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The reproductive cycle of the flowered racer (*Platyceps florulentus*) from a rural area to the east of the Nile Delta, Egypt.

Amany A. Abd El-Aziz*, Hala G. El-Tantawi, Sohail Soliman and Kareem M. Soliman
Department of Zoology, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt
***Corresponding Author: amanyadel@sci.asu.edu.eg**

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ABSTRACT
The present piece of work aims to study the reproductive cycles of both sexes of the flowered racer, (*Platyceps florulentus*), from a rural area to the east of the Nile Delta, Egypt. In males, spermatogenesis started in winter, continued in spring, peaked in summer, and ceased in autumn. The lowest relative weight of testes was recorded in autumn. It gradually increased until it reached a maximum value in summer when sperms were observed in smears of the caudae epididymis. In females, the previtellogenic phase started in winter and continued in spring. The vitellogenic and postvitellogenic phases were recorded in summer and autumn, respectively. The lowest relative weight of ovaries was recorded in autumn. It gradually increased until it reached a maximum value in summer when ovulation occurred. It is clearly evident, that the reproductive cycle of males, in the present study, is of the vernal type, and that of females is of the prenuptial type. The reproductive cycles of both sexes occured once a year and coincided with each other. The mating season of this snake species was in summer when environmental conditions were most favorable. The results of the present study are of special interest to wildlife conservationists since they help manage the natural populations of this snake species.

INTRODUCTION

Snakes form an important element in the ecosystem in which they live. They are, however, vulnerable to population declines due to several intrinsic and extrinsic factors. One of the intrinsic factors is the slow reproductive cycle **(Webb *et al.*, 2002)**. An example of extrinsic factors is the lack of suitable conservation measures, perhaps due partly to their bad reputation as harmful organisms to both humans and animals **(Andrews and Gibbons, 2005).**

The geographic distribution of the flowered racer, *Platyceps florulentus* (Geoffroy, 1827) is confined to several African countries that include Egypt, Sudan, Ethiopia, and Eritrea. In Egypt, it is present in the Nile Valley and Delta as well as the Suez Canal area. It is also present in newly reclaimed areas, east and west of the Nile Delta, including North Sinai **(Marx, 1968; Saleh, 1997; Baha El Din, 2006; Ibrahim, 2013)**. The habitats of this diurnal and crepuscular snake species are largely associated with River

Nile and include cultivated lands, uncultivated fields, canal banks, and margins of wetlands. The snake is also found in ruins, old houses, and semi-deserts **(Baha El Din, 2006)**. Despite the wide distribution of this species, little information is available on its reproductive cycle. Information on the life histories of other serpents of Egypt is also scanty.

The aim of the present work was to study the reproductive cycles of both sexes of this snake species based on ecological, histological, and histochemical aspects. Sperm parameters were also used as a tool for evaluating the reproductive activity of males. A better understanding of how snakes reproduce is important for the proper management of their wild populations that are increasingly threatened by human activities.

The present work is a part of an inclusive study that covers the populations of snake species of the study area, their reproductive cycles, and their use as potential bioindicators of environmental pollutants. A rural area with extensive agricultural fields, irrigation canals, and ditches, located to the east of the Nile Delta, was chosen to collect snake specimens of the present study on a monthly basis.

MATERIALS AND METHODS

1. The study area

Sampling of snakes was carried out in Al- Sahayra district (30° 44` N and 31° 28` E), Diarb Negm city area, Al Sharqiya Governorate, Egypt. It is an agricultural area located to the East of Nile Delta, some 108 km to the northeast of Cairo (Fig.1). The environmental conditions in such agricultural areas are more stable than those of wild areas of the country. The Nile secures an abundant and continuous supply of irrigation water that allows the cultivation of crop plants throughout the year. Wheat, corn, rice, cotton, beetroot and alfalfa were the dominant cultivations of the study area in the different seasons of the year. The climate and vegetation of the Nile Valley and Delta are affected by the presence of the Nile which creates an area of almost subtropical conditions in lowlands **(Zohary, 1973)**. Without the presence of this river the climate of the area would be of the tropical desert type. The monthly values of temperature and precipitation during the study period were provided by the Central Laboratory for Agricultural Climate, Dokki, Giza (Fig. 2).

2. Sampling of snakes

Only adult snakes (with snout-vent lengths of approximately 474-539 mm) are included in the present study. Snake were sporadically collectes over a period of 19 months that extended from December 2019 to June 2021. Field trips were stopped for several months due to the Governmental lockdown regulations connected with COVID-19 pandemic. The actual sampling months were thus 12 months divided into three periods; i.e., December 2019, January-March 2020, July-November 2020, and April-June 2021. All months of the year are thus represented in this interrupted sampling period. A total of 25 adult individuals of both sexes were collected throughtout the study period. To interpret results on a seasonal basis, collected snake specimens were grouped according to the sequence of months of the year, regardless of chronology, as follows:

- Specimens collected in December, January, and February were grouped for winter.
- Specimens collected in March, April, and May were grouped for spring.
- Specimens collected in June, July, and August were grouped for summer.

- Specimens collected in September, October, and November were grouped for autumn.

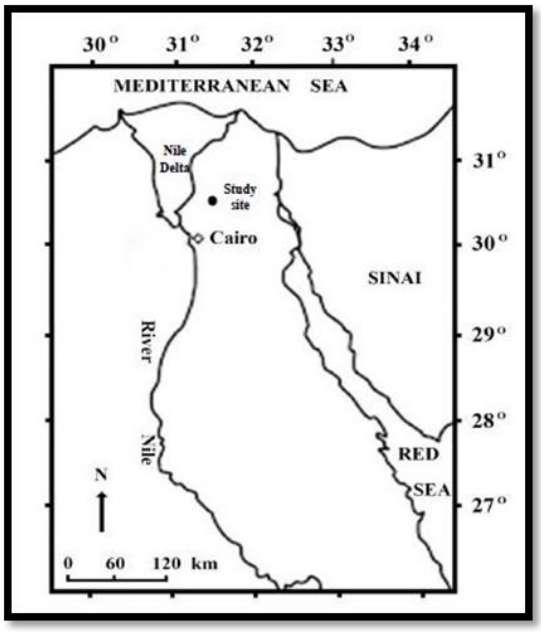

Fig. 1. A map showing the location of the study site.

