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Female reproductive cycle of the lacertid lizard *Acanthodactylus schreiberi syriacus* (Reptilia: Squamata) from Lebanon

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Abstract. We studied the female reproductive cycle in a population of the oviparous lizard *Acanthodactylus schreiberi syriacus* from sandy beaches of Tyre, Lebanon. Females emerged from hibernation in mid-April, one month later than males. They reached sexual maturity at 57 mm snout-vent length and had smaller body sizes than males. Fertilization started in May. Among 33 gravid females, only one female showed evidence for two clutches produced in the same reproductive season whereas most of females laid one clutch per season. Mean clutch size was 2.5 ± 1.0, ranging from 1-4. We found no significant correlation between female body size and clutch size.

Keywords. Vitellogenesis, oviductal eggs, ovaries, clutch, histology, oviparity.

Introduction

Schreiber's fringe-fingered lizards *Acanthodactylus schreiberi* Boulenger, 1878 are oviparous lizards found in the Eastern Mediterranean and include three subspecies: *A. s. schreiberi* from Cyprus, *A. s. ataturi* Yalcinkaya et Gocmen, 2012 from Turkey, and our studied population *A. s. syriacus* Boettger, 1878 which distribution is restricted to the Mediterranean coastal areas of Haifa and Tyre (Salvador, 1982; Hraoui-Bloquet et al., 2002). The genus *Acanthodactylus* includes more than 40 species distributed from North Africa to the Middle East through the Arabian Peninsula and occurs in the west as far as Pakistan and Afghanistan (Salvador, 1982; Arnold, 1983). One species, *A. erythrurus* (Schinz, 1833) reaches the Iberian Peninsula from Morocco (Harris et al., 2004). Taxonomically it is a very complex group, since most species are morphologically similar and often show intraspecific variability (Blanc and Cariou, 1980; Blanc, 1984; Werner et al., 2012). Systematics of *Acanthodactylus* species was investigated in order to clarify their phylogenesis (Arnold, 1983; Bons and Geniez 1995; Harris et al., 2000; Carretero et al., 2011). Recently, Tamar et al. (2014) revised the taxonomy of *A. s. syriacus* and assigned it to *A. boskianus asper* (Audouin, 1829). *Acanthodactylus schreiberi* being restricted to Cyprus and Turkey.

Several studies have reported information about reproduction of *Acanthodactylus* species (Busack and Klosterman, 1987; Al-Johany and Spellerberg, 1988; Carretero and Llorente, 1995; Goldberg, 2013). Phylogeny and/or environment influence the reproductive pattern in lizards (Bons and Saint-Girons, 1982; Blanc, 1984; Castilla et al., 1992). *Acanthodactylus schreiberi* was classified as endangered (Hraoui-Bloquet et al., 2009). They are threatened by coastal urbanization. In 1998, Tyre coast was declared as nature reserve. In this paper, we studied the female reproductive pattern of a population of *A. s. syriacus* from Tyre - Southern Lebanon, by histological examination of museum specimens and compared it with reproductive characteristics of other populations of *Acanthodactylus* species from different geographical localities.

Materials and Methods

The study area is located in the Mediterranean coast of Tyre (Sour; 33°15′E, 35°12′N), Southern Lebanon.

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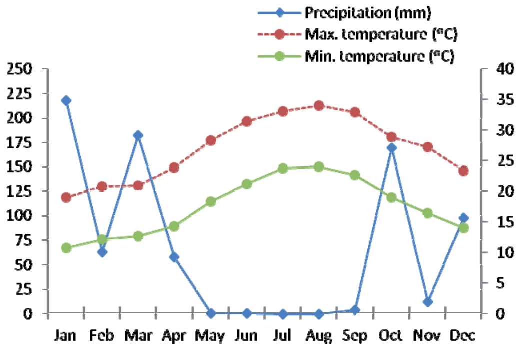

Figure 1. Monthly variation of mean precipitation (left axis) and mean minimum and maximum air temperatures (right axis) during the studied year.

It consists of sandy dunes with sparse vegetation of mainly Juncaceae, Asteraceae and Brassicaceae families, with mean annual temperature of 21.22°C and total annual precipitation of 808.84 mm (30 years mean for the period 1964-1995 with missing data for the two years, 1976 and 1982). The corresponding values for the studied year 1996 were 22.2°C and 804 mm, respectively. Monthly variations of mean precipitation and temperature during the studied year are shown in Fig. 1. Climatological data were obtained from Beirut located at about 70 km from Tyre with similar climate characteristics.

