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The Female Reproductive Cycle of the Spotted Toad-headed Agama, *Phrynocephalus maculatus* (Sauria: Agamidae) in Iran

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The Spotted Toad-headed Agama, *Phrynocephalus maculatus*, is a member of the Agamidae family distributed in the central and south-eastern deserts of Iran. Iranian specimens are rare in collections. In this research, the female reproductive cycle of this species was studied from April 5 to August 5, 2013. Totally, 15 adult females were collected by hand at midday from southern parts of Damghan County, located in Semnan Province of Iran. Ovaries were removed and processed for histological and morphometric studies. The oogenic cycle begins from early April, mating occurs at the beginning of May, with oviposition occurring from late May to mid July. Females lay 2-3 eggs per clutch with the possibility of producing a secondary clutch later in the season. Maximum reproductive activity occurs in May and early June and reduces from early July and ends in August. There was no significant difference between the right and left side of the reproductive system. Hence, oogenesis occurs from April through July, *P. maculatus* follows an associated reproductive cycle typical for temperate species.

Key words: Lizard, Agama, *Phrynocephalus maculatus maculatus*, oogenesis, reproduction.

INTRODUCTION

Toad-headed lizards of the genus *Phrynocephalus* (Agamidae) include more than 40 species distributed from north-western China to Turkey and they are major components of the central Asian desert fauna (Zhao et al., 2011). The Spotted Toad-headed Agama (Black-tail Toad-headed Agama), *Phrynocephalus maculatus*, is a member of this family, also known as the chameleons of the Old World due to their striking ability to change their body color (Firouz, 2005). Therefore, the body color of this lizard is significantly variable, but typically has distinct brown bars across the body and tail. It also tends to match the color of its background and lizards found on pale coastal sands are known to be paler and less patterned than those on red, inland sands (Hellyer & Aspinall, 2005). This agama inhabits desert regions, desiring harder sandy surfaces (Hellyer & Aspinall, 2005). The activity of this species occurs during all but the hottest hours of the day, scurrying across the sand and in the hunt for its insect prey. During the hottest periods to limit contact with the sand, it will stand high on extended legs trying to balance on fingertips and heels while using the tail as a prop (Hellyer and Aspinall, 2005). The spotted toad-headed agama is capable of sinking rapidly into the sand through vibrating the body in a process called 'shimmy burial', and this behavior is used to escape from predators or create a nocturnal shelter (Firouz, 2005; Hellyer & Aspinall, 2005).

This species originates from Iran, Iraq, Afghanistan, Pakistan Turkmenistan, Syria, Oman, northern Saudi Arabia, Kuwait and the United Arab Emirates. The subspecies Black-tailed toad agama, *P. maculatus maculatus* Anderson, 1872 are scattered on the Central Plateau of Iran, at elevations between 500 and 3000 m, east through southern Afghanistan and Baluchistan, and extending into Nushki, Pakistan (Anderson, 1999). Along with many other species of reptiles in the Middle East region, *P. maculatus* is a fairly understudied species. This species has yet to be assessed by the IUCN.

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Consequently, it is currently unclear whether there are many major threats to the species or not (Alsharhan *et al*., 2008). Since no data is available regarding the reproduction of this species, this research was conducted to characterize its reproductive cycle through focusing on its oogenic cycle in Iran.

Study Area

All specimens were collected from the four following stations: Hassan Abad, Saleh Abad, Alian and Yazdan Abad villages located in the south of Damghan County, Semnan Province (54°19'E, 35°55'N). Damghan is situated 1170 m above sea level and north of the Central Kavir Desert (Figs. 1 & 2). The annual average temperature is 17.2°C. The study area is composed of alkaline saline soils containing clay and sand. The dominant plant species are *Tamarix* sp., *Salsola* sp., *Alhaji* sp., *Peganum* sp., *Atriplex* sp. and *Astragalus* sp.

Sampling: sampling was conducted periodically every month during the activity period of this species from April 5 to August 5, 2013. All specimen collections were by hand, from 9 AM to 4 PM. In total, 15 adult and mature females were captured (three specimens per sampling period). To study the frequency of clutch deposition, some specimens were kept in terrarium.