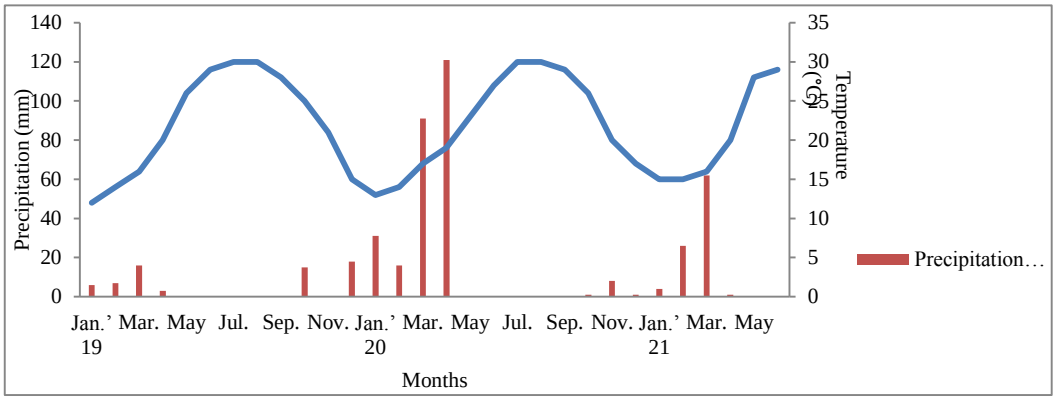

Fig. 2. Monthly average temperature and precipitation in Diarb Negm area during the whole study period (December 2019 to June 2021, inclusive).

3. Histological and histochemical preparations

Collected snakes were transferred alive to the laboratory in the Department of Zoology, Faculty of Science, Ain Shams University, Cairo. Snakes were euthanized with chloroform and the right testes and ovaries were dissected out of snakes for the histological and histochemical study. The organs were cleaned of adhering connective tissues, fixed in Bouin's solution for 24 hours, dehydrated in ascending grades of ethyl alcohol, and then cleared in terpineol for at least 3 days. The organs were then embedded in three changes of paraffin wax; one hour each. Transverse sections, 5 µm thick, were cut by a rotary microtome, and the sections were mounted on clean microscopic slides.

The sections of testes were divided into two sets; the first set was stained with Harris hematoxylin and eosin **(Bancroft and Stevens, 1990)**, and the second one was stained with Mercury bromophenol blue **(Humason, 1962)**. The ovarian sections were similarly divided into two sets; the first set was stained with Harris hematoxylin and eosin, and the second one was stained with periodic acid Schiff's reagent **(Humason, 1962).**

Sections of both testes and ovaries were examined under a light microscope (B-500T, OPTIKA, Ponteranica, Italy) to identify the seasonal changes in their histological structure. The slides were photographed by a digitalized light microscope with a camera, at the Regional Center for Mycology and Biotechnology, Al Azhar University, Cairo, Egypt.

The terms used by **Loebens *et al*. (2017)** and **Afsharzadeh *et al*. (2015)** for describing the microscopic structure of the testes and ovaries, respectively, are used in the present work.

4. Sperm parameters

After necropsy, the right cauda epididymis was immediately excised and cleaned of adhering connective tissues. It was then ruptured, using a pair of small scissors, to release sperms into a 35-mm petri dish containing 2 ml of a physiological saline solution (0.9%) **(Rekha and Chandrashekara, 2014)** at room temperature.

4.1. Sperm count

Sperms were counted, by the use a hemocytometer, under a light microscope at a magnification power of X400 (objective lens). The number of sperms was counted in the four corner squares as well as in the central one ($1mm^2$). The mean value was calculated and the number of sperms/ml was calculated by multiplying the mean value by 10^6 **(Zimmerman and Mitchell, 2017)**.

4.2. Sperm motility

Motility of spermatozoa was estimated by placing a drop of sperms suspended in a saline medium under a coverslip at room temperature, and sperms were examined under a light microscope (X400). On each slide, 100 sperm cells were counted. Sperm motility was expressed as the percentage of motile sperms of the total number of counted sperms **(Zimmerman and Mitchell, 2017)**.

4.3. Sperm abnormalities

Spermatozoa were prepared for the examination of the sperm abnormalities by placing and smearing drops of sperms in saline medium on clean microscopic slides. Smears were then air-dried and fixed in methanol. After fixation, samples were stained with 1% aqueous Eosin-Y solution for 1 hr, washed with distilled water, and covered with coverslips **(Narayana *et al*., 2005)**. Morphology was evaluated by observing 100 sperm cells under oil-immersion lenses (X1000). Sperm abnormalities were recorded as percentages of the total sperm counted **(Fahrig *et al.*, 2007)**.

5. Statistical analysis

Descriptive statistics including means, standard deviations, and range (minimum and maximum) were calculated. Kruskal-Wallis test was used to compare seasonal variation in the relative weights of gonads using GraphPad prism program version 4.03 (software; San Diego, CA, USA). A *p*-value of <0.05 was considered to be statistically significant **(Turner and Thayer, 2001)**.

RESULTS

1. Males

1.1. Male reproductive cycle

The histological examination of testes of male snakes collected throughout different seasons of the study period indicated that the reproductive cycle of male *P. florulentus* could be divided into five successive stages as follows: stage I (winter), stage II (Early spring: March and April), stage III (late spring: May), stage IV (summer), and stage V (autumn). Each one of these stages had its diagnostic histological features as follows:

Stage I: It showed oval or rounded seminiferous tubules surrounded by thin basal lamina. The tubules showed irregular layers of spermatogenic cells (Fig. 3a). Only spermatogonia and primary spermatocytes were identified (Fig. 4a). Examination of testis sections stained with bromophenol blue stain revealed the presence of Sertoli cells and many interstitial cells (Fig. 4a).