A total of 150 *A. s. syriacus* lizards were captured from April to November 1996. Snout-vent length (SVL) of each lizard was measured immediately with a caliper to the nearest 0.01 mm. All lizards were anesthetized, dissected and the gonads removed. Enlarged ovarian follicles and oviductal eggs of females were counted and measured then stored in Bouin'solution. Later in the laboratory, the ovaries were subject to histological analysis. They were dehydrated in increasing concentrations of ethanol and kept in butanol until paraffin embedding using standard protocol. They were sectioned with a rotary microtome at 5 µm and stained using hematoxylin and eosin. Ovary slides were examined for the presence of vitellogenic follicles or corpora lutea. Female reproductive cycle was determined based on the size and the state of their ovarian follicles and the presence of eggs in their oviducts. Four stages were noted in the ovarian cycle: (i) Non vitellogenic follicles; (ii) Vitellogenic follicles (2.25 - 5.5 mm); (iii) Enlarged vitellogenic follicles ≥ 5.5 mm; (iv) Oviductal eggs. Females from May which they have laid their oviductal eggs and presented corpora lutea in their ovaries were counted as oviductal eggs. They had previtellogenic follicles in the germinal epithelium with non vitellogenic follicles. The smallest SVL of females containing enlarged follicles was considered the minimum size at sexual maturity. Females with inactive gonads were considered as subadults. The production of a second clutch was determined by concomitant presence of enlarged follicles and oviductal eggs or the presence of two distinct sets of corpora lutea. The collected lizards were deposited at the Natural History Museum of the Lebanese University. We used the software SPSS 20.0® for statistical analysis.

Results

Our sample consisted of 58 adult females, 74 adult males and 18 subadults (Fig. 2). The mean SVL was 66.36 mm ± 4.33 SD (range = 57 - 77 mm) for females, 72.27 mm ± 7.85 SD, (range = 54 - 86 mm) for males. The independent t-test results showed significant difference between male and female body sizes, $t_{(130)} = 5.15$, $p < 0.001$, therefore, males have larger body sizes than females. The smallest reproductively active female measured 57 mm in SVL and was from August. Fifteen immature females were found with SVL < 55 mm. Their

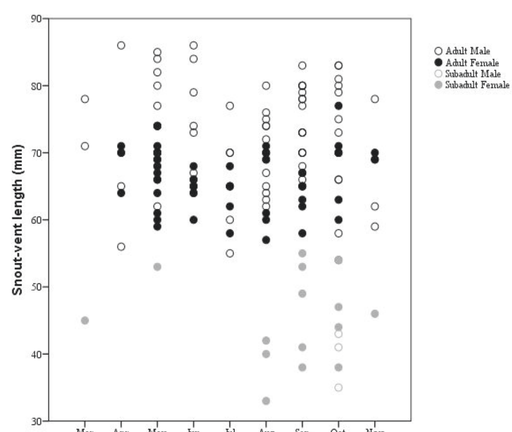

Figure 2. Monthly distribution of snout-vent length for *Acanthodactylus schreibeiri syriacus* lizards.

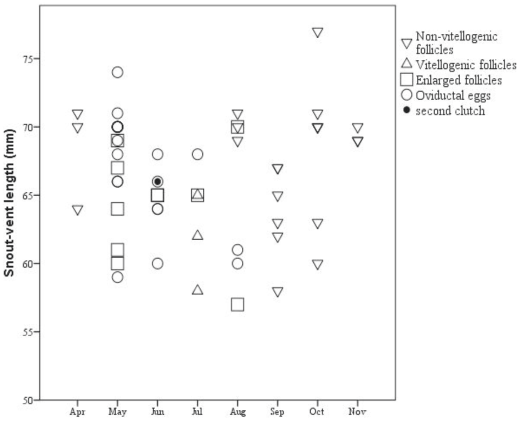

Figure 3. Female reproductive cycle of *Acanthodactylus schreiberi syriacus* lizards. The solid circle indicates female which produced two clutches.

mean SVL was 45.20 mm ± 6.66 SD (range = 33 - 55 mm).

Females of *A. s. syriacus* spend four months in hibernation from December to March. They emerged from hibernation in April. The three females collected in April (Apr, 13) had non-vitellogenic follicles (Fig. 2). The first enlarged follicles as well as the first oviductal eggs were seen in May (May, 27). Thus, fertilization started in May. There was an asynchronisation among females during the active reproductive period, from May to August. Among 38 females examined, 21 females showed eggs in their oviducts whereas 11 females had enlarged follicles in their ovaries and 3 had vitellogenic or non vitellogenic follicles (Fig. 3). None of the females exhibited concomitant presence of enlarged follicles and oviductal eggs. However, one female from June (Fig. 3; solid circle) 66 mm SVL with nor enlarged ovarian follicles and neither oviductal eggs revealed two distinct sets of corpora lutea. No atretic follicles were found in any case. The minimum diameter of vitellogenic follicles was 2.25 mm. We found no differences between the number of vitellogenic follicles (≥ 2.25mm) and oviductal eggs with respect to female body size (ANCOVA, $F_{1,34} = 0.26$, $P = 0.61$, n = 37). Therefore, these data were pooled for estimating clutch size. The mean clutch size was 2.60 ± 1.0, range = 1 - 4. Most of females (38%) laid 2 eggs. About 24% laid 3 or 4 eggs while 14% one egg. The total number of eggs produced over the entire season was 96 eggs**.** Clutch sizes were not affected with female body size ($R_{Spearman} = 0.19$, $p = 0.24$). However, we found a significant correlation between egg sizes and female body size ($R_{Spearman} = 0.55$, $p < 0.001$). All specimens collected in September (Sept, 4), October (Oct, 5 - Oct, 20) and November (Nov, 9) had non-vitellogenic follicles (Fig. 3).