MATERIAL AND METHODS

The specimens were transferred alive to the Zoology Laboratory of Islamic Azad University, Damghan Branch and their W (Weight), SVL (Snout-Vent Length), TL (Tail Length) and HL (Head Length) were measured. Then, they were killed by chloroform and dissected. ROD (Right Ovary Diameter), LOD (Left Ovary Diameter), ROW (Right Ovary Weight), LOW (Left Ovary Weight), RFN (Right Follicles Numbers), LFN (Left Follicles Numbers), MaxRFD (Right-Follicles Maximum Diameter), MaxLFD (Left Follicles Maximum Diameter), MinRFD (Right Follicles Minimum Diameter), MinLFD (Left Follicles Minimum Diameter), MRFD (Right Follicles Mean Diameter), MLFD (Left Follicles Mean Diameter), ROEL (Right Oviductal Egg Length), LOEL (Left Oviductal Egg Length), ROEWe (Right Oviductal Egg Width), LOEWe (Left Oviductal Egg Width), ROEW (Right Oviductal Egg Weight), LOEW (Left Oviductal Egg Weight) and FLD (Follicular Layer Diameter) were measured. Length, width and diameter measurements were taken with a dial caliper with an accuracy of 0.02 mm. Weight was measured by a scale with an accuracy of 0.001 g. Gonads, once removed, were examined for metric and meristic characters. The number, weight and diameter of immature, growing and mature follicles and oviductal eggs were studied in right and left ovaries, independently. After fixing the ovaries in 10% formalin, tissues were dehydrated, cleared in Xylene, infiltrated and embedded with paraffin. Sections were made at 5–7 microns, deparaffinized, re-hydrated, stained (Hematoxylin & Eosin) and mounted. The sections were investigated through light microscopy at 100× and 400× magnification. Photographs were prepared by a digital camera. Data was analyzed by SPSS 18 software, one-way ANOVA and Tukey test to compare biometric data among monthly samples ($P< 0.05$).

RESULTS

In this study, the maximum SVL, TL and HL of samples were 59.79, 85.93 and 14.32 mm, respectively. SVL of the smallest mature female was 39 mm.

Phrynocephalus maculatus within the study region hibernates from early October to early April. Emergence of the specimens occurs in early April and oogenesis begins. Mating is observed in early May. The ovaries are paired and vesicular and consist of 3-7 follicles. Three types of follicles were observed: immature, growing and mature. The small and growing follicles were observed in April with an immediate increase in their ovary size in May and mature follicles were observed from early May to late June. The mean diameter of ovary in April, May and June were 2.45, 5.33 and 4.29 mm, respectively.

FIGURE 1. *Phrynocephalus maculatus* in Hassa Abad, Damghan, Iran.

FIGURE 2. Map showing the sampling site (Damghan County) in northern Iran.

FIGURE 3. Saleh Abad station in Damghan County.

The follicular layer is multilayered and polymorphic (Fig. 4). The diameter of follicular layer is 40.60-70 μm. The diameter of the nucleus varies between 26-56 μm in immature and growing follicles.

Oviductal eggs were observed between 5 May and 5 July. Frequently, two oviductal eggs were observed in mature females. There existed three oviductal eggs in one single specimen (14.61 × 7.35, 14.88 × 7.70 and 14.31 × 8.25 mm) on 5 June (Figs. 5 & 6). The weight, length and width of the largest oviductal egg were 1.043 g, 21.97 and 11.13 mm respectively. Eggs are yellowish white and oval shaped and oviposition occurs from late May to mid July. The numbers of laid eggs varied between 2 to 3 per clutches incubated for around six to seven weeks in a burrow.

The weight, SVL, HL and TL of the smallest juvenile obtained in early August were 2.00 g, 34.86 mm, 8.94 mm and 56.98 mm, respectively. Generally, the juveniles were observed from early August to September.

The statistics analyses of body size and ovarian macroscopic and microscopic characters are presented in Tables 1 and 2, respectively. Analysis of variances of characters shows that the following characters including weight and diameter of left and right ovaries, maximum, minimum and mean diameter of follicles and follicular layer have significant differences among groups ($P < 0.05$, Tables 1 and 2).

Table 3 shows the statistics analyses of oviductal eggs characters. The maximum activity of oogenesis takes place in May and June (Figs. 7 & 8). No significant differences have been observed in the ovarian characters of the left and right side of the body (paired t-test, $P > 0.05$ in all cases).

