



Some Biological Aspect and Gonads Histology of *Sepia savignyi* (Blainville, 1827), in the Gulf of Suez, Egypt.

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ABSTRACT

This work is the first histological description of gonad maturation of *Sepia savignyi* and the beginning of future research on its basic biology. A total of 452 samples, ranged in dorsal mantle length from 3.9 to 29 cm with average 16.35 ± 1.50 and total weight ranged from 19 to 1826.8 gm, with average 606.79 ± 110.7 , of which 269, female and 183 male were examined between September 2014 to May 2015 obtained from the Gulf of Suez, Egypt. The size at first sexual maturity (DML₅₀) was 11.9 and 8.4 cm for female and male, respectively. The Gonadosomatic indices show that the females and males have two peaks in the year-round spawning period the first is in December (4.1 %) and (2.4%) and the second is in April (3.5%) and (2.2%) for female and male, respectively. Five stages of gonad maturation were established for the female and male, namely immature (I), maturing (II), mature (III), spawning (IV) and spent (V) based on macroscopic examination. Histological examination showed that each ovary and testis had various stages of oocyte and spermatocytes. Oogenesis of oocyte demonstrated seven stages of maturity in females as oogonia, early primary oocyte, late primary oocyte, previtellogenic oocyte, vitellogenic Oocyte, advanced vitellogenic oocyte and ripe oocytes. Spermatogenesis of testes divided into spermatogonia, primary and secondary spermatocytes, spermatid and spermatozoa.

INTRODUCTION

Cuttlefish are marine animals belong to Class Cephalopoda. They are widespread throughout worldwide oceans inhabiting the warm, tropical shallows to the cold depths. In the Gulf of Suez, cephalopod fishery is economically very important, due to its high commercial values in the national and international markets. The cuttlefish's catch in the Gulf of Suez composed of four species; *Sepia savignyi*, *S. pharaonis*, *S. dollfusi*, and *S. trygonina* but *S. savignyi* was the most abundant species.

Knowledge of reproduction is important in solving some fishery management questions such as the determination of spawning stock. Moreover, a correct identification of mature individuals in the population is thus a crucial step for a

precise estimation of spawning stock biomass, and finally for assessing the status of the stock and establishing harvest level. Although, there are some data, regarding reproductive biology of cuttlefish all over the world (Önsoy and Salman, 2005; Ghazvineh *et al.*, 2012; Dursun *et al.*, 2013; and Salman, 2015), there is no any histological studies on *Sepia savignyi* in the Gulf of Suez. Few studies have contrasted the morphological maturity scale with the histological analysis in other cephalopod species such as squid *loligo vulgaris* Sauer and Lipinski. (1990), *Sepia. dollfusi* Gabr (1998), *Octopus vulgaris* Gonçalves *et al.*, (2002) and *Octopus vulgaris* Cuccu *et al.*, (2013).

This work is the first study on the histological description of gonad maturation of *Sepia savignyi* from the Gulf of Suez, Egypt. Which the beginning of future research on its basic biology. It is intended to estimate the size at first maturity of *Sepia savignyi*, which used as a basis of the minimum legal size determination. Also, it assesses the reproductive biology of *Sepia savignyi* based on the morphological and histological description of the gonadal development and determination of the spawning season during the investigated period.

MATERIALS AND METHODS

Samples of *Sepia savignyi* (269 females and 183 males) collected from the Suez Gulf (El- Ataka port) during the fishing season from September 2014 to May 2015. In the laboratory, the dorsal mantle lengths (DML) were measured within 0.1 cm, total body weights (TW) within 0.01 g, and gonad weights (GW) within 0.01 g. Specimens were dissected to determine sex and maturity stage.

Length at first sexual maturity ($L_{50\%}$)

The length at which 50% of *Sepia* reaches their sexual maturity was estimated by fitting the percentage maturity against mid-lengths (king, 1995).

Gonadosomatic indices (GSI)

Gonado-somatic index (GSI) was determined by using the following equation:

$$GSI = (\text{Weight of gonad} / \text{Body weight}) \times 100$$

Gonad Histology

Small pieces of gonad were fixed for 48 h in Bouin's solution and then transferred to 70% ethyl alcohol for preservation. Clearing and paraffin embedding were performed using standard histological techniques; sections were cut at 7 μm thickness by using automatic microtome LKB and stained by Hematoxylin and eosin Bancroft and Stevens (1996). The tissues were investigated using a Leica microscope, equipped with a digital camera. Maturity stages for each sex were determined by using Gabr *et al.* (1998) scale with some modifications.