Discussion

Females of *A. s. syriacus* reached maturity at 57mm SVL as we considered females with enlarged follicles in their ovaries as reproductively active. There is a difference in the attainment of sexual maturity in different *Acanthodactylus* species. Females of *A. boskianus* reached sexual maturity at 48 mm SVL determined by the early yolk deposition (Goldberg, 2013), and those of *A. schmidti* Haas, 1957 at 71 mm SVL, determined by the presence of oviductal eggs (Al-Johany and Spellerberg, 1988). However, they both mature from their first year of age (Al-Johany and Spellerberg, 1988; Roobas and Feulner, 2013). *Acanthodactylus erythrurus* reached maturity in the first or second year of age depending on the individual (Carretero and Llorente, 1995). Similar observations were found in *A. s. syriacus* females. For hatchlings born in August and September, sexually mature size would be reached one year later, end of summer or autumn an inappropriate time for the reproductive activity. Their first breeding would be in the subsequent spring (second year of age).

Acanthodactylus schreiberi syriacus showed a common reproductive pattern of temperate region. Since the first oviductal eggs were seen in May, it is most probably that vitellogenesis commenced immediately thereafter. Females emerged from hibernation in spring and started vitellogenesis, followed by a period of gravid state and subsequent period of sexual rest (Fig. 4). This sequence of events is similar to that observed among *Acanthodactylus* species (Fig. 4A). However, the beginning and the end of the reproductive period as well as its time duration varied among species (Fig. 4A). This might be explained by the environmental factors (such as temperature and precipitation) and phylogenetic factors which influence the timing of the reproductive season (Carretero, 2006). Females of *A. s. syriacus* emerge from hibernation one month later than males (Fig. 4B). Their reproductively active period lasted from May to August. Simultaneously, males undergo vernal spermiogenesis, followed by a short period of regression in September and subsequent testicular recrudescence in October and November (Fig. 4B, Hraoui-Bloquet et al. 2007).

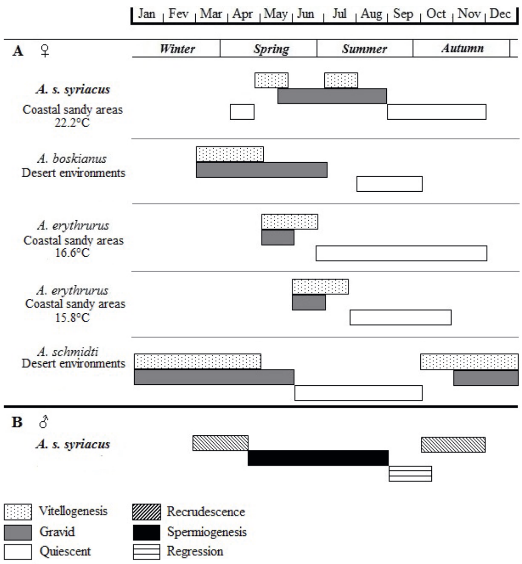

Figure 4. (A) Comparative study of the female reproductive cycle of *Acanthodactylus schreiberi syriacus* with that of *A. boskianus* (Goldberg, 2013), *A. erythrurus* (Carretero and Lorente, 1995), and *A. schmidti* (Al-Johany and Spellerberg, 1988). (B). Male reproductive cycle of *A. s. syriacus.*

The number of clutch and number of eggs produced per clutch may act as a limiting factor in our studied population. Most of females produce one clutch (2 eggs) in a reproductive season. A second clutch can be laid, if environmental conditions were satisfied. Thermal characteristics of climate influence the number of clutches (Castilla et al., 1992) and its impact on the lizards' body temperatures influence the reproductive pattern (Castilla et al., 1999). The possibility of two clutches was reported in *A. erythrurus* from Mediterranean coast of Spain (Carretero and Lorente, 1995). No evidence of multiple clutches was reported in *A. boskianus* specimens (Goldberg, 2013). In Turkey and Jordan, *A. boskianus* laid multiple clutches (Baran and Atatür, 1998; Disi et al., 2001). Sperm storage crypts were found in the infundibulum of the female lizard *A. scutellatus* (Audouin, 1827) (Bou-Resli et al., 1981). They may facilitate the production of multiple clutches. Zotos et al. (2012) showed evidence of sperm storage in *A. s. schreiberi* from Cyprus by isolating mated females and examination of their possibility of laying multiple clutches without further copulation.

Precipitations influence the relative abundance and the availability of food. The range of the prey size of *A. s. syriacus* specimens, their taxonomic diversity and the absence of a close relationship between prey and predator size emphasize their alimentary opportunism (Lahoud-Hokayem et al., 2006). Our results showed low reproductive output of the female reproductive pattern of *A. s. syriacus* which reflects an adaptive response to the environmental conditions rather than phylogenetic constraints on the reproductive patterns.

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