's Rock Lizard *Phoenicolacerta kulzeri* (Reptilia: Lacertidae)

Fida Nassar*

Department of Life and Earth Sciences, Laboratory of Georesources, Geosciences and Environment(L2GE), Faculty of Sciences 2, Fanar Campus, Lebanese University, Lebanon

ARTICLE INFO

Article history
Received: 31 October 2021
Accepted: 8 November 2021
Published Online: 19 November 2021

Keywords:
Oviparity
Sexual dimorphism
Clutches
Vitellogenic follicles
Ovarian cycle

ABSTRACT

The present study aims to determine, by histological examination, the female reproductive cycle of specimens of Kulzer's rock lizards collected in a mountainous region in Lebanon. Females of *Phoenicolacerta kulzeri* followed a seasonal reproductive pattern. Winter hibernation period lasted for 5 months. Females of *P. Kulzeri* exhibited a reproductive activity during spring characterized by the presence of vitellogenic follicles in the ovaries and eggs in the oviducts. Oviposition occurred throughout the springtime. Up to two clutches were produced with an average clutch size of 2.9 eggs. Clutch size was significantly correlated to female body size. The attainment of sexual maturity was at a minimum size of 46 mm snout-vent length. The mean body size of adult females was 55.0 mm and did not differ significantly from that of adult males. Females showed a period of sexual rest in summer and none of them showed a reproductive activity in autumn. The female reproductive activity of *P. kulzeri* was well-synchronized with that of the males in spring; however, an autumnal asynchrony was observed between the female and male reproductive cycles, since males continued to exhibit a spermatogenetic activity.

1. Introduction

The Lacertidae family is a diverse family of wall lizards of about 40 genera with more than 180 species widely distributed across Europe, the Middle East and North Africa [1]. Some of them reached Eastern Asia [1,2]. Lebanon, with its Mediterranean climate characterized by a wet cool winter and dry warm summer, has different species of lacertid lizards [1,3] including *Acanthodactylus Schreiberei* which distribution is restricted to the Mediterranean coastal areas, *Ophisops elegans* very common in Lebanon with a wide distribution in Turkey reaching Southwest Asia and *Parvilacerta fraasii* endemic to Lebanon. Two more species that belong to the genus *Phoenicolacerta,* are also found in Lebanon [1,3]. They were previously classified and known as species of the genus *Lacerta* [4]. The Lebanon lizard, *Phoenicolacerta laevis* is the most widespread species in the Middle East and extends from Lebanon and Jordan to Turkey and Georgia [1,5] and the Kulzer's rock lizard, *Phoenicolacerta kulzeri* (Müller & Wettstein, 1932)

**Corresponding Author:*
Fida Nassar,
Department of Life and Earth Sciences, Laboratory of Georesources, Geosciences and Environment, Faculty of Sciences 2, Fanar Campus, Lebanese University, Lebanon;
Email: fida.nassar@ul.edu.lb

DOI: https://doi.org/10.30564/jzr.v3i4.4065

comprises three subspecies: two of them *Phoenicolacerta kulzeri petraea* and *Phoenicolacerta kulzeri khazaliensis* are found in Southern Jordan [6,7] and our studied subspecies *Phoenicolacerta kulzeri kulzeri* found in rocky areas of high altitude mountains in Lebanon [3], presumably its distribution extends to neighbouring countries [8].

Molecular and phylogenetic studies showed close relationships between *P. laevis* and *P. kulzeri* but they were considered as distinct species [4,9-11]; The genus *Phoenicolacerta* includes two other species, the Syrian rock lizard *Phoenicolacerta cyanisparsa,* found in Syria Arab Republic and Turkey [12]; and the Troodos rock lizard *Phoenicolacerta troodica* endemic to Cyprus [13]. *Phoenicolacerta kulzeri* was classified as endangered species with a population trend decreasing [8], while the population trend of *P. cyanisparsa, P. troodica* and *P. laevis* is stable [12-14].