RESULTS AND DISCUSSION

Length at first sexual maturity

Size at first sexual maturity (l_{50}) has been considered as a reference point of minimum legal size to prevent stock destruction. It has been employed by many fisheries managers as a management measure of fish stock. The smallest mature female was 7.5 cm in ML and the lengths from 3.9 to 6.5 cm are immature. The size at maturity (ML_{50}) was 11.9 cm for females. Maturity increased with increased length and the dominant mature female showed from lengths 7.5 to 29.5 cm. The percent of maturity in males increase in length where lengths 4.5 and 5.5 cm were completely immature. The smallest mature male was 6.5 cm and the ML_{50} was 8.4 cm for ML

(Fig. 1) .Throughout the species range, males of sepia species reach maturation at smaller sizes than females Gabr *et al.*, (1998) which found that the size at maturity for *S. pharaonis* was 61 mm ,ML for males and 122 mm, ML for females, while for *S. dollfusi* was recorded in 7.5 and 8.4 cm for male and female respectively Önsöy and Salman (2005). recorded(ML_{50}) of *S. officinalis* in 13 cm ML for females and 9cm for males Difference in values in size at first sexual maturity between species might occur due to temperature according to different environments. Actually, common cuttlefish, which stay in warm waters, are maturing earlier than those that are in cold waters, according to Boletzky (1988).

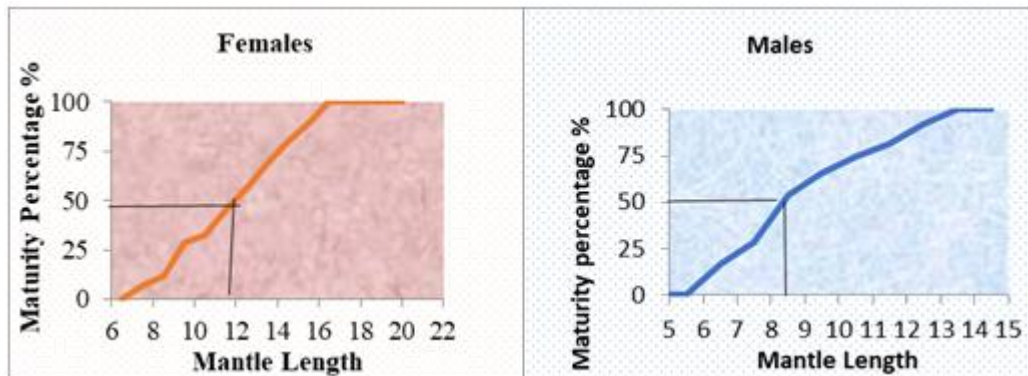


Fig. 1: Female and male distribution of 50% of the population (ML_{50}) at the size of maturity of *S. savignyi* obtained from the Gulf of Suez during the fishing season from

Our results are practically coincident with Mehanna and El-Gammal (2010) from the Gulf of Suez (ML_{m50} 9.51 cm ML male; female 10.22 cm, ML of *S. savignyi*) El-Sherbeny (2009) from the Gulf of Suez recorded L_{50} for the sexes combined 13.7cm ML, for *S. savignyi*. Our result would not conform to this because sex must be separate.

Gonadosomatic index (GSI)

GSI is important for deciding the reproductive cycle of *S. savignyi* in the Gulf of Suez. It implies that the females and males have two peaks in the year-round spawning period where firstly is in April (3.5%) and (2.2) and the second is in December (4.1 %) and (2.4) for female and male, respectively. (Fig. 2)

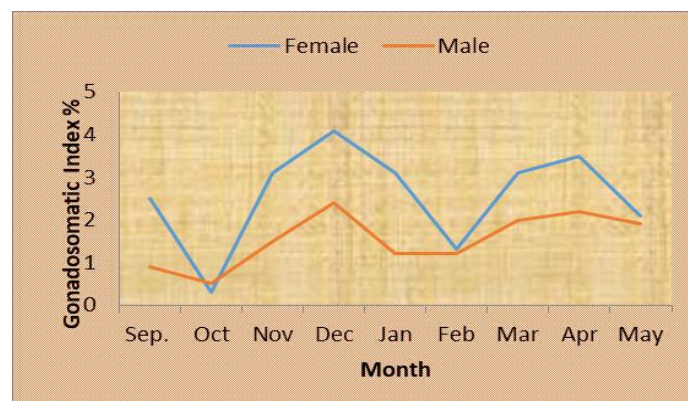












Fig. 2: Monthly female and male gonadosomatic index values of *S. savignyi* obtained from the Gulf of Suez during the fishing season from September 2014 to May 2015.