Stage II: During this stage, spermatogonia, primary spermatocytes and early spermatids greatly increased in number, and appeared densely crowded together (Fig. 3b). Connective tissues and interstitial cells were less abundant in the testicular tissue (Fig. 4b).

Stage III: This stage was characterized by the presence of spermatogonia, primary spermatocytes, late spermatids and a small number of early spermatozoa. Various spermatogenic cells at different developmental stages were usually gathered in the form of several spermatogenic pyramid-shaped clusters. The second feature of this stage was a noticeable increase in nuclear basophilia (Figs. 3c and 4c). Thin tunica albuginea and very few Sertoli and interstitial cells were noticed (Fig. 4c).

Stage IV: It was the reproductive stage of the cycle. The testis showed abundant connective tissues with many interstitial cells (Fig. 3d). The tubular cavity had a wide diameter and was filled with sperms indicating that male snakes were sexually active during this stage. In bromophenol blue stained sections, sperm nuclei appeared dark blue (Fig. 4d).

Stage V: It is characterized by a noticeable reduction in the diameter of seminiferous tubules and consequently the size of the testis (Figs. 3e and 4e). Abundant connective tissue vascularized with blood vessels and interstitial cells in the intertubular spaces were remarkably observed (Fig. 3e). Number of sperms in lumena of seminiferous tubules was greatly reduced (Fig. 4e).

1.2. Seasonal changes in relative testis weight (RTW)

RTW was calculated as follows: (the average individual testis weight in grams/mean body weight in grams) X 100. RTW significantly increased ($P < 0.05$) in summer (0.91%) compared with autumn (0.37%) (Fig. 5).

1.3. Seasonal changes in sperm parameters

Sperms were only observed and counted in summer (195 X 10^6/ml). No sperms were observed in other seasons. Signs of abnormalities were only observed in 2% of the total counted sperms, and the percentage of motile sperms was as high as 91% of total counted sperms (Table 1).

Table 1. Seasonal changes in mean sperm parameters of adult males of *P. florulentus* collected during the study period from a rural area east of Nile Delta. The mean is followed by ± S.D., range (in parentheses), and number of specimens.

Sperm parameters / seasons	Sperm count ($x10^6$/ml)	Sperm motility %	Sperm abnormality %
Winter	0 ± 0 (0 – 0),3	----------	----------
Spring	0 ± 0 (0 – 0),5	----------	----------
Summer	195 ± 35.3 (170 – 220),2	91.5 ± 2.1 (90 – 93), 2	2 ± 1.4 (1 – 3),2
Autumn	0 ± 0 (0 – 0),4	----------	----------

2. Females

2.1. Female reproductive cycle

The ovarian parenchyma was divided into two regions; an outer cortex and an inner medulla, and was surrounded by a dense connective tissue layer; the tunica albuginea. The cortex contained different stages of oogenic, or ovarian follicles, and the medulla contains masses of individual fibrocytes and blood vessels. During the process of folliculogenesis, or the maturation of oogenic follicles in the cortex layer, follicles passed through four stages; namely, follicle I (stage 1), follicle II (stage 2), follicle III (stage 3), and corpus luteum (stage 4). These four stages differed from each other in the characters of each of the granulosa layer, theca layer, vitelline membrane, and vitellus deposition.

The reproductive cycle of female *P. florulentus* was divided into the following three successive phases, based on both morphometric measurements and the percentages of different types ovarian follicles present: 1. previtellogenic phase that started in winter and continued to spring, 2. vitellogenic phase in summer, and 3. postvitellogenic phase in autumn.

1. Previtellogenic phase: This phase started in winter and continued in spring. In winter, the mean percentages of oogenic follicles of types I and II (stages 1 and 2) were 33% and 67%, respectively (Table. 2). No follicles of type III were observed (Figs. 6a and 7a). Follicle I was surrounded by a dense theca layer and lined by the granulosa formed of three cell types arranged in many layers: small, pyriform and intermediate cells. The small cells were cuboidal with rounded central nuclei and granular cytoplasm. The pyriform cells were large with pale cytoplasm and voluminous nuclei containing clumps of heterochromatin. The intermediate cells were rounded with clear cytoplasm and rounded darkly stained nuclei (Fig. 8a). Follicle I was also filled with clear ooplasm enclosed within a thick vitelline membrane. Follicle II was surrounded by a theca layer less dense than that of follicle I, and also lined by granulosa layers formed of three cell types: small, pyriform and intermediate, arranged in three layers (Fig. 8b). Follicle II was also filled with ooplasm which was enclosed within a thin vitelline membrane, and contained yolk granules (vitellus granules). Yolk granules were intensely stained with PAS. The accumulation of yolk started at the periphery of the oocyte (Figs. 6a and 7a). In spring, there was a great increase in the percentage of follicle II since it formed 97% of

follicles observed. Follicle I only formed 3% of observed follicles during this season (Table. 2 and Figs. 6b and 7b).