Like most of lizards of the Lacertidae family, *Phoenicolacerta* species are all oviparous. However, the genus *Eremias* contains both viviparous and oviparous species and one lacertid species the European common lizard *Zootoca vivipara* exhibit reproductive bimodality [15]. Oviparous species can produce multiple clutches during the breeding season. The stages of embryonic development at the time of oviposition depend on the lacertid species and vary from stage 22 - 34 [15] (early organogenesis - late organogenesis) according to the classification of Dufaure and Hubert [16]. Reproductive cycles have been well-documented in lizards [17,18]. They were classified as either seasonal or continuous reproductive cycles based on relationships between gametogenesis, mating behavior and hormones production [19,20]. Lizards inhabiting temperate zones and high elevations show in general a seasonal reproductive pattern, while others from tropical regions display a continuous spermatogenic cycle in which species breed continuously throughout the year [17,18]. However, each species brings its own characteristic and variations in the reproductive pattern such as the length of the reproductive activity, attainment of sexual maturity, clutch size. Therefore, the objective of this study was to determine the reproductive characteristics of the female reproductive cycle of *P. kulzeri* lizards. This will contribute to better understand the reproductive strategy of this species knowing that males of *P. kulzeri* from the same herpetological collection, exhibited spermiogenesis throughout all the reproductive period [21].

2. Material and Methods

Female reproductive organs (ovaries and oviducts) of 57 specimens of *P. kulzeri* were processed for histological analysis. They were removed from lizards collected in 2000, from April to November, and in 2001, from April to September, and deposited at the Natural Museum of the Lebanese University by Souad Hraoui-Bloquet. No specimens were collected during the hibernation winter which lasted from November to March. The area of collection was the mountainous region of Mahrouka - Sannine with an altitude of around 2000 m (34° 00'N, 35° 52'E). The habitat was described to be predominantly rocky with sparse vegetation, with a mean annual temperature of 14.9°C and a total precipitation of 44.8 mm [21] (10 years mean for the period 1998-2007). Snout-vent length (SVL) of each specimen was measured to the nearest 0.01mm using a caliper. Ovarian follicles (yolky and enlarged follicles) and oviductal eggs were counted and measured. They were fixed in Bouin's solution, dehydrated in increasing concentrations of ethanol, starting in 70% and ending in absolute ethanol. The clearing step was performed with butanol. Samples were kept in butanol until paraffin embedding using standard protocol. The blocks of paraffin-embedded ovaries were cut into sections of 5µm thickness using a rotary Reichert Jung microtome. Sections were stained with the routine combination of the two histological dyes, haematoxylin followed by eosin counterstain. Ovary slides were examined with a light Olympus CHB microscope for the state of follicles, vitellogenic or atretic follicles and the presence of *corpora lutea* which indicate recent oviposition. Five various female reproductive stages were determined based on the size and the type of the ovarian follicles and the presence of eggs in the oviducts, as shown in Table 1. The software SPSS 20® was used for statistical analysis. All variables were log transformed and tested for normality. Analysis of variance by means of ANCOVA was run using the logarithm of SVL as a covariate. ANCOVA was used when all assumptions were met. Spearman's rank order correlation was run to determine the relationship between clutch size and female body size.

Table 1. Main characteristics of the ovaries and oviducts used to establish the different stages of the female reproductive cycle of the specimens of *P. kulzeri* collected in the mountainous region of Sannine in Lebanon

Main characteristics	Stage
Vitellogenic follicles (2.25--5.5 mm) with yolk accumulation	**Vitellogenesis**
Enlarged ovarian follicles ≥ 5.5 mm and vitellogenic follicles	**First Clutch**
Eggs in the oviducts and vitellogenic follicles	
Enlarged ovarian follicles, *corpora lutea* and vitellogenic follicles	**Second Clutch**
Enlarged follicles, eggs in the oviducts and vitellogenic follicles	
Degeneration of the ovarian follicles	**Atresia**
Non-vitellogenic follicles	**Quiescent**

