Reproductive activity of the sand boa, *Eryx jayakari* throughout the year in Riyadh region of Saudi Arabia

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Abstract: The biological reproductive aspects of the sand boa, *Eryx jayakari*, in Riyadh region of Saudi Arabia were investigated over a period of one year. Study of the reproductive cycles of male and female *Eryx jayakari* revealed that the breeding season is relatively short (2-2.5 months), since it extends from March to May. Thereafter, females lay the eggs during July, and hatching probably takes place during early July till mid-September. However, offspring were seen and collected at end of October. Thereafter, no evidence for sexual activity was observed, this may indicate just a single clutch during the year. Relative testis weight to body weight was increased ($\bar{x}$ = 0.083 %) during reproductive activity where maximal expansion of seminiferous tubules was also attained ($\bar{x}$ diameter = 457 µm) and testis volume ($\bar{x}$ = 380 mm³) during May. On the other hand, the ovarian activity was the highest throughout April and May where ovarian parameters were greater and an average egg diameter of 27 mm was recorded. A positive correlation was observed between the weight of fat bodies in the male and female *Eryx jayakari* and their reproductive activity. Maximum weight of fat bodies was reached during May ($\bar{x}$ = 6.3 g and 15.4 g in males and females, respectively).
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1. Introduction

The available studies concerning reproductive cycles of reptiles in Arabian Peninsula are scanty. Generally, reproductive activity in snakes is characterized by seasonality (i.e., throughout Spring or Summer) (Feriche *et al*., 2008). As other reptiles in desert areas, they have a short breeding season (Rodiny, 2006). Reproductive cycle in snakes could be investigated through tracking the stages of gonadal growth and structure (Al-Sowelim, 2001). Climatic conditions (e.g. rainy seasons) play a pivotal role in determining the breeding season in many reptiles inhabiting desert areas. Some snakes are laying eggs as one clutch per year (Al-Shammari, 2007) or two clutches annually (Mazuch, 2006). The time required for egg hatching is quite different according to species, it reached to four months in pyramids viper, *Echis pyramidum* (Al-Shammari, 2007), whereas it was 2-5 months in horned viper, *Cerastes gasperettii* (Al-Sowelim, 2001). Castilla and Bauwens (1990) mentioned that fat body weight was correlated with reproductive activity in most reptiles where it decreased significantly following mating and increased again before getting into winter hibernation. Al-Sowelim (2001) reported that the breeding season of horned viper, *Cerastes gasperettii*, in central region of Saudi Arabia occurred in April and May. Al-Shammari, (2007) using *E. pyramidum* in southern region of Saudi Arabia showed that the period of reproductive activity in both sexes extended for 5 months (March till July in males, May till September in females) and mating occurred during June and juveniles were seen during November and December. Weaver (2010) found that the male snake *Hypsiglena chlorophaea* in U.S. had a peak of reproductive activity from mid-May till end of June and offspring were seen during mid –August. The present investigation was undertaken to determine the reproductive activity of the sand boa, *Eryx jayakari*, in Riyadh region of Saudi Arabia by tracking the changes in gonadal size and structure throughout months of the year and determining the reproductive cycle and other related aspects.

2. Material and Methods

A total number of 83 specimens of adult sand boa, *Eryx jayakari* (58 males and 25 females) were collected from the study area, Riyadh region of Saudi Arabia by monthly field trips. Ten specimens of the snake (males and females) were obtained each month for a duration of one year. The collected samples were then transferred to the reptilian laboratory in Zoology Dept., College of Science, King Saud University where they were killed immediately by freezing. Body weight and dimensions were recorded before dissecting the snakes. Right testes and ovaries were excised, weighed and fixed in Bouin s fluid (10 %). Paraffin sections (5-7 µm thick) were prepared according to Bancroft and Stevens (2008) which stained with

hematoxylin and eosin. Measurements of seminiferous tubules were determined by using Cool Scope (Nikon, Japan), volume of the testis was estimated according to the formula of Castilla and Bauwens (1990):

$V = 3/4\pi a^2 b$

where:

V = volume

a = ½ of the shortest diameter

b = ½ of the longest diameter

$\pi = 3.14$

Testis diameter was measured by using Vernier caliper. Spermatogenic stages of Seminiferous wall were determined according to the method of Flores *et al* (1990). Concerning ovaries, the size changes throughout months of the year were recorded using Vernier caliper, additionally, relative ovarian weight to body weight, diameter of the ovary and egg diameter were recorded. During dissection, fat bodies were removed from both sexes and weighed.