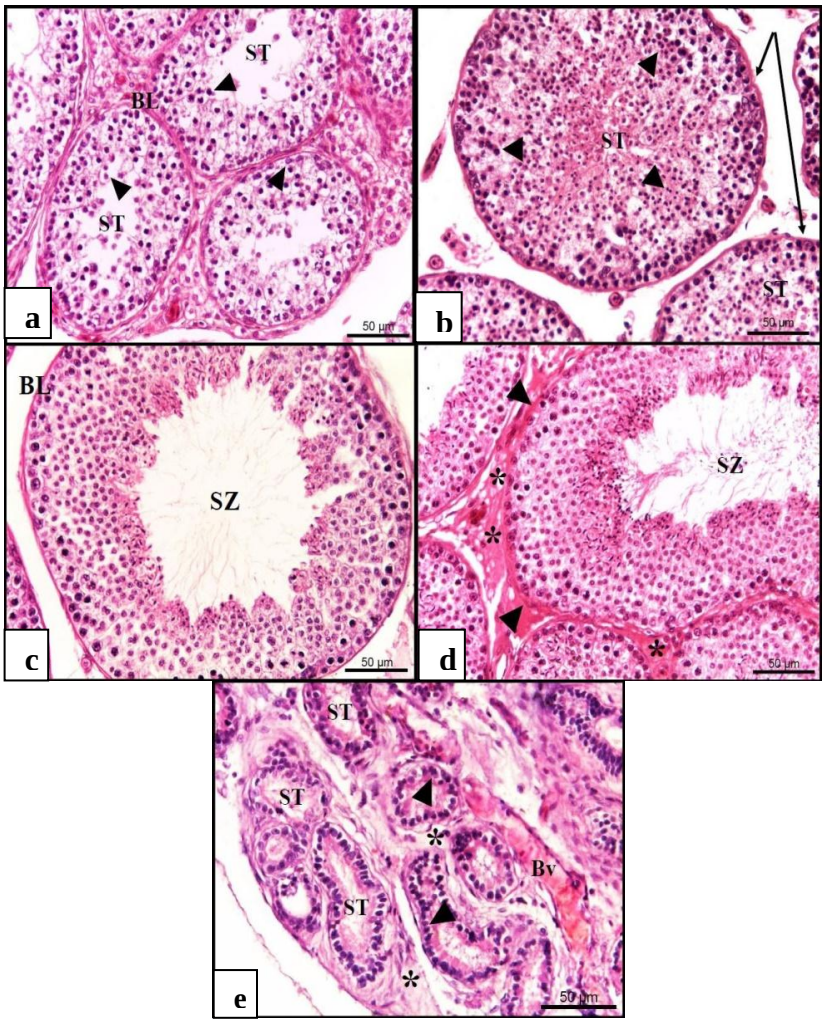

Fig. 3. Photomicrographs of transverse sections of the testis of *P. florulentus* showing different stages of its reproductive cycle. **(a)** Stage I (winter) showing oval or rounded seminiferous tubules (ST) surrounded by thin basal lamina (BL). The tubules are filled with irregular layers of spermatogenic cells (arrow head). **(b)** Stage II (early spring), showing enlarged seminiferous tubules (ST) with thin basal lamina (arrow), active spermatogenic cells (arrow heads), with complete absence of spermatozoa. **(c)** Stage III (late spring) showing the presence of few early spermatozoa (SZ) in the lumen of the large seminiferous tubule, and thin basal lamina (BL). **(d)** Stage IV (summer) showing narrow intertubular spaces and abundant connective tissue (*), thickened basal lamina (arrow heads), presence of mature sperms (SZ) in the lumen of the seminiferous tubule. **(e)** Stage V (autumn) showing irregular shrinked seminiferous tubules (ST), inactive one layer of the germinal epithelial cells (arrow heads), presence of large blood vessels (Bv), and narrow intertubular spaces (*). [H&E stain]

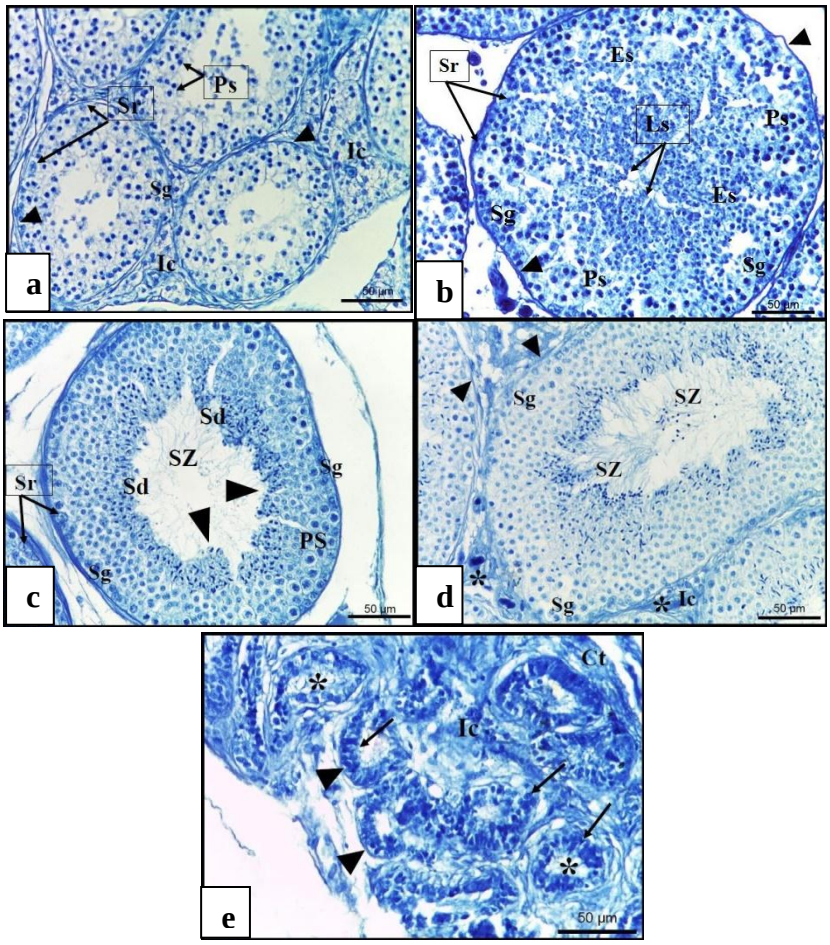

Fig. 4. Photomicrographs of transverse sections of the testis of *P. florulentus* showing different stages of its reproductive cycle. **(a)** Stage I (winter) showing seminiferous tubules surrounded by thin basal lamina (arrow head) with interstitial cells in-between (Ic). The tubules are lined with germinal epithelium including spermatogonia (Sg), primary spermatocytes (Ps) and large Sertoli cells (Sr). **(b)** Stage II (early spring), showing seminiferous tubules surrounded by thin basal lamina (arrow head). The tubules are lined with groups of germinal epithelia including spermatogonia (Sg), primary spermatocytes (Ps), a lot of early spermatids (Es), and smaller late spermatids (Ls) filling the center of the seminiferous tubules, and various Sertoli cells (Sr). **(c)** Stage III (late spring), showing the presence of pyramidal groups of stratified germinal epithelia (arrow heads), basal spermatogonia (Sg), large primary spermatocytes (PS), many mature spermatids (Sd) and few early spermatozoa (SZ) in the expanded lumen of the seminiferous tubule. Few Sertoli cells (Sr) are present in the seminiferous tubule. **(d)** Stage IV (summer) showing narrow intertubular spaces (*) enclosing interstitial cells (Ic), the lumen of the seminiferous tubule filled with mature sperms with tails and dark heads (SZ), and the presence of few faintly stained spermatogonia (Sg). **(e)** Stage V (autumn) showing the seminiferous tubules with few sperms in the small lumen (*) surrounded by a thin basal lamina (arrow head), existence of thick connective tissues (Ct), various interstitial cells (Ic) in the intertubular spaces, and the presence of one or two layers of inactive darkly stained germinal epithelial cells (arrow). [Bromophenol blue stain]