3. Results and Discussion

The female of *P. kulzeri* with the smallest SVL and exhibiting enlarged ovarian follicles was considered to be sexually mature and reproductively active. Sexually maturity in lacertids is attained at a minimum body size of the lizard and is independent from the age [17]. The smallest reproductively active female exhibiting enlarged ovarian follicles, measured 46 mm in SVL and was from June (Figure 1). Consequently, only females having SVL ≥ 46 mm were considered to be adults. No differences were found in female adult stages between both years (24 females from 2000 and 29 from 2001) with respect to body size (ANCOVA, $F_{(1,50)} = 0.9$, $p > 0.01$). Therefore, the data of both years were pooled for subsequent analysis. The SVL for adult females ranged from 46 to 65 mm, with mean SVL = 55.09 mm ± 4.25 SD, n = 53 and the SVL for subadults ranged from 40 to 45 mm with mean SVL = 42.75 mm ± 2.63 SD. Mean SVL was 53.50 mm ± 4.55 SD for adult males [21]. An independent t-test was conducted to compare SVL in females and in males.

There was no significant difference between adult male and female body sizes, $t_{(106)} = -1.84$, $p > 0.05$. Similar observation, with no sexual body size dimorphism was reported in sympatric lacertid *Ophisops elegans* [22]; However, sexual dimorphism is more common in lizards with the majority of males larger than females [23]; Variation in body size may be explained by sexual selection [23-25] where the males maintain larger body size to compete for territory and access to females [24,25]. Females with larger body size than males are common. This might be due to the effects of their reproductive mode and their lineage identity [25,26]. Environmental factors such as temperature, water and food availability may also be implicated in the variation in body size in reptiles [22,25,26].

No difference was found between the number of enlarged follicles and oviductal eggs with respect to female body size (ANCOVA, $F_{(1,9)} = 1$, $p = 0.34$). Therefore, these data were pooled for estimating clutch size and the number of eggs produced per clutch. The mean clutch size was 2.92 ± 1.27, range = 2-6. However, there was a significant correlation between clutch size and female body size ($R_{\text{Spearman}} = 0.75$, $p = 0.00$, n = 27). The larger females produced up to two clutches whereas smaller females laid only a single clutch. This increase in egg number with female body size is a common occurrence in most lizards including lacertids with multiple clutch sizes [27], where clutch size is adjusted to the changing body size of the female as she grows [28].

The reproductive activity of *P. kulzeri* lizards lasted from April to October. No specimens were collected during the hibernation period between November and March. The first females with enlarged follicles were observed in April. This the first clutch was produced in April. Six of the 8 females examined, exhibited their first enlarged follicles in their ovaries; the two remaining were in vitellogenesis (Figure 1). Among 21 females examined in May and June, 3 females showed eggs in their oviducts whereas 2 females had enlarged follicles in their ovaries and 16 exhibited concomitant presence of enlarged follicles and oviductal eggs indicating a possible second clutch. Nineteen females out 24 females' specimens collected from July through October had mainly non-vitellogenic follicles. Two females showed atretic follicles in their ovaries and three showed early vitellogenesis in September and October (Figure 1). Winter hibernation period lasted for 5 months, from November to March. Mating occurred most probably upon emergence from winter hibernation. Females of *P. kulzeri* exhibited a seasonal reproductive cycle that is common to that of most lacertid lizards from temperate region. After they emerged from hibernation in spring, females of *P. kulzeri* undergo vitellogenesis, followed by a sexual reproductive activity (egg laying period) and a subsequent period of sexual rest (Figure 2). Simultaneously, males of *P. kulzeri* exhibited continuous spermatogenetic activity which lasts from April to October. Similar pattern of reproduction was described in the sympatric lizard *P. laevis* [29-31] (Figure 2) and in mountainous populations of lacertids such as *Podarcis vaucheri* [32,33] and *Takydromus hsuehshanensis* [34]. However, no autumnal reproductive activity was described in none of these species. Indeed, production of eggs by the females showed a seasonal pattern while males produce sperm at all times of the reproductive period [29-31]. Differences in the reproductive pattern and reproductive characteristics in each population could reflect phylogenetic constraints on the reproductive pattern or an adaptive response to the environmental conditions.

4. Conclusions

This study highlights the specific reproductive characteristics and activities in females of the oviparous lacertid lizard *P. kulzeri* and contributes to a better knowledge of the reproductive biology of this species. Females of *P. kulzeri* exhibited a seasonal reproductive pattern with maximum activity in spring. Each female produced up to two clutches per breeding season. Clutch size was correlated with female body size.