TABLE 1. Statistics analyses of body size and ovarian macroscopic characters in *Phrynocephalus maculatus* (For abbreviations, see Materials and Methods)

Characters	N	Minimum	Maximum	Mean ± SE	Std. Deviation	F	Sig.
W (g)	15	3.93	9.36	5.20 ±0.39	1.53	1.801	0.205
SVL (mm)	15	39.00	59.79	50.34 ± 1.29	5.01	2.387	0.121
LCD (mm)	15	69.98	85.93	78.53 ± 1.06	4.12	1.304	0.333
HL (mm)	15	11.34	14.32	12.19 ± 0.19	0.75	1.353	0.317
HW (mm)	15	10.10	14.37	11.51 ± 0.27	1.07	0.517	0.726
ROD (mm)	15	2.10	5.49	3.942 ± 0.27	1.07	12.449	0.001
LOD (mm)	15	2.30	5.90	3.96 ± 0.28	1.10	13.282	0.001
ROW (g)	15	.007	1.30	0.32 ± 0.10	0.39	38.769	0.000
LOW (g)	15	.005	1.10	0.30 ± 0.09	0.35	83.365	0.000

TABLE 2. Statistics analyses of ovarian microscopic characters in *Phrynocephalus maculatus*

Characters	N	Minimum	Maximum	Mean ± SE	Std. Deviation	F	Sig.
RFN	15	3.00	7.00	4.46 ± 0.29	1.12	3.545	0.048
LFN	15	3.00	6.00	4.20 ± 0.26	1.01	4.250	0.029
MaxRFD (mm)	15	1.08	5.43	2.66 ± 0.34	1.34	7.388	0.005
MaxLFD (mm)	15	1.00	4.65	2.03 ± 0.25	0.99	3.613	0.045
MinRFD (mm)	15	0.43	1.66	0.90 ± 0.09	0.37	6.141	0.009
MinLFD (mm)	15	0.33	1.43	0.78 ± 0.09	0.35	14.533	0.000
MRFD (mm)	15	1.54	3.90	2.26 ± 0.15	0.60	3.830	0.039
MLFD (mm)	15	1.06	3.50	2.35 ± 0.20	0.77	7.295	0.005
FLD (μm)	15	40.60	70.00	54.35 ± 2.71	10.50	15.396	0.000

TABLE 3. Statistics analyses of oviductal eggs characters in *Phrynocephalus maculatus*

Characters	Minimum	Maximum	Mean ± SE	Std. Deviation
ROEN	1.00	2.00	1.33 ± 0.33	0.57
LOEN	1.00	1.00	1.00 ± 0.00	0.00
ROEL (mm)	14.46	21.97	16.97 ± 2.49	4.32
LOEL (mm)	14.17	20.27	16.44 ± 1.92	3.33
ROEWe (mm)	7.80	11.13	9.00 ± 1.06	1.84
LOEWe (mm)	7.70	10.17	8.55 ± 0.80	1.39
ROEW (g)	0.64	1.043	0.78 ± 0.12	0.22
LOEW (g)	0.54	1.014	0.73 ± 0.14	0.24