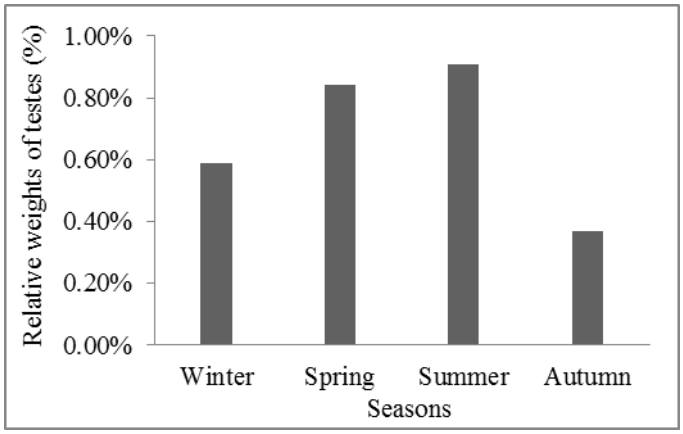

Fig. 5. Seasonal changes in mean relative testis weight of adult males of *P. florulentus* collected during the study period from a rural area east of Nile Delta.

2. Vitellogenic phase: During this phase, follicles of types II and III (stages 2 and 3) were observed. No follicles of type I were present (Figs. 6c and 7c). The mean percentages of oogenic follicles of both types were 50% (Table. 2) Follicle III was surrounded by a thin theca layer and lined by a granulosa layer formed of a single cell type arranged in 1-2 layers of small cuboidal cells with rounded nuclei and granular cytoplasm (Fig. 8c). Follicle III was also filled with ooplasm that contained a large number of yolk granules. The vitelline membrane was absent in this stage (Figs. 6c and 7c).

3. Postvitellogenic phase: During this phase, oogenic follicles of type II (stage 2) and corpora lutea (stage 4) were observed. The mean percentages of these two stages were 51% and 49%, respectively (Table. 2). No follicles of types I and III were observed (Figs. 6d, 7d). The follicles were surrounded by dense connective tissues. The corpus luteum was formed as a result of the collapse of the wall of follicle III after ovulation and was intensely-stained. The granulosa cells of ovulated follicle invaded the follicular cavity forming a central mass of luteal cells with rounded to oval nuclei. There were also numerous large macrophages with central rounded nuclei, indicating a phagocytic activity so that the vacuolization was markedly noticed (Figs. 6d, 7d, 9a and 9b).

2.2. Seasonal changes in relative ovary weight (ROW)

ROW was calculated as follows: (the average weight of the ovary of each individual snake in grams/mean body weight in grams) X 100. ROW significantly increased ($P < 0.05$) in summer (0.58%) compared with autumn (0.08%) (Fig. 10).

Table (2). Seasonal percentages of different types of ovarian follicles in adult females of *P. florulentus* collected during the study period from a rural area east of Nile Delta.

Percentages of average numbers of different follicle types per individual female																			
Winter					Spring					Summer					Autumn				
Total average	F 1		F 2		Total average	F 1		F 2		Total average	F 2		F 3		Total average	F 2		Cor. L .	
	No	%	No	%		No	%	No	%		No	%	No	%		No	%	No	%
12	4	33	8	67	11.3	0.3	3	11	97	13.3	6.7	50	6.7	50	7.8	4	51	3.8	49

*Number of different types of follicles is considered as the average number per individual female calculated on a seasonal basis.

