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Biology of Reproduction and Histological Examination of Female Egyptian Sole, *Solea aegyptiaca* (Chabanand, 1927) from Wadi El-Rayan Lake, El-Fayoum, Egypt

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ABSTRACT

The present study examined the reproductive biology and histological characteristics of *Solea aegyptiaca* in the Lower Lake of Wadi El-Rayan, El-Fayoum, Egypt. The investigation aimed to estimate biological features such as the maturity stages throughout the spawning season, gonadosomatic index (GSI), fecundity, egg diameter, and spawning, as well as histological characteristics of the ovaries, including the previtellogenesis and vitellogenesis phases. Monthly fish samples were collected from the 3rd depression of Wadi El-Rayan Lake from October 2022 to September 2023. This study revealed that the spawning period of *Solea aegyptiaca* extended from late December to early March. The peak GSI value in females was recorded at 10.6 in January, while the minimum value was 1.2 in August. In males, the highest GSI value was 0.17 in late December, with the lowest value recorded at 0.042 in September. The frequency distribution of egg diameters consisted of 10 groups. The first four groups represented small and transparent oocytes (previtellogenesis phase), while the second group consisted of yolky oocytes (vitellogenesis phase). In addition, a strong relationship was recorded between the absolute fecundity of fish and their length and weight. For the absolute fecundity, a range from 33,635 to 90,520 was detected in egg production, corresponding to fish weights of 55.5 and 180 grams, respectively. Furthermore, relative fecundity was directly proportional to fish length, with egg production varying from 1,367 to 2,695.25 eggs per centimeter for fish measuring between 19 and 27 cm. Similarly, relative fecundity was directly proportional to fish weight, ranging from 440 to 650 eggs per gram of gutted weight for fish between 55.5 and 180 grams. Oogenesis was divided into the previtellogenesis and vitellogenesis phases, with sub-stages including immaturation, maturation, vacuolization, yolk deposition, ripening, and spawning. Our findings suggest that *Solea aegyptiaca* has an extended spawning period with fractional spawning.

INTRODUCTION

Wadi El-Rayan Lake is located in the northern region of the El-Fayoum area, extending from latitude 22° to 23°8' N (Fig. 1). It is approximately 140km from Cairo. The lake serves as a reservoir for agricultural drainage water collected from the surrounding lands. Wadi El-Rayan lakes are considered one of Egypt's important fishery resources. The lakes were formed in 1973 and are divided into two large basins: Rayan

Lake 1 (Upper Lake) and Rayan Lake 2 (Lower Lake). The connecting area between the two basins forms a shallow cataract (waterfall), which facilitates the continuous flow of water from Lake Rayan I to Lake Rayan II. The maximum depth of Upper Lake is 22.0 meters, while the Lower Lake reaches a depth of 35.0 meters. The salinity of the Upper Lake is less saline (1.52–1.94g/ l) compared to the Lower Lake (14.3–17.33g/ l). The increasing salinity in the Lower Lake has contributed to the significant deterioration of its aquatic environment **(Aboul-ela & Khalil, 1988; Saleh *et al.*, 1988; Konsowa & Abd Ellah, 2002a, b)**.