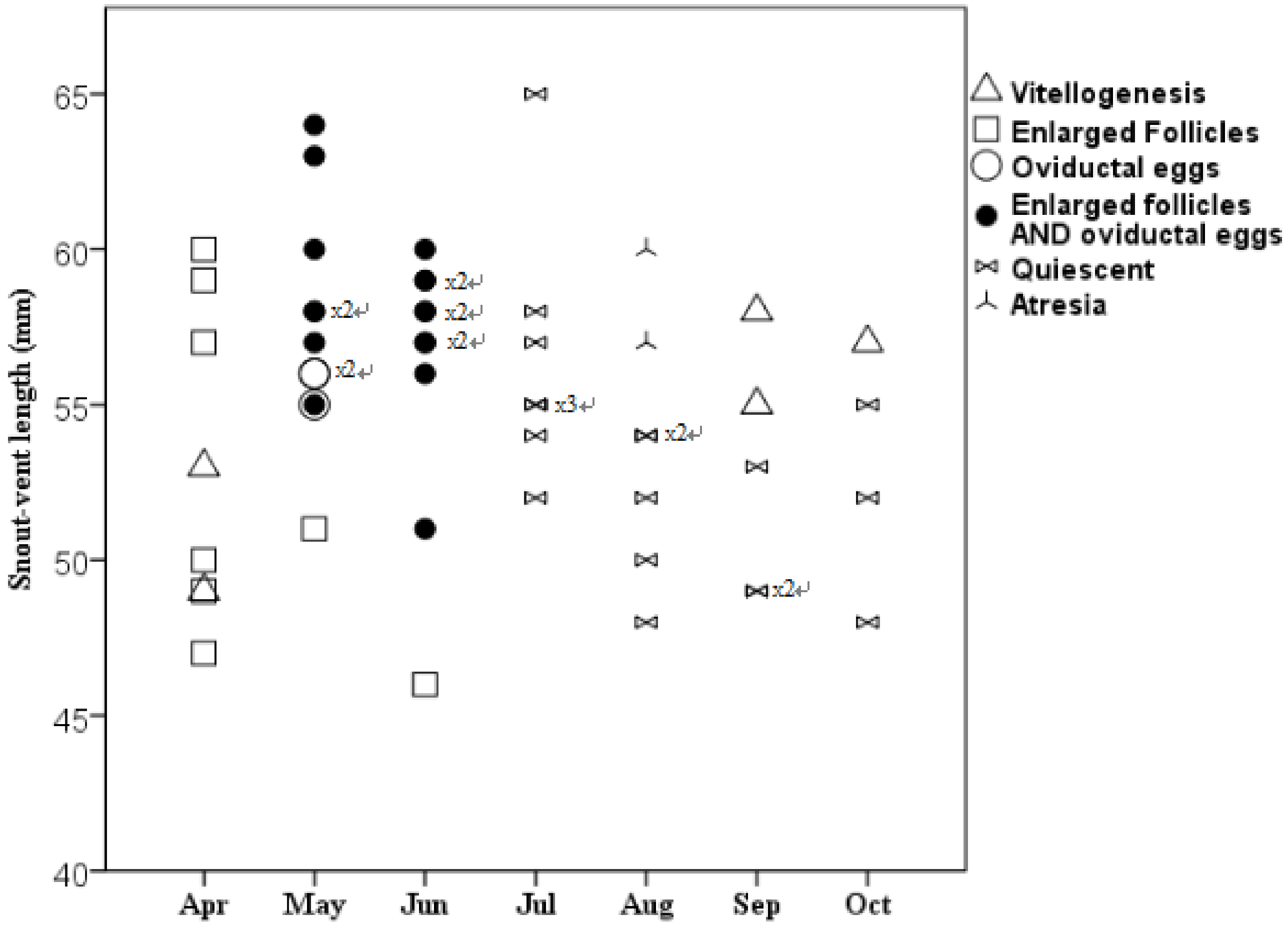

Figure 1. Female reproductive cycle of *P. kulzeri*. Females deposited their first clutch (enlarged follicles or oviductal eggs) in April and May. A second egg clutch (enlarged follicles and oviductal eggs) was laid in May and June. Numbers on the right indicate the number of specimens with a same stage and having a same SVL.