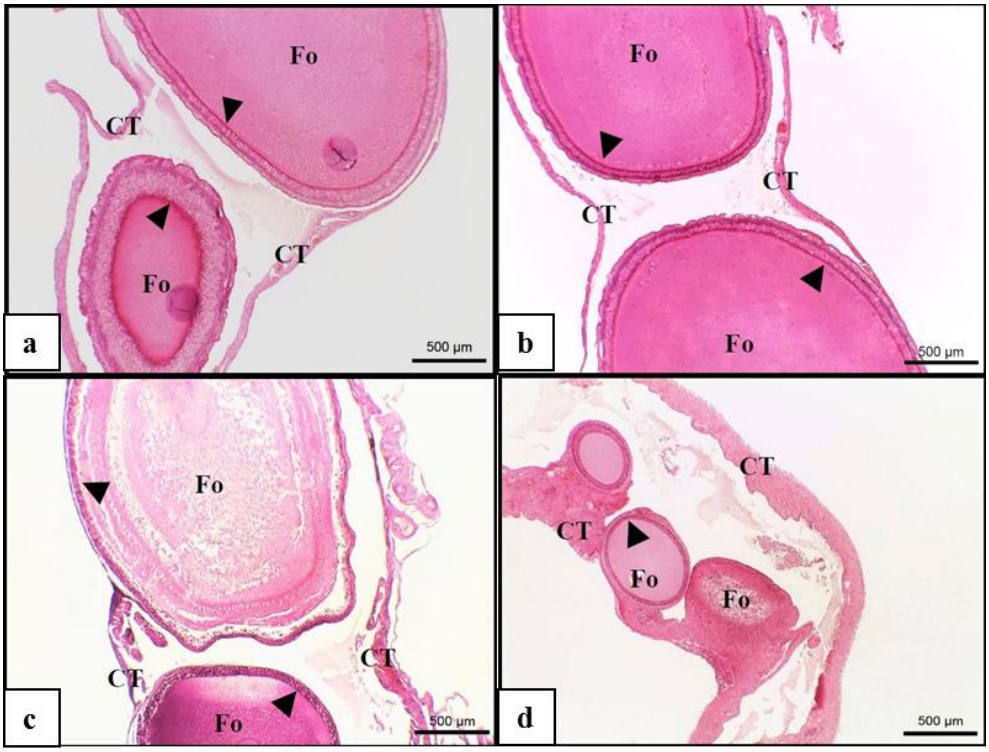

Fig. 6. Photomicrographs of a longitudinal section of the ovary of *P. florulentus* showing different stages of its reproductive cycle. **(a)** The previtellogenic phase (winter), showing oval or rounded follicles (Fo) surrounded by a dense connective tissue (CT). The follicles are lined with different layers of granulosa cells (arrow head). **(b)** The previtellogenic phase (spring), showing oval or rounded follicles (Fo) surrounded by delicate connective tissue (CT). The follicles are lined with multiple layers of granulosa cells (arrow head). **(c)** The vitellogenic phase (summer), showing oval or rounded follicles (Fo) surrounded by a dense connective tissue (CT). The follicles are lined with multilayers of granulosa cells (arrow head). **(d)** The postvitellogenic phase (autumn), showing oval or rounded follicles (Fo) surrounded by dense connective tissue (CT). The follicles are lined with multilayers of granulosa cells (arrow head). [H&E stain]

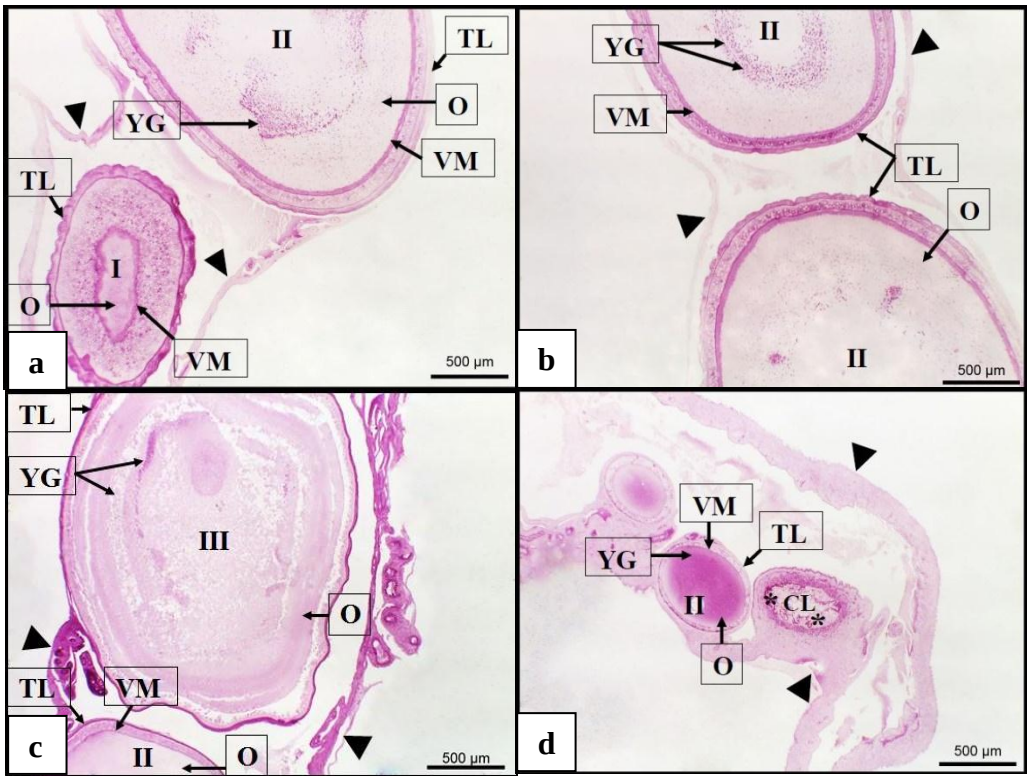

Fig. 7. Photomicrographs of a longitudinal section of the ovary of *P. florulentus* showing different stages of its reproductive cycle. **(a)** The previtellogenic phase (winter) showing the follicles I and II surrounded by dense connective tissues (arrow head). Each follicle is surrounded by a theca layer (TL) which is more dense in follicle I than in follicle II. Each follicle is filled with ooplasm (O) which is clear and less stained in follicle I with yolk granules (YG) and more stained in follicle II. The ooplasm is enclosed in a thick vitelline membrane (VM) in follicle I, and a thin one in follicle II. **(b)** The previtellogenic phase (spring) showing the follicles surrounded by a dense connective tissue (arrow head). Follicle II is enclosed in a less dense theca layer than follicle I. Each follicle is filled with ooplasm (O) with densely stained yolk granules (YG). The ooplasm is enclosed in a thin vitelline membrane (VM). **(c)** The vitellogenic phase (summer) showing follicles II and III surrounded by dense connective tissues (arrow head). Follicle II is surrounded by less dense theca layer than follicle III. Each follicle is filled with ooplasm (O) and many densely stained yolk granules (YG). The vitelline membrane (VM) which encloses the ooplasm is thin in follicle II and absent in follicle III. **(d)** The postvitellogenic phase (autumn) showing the follicules surrounded by dense connective tissues (arrow head). Follicle III is enclosed in a less dense theca layer (TL), and is filled with ooplasm (O) and densely stained yolk granules (YG). The ooplasm is lined with a thin vitelline membrane (VM). Irregular corpus luteum (CL) with discontinuous membranes (*) is observed. [Periodic acid-Schiff stain]