2.1. Statistical analysis:

All data were expressed as means ± standard error (M ± SEM). Analysis of variance (ANOVA) was performed using SPSS (Version 11.5) to check monthly effects ($P \leq 0.05$) on studied characters.

3. Results

3.1. Male reproductive cycle:

The variability in sexual activity of the sand boa, *Eryx jayakari* males could be classified into the following 4 phases:

3.1.1. Quiescence phase:

This stage was extended from October till February where the testis had a declined volume ($\bar{x}$ = 40 mm³), the relative testis weight to body weight reached $\bar{x}$ = 0.045 %. The average diameter of seminiferous tubules was116 µm (Table-1 and Fig.1a,b), without observable spermatocytes.

3.1.2. Starting activity phase:

This stage commenced at March where testis volume was increased ($\bar{x}$ = 52 mm³), the $\bar{x}$ diameter of seminiferous tubules was almost doubled ($\bar{x}$ = 248.7 µm) compared with the previous stage and the relative testis weight to body weight has not been changed drastically ($\bar{x}$ = 0.040%). A slightly series of cellular divisions in the spermatogenic epithelium of the seminiferous tubule wall was observed (Table 1).

Table 1: Testicular parameters and fat body weight (Wt) throughout months of the year in *Eryx jayakari* males

Months	No. of Males	relative testis Wt. to body Wt.%	Testis volume (mm^3)	seminiferous tubule (µm) diameter	Fat body Wt (g)
March	7	0.045±0.003	52±18	248.7±45	0.87±0.41
April	8	0.083±0.029	56±25.9	410±35*	1.37±0.29*
May	10	0.24±0.023*	380±17.5*	457±20	6.3±0.89*
June	7	0.058±0.031*	233±10.6*	148±44*	1.61±0.89*
July	9	0.048±0.013	194±10*	127±23	1.9±0.85
Aug.	8	0.050±0.018	48±15.2	120±12	2.36±1.08
Sept.	3	0.036±0.02	68±29	129±31	2.23±1.7
Oct.	6	0.039±0.002	40±9.1	116±24	1.4±1.04

*P<0.05 Values are Means ± S. E.

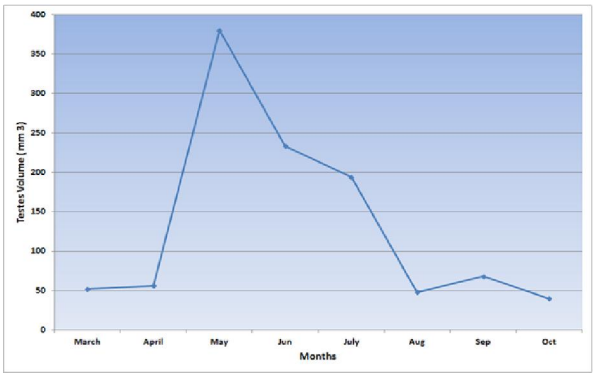

Fig. 1a: Average testis volume of the *Eryx jayakari* males throughout monthes of the year.

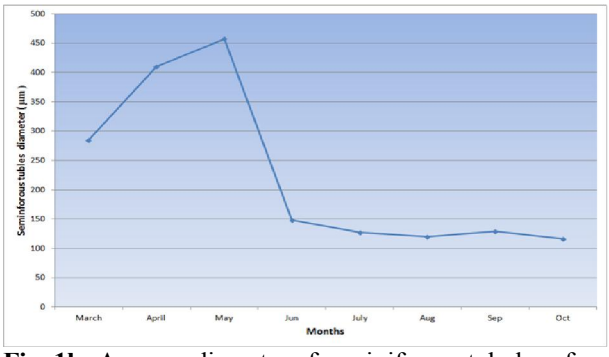

Fig. 1b: Average diameter of seminiferous tubules of *Eryx jayakari* testis throughout monthes of the year.

3.1.3. Maximal activity phase:

This stage occurred throughout May where all measured testis parameters were significantly (P≤0.05) the highest ($\bar{x}$ volume = 380 mm^3, the relative testis weight to body weight reached $\bar{x}$ =0.083% and $\bar{x}$ diameter of seminiferous tubule=457µm). (Table 1 and Fig. 1a,b,c and Fig. 2).

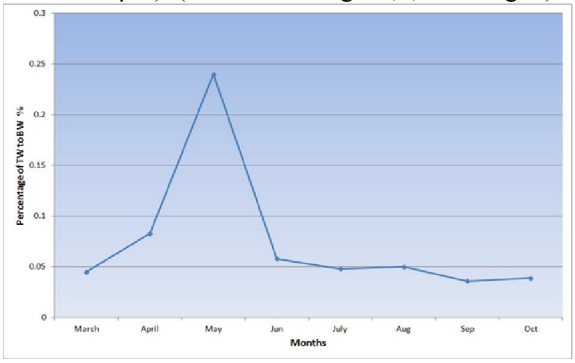

Fig. 1c: Average reletive testis weight (TW) to body weight (BW) in *Eryx jayakari* males throughout monthes of the year.