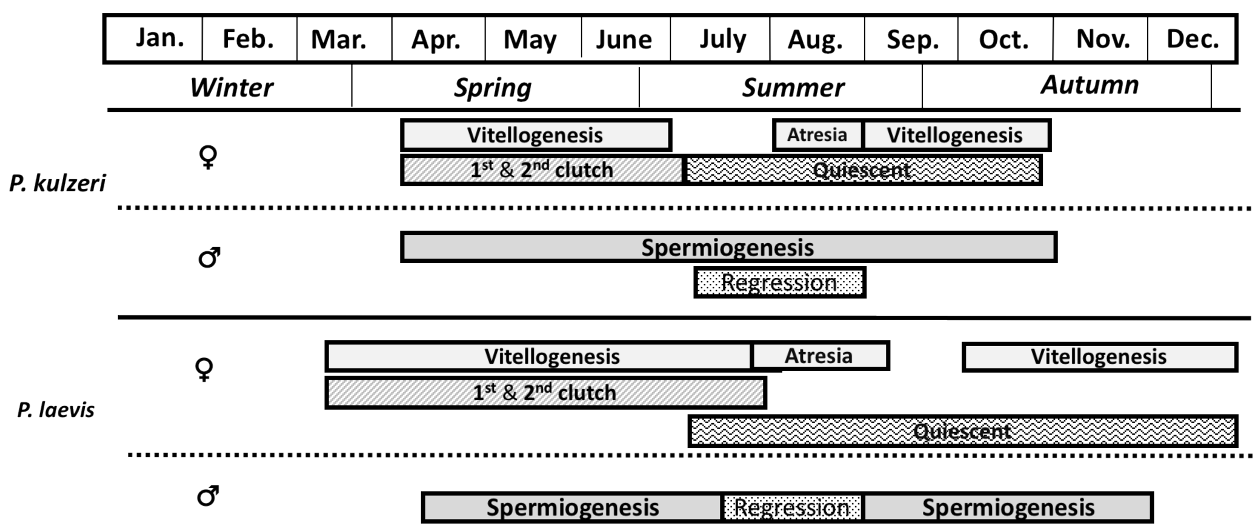

Figure 2. Female and male reproductive cycles of *P. kulzeri* compared with those of the sympatric *P. laevis*. Females of *P. kulzeri* and *P. laevis* showed seasonal activity while males had more or less continuous spermiogenesis.

References

[1] Uetz P, Freed P, Hosek J. The Reptile Database [R]. 2020. Available at http://www.reptile-database.org.

[2] Ananjeva NB, Orlov NL, Khalikov RG, Darevsky IS, Ryabov SA, Barabanov AV. [M]. The Reptiles of Northern Eurasia. 2006; 79-112.

[3] Hraoui-Bloquet S, Sadek R, Sindaco R, Venchi A. The herpetofauna of Lebanon: new data on distribution [J]. Zoology in the Middle East; 2002; 27(1): 35-46. DOI: https://doi.org/10.1080/09397140.2002.10637939.

[4] Arnold EN, Arribas O, Carranza S. Systematics of the Palearctic and Oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera [J]. Zootaxa; 2007; 1430: 1-86. DOI: https://doi.org/10.11646/zootaxa.1430.1.1.

[5] Tarkhnishvili D, Gabelaia M, Kandaurov A, Bukhnikashvili A, Iankoshvili G. Isolated population of the Middle Eastern *Phoenicolacerta laevis* from the Georgian Black Sea Cost, and its genetic closeness to populations from southern Turkey [J]. Zoology in the Middle East; 2017; 63(4): 311-315. DOI: https://doi.org/10.1080/09397140.2017.1361191.

[6] Bischoff W, Muller J. Revision des levantinischen *Lacerta laevis/kulzeri*-Komplexes: 2. Die Petra-Eidechse *Lacerta kulzeri petraea* ssp.n. [J]. Salamandra; 1999; 35(4): 243-254.

