Reproductive biology of the horned viper, *Cerastes cerastes gasperettii* in the central region of Saudi Arabia

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**Abstract** The reproductive biology of the horned viper, *Cerastes cerastes gasperettii*, in Riyadh region of Saudi Arabia was investigated over a period of one year. Study of reproductive cycle of male and female *C. c. gasperettii* revealed that the breeding season is relatively short (April and May). Thereafter females laid eggs by mid of July and hatching probably had taken place by the end of September. No activity was observed during winter, this may indicate just a single clutch per year. Relative testis weight to body weight was drastically increased ($X = 0.88\%$) during the peak of reproductive activity (May) where maximal expansion of seminiferous tubules was also attained during April and May ($X = 209 \mu m$ and $191 \mu m$, respectively). Likewise, the ovarian activity was the highest during May where ovarian parameters were greater in terms of relative ovarian weight to body weight and ova diameter being 0.46% and 2.29 mm, respectively. Fat body weight was increased drastically just before the peak of reproductive activity then started to decline during June. It could be concluded that the harsh desert conditions and similar environments certainly affect reproductive activity of Saudi Arabian reptiles including snakes.

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1. Introduction

Although a considerable number of studies have been conducted on reproductive aspects of reptiles from Saudi Arabia and other similar environments, fewer ones have tackled the reproductive biology of snakes. White et al. (1982) and Feriche et al. (2008) reported that reproductive activity of snakes was characterized by seasonality. As other reptiles in desert areas, snakes have a short breeding season (Rodiny, 2006).

Environmental conditions play a significant role in determining the breeding season in many reptiles including snakes inhabiting desert areas. Al-Johany et al. (1997) reported occurrence of reproductive season of the skink, *Scincus mitranus*, in May and June in the central region of Saudi Arabia. The authors observed young lizards in late September indicating one clutch during the reproductive season. In the same area, Al-Sadoon (2001) obtained a short reproductive season in...
*Uromastyx aegyptius* (May till early July) and collected offspring during October which pointed out to one clutch per annum. Also, Al-Sadoon et al. (2014) working on the worm lizard, *Diplometopon zarudnyi* in Riyadh region of Saudi Arabia reported a relatively short breeding season (April to June), females laid eggs during July, hatching took place during August and offspring were seen at mid-October, which indicated just a single clutch during the year. 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 four months in pyramids viper, *Echis pyramidum* (Al-Shammari, 2007). He stated that the period of reproductive activity in both sexes of pyramids viper in southern region of Saudi Arabia extended for 5 months (March till July in males, May till September in females), as mating occurred during June and juveniles were seen during November and December. Weaver (2010) found that the male snake *Hypsiglena chlorophaca* in U.S. had a peak of reproductive activity from mid-May till the end of June and offspring were seen during mid-August. Aldridge and Semlitsch (1992) reported that ovulation and oviposition in the female snakes, *Tantilla coronata* occurred during June, whereas spermatogenesis in males started during May and culminated during July and August. The authors also pointed out that the mating season of this species extended form May till September. 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. Therefore, the present investigation was undertaken to determine the reproductive cycle and mating season in both sexes of *Cerastes cerastes gasperettii*. Also, tracking oviposition and hatching, in addition to describing morphological and histological changes of the gonads in relation to months of the year.

2. Materials and methods

The Arabian horned viper, *C. c. gasperettii* may reach 85 cm in length. The body is fat, the tail is short and distinct, the head is flat, wide and triangular in shape. There may or may not be a horn above each eye. Although they are more common in well-vegetated desert, they are also found in most sandy areas. They are nocturnal and feed on small vertebrates (Fig. 1). A total number of 100 specimens of adult horned viper, *C. c. gasperettii* (48 males and 52 females) were collected from the study area, Riyadh region of Saudi Arabia by monthly field trips. Riyadh region has a continental weather that is extremely hot in summer and cold in winter. The temperature ranges from a maximum of approximately 49 °C in mid-summer (August) to below freezing temperatures (January). The relative humidity ranges from 15% in July to 71% in January. A mean annual precipitation ranges from 16.5 mm in December to 1.7 mm in March. About 5–6 specimens from each sex were obtained each month for a duration of 12 months (March till October) (Fig. 2). The collection techniques for snakes were generally based on searching the area on foot. Most of the animals were collected between dawn and mid-morning, or shortly before sunset. Following hunting of the snakes, body temperature was immediately recorded as well as air and ground temperatures. The collected snakes were then transferred to the reptilian laboratory in Zoology Dept., College of Science, King Saud University, Riyadh, Saudi Arabia where they were killed immediately by freezing, then body weight and dimensions were recorded before dissecting the snakes. Right ovaries and testes were excised (left gonads were atrophied), weighed and fixed in Bouin’s fluid (10%). Paraffin sections (5–7 µm thick) were prepared according to Bancroft and Stevens (2008), then stained with hematoxylin and eosin. Measurements of seminiferous tubules were determined by using Cool Scope (Nikon, Japan). Volume of each testis was estimated according to the formula of Castilla and Bauwens (1990):

\[ V = \frac{3}{4} \pi a^2 b \]

where:

- \( V \) = volume.
- \( a = \frac{1}{2} \) of the shortest diameter.
- \( b = \frac{1}{2} \) of the longest diameter.
- \( \pi = 3.14 \).