Various authors confirmed that there are two peaks of GSI in numerous *Sepia* species such as. Gabr *et al.*, (1998) in Suez canal observed that *S. dollfusi* have two beaks in January-April also *S. pharaonis* attains two beaks in March-June Onsoy and

Salman (2005) observed that the spawning period of *S. officinalis* covers the whole year with two maximum peaks in March and June. Ghazvineh *et al.*, 2012 attains two peaks for *S. Pharaonis* in May and June Persian Gulf in Iran and Dursun *et al.*, (2013) found that the mean monthly female GSIs showed two annual peaks, in spring (May) in addition in autumn (November) for pink cuttlefish *Sepia orbignyana* in the Aegean Sea. Our results did not conform to Akyol *et al.*, (2011) recorded one beak of *Sepia officinalis* and varied in spawning seasons. And, Riad. R. *et al.*, (2015) found that the mean monthly of GSI demonstrated one annual peak, early Spring and in Summer, and sometimes extended to Autumn (with one peak) of *Sepia pharaonis* in the Gulf of Suez. This difference is may be due to environmental conditions (temperature, salinity, PH, organic matter, etc.).

Macroscopic maturity stages of *Sepia savignyi* in the Gulf of Suez as follows:

Maturity stage	Female	Macroscopic maturity stages
Stage I (Immature)	Ovary very small, disc shape and occupying the posterior mantle as a whitish patch in cuttlefish. Nidamental glands appear as very fine transparent strip, small in size, accessory nidamental gland not apparent.	
Stage II (Maturing)	Ovary occupies nearly half of the posterior body cavity. Individual ova visible. Ovary with uniform sized maturing yellowish oocytes. This stage is very brief. Nidamental glands larger, thicker, pearl-shaped and creamy white, while the accessory nidamental gland was big and orange	
Stage III (Mature)	Ovary very prominent with plenty of translucent eggs and occupies an entire posterior mantle cavity. Nidamental glands large, thick and creamy white. Accessory nidamental glands were orange in color. Smooth, transparent, mature eggs, the distal part of the ovary with striated eggs and small eggs.	
Stage IV (Spawning stage)	The gonad decrease in volume. Ovary with few striated loose eggs and few medium to small eggs attached to the connective tissue of the ovary, nidamental glands flabby, accessory nidamental glands orange	
Stage V (Spent stage)	Gonad was gelatinous materials, free of eggs and yellowish in color. Nidamental glands were flaccid.	

Maturity stage	Male	Macroscopic maturity stages
Stage I (Immature)	The testis was thin, flat and the Spermatophoric sac not visible	
Stage II (Maturing)	The testis was larger than the previous stage and thicker. The spermatophoric sac was visible and empty	
Stage III (Mature)	Creamy white testis large and fully developed and the spermatophores packed in a spermatophoric sac.	
Stage IV (Spawning stage)	Spermatophoric sac swollen and full of spermatozoa	
Stage V (Spent stage)	Testis was small, flaccid and empty of spermatozoa	

Monthly Distribution of Maturity Stages

Concerning female *S. savignyi* the happening of spawning stage was observed in September, December, January, February, March, April and May with a high percentage in December (48.2 %) and April (44 %). The spent stage was recorded a high value in January, (35.7 %,) and May (31%).

In the male, *S. savignyi*, the occurrence of spawning stage was seen in November, December, January, February, March, April and May with a high percentage in December (33.8%) and in April (37.5 %). The spent stage was founded in January, February and May were highest percentages in May (33.4 %), (Fig. 3). By studying both the percentage distributions of gonadal maturation stages and the monthly variations of the GSI, we suggest an extended spawning period for *Sepia savignyi* population in the Gulf of Suez. This is similar to the previous described for other cuttlefish species in different areas. Our result agrees with (Gabr *et al.*, 1998; Önsoy and Salman., 2005; Ghazvineh *et al.*, 2012 and Dursun *et al.*, 2013) recorded prolonged spawning season.