[7] Modrý D, Necas P, Rifai L, Bischoff W, Hamidan N, Amr Z. Revision of the Levantine "*Lacerta*" *laevis*/*kulzeri*- Complex: 3. The Rock Lizard of Wadi Ramm, *Phoenicolacerta kulzeri khazaliensis* ssp. n. [J]. Vertebrate Zoology; 2013; 63(3): 307-312.

[8] Disi AM, Hraoui-Bloquet S, Sadek R, Werner Y. *Phoenicolacerta kulzeri*. *The IUCN Red List of Threatened Species* 2006: e.T61524A12503047. https://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T61524A12503047.en.

[9] Beyerlein P, Mayer W. Lacerta kulzeri-its phylogenetic relationships as indicated by DNA sequences [J]. Natura Croatica 1999; 8(3):181-187.

[10] Tosunoğlu M, Göçmen B, Atatür MK, Çevik IE. Morphological and serological investigations on *Lacerta laevis* Gray, 1838 (Sauna: Lacertidae) populations from Anatolia [J]. Zoology in the Middle East; 2001; 23(1): 55-60. DOI: https://doi.org/10.1080/09397140.2001.10637867.

[11] Tamar K, Carranza S, In den Bosch H, Sindaco R, Moravec J, Meiri S. Hidden relationships and genetic diversity: Molecular phylogeny and phylogeography of the Levantine lizards of the genus *Phoenicolacerta* (Squamata: Lacertidae) [J]. Molecular Phylogenetics and Evolution; 2015; 91: 86-97. DOI: https://doi.org/10.1016/j.ympev.2015.05.002.

[12] Tok V, Ugurtas I, Sevinç M, Crochet PA, Kaska Y, Kumlutaş Y, Avci A, Sindaco R. 2009. *Phoenicolacerta cyanisparsa . The IUCN Red List of Threatened Species* 2009: e.T61520A12501576. DOI: https://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T61520A12501576.en.

[13] Lymberakis P. *Phoenicolacerta troodica*. *The IUCN Red List of Threatened Species* 2009: e.T157276A5067952. DOI: https://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T157276A5067952.en.

[14] Crochet P-A, Lymberakis P, Hraoui-Bloquet S, Sadek R, Werner YL, Tok V, Ugurtas, IH, Sevinç M, Kaska Y, Kumlutaş Y, Kaya U, Avci A, Üzüm N, Yeniyurt C, Akarsu, F. 2009. *Phoenicolacerta laevis* (errata version published in 2016). *The IUCN Red List of Threatened Species* 2009: e.T164570A86642956.

[15] Braña F, Bea A, Arrayago, M. J. Egg Retention in Lacertid Lizards: Relationships with Reproductive Ecology and the Evolution of Viviparity. [J]. Herpetologica; 1991; *47*(2), 218-226. http://www.jstor.org/stable/3892736.

[16] Dufaure JP, Hubert J. Table de développement du lézard vivipare : *Lacerta (Zootoca) vivipara* Jacquin. [J]. Archives d'Anatomie Microscopique et de Morphologie Expérimentale ; 1961 ; 50 : 309-328.

[17] Carretero MA. Reproductive cycles in Mediterranean lacertids: plasticity and constraints [M]. 2006; 33-54.

[18] Van Dyke JU. Cues for reproduction in squamate reptiles [M]. 2014; 109-143.

[19] Lovern MB. Hormones and Reproductive Cycles in Lizards [M]. 2011; 321-353. DOI: https://doi.org/10.1016/B978-0-12-374930-7.10012-3.

[20] Lofts B. Reptilian Reproductive Cycles and Environmental Regulators. [M] 1978; 37-43. DOI: https://doi.org/10.1007/978-3-642-66981-1_7.

[21] Rizk K, Nassar F. Male reproduction cycle of Kulzer's Rock Lizard, Phoenicolacerta kulzeri (Müller & Wettstein, 1932), in Lebanon (Reptilia: Lacertidae) [J]. Zoology in the Middle East; 2015; 61(4): 318-323. DOI: https://doi.org/10.1080/09397140.2015.1101920.