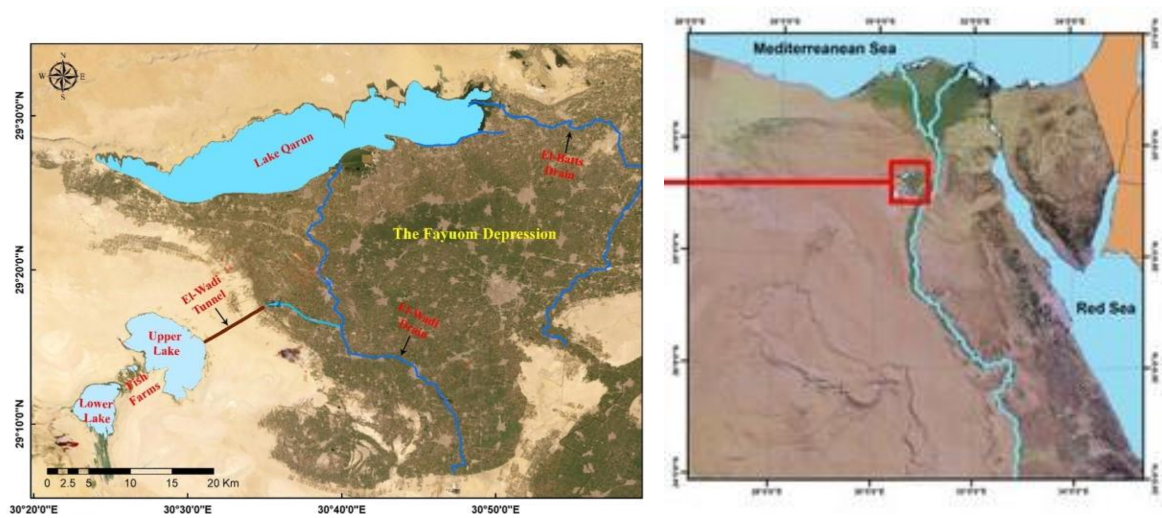

Fig. 1. A map of Egypt indicating the location of El-Fayoum Depression and Wadi El-Rayan lakes (the map was downloaded from Google Earth)

The Egyptian sole, *Solea aegyptiaca*, belongs to order: Pleuronectiformes and family: *Soleidae*. It is the most important sole species found in Wadi El-Rayan Lake. Generally, this species inhabits shallow, sandy, and sandy/muddy environments **(Froese & Pauly, 2007)**.

The sole fish is highly sought after by customers due to its distinctive and excellent flavor. However, due to the progressive increase in salinity in the third lake (Upper Lake) of Wadi El-Rayan, the Lakes and Fish Resources Protection and Development Agency, in collaboration with the National Institute of Oceanography and Fisheries Egypt (NIOF), made the first attempt to transplant *Solea aegyptiaca* into the third depression of Lake Wadi El-Rayan in 2008. The fish successfully acclimatized and reproduced in the new environment of El-Rayan. According to the official statistics, the catch of sole gradually increased from 239 metric tons (MT) in 2015 to approximately 346 MT in 2017. However, the annual catch of sole then declined gradually to 161 MT in 2021 **(Fish Statistic Yearbook, 2021)**.

Understanding the reproductive biology of *Solea aegyptiaca* plays an important role for scientists in aquaculture and hatchery management. The reproductive biology and morphological differentiation of this species have been studied by various researchers, including **Zaki and Hamza (1986)**, as well as studies from other locations worldwide **(Rajaguru, 1992; Vallisneri *et al.*, 2001; Türkmen, 2003; Narimatsu & Hirobumi, 2005; García-López *et al.*, 2006c)**, and more recent work on *S. aegyptiaca* by **Mehanna (2007)**, **Ahmed *et al.* (2010)** and **Gaber (2015)**.

On the other hand, histological observations on the development of oocytes in the gonads were explained by **Wallace and Selman (1981)**. In this context, **Abd El-Kawi (1995)** succeeded in inducing the spawning of *Solea vulgaris* from Lake Qarun. Several studies on the gonadal histology of sole species have examined *Solea aegyptiaca* in Lake Qarun **(El-Husseiny, 2001)** in addition to *Microchirus azevia* **(Afonso-Dias *et al*., 2005)**, and *Solea senegalensis* **(García-López *et al.*, 2005, 2006b, 2007)**. Furthermore, **Assem *et al.* (2012)** successfully induced breeding in *Solea* species caught from the Mediterranean Sea. Additionally, **Cerim and Ateş (2019)** studied the reproductive biology of female *Solea solea*.

This study aimed to provide information about *Solea aegyptiaca* in its new environment, the Lower Lake of Wadi El Rayan Lake. This study included morphological changes, the gonado-somatic Index (GSI), egg diameter, and fecundity during spawning season. A histological examination of the gonads provides information on the reproductive pattern of this species, offering scientific insights and advice regarding its population in this lake.