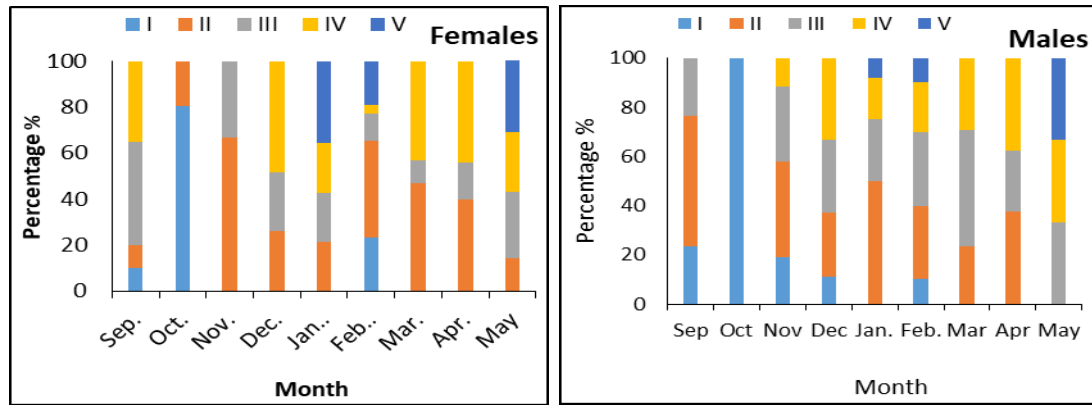
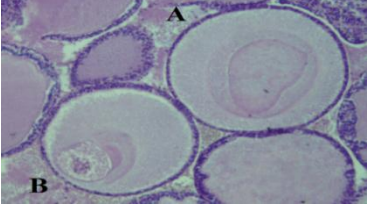
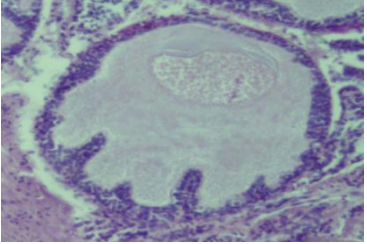
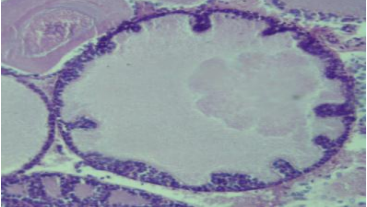


Fig. 3: Monthly percentage change in the female and male gonadal stages of *S. savignyi* collected from the Gulf of Suez during the fishing season from September 2014 to May 2015

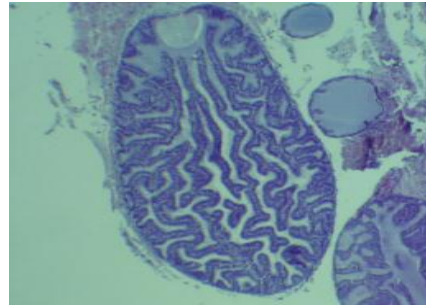
The microscopic character of *Sepia savignyi* Oogenesis of female, oocyte stages.

There are seven stages of oocyte maturation based on cell types and their proportions, the thickness of the follicular layers of the oocytes follicle invaginations.

stages of oocyte maturation	The microscopic character of <i>Sepia savignyi</i> oocyte stages	
Stage 1 Oogonia (OO)	Oogonia are small round cells, with visible cytoplasm. These cells are embedded in the gelatinous material. The nucleus in the center of the cell. It's diameter of oocytes about 2 mm in average	
Stage 2 Early Primary Oocyte (EPO)	Oocytes are associated with one or several follicles. They have a round shape nucleus, which migrates to animal pole. At this stage diameter of oocytes about 4mm on average.	
Stage 3 – Late Primary Oocyte	Oocytes are surrounded by a layer of flat follicle cells. The oocyte diameters vary between 4mm and 6mm. The portion of the cell occupied by the cytoplasm is larger than the one occupied by the nucleus	

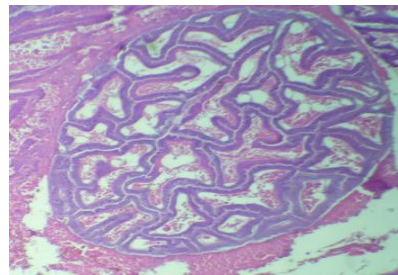
Stage 4 –
Previtellogenic
Oocyte (PVO)