[22] Nassar F, Sadek R, Hraoui-Bloquet S. Male reproductive seasonality of the Snake-eyed Lizard, *Ophisops elegans* Ménétries, 1832, from Lebanon (Reptilia: Lacertidae) [J]. Zoology in the Middle; 2016; 63(1):1-7. East. DOI: http://dx.doi.org/10.1080/09397140.2017.1269

396.

[23] Cruz-Elizalde R, Ramírez-Bautista A, Rosas Pacheco LF, Lozano A, Rodríguez-Romero FJ. Sexual dimorphism in size and shape among populations of the lizard *Sceloporus variabilis* (Squamata: Phrynosomatidae) [J]. *Zoology;* 2020; *140*, 125781.
DOI: https://doi.org/10.1016/j.zool.2020.125781.

[24] Olsson M, Shine R, Wapstra E, Uivari B, Madsen T. Sexual dimorphism in lizard body shape: the roles of sexual selection and fecundity selection [J]. Evolution; 2002; 56(7):1538-42.
DOI: https://doi.org/10.1111/j.0014-3820.2002.tb01464.

[25] Cox RM, Kahrl A. Sexual selection and sexual dimorphism [M]. 2015: 78-108.

[26] Roitberg ES, Orlova VF, Bulakhova NA, Kuranova VN, Eplanova GV, Zinenko OI, Arribas O, Kratochvíl L, Ljubisavljević K, Starikov VP, Strijbosch H, Hofmann S, Leontyeva OA, Böhme W. Variation in body size and sexual size dimorphism in the most widely ranging lizard: testing the effects of reproductive mode and climate [J]. Ecology and evolution; (2020); 10(11), 4531-4561.
DOI: https://doi.org/10.1002/ece3.6077.

[27] Roig JM, Carretero MA, llorente GA. Reproductive cycle in a pyrenean oviparous population of the common lizard (*Zootoca vivipara*) [J]. Netherlands Journal of Zoology; 2000; 50(1): 15-27.
DOI: https://doi.org/10.1163/156854200X00243.

[28] Du W, Ji X, Shine R. Does body volume constrain reproductive output in lizards? [J]. Biology Letters; 2005; 1(1):98-100.
DOI: https://doi.org/10.1098/rsbl.2004.0268.

[29] Hraoui-Bloquet S. Le cycle sexuel des mâles chez *Lacerta laevis* dans les montagnes du Liban [J]. Amphibia Reptilia; 1985; 6(2):217-227.
DOI: https://doi.org/10.1163/156853888X00594.

[30] Hraoui-Bloquet S. Le cycle sexuel des femelles chez *Lacerta laevis* Gray 1838 dans la montagne du Liban [J]. Amphibia-Reptilia; 1987; 8(2):143-152.
DOI: https://doi.org/10.1163/156853887X00405.

[31] Hraoui-Bloquet S, Bloquet, G. Le cycle sexuel des mâles chez *Lacerta laevis* sur la côte du Liban et comparaison avec les lézards de montagne [J]. Amphibia Reptilia; 1988; 9(2): 189-195.
DOI: https://doi.org/10.1163/156853888X00594.

[32] Mamou R, Moudilou E, Amroun M, Exbrayat J-M. Reproductive cycle of male wall lizard, *Podarcis vaucheri* (Reptilia: Sauria: Lacertidae), in Djurdjura, Northern Algeria [J]. Basic and Applied Herpetology; 2017; 31:77-89.
DOI: https://doi.org/10.11160/BAH.77.

[33] Mamou R, Moudilou EN, Ghoul A, Exbrayat J-M. Cycle de reproduction des femelles de *Podarcis vauvcheri* (Reptilia, Lacertidae) du Djurdjura, nord Algérie [J]. Bulletin de la Société Herpétologique de France; 2019; 169(3): 11-26.

[34] Huang W-S. Reproductive Cycles of the Grass Lizard, *Takydromus hsuehshanensis*, with Comments on Reproductive Patterns of Lizards from the Central High Elevation Area of Taiwan [J]. Copeia; 1998; 4(30): 866-873.
DOI: https://doi.org/10.2307/1447333.