MATERIALS AND METHODS

-Samples for biological studies

Samples of *Solea aegyptiaca* were collected monthly, with approximately 240 fish from the third depression (Lower Lake) of Wadi El-Rayan Lake, Fayoum, during the spawning season from October 2022 to September 2023. For each fish, we recorded the sampling date, sex, and stage of sexual maturity. The total length, total weight, and gutted weight (weight without viscera) of each fish were measured to the nearest 0.1cm and 0.1g, respectively. The fish were then dissected to determine the internal gonadal maturity stages morphologically, following the description provided by **Assem (1995)**.

The gonads, after being removed, were weighed to the nearest 0.01g. The ovaries were then preserved in 10% formalin for further studies. Maturity stages were determined by visual examination with the naked eye and via microscopy in young specimens. To determine the spawning season:

Gonadosomatic index (GSI): The Gonadosomatic Index was used to assess gonadal development in *Solea aegyptiaca*. The GSI was calculated for all specimens using the formula developed by **Gibson and Ezzi (1980)**, which expresses the gonad weight as a percentage of the gutted fish weight, as shown in the following equation:

GSI = (gonad weight (g) / body weight (g)) × 100.

Maturity stages throughout the spawning season: The maturity stages of female *S. aegyptiaca* were determined by examining the external appearance of the gonads, based on the shape, size, and color of the ovaries throughout different months of the year **(Kirollus, 1977; Zaki *et al.*, 1995, 1996)**.

Egg diameter: The egg diameter was estimated by measuring 100 oocytes from a certain part of the ovary. Oocytes were carefully separated from the ovarian wall and any connective tissue. Their diameters were measured using an Olympus CX41 optical microscope with a micrometer eyepiece. The oocytes were classified based on their size and transparency during ovarian proliferation.

Fecundity (F): Absolute fecundity (aF), or total fecundity, was calculated by counting the total number of ripe eggs in the ovary. A 0.01g sample of the ovary was weighed, and all ripe oocytes were counted under a light microscope. Relative fecundity (rF) was determined by measuring the number of ripe eggs per unit weight or length of the fish, according to **Kume and Joseph (1969)** and **Kirollus (1977)**.

Samples for histological studies: The fish were rapidly dissected, and the gonads were carefully removed. The gonads (testes and ovaries) were fixed in aqueous Bouin's solution for 48 hours **(Abdel-Kawi, 2002)**, then dehydrated in ascending concentrations of ethyl alcohol (from 70 to 99%). The samples were cleared in xylene and were embedded in paraffin wax. Transverse sections of the gonads were cut to a thickness of 5-7µm. The sections were stained with hematoxylin and eosin and were mounted with Canada balsam. Each slide was examined under a light microscope (Olympus CX41), and photographs were taken using an OPTICA digital camera.

RESULTS

1. Biological features

We observed differences in morphological changes between males and females of *S. aegyptiaca* during the spawning season. We classified them according to the shape, color and size of the ovary during different months. While in males, there is no formal appearance due their small size.

1.1- Maturity stages throughout spawning season

In the present study, we followed previous studies to describe the maturity stages of *Solea aegyptiaca* as follows:

Female

Immature stage: The ovaries were small, occupying about one-third of the body cavity. Their color was pink, and the eggs were barely visible.

Nearly ripe stage: The ovary was large, extending along two-thirds of the body from the ventral side toward the caudal region of the body cavity. The eggs were easily identified and had changed to a yellowish-white color, although a few still retained a pink hue.

Ripe or mature stage: The ovary filled most of the body cavity, and the eggs were large and yellow. The eggs could be easily extruded by gently pressing on the abdomen.

Spawning stage: Spawning occurred, and the eggs could be easily extruded. This stage was observed from mid-December to late March.

Spent stage: The ovary became small again, regained its pink color, and the tissue was completely flaccid. The eggs in the ovary were small and few.