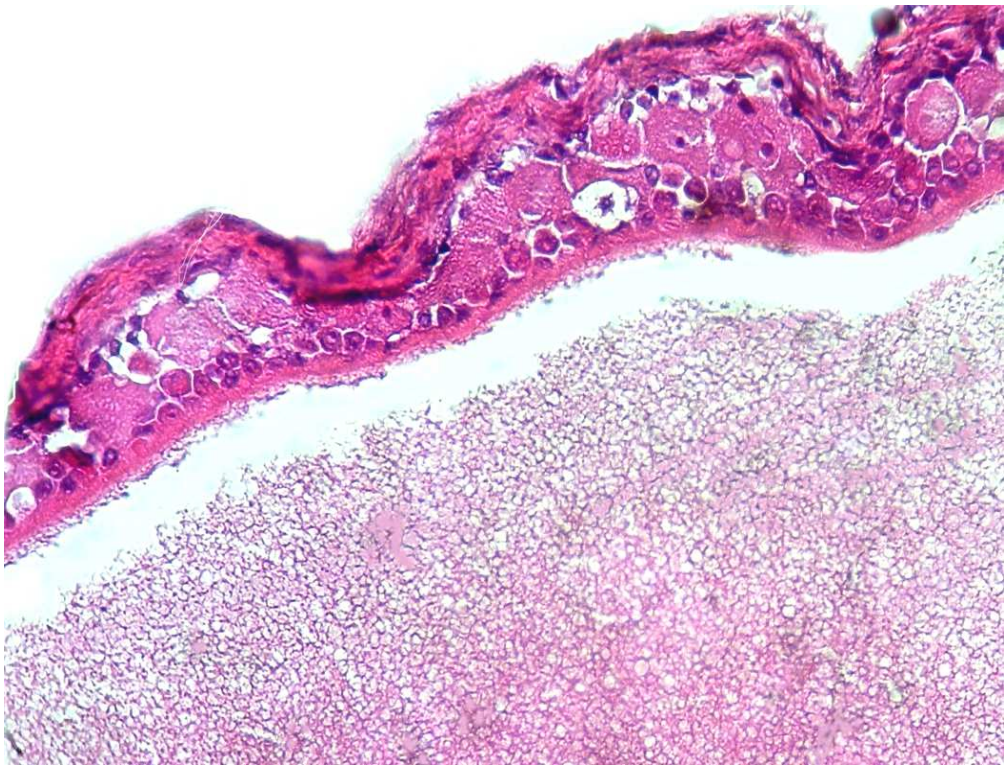

FIGURE 4. The follicular layer of *Phrynocephalus maculatus* in June 2013 (1000× magnificent).

FIGURE 5. Mature female *Phrynocephalus maculatus* having three oviductal eggs in abdomen in 5 June, 2013.

FIGURE 6. Oviductal eggs of *Phrynocephalus maculatus* in 5 June, 2013.

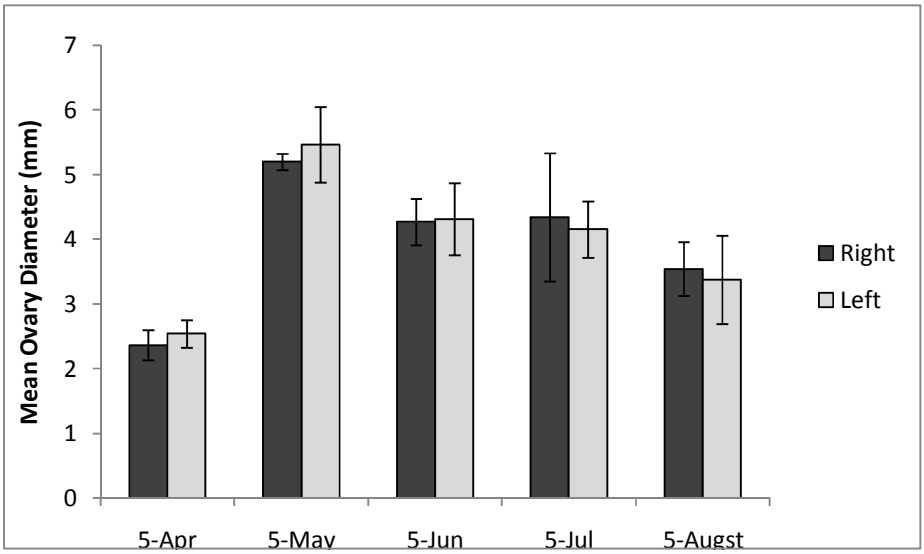

FIGURE 7. Mean Ovary Diameter in *Phrynocephalus maculatus* from April to August 2013.

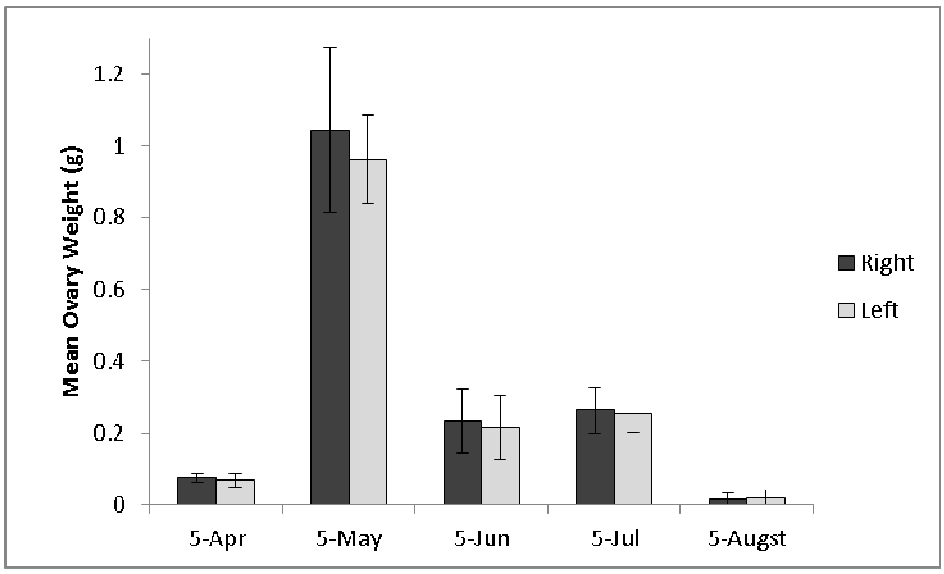

FIGURE 8. Mean Ovary Weight of *Phrynocephalus maculatus* from April to August 2013.