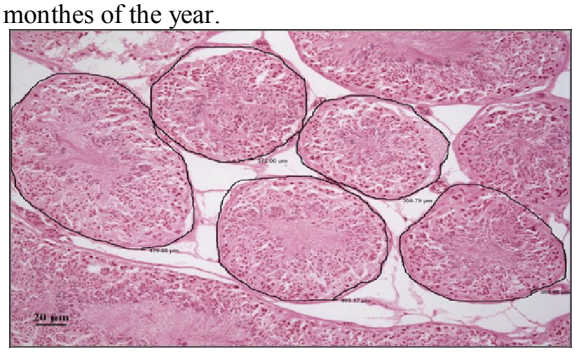

Fig. 2: Transferse section in *Eryx jayakari* Testis showing active seminiferous tubules during May (60X).

3.1.4. Regression phase:

This phase extended from July till September where testis parameters decreased once again to be approximately closer to those of the quiescence phase with disappearance of spermatocytes. Moreover, testes appeared atrophied (Table 1 and Fig. 1a,b,c).

3.2. Female reproductive cycle:

Four phases of ovarian activity of *Eryx jayakari females* were identified as follows:

3.2.1. Quiescence phase:

This phase was extended from October till February where relative ovarian weight was minimal ($\bar{x}$ =0.012%) and the $\bar{x}$ diameter of the ovary was 10 mm. Few smaller-sized eggs were observed on the surface of the ovary by naked eye.

3.2.2. Recrudescence phase:

Upon completion of hibernation, females appeared during March where relative ovarian weight ($\bar{x}$ =0.68%) was increased drastically. The ovary resembled mulberry with an average diameter of $\bar{x}$ = 31.5 mm, meanwhile, $\bar{x}$ of egg diameter was 8.75 mm. (Table 2 and Fig 3a,b,c).

3.2.3 Maximal activity phase:

This phase in the females corresponded with that of the males (April and May). Relative ovarian weight to body weight increased significantly (P≤0.05) to reach ($\bar{x}$ =1.02%) (Fig 3a). Average diameter of the ovary attained $\bar{x}$ = 40.5 mm (Fig 3b), meanwhile $\bar{x}$ of egg diameter was increased significantly ($\bar{x}$ = 27 mm) (Table 2 and Fig 3c). It was observed that females were disappeared by end of May till start of July for oviposition.

3.2.4 Regression phase:

This phase occurred during August and September where relative ovarian weight to body weight declined to $\bar{x}$ =0.062% (Fig 3a). Meanwhile, ovarian diameter decreased to $\bar{x}$ = 16.5 mm (Table 2 and Fig. 3b). No eggs were observable on the surface of the ovary due to its atrophy.

Table 2: Ovarian parameters and fat body weight throughout monthes of the year in *Eryx jayakari* females.

Months	No. of females	Relative ovarian Wt. to body Wt.%	Ovarian (mm)diameter	Egg diameter (µm)	Fat body Wt (g)
March	2	0.68±0.08	31.5±3.5*	8.75±.35	4.7±1.3
April	3	1.08±0.52*	34±3	20.3±2.08	10.7±3
May	2	1.168±0.25*	40.5±2.1*	27±2.8*	15.4±1.2*
June	3	0.33±0.04	19.3±1	-	3.2±.73
July	3	0.19±0.05	15.3±4.5	-	3.6±.9
Aug.	3	0.15±0.04	17.3±3.5	-	3.8±.8
Sept.	6	0.14±0.03	15.2±4.3	-	2.5±1.7
Oct.	3	0.012±0.01	10±2.6	-	4.1±1.6

P<0.05 Values are Means ± S. E. *

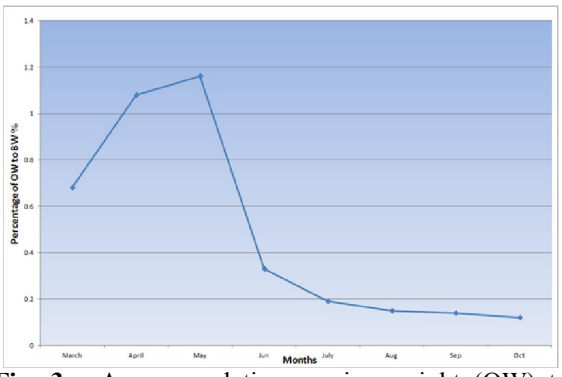

Fig. 3a: Average reletive ovarian weight (OW) to body weight (BW) in *Eryx jayakari* females throughout monthes of the year.