Relative testis or ovary weight to body weight was calculated and seminiferous tubules diameter and ovum diameter were measured in µm from histological sections. During dissection fat bodies were removed from both sexes and weighed.

2.1. Statistical analysis

All data were expressed as means ± standard errors (M ± S. E.). One-way ANOVA was performed using SPSS (Version 11.5) to check monthly effects (\( P < 0.05 \)) on studied characters.

3. Results

3.1. Breeding season

Reproductive activity of the horned viper was observed to occur throughout a short period of the year (April and May) where climatic conditions were somehow improved after termination of the winter season.

Seminiferous tubules of adult male testes were stuffed with spermatozoa starting from early April till the end of May. Likewise, oviducts were filled with eggs during June where
females laid eggs by mid-July. End of July denoted out of the breeding season, since testes started to atrophy and oviducts contained no eggs. Mating process was hard to be watched in the natural habitats, however, traces of males and females in mating position were seen on the ground surface. Juveniles were seen by the end of September with smaller sizes but phenotypically looking their parents alike, highly active in searching for their feed even much more than adults.

3.2. Male reproductive cycle

The variability in sexual activity of the horned viper, *C. c. gasperettii* males could be classified into the following 4 phases:

(1). **Quiescence phase**: Quiescence phase was extended from October till February, where testis had a declined volume ($\bar{X} = 10 \text{ mm}^3$) (Fig. 3), seminiferous tubule

![Figure 2: Distribution of *Cerastes cerastes gasperettii* in the study area.](image-url)
diameter ($\bar{x} = 40 \mu m$) (Fig. 4) without observable spermatocytes and relative testis weight to body weight ($\bar{x} = 0.11\%$) (Fig. 5).

(2). **Recrudescence phase**: Recrudescence phase occurred during March where testis volume started to increase ($\bar{x} = 80.6 \text{ mm}^3$) (Fig. 3), seminiferous tubules diameter increased by three folds ($\bar{x} = 132.2 \mu m$) and relative testis weight increased more than twice ($\bar{x} = 0.27\%$) (Fig. 5) when compared with the earlier stage. A slightly series of cellular divisions in spermatogenic epithelium of seminiferous tubule wall was observed.

(3). **Maximal activity phase**: Maximal activity phase of the horned viper males occurred throughout April and May where all estimated characters were drastically increased (Table 1) and (Figs. 3–5). A considerable number of spermatozoa existed within the lumen of seminiferous tubules during May (Fig. 6).

(4). **Regression phase**: Regression phase was extended from July till October where all characters clearly declined to be closer to the quiescence phase with disappearance of spermatocytes and presence of fewer spermatozoa (Table. 1).

3.3. Female reproductive cycle

Four phases of ovarian activity of *C. c. gasperettii* females were identified as follows:

(1). **Quiescence phase**: Quiescence phase was extended from October till February same as found in *C. c. gasperettii* males, where relative ovarian weight was minimal ($\bar{x} = 0.088\%$) (Fig. 7). Fewer small-sized eggs were observed on ovarian surface with an average diameter of 788 $\mu m$.

(2). **Recrudescence phase**: March was the month of commencement of reproductive activity in *C. c. gasperettii* females similar to male in that respect. All studied characters were almost doubled when compared to the previous stage (Table 2 and Fig. 8).

(3). **Maximal activity phase**: Maximal reproductive activity phase of *C. c. gasperettii* females had taken place throughout April and May, same as in males, maximum ovum diameter was recorded ($\bar{x} = 2.15 \text{ mm}$), meanwhile relative ovarian weight was drastically increased (0.5%) (Table 2 and Fig. 8). By the end of June elongated and yellowish eggs were found in oviducts following dissection. Number of eggs in each oviduct ranged from 4 to 8 eggs being higher in the right oviduct.

(4). **Regression phase**: Regression phase occurred during August and September where studied characters were drastically declined (Table 2). Newly hatched juveniles were seen by the end of September and early beginning of October.

3.4. Fat body cycle

Average fat body weight started to increase during Autumn (September–October), this increase was culminated before breeding season was started (April–May), then weight was obviously decreased during Summer (June–July) with decline being much more in females than males due to utilization of fats in yolk formation of the ova (Tables 1 and 2 and Figs. 9 and 10).