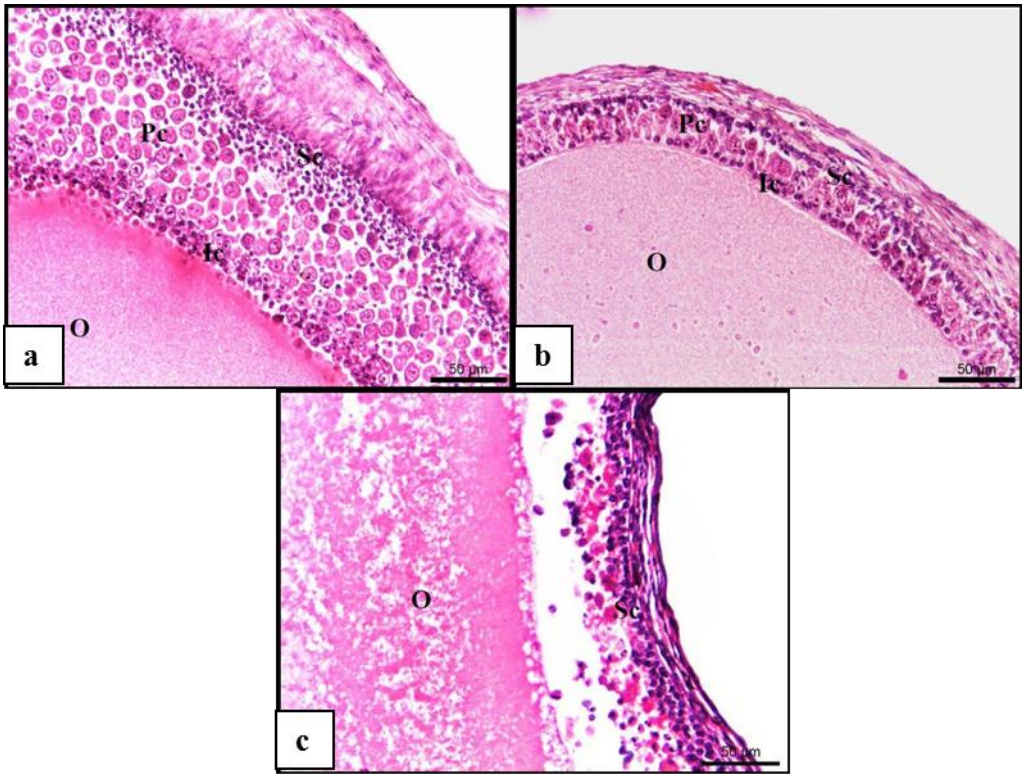

Fig. 8. Photomicrographs of a magnified part of the ovary of *P. florulentus* showing: **(a)** Follicle I lined with granulosa formed of three cell types arranged in many layers; small, pyriform and intermediate cells. The small cuboidal cells (Sc) have rounded nuclei and granular cytoplasm. The pyriform large cells (Pc) show pale cytoplasm and voluminous nuclei with clumps of heterochromatin. The tntermediate rounded cells (IC) have clear cytoplasm and rounded nuclei. Clear homogeneous ooplasm (O) is observed. **(b)** Follicle II lined with granulosa formed of three cell types arranged in many layers; small, pyriform and intermediate cells. The small cuboidal cells (Sc) have rounded nuclei and granular cytoplasm. The pyriform large cells (Pc) with pale cytoplasm and voluminous nuclei with clumps of heterochromatin. The intermediate rounded cells (IC) with clear cytoplasm and rounded nuclei. The follicle is filled with distinct granulated ooplasm (O) with vitellus granules. **(c)** Follicle III lined with thin granulosa formed of a single small cell type arranged in 1-2 layers. The small cuboidal cells (Sc) have rounded darkly stained nuclei and granular cytoplasm. The ooplasm (O) with less homogeneous vitellus granules.

[H&E stain]

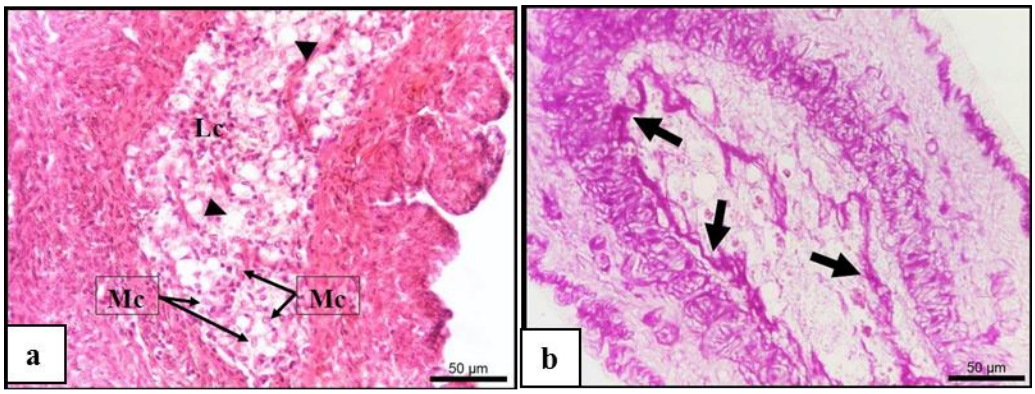

Fig. 9. Photomicrographs of a magnified part of an ovary section of *P. florulentus* showing: **(a)** the corpus luteum with the granulosa cells of ovulated follicle invading the follicular cavity and forming a central mass of luteal cells (Lc) with circular to oval nuclei. Numerous large macrophage cells (Mc) and many vacuoles are noticed (arrow head). [H&E stain]. **(b)** The irregular corpus luteum with a darkly stained folded wall of the follicle after ovulation (arrows). [Periodic acid-Schiff stain]

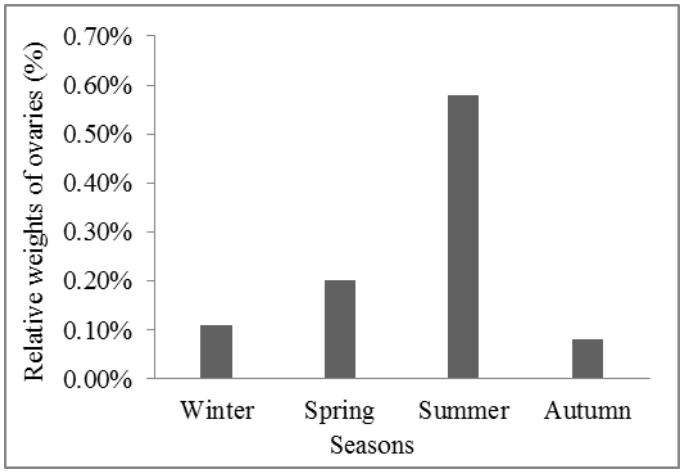

Fig. 10. Seasonal changes in mean relative ovary weight of adult females of *P. florulentus* collected during the study period from a rural area east of Nile Delta.