DISCUSSION

The reproduction timing can considerably influence the offspring's fitness along with the fitness of the parents particularly in species inhabiting extreme environments like deserts (Zhao et al., 2011).

The most significant feature of agamid lizards is that they are mostly egg layers producing a clutch of one to seven eggs incubated for almost six to eight weeks in a burrow (Halliday & Adler, 2002; Ananjeva et al. 2006). Still, a small portion of them such as *Phrynocephalus erythrurus* are viviparous (Ananjeva et al, 2011). Blanford (1876) reported finding two eggs in oviducts of each of two females collected near Bam in late April.

The uniqueness of this study comes from the fact that the oogenic cycle of *P. maculatus* are being reported for the first time from Iran and west southern Asia. The oogenesis occurs in the spring and early summer from April through late July and both ovaries are active in reproductive season.

The results show the three following oogenic cycle phases for *P. maculatus*: Inactive phase: possessing small follicles from September to March, 2- Vitellogenic phase: possessing growing and mature oocytes from early April to mid-May and 3- Oviductal egg phase: from late May to late July. A multilayer and polymorphic follicular layer similar to other agamids including *Laudakia caucasia* is reported for *P. maculatus* and the small cells of the granulosa layer are differentiated into three distinct cell types: small, intermediate and the large pyriform cells (Bahar Ara et al., 2009).

The morphology, oogenesis and folliculogenesis of the ovaries of *P. maculatus* are related to the general squamata pattern designated for oviparous reptiles (Moodley & Van Wyk, 2007). A sympatric agama within the study area is the *Phrynocephalus scutellatus* where the largest and smallest SVL of reproductive females were recorded as 55 mm and 45 mm respectively (Rastegar Pouyani, 1997). The maximum reproductive activity tends to start in June which is followed by a reduction from July to August and finally terminates in September (Rastegar Pouyani, 1997).

Trapelus agilis, another sympatric agama in the study area, demonstrated oviposition for three times with 6-14 eggs per clutch. Early May is reported to be the peak of female reproduction of the *T. agilis* species (Rastegar Pouyani, 1997).

A variation of 2 to 3 eggs per clutch was observed in *P. maculatus* with females having no more than one developing egg in a single ovary at a time. Yet another sympatric agamid within the study region is *Laudakia caucasia* laying 4 to 5 eggs per clutch along with a later peak female reproductive cycle in June compared to that of *P. maculatus* (Yazdanpanahi, 2000; Bahar Ara et al., 2009).

Once deposition occurs, the egg shells harden and consequently, this would lead to a better protection against desiccating environmental conditions and invertebrate predators than a soft shell.
The sympatric agama within the study region, *Phrynocephalus scutellatus* has the largest and smallest SVL of reproductive females as 55 mm and 45 mm, respectively (Rastegar Pouyani, 1997). Also, the maximum reproductive activity takes place in June with a reduction from July to August and finally ending in September (Rastegar Pouyani, 1997).
In *Phrynocephalus przewalskii*, the vitellogenesis process for females commences in April and contains oviductal eggs from May to June. A significant decrease in gonad volume occurs in July reaching its minimum volume from August to September. Hatching occurs during summer and early fall with a mean recorded clutch size of 2.7 ± 0.9 SE (Zhao et al., 2011).
The SVL of two other smallest reproductive females of toad-headed agamas, *Phrynocephalus frontalis* and *Phrynocephalus versicolor*, from North China have recorded 43.5 mm and 44.2 mm, respectively (Qu et al., 2011). A single clutch of 2 to 6 eggs per breeding season was laid by females of both species. An evident trade-off between the size and number of eggs was observed in both species (Qu et al., 2011). The smallest reproductive female size for *P. maculatus* species was 39 mm.
The results of this study indicate that the reproductive cycle of *P. maculatus* follows an associated reproductive cycle typical of species from temperate areas that occur during the well-defined periods in which oocytes are not found in the ovaries all year round.

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