4. Discussion

The reproductive cycle of male and female *C. c. gasperettii* is just one seasonal cycle per year. This finding is consistent with that of White et al. (1982) and Mitchell and Zug (1984) on snakes genus Nerodia in warmer areas. This reproductive activity of *C. c. gasperettii* was proven to be a short period
(April and May), then it started to decline in June which was in agreement with the findings of Aldridge et al. (1995) on females of the snake *T. coronata* and *Nerodia rhohifer* who reported that ovulation in these species occurred during May and June. In the present study, eggs were observed by the end of June where followed by oviposition in mid-July which was in accordance with the findings of Solorzano and Cerdas (1988) working on Serpents Alcilgelh in Costa Rica as well as with those of Aldridge and Semlitsch (1992) who found that oviposition in the snake *T. coronata* occurred during June and early July.

In terms of mating season of *C. c. gasperettii*, it is consistent with Al-Farraj (1993) who reported that reproductive activity of *S. mitranus* occurred from May till July in Riyadh region of Saudi Arabia. However, our results in that respect were contradictory to those of Al-Johany (1986) and Dehlawi (1986) working on the lizards *Acanthodactylus schmidti* and *Acanthodactylus boskianous*, respectively in Saudi Arabia. This discrepancy might be attributed to diurnal activity of these lizards whereas *C. c. gasperettii* is a nocturnal species. Al-Johany (1986) and Al-Farraj (1993) reported that breeding season of numerous reptiles dwelling desert areas was synchronized with winter (rainy season) and controlled by ambient temperature, photoperiod, rainfall rate, humidity and availability of stored food. These observations supported our findings where

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**Table 1** Means of testicular parameters and fat body weight throughout months of the years in *C. c. gasperettii* males.

| Month | Sample no. | Body weight (X g) | % of testis weight to body weight | Seminiferous tubule diameter (X μm) | Testis volume (X mm³) | Fat body weight (X g) |
|-------|------------|-----------------|----------------------------------|----------------------------------|-----------------------|---------------------|
| MAR   | 6          | 89.8            | 0.27                             | 132.2                            | 80.6                  | 6.1                 |
| APR   | 6          | 60              | 0.78                             | 209.1                            | 283                   | 5.45                |
| MAY   | 6          | 83.6            | 0.88                             | 191.4                            | 338                   | 3.42                |
| JUN   | 6          | 92.8            | 0.25                             | 93.5                             | 42                    | 2.9                 |
| JUL   | 6          | 99.7            | 0.13                             | 90                               | 18.2                  | 1.39                |
| AUG   | 6          | 73              | 0.16                             | 58.2                             | 22                    | 2.5                 |
| SEP   | 5          | 89              | 0.11                             | 59.9                             | 17.5                  | 2.44                |
| OCT   | 5          | 62.3            | 0.11                             | 40                               | 10                    | 3.5                 |
| X ± SE| 48         | 67.2 ± 15.5     | 0.20 ± 0.074                     | 109.28 ± 6.2                     | 0.22 ± 0.083          | 3.46 ± 0.083        |

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**Table 2** Means of ovarian parameters and fat body weight throughout months of the years in *C. c. gasperettii* females.

| Month | Sample no. | Body weight (X g) | % of ovary weight to body weight | Ova diameters (X μm) | Fat body weight (X g) |
|-------|------------|-----------------|---------------------------------|---------------------|---------------------|
| MAR   | 6          | 118.2           | 0.23                            | 1784.5              | 7.8                 |
| APR   | 6          | 89.3            | 0.47                            | 1999.5              | 6.9                 |
| MAY   | 6          | 207.8           | 0.46                            | 2291.6              | 12.5                |
| JUN   | 7          | 72.2            | 0.26                            | 1333                | 1.82                |
| JUL   | 8          | 194.1           | 0.14                            | 850                 | 1.32                |
| AUG   | 6          | 111.6           | 0.13                            | 851.9               | 2.45                |
| SEP   | 8          | 87.4            | 0.129                           | 854.6               | 2.97                |
| OCT   | 5          | 147.9           | 0.088                           | 788                 | 6.04                |
| X ± SE| 52         | 94.8 ± 23.9     | 0.168 ± 0.0049                  | 0.22 ± 0.080        | 5.22 ± 0.05         |

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**Figure 6** Seminiferous tubule of *C. c. gasperettii* testis during May showing the lumen filled with spermatozoa (400×).

**Figure 7** Average relative ovarian weight to body weight in *C. c. gasperettii* females during months of the year.
breeding season of C. c. gasperettii occurred following completion of hibernation (i.e., end of raining season). Since juveniles of C. c. gasperettii were only seen in September, this indicates that this species has only one clutch for laying eggs during its reproductive season. These results are consistent with those of Al-Sadoon et al. (2014) who reached to almost the same conclusion concerning the worm lizard, D. zarudnyi derived from the same locality as the horned viper. An inverse relationship was found in the present study between fat body weight and oogenesis and vitellogenesis throughout May and June. This is in accordance with the findings of Castilla and Bawens (1990). It could be concluded that the reproductive cycle of the male and female horned vipers, C. c. gasperettii in Riyadh region of Saudi Arabia proved to be a relatively short period and greatly variable throughout months of the year. The harsh desert conditions certainly affect reproductive biology of Saudi Arabian reptiles.

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