The follicle epithelium initiates the oocyte embedding by intensive multiplication of the follicle cells (two layers of follicular cells, the inner one consisting of cuboidal cells, the outer one of flat follicular cells), with a subsequent displacement of the nucleus at the polar zone of the cell. In a few oocytes, the follicle cells form a syncytium (folds). These folds can penetrate deep inside the cell and encroach the cytoplasm. Oocyte size increases (7mm to 8mm). There is an initiation of nucleoli degeneration and the first production of yolk globules.



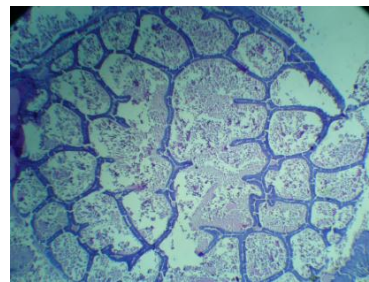
Stage 5 –
Vitellogenic
Oocyte (VO):

This stage is characterized by a very strong oocyte diameters increase. The follicular epithelium is active in vitellogenesis. The follicular folds are displaced towards the periphery of the oocytes by the formation of the yolk. Oocyte sizes range from 9mm to 1.2cm. In some oocytes, the nuclei in the polar zone are still visible.



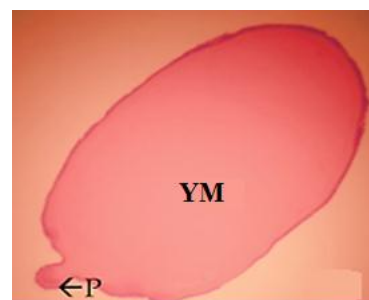
Stage 6 –
Advanced
Vitellogenic
Oocyte (AVO)

These oocytes reach the maximum size (1.5cm). The cytoplasm is filled with yolk granules; the folds are beginning reabsorbed towards to the cell wall.



Stage 7 – Ripe
Oocytes (RO):

Oocytes are issued by the cytoplasm is filled with yolk granules and the folds are completely reabsorbed, involved and protected by the chorion. At the end, they have a distal chorionic a cellular long filament peduncle. These oocytes are ready for ovulation. Oocytes ranged in size from 1cm to 1.5cm



Spermatogenesis of male Spermatogenesis takes place within the seminiferous tubules, the functional units of the testis. From the periphery to the center of each seminiferous tubule in the mature testis can be found spermatogonia, spermatocytes, spermatids, and spermatozoa, respectively (Fig. a).

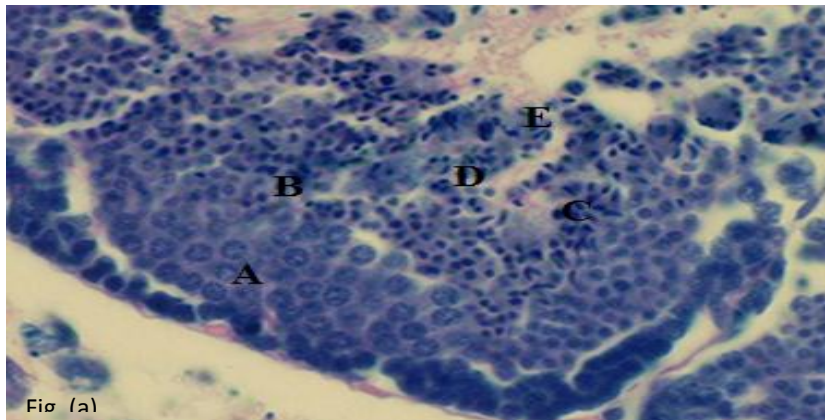


Fig. (a) Microscopic male *Sepia savignyi* at different maturity stages: Transverse section of *Sepia savignyi* testis. A = spermatogonia B = primary spermatocyte, C= secondary spermatocyte, D= spermatid and E= spermatozoa. (4 X).