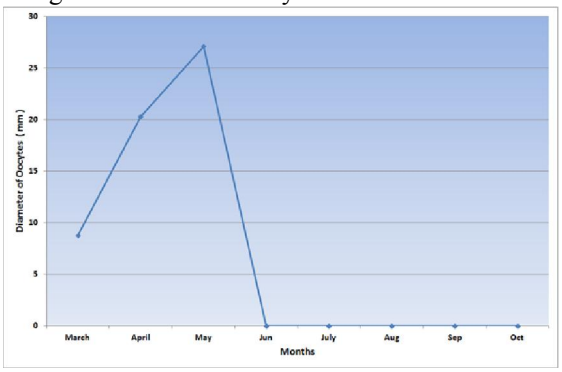

Fig. 3b: Average reletive ovarian diameter in *Eryx jayakari* females throughout monthes of the year.

Fig. 3c: Average reletive egg diameter in *Eryx jayakari* females throughout monthes of the year.

3.3 Fat body cycle:

Fat bodies represent the stored adipose content which considered as energy storage. This fat content is fluctuated according to food availability and organism activity. Upon completion of hibernation, fat bodies of *Eryx jayakari* started to increase where it reached its maximum weight significantly (P≤0.05) during May ($\bar{x}$ = 6.3 g and 15.4 g in males and females, respectively). (Tables 1and 2 & Fig. 4a,b).

Fig. 4a: Average fat body weight in *Eryx jayakari* males throughout monthes of the year.

Fig. 4b: Average fat body weight in *Eryx jayakari* females throughout monthes of the year.

4. Discussion

The reproductive cycle of the *Eryx jayakari* is just one seasonal cycle per year. This finding is in agreement with that of Rodiny (2006) and Feriche *et al*., (2008). This reproductive activity was proven to be a short period (2-2.5 months) extended from end of March till end of May in Riyadh region of Saudi Arabia (S.A.) where diameters of seminiferous tubules started to increase from mid-March while it reached its maximal activity significantly (P≤0.05) during May. This is in an accordance with Al-Enaizi (2003) who studied the ultrastructure and differentiation of the germinal epithelium in the testis of *Eryx jayakari* in Riyadh region of S.A**.** Also this finding is in an agreement with that reported by Al-Sweilem (2001) on *C. gasperettii* in central region of S.A. which sharing *Eryx jayakari* the same ecological habitat and the same nocturnal activity. On the contrary, it was in disagreement with Al-Shammari (2007) using *E.pyramidum* in the southern area of S.A. who reported a longer reproductive period (5 months) extended from March till end of July which attributed to no hibernation practiced by this species of reptiles. It was found that *Eryx jayakari* was oviparous species, meanwhile Lanza and Nistri

(2005) reported that *E.colubrinus,* belonging to the same genus and living in Africa, was an ovo-viviparous species, whereas it agreed with Al-Sweilem (2001), Al-Kahtani, (2003) and Al-Sweilem (2001). It was found that *Eryx jayakari* females laid just one egg clutch only annually which was in agreement with the findings of Al-Sweilem (2001) and Al-Shammari (2007). Egg hatchability of *Eryx jayakari* was found to occur from early July till middle of September (2-3 months), since offspring were seen and collected by the end of October where they were phenotypically resemble their parents. This period was reported to be appropriate for emerging of juveniles of Arabian Peninsula reptiles, which had a hibernation (Al-Sweilem, 2001, Al-Kahtani, 2003, Rodiny 2006). Gonadal development of *Eryx jayakari* started on March which corresponded with completion of hibernation where improvement of climatic temperature and food availability were realized. Fat bodies of *Eryx jayakari* males and females increased significantly ($P \leq 0.05$) following hibernation at a stage just preceding the mating process, then this fat storage was declined during reproductive activity. It is worthy to mention that reptilian males do not require a higher level of fat storage for the sake of the reproductive process (Aubret *et al*., 2002), whereas females do require greater level. This explain the difference between males and females in fat storage (6.3 g and 15.4 g, respectively).

It could be concluded that the reproductive cycle of the male and female Sand Boa, *Eryx jayakari* in Riyadh region of S.A proved to be a relatively short period and greatly variable throughout months of the year. The desert conditions certainly affected Saudi Arabian reptiles reproductive biology.

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