DISCUSSION

The reproductive cycles of reptiles are intimately associated with environmental conditions prevailing the habitats in which they live. The results of the present study indicate that ambient temperature and the availability of food might play key roles in determining the onset, duration, and cessation of different stages of the reproductive cycles of both sexes of this snake species.

It was clearly noticed that the testes and ovaries of *P. florulentus* had the normal microscopic structure known for male **(Aldridge and Sever, 2016)** and female **(Guraya, 2013)** snakes**.**

The reproductive cycles of both sexes of *P. florulentus* are synchronous. In males, spermatogenesis started in winter, continued in spring, reached its peak in summer when sperms were observed in smears of caudae epididymis, and stopped in autumn. There were also parallel changes in the relative testis weight that had a minimum value in autumn and a maximum value in summer.

In females, the previtellogenic phase started in winter and continued in spring, the vitellogenic phase occurred in summer, and the postvitellogenic phase was in autumn. There were also parallel changes in the relative ovary weight that had a minimum value in autumn and a maximum value in summer when ovulation occurred.

The synchronization of the reproductive cycles of both sexes of this snake species might be correlated to changes in environmental factors, such as temperature and availability of food, in the habitat in which they live. Such factors are suggested to affect the hormonal activity, and therefore, the activities of reproductive organs **(Amer and Elshabka, 1978).**

According to **Licht (1972),** changes in environmental temperature generate seasonal reproductive cycles and might alter the physiological response of pituitary gonadotrophs. The spermatogenic activity, for example, reached its peak after the end of brumation in snakes since it stops or slows down under low environmental temperature (**Saint Girons, 1982)**. During brumation, snakes exhibit a slowing down of all metabolic and physiological activities to maintain an energy balance **(Holden *et al*., 2021)**. The suitable environmental temperature might also be an important factor for successful incubation of eggs **(Cunnington and Cebek, 2005).**

Rainfall might also influence the reproductive activities of snakes through its effect on the vegetation where snakes' prey live. Undoubtedly, Prey provide snakes with energy required for reproductive activities such as the production of sperms **(Shiravi *et al*., 2012; Aldridge *et al*., 2020)**. The reproductive cycles of snakes in many parts of the world, especially tropical regions, are closely correlated with the rainy season and the availability of prey **(Henderson and Hoevers, 1977; Pizzatto and Marques, 2006; Shine and Brown, 2008; Barros *et al*., 2014; García-Cobos *et al*., 2020)**. In the present study, prey items were only recovered from the alimentary canals of snake specimens collected in summer and autumn **(unpublished data)**. This could be interpreted on the basis of the increased frequency of food intake in these seasons to increase body fats prior to brumation in winter **(Aleksiuk and Cowan, 1969)**. During brumation, environmental temperature drops, metabolism of snakes slows down and the rate of utilization of nutrients decreases **(Aleksiuk, 1970)**. Rainfall might also make the soil more humid and suitable for the incubation of eggs. A certain degree of soil humidity is necessary to prevent egg desiccation and to prepare eggs for hatching **(Saint Girons, 1982).**

The effect of rainfall on the reproductive cycle of *P. florulentus* of the present study is, however, questionable since the snake lives in a stable agricultural environment where irrigation water, vegetation, and consequently prey, are available all the year round. In this type of habitat, it is easy for snakes to find suitable nest sites with apporpriate amount of humidity without a need to wait for rainfall. Mammal burrows, tree hollows, spaces under rocks and crevices, which are suitable nest sites for oviparous snakes **(Whitaker *et***

***al*., 2004),** are available in such agricultural habitat. Moreover, The peak of the rainy season in Egypt extends through winter and early spring; several months before the breeding season of this species. The effect of rainfall on the reproductive cycles of snakes in this habitat type is thus not certain and must be carefully examined.

The reproductive activities of one sex do not only depend on environmental abiotic factors but might also be influenced by the reproductive cycle of the other sex. In snakes, which have no prolonged brumation, the sexual activity of males was reported to be influenced by that of females (**Saint Girons, 1982)**.

The timing of the different stages of the reproductive cycle of males of the present study could generally be compared with that recorded for other snake species of the Middle East such as the grass snake, *Natrix natrix* **(Faghiri *et al*., 2011)** and the dice snake, *Natrix tessellata* (**Savasari *et al*., 2015)** in northern Iran. The reproductive cycle of females could also be compared with that of other serpents of the region such as the Iran viper, *Vipera albicornuta* **(Afsharzadeh *et al*., 2015)**. Synchronization between the reproductive activities of both males and females of the Schokari sand racer, *Psammophis schokari*, in Israel was recorded in spring **(Goldberg, 2015)**.

Sperm parameters were also used to support the results of histological examination of testes. These parameters were used by other authors to evaluate the reproductive potential of snakes (**Moshiri *et al*., 2014)**.

Our findings revealed that males produced spermatozoa just before mating in summer. The male reproductive cycle of *P. florulentus* could thus be referred to as a vernal cycle **(Savasari *et al*., 2015)**. Ovulation in females of this species follows the peak of spermatogenesis in males so that, the female reproductive cycle could be referred to as a prenuptial cycle **(Afsharzadeh *et al*., 2015)**.

The results of the present study are of special interest to wildlife conservationists since they help manage the natural populations of this snake species.

CONCLUSION

The male reproductive cycle of *P. florulentus* is of the vernal type and the female reproductive cycle is of the prenuptial cycle. Reproductive cycles of both sexes of the flowered racer had completed within one year; i.e, they occurred once a year. The mating season occurred in summer.

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