Microscopic maturity stages of *Sepia savignyi* (Females and Males) in the Gulf of Suez as follows:

Maturity stage	Female	Male
Stage I (Immature)	Oocytes of young female <i>Sepia savignyi</i> compactly filled the ovaries. Several growing showed spherical nucleus by developing a layer follicle cells. Oocytes were partially or entirely surrounded by a single layer of follicle cell. (Plate B: Fig. 1)	Seminiferous tubules are well defined, but small and empty. Only spermatogonia are present in low numbers (Plate C: Fig. b).
Stage II (Maturing)	At this stage, the oocyte began to develop, showing the continuous growing layer of follicular cells, and the follicle cell of oocytes are folded and most penetrate into the oocytes to form a syncytium (Plate B: Fig. 2.).	Seminiferous tubules are clearly defined along the testes. Spermatogonia, primary and secondary spermatocytes and spermatids are present. Spermatozoa are more evident and visible in all seminiferous tubules (Plate C: Fig. c).
Stage III (Mature)	In this stage, the majority of oocytes are transparent beside other oocytes of different vitellogenin stages. The follicular syncytium degenerate completely and mature eggs are ready for the ovulation . (Plate B: Fig. 3).	Seminiferous tubules are large and well defined. There are no empty spaces between cells. All types of cells are present with abundant spermatozoa in the central lumen (Plate C: Fig. d).
Stage IV (Spawning stage)	The most oocytes of this stage in vitellogenic stage and few oocytes in previtellogenic stages besides ripe stages in a few numbers. (Plate B: Fig. 4).	Only a few primary and secondary spermatocytes, sperm-atids and spermatozoa are present, dispersed throughout the seminiferous tubules (Plate C: Fig. e).
Stage V (Spent stage)	In this stage, gelatinous material is completely free from oocytes (Plate B: Fig. 5)	The lobule is empty of spermatozoa, some of the spermatogonia and seminiferous tubules are flaccid and empty of sperms (Plate C: Fig. f).

Spermatogonia:- These are the largest male cells and they occur in a crypt. They resemble oogonia, but they do not develop a chromatin nucleus. The lack of clear, distinctive morphological features between oogonia and spermatogonia is well known (Plate C: Fig. b).

Spermatocytes:--**Primary spermatocyte stage:** -This stage characterized by a large nucleus and has a small amount of cytoplasm (Plate C: Fig. c).

-**Secondary spermatocyte stage:** - This stage demonstrated cells where nuclei well separated and stain darkly (Plate C: Fig. d).

Spermatid stage: - They have small nuclei, are close together, occur in crypts, and they stain dark red (with Hematoxylin and eosin stain) (Plate C: Fig. e).

Spermatozoa: -Spermatozoa are spermatids with tails, which stain blue (hematoxylin); the tails stain pink (eosin) though these can be difficult to see. Spermatozoa in sexually mature males occur in crypts (Plate C: Fig. f).

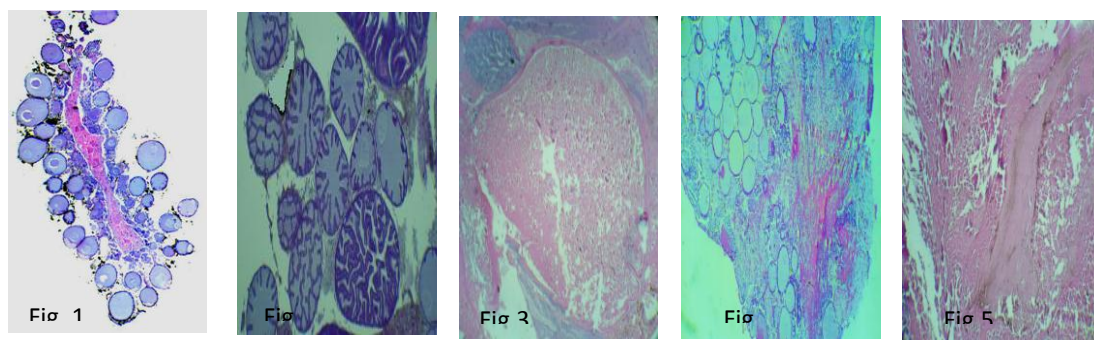


Plate B: Microscopic female *Sepia savignyi* at different maturity stages. From left to right respectively, Cross section of ovary showing Fig (1) Immature stage (4 X). Fig (2) Maturing stag (4 X). Fig (3) Mature stage (4X), Fig (4) Spawning stage (4X), and Cross section of ovary showing Fig (5) Spent stage (4X) stained with Hematoxylin and Eosin.