1.2- Gonadosomatic index (GSI)

The gonadosomatic index (GSI) of female and male is represented in Fig. (2a). The GSI of females showed peak values in December and January, recorded at 9.58 ± 1.5 and 10.6 ± 1.9, respectively. It then gradually decreased to 5.38 ± 3.2 in March. After spawning, the GSI sharply declined, reaching a minimum value of 1.2 in August. These findings indicate that the spawning season began in December and continued through March.

On the other hand, GSI of males was detected in spite of the small size of the testes. The GSI of males recorded the highest value (0.17) in December, as shown in Fig. (2b). The lowest GSI value for males was recorded (0.04) in September, and it started to increase slightly from October (0.065) to December (0.16).

The lowest GSI value for females (1.2) was recorded in September, after which it began to increase, reaching 9.58 in December and peaking at 10.6 in January. This indicates that *Solea aegyptiaca* in the Lower Lake of Wadi El-Rayan spawns during the winter months.

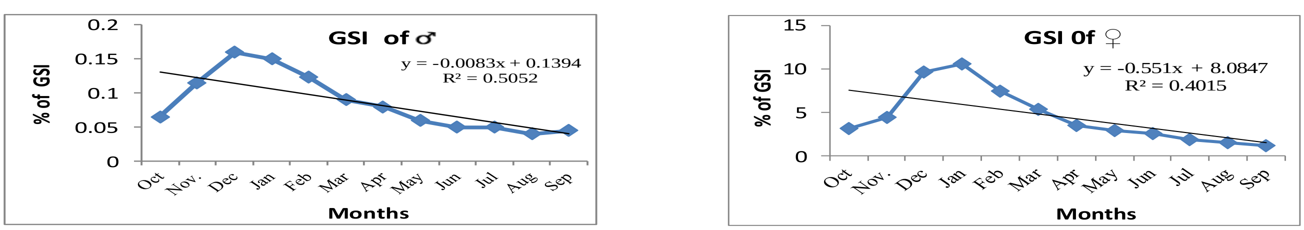

Fig. 2a, b. Monthly variation in gonado-somatic indices (GSI values) of *S. aegyptiaca* females and males during an annual cycle from October 2022 to September 2023

1.3 Fecundity

The relationship between fecundity (both absolute and relative) and body size (total length in cm and gutted weight in g) of *Solea aegyptiaca* was calculated. The results, shown in Table (1), indicate that the number of eggs gradually increased with fish length and weight. For example, a fish measuring 19cm (55.5g) spawns approximately 33,635 eggs, while a fish measuring 27.0cm (180g) spawns a maximum of about 90,520 eggs.

Table 1. The relationship between absolute fecundity (aF), calculated fecundity (cF) and relative fecundity (rF) with the length and gutted weight of *S. aegyptiaca* fish in the Lower Lake of Wadi El-Rayan during the spawning period of October 2022 to September 2023

No	L (cm)	Gutted Wt.(g).	a F	cF (gutt. Wt	rF. / gutt.Wt.	cF(L	rF/ L
5	19	55.5	33635	24420	440	25973	1367
8	20	59.0	41533	28910	490	33240	1662
3	21	64.5	43290	25155	390	30876.3	1470.3
4	22	71.5	50300	32175	450	45053.8	2047.9
6	23	102	65520	56100	550	50348.15	2189.05
8	24	150	70958	93000	620	56112	2338
6	25	164	82130	103812	633	63062.5	2522.5
3	27	180	90520	117000	650	72771.75	2695.25

a- Relationship of fecundity with length

The relationship between absolute fecundity (aF) and length (L) is plotted in Fig. (3). It is represented by growth regression equation, which can be written as: Ln aF= 7.989+ 0.131 L. adj R^2 = 0.956 correlation coefficient 0.987. The relative fecundity/Length (rF/L) gradually increased from 1367 to 2695.25 eggs per cm. the relationship of relative fecundity against length is plotted in Fig. (4) and is represented by the following growth regression: LN rF/L = 5.571+ .089 L (adj.R^2= 0.867)