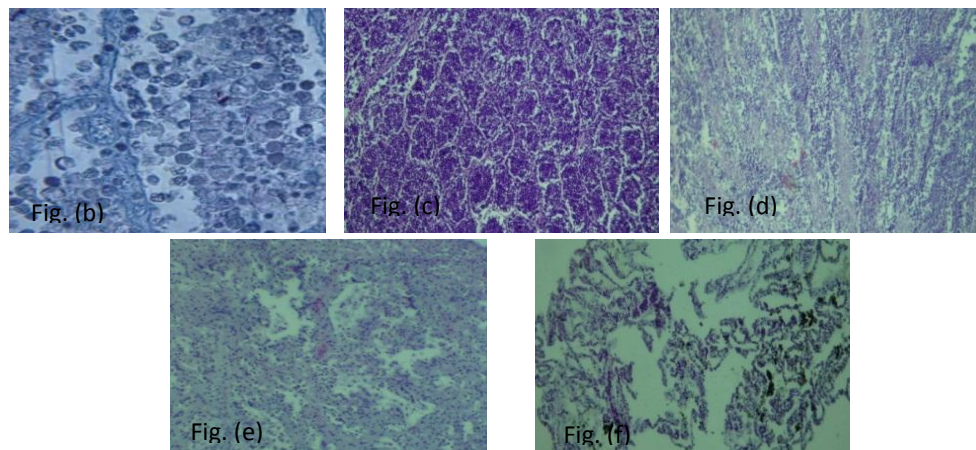


Plate C: Figures (b) (c) (d) (e) and (f) showing Cross section of testis immature stage (4 X). , maturing stage (4X), mature stage (4X), spawning stage (4X).and Spent stage (4X) respectively stained with Hematoxylin and Eosin

Virtually macroscopic and microscopic studies of cephalopods are shown in *Loligo species*. Gabr *et al.*, (1998) the only author which examined the histology of gonad maturity stages of two species *S. dollfusi* and *S. pharaonis* without represented oogenesis and spermatogenesis for ovary and testis, respectively.

The gonad maturation of *S. savignyi* is relatively similar to *S. pharaonis* and *S. dollfusi* Gabr *et al.*, (1998) and *Sepia elegans* Salman (2015). (Gonçalves *et al.*, 2002;

Cuccu *et al.*, 2013; and Sieiro *et al.*, 2014), proposed histological approaches for the verification of the macroscopic maturity stages in several cephalopods species. It was possible to observe the nucleolus of *S. savignyi* until the initiation of the development of the first yolk granules. Our studies also, reported that sizes of oocytes, the morphology of nucleus, and the relationship between oocytes and the follicle cells play a role in oocyte development these have been used to define of oocyte development. It is the first study which illustrates the seven stages (1. Oogonia, 2. Early primary oocyte, 3. Late primary oocyte, 4. Previtellogenic Oocyte, 5. Vitellogenic Oocyte, 6. Advanced Vitellogenic Oocyte, 7. Ripe oocyte) of oocyte oogenesis development in female *S. savignyi*.

In an early stage the oocytes are surrounded by a simple layer of follicle cells, then the formation of the follicle cells of a syncytium, followed by vitellogenesis. Finally, the follicular syncytium degenerates and the mature eggs are ready for the ovulation Arnold and Williams-Arnold (1977). In this study, the gonadal development of female *S. savignyi* was divided into five maturity stages as stage I immature, stage II (maturing), stage III (mature), stage IV (spawning), and V (spent), which defined by observations of the oocytes that appear in its ovary.

The individuation of ripe oocytes bearing a peduncle, not obvious to the naked eye. It is known that the peduncle is necessary during spawning when the eggs are interwoven to form the strings Mangold (1983) that attach to the shelters by means of secretions Frösch and Marthy (1975). The ovaries of spent specimens (stage V) contained post ovulatory follicles. Overall, the scales proposed in this research allow easy identification of different phases of sexual maturation for *S. savignyi* and can be an important tool to define evaluation models for sound management of the species. The present work provides the first reported on the microscopic of spermatogenesis of the *Sepia savignyi* exhibiting the details of the morphological characteristics, the pattern of lobules in the spermatogonia, spermatocytes, spermatids, and spermatozoa stage. There has not been any record of details of spermatogenesis in the cuttlefish. However, many studies on spermatogenesis in other species of cephalopod family groups are available such as *Loligo sp.* Laptikhovsky and Arkhipki (2001) and Squid species. Sirinupong (2012).

Histologically, the testis of *S. savignyi* has a tubular structure, as it is certainly observed that the spermatogonia are associated with the tubular basement membrane and different maturation stages of spermatocytes are sorted into the lumen, so it is possible to detect all maturation stages in any section of the tubule. Several authors consent with this description in several cephalopods Budelmann *et al.*, (1997) and Olivares-Paz *et al.*, (2001)

The labeling of the stages of gonadal development in male *S. savignyi* is five stages which differ than Gabr *et al.* (1998) who observed four stages of the gonadal development in the cuttlefish of *Sepia pharaonis* and *S. dollfusi*.

. In mature males, the spermatozoa are full the lobules with represented spermatocytes and spermatids as other cephalopods in Mediterranean areas Guerra, (1988), comparable to that found by Silva *et al.* (2002) in the Gulf of Cádiz and Otero *et al.* (2007) for the Galician waters. The flaccid appearance of the testis in the spent specimens corresponds to the large spaces inside the tubules as a result of the small number of germinal cells Cuccu *et al.*, 2013).

This paper deals a detailed description of the macroscopic and microscopic structure of gonad *S. savignyi*. In addition, we suggest a simple microscopic scale that

can be utilized to estimate the maturity degree of the gonads, which can be used in the following research.

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ARABIC SUMMARY

بعض النواحي البيولوجية والهستولوجية لمناسل سيبيا سافيني (*Sepia savignyi* (Blainville, 1827) في خليج السويس , مصر.

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هذا العمل يعتبر الوصف النسيجي الأول لنضج المناسل في سيبيا *Sepia savignyi* مع القاء الضوء علي بعض خواصها البيولوجية الأساسية. تقع سيبيا سافيني تحت فئه سيبيدا وتنتمي الي فصيلة الراسقدميات وهو النوع الشائع في خليج السويس ويتم تجميعها بحرفة الجر. وتعتبر السيبيا ذات اهمية اقتصادية كبيرة في مصر وذلك لارتفاع سعرها وكثرة الطلب عليها في الاسواق.

تمت دراسة ٤٥٢ عينة ، تراوحت في طول الوشاح الظهري من ٣.٩ إلى ٢٩ سم بمتوسط 16.35 ± 1.50 ووزن إجمالي من ١٩ إلى ١٨٢٦.٨ جم ، بمتوسط 606.79 ± 110.7 ، تم فحص ٢٦٩ أنثى و ١٨٣ ذكر في موسم الصيد الممتد من سبتمبر ٢٠١٤ حتى مايو من ميناء الاتكة بخليج السويس ، مصر. بلغ الطول عند النضج الجنسي الأول (DML₅₀) ١١,٩ و ٨,٤ سم للإناث والذكور على التوالي. ولقد أظهرت مؤشرات معامل النضج الجنسي أن الإناث والذكور لديهم ذروتين في فترة وضع البيض على مدار السنة الأولى في شهر ديسمبر (٤.١٪) و (٢.٤٪) والثانية في شهر أبريل (٣.٥٪) و (٢.٢٪) للإناث. والذكور على التوالي. ولقد تم تقسيم مراحل النضج الجنسي للمناسل بناء على الشكل والحجم واللون الي خمس مراحل على أساس الفحص العيني لكلا من الإناث والذكور وهي كالآتي ، مرحلة المناسل غير الناضجة (I) ، مرحلة قبل النضج (II) ، مرحلة النضج (III) ، مرحلة وضع البيض (IV) و مرحلة ما بعد رمى البيض (V)

تم تقسيم مراحل نضوج البيض الي سبعة مراحل بناء على الفحص الهستولوجي في الإناث وهي مرحلة امهات البيض ، مرحلة البويضات الأولية المبكرة ،مرحلة البويضات الأولية المتأخرة ، مرحلة البويضات قبل اضافة المح ، مرحلة البويضات في مرحلة تكوين المح ، مرحلة المح المتقدمة و مرحلة النضج. بينما مراحل تكوين الحيوان المنوى من الخصيتين في ذكور السيبيا كانت كالآتي مرحلة امهات المنى ومرحلة الخلايا المنوية الاولية ومرحلة الخلايا المنوية الثانوية ومرحلة الطلائع المنوية ومرحلة الحيوانات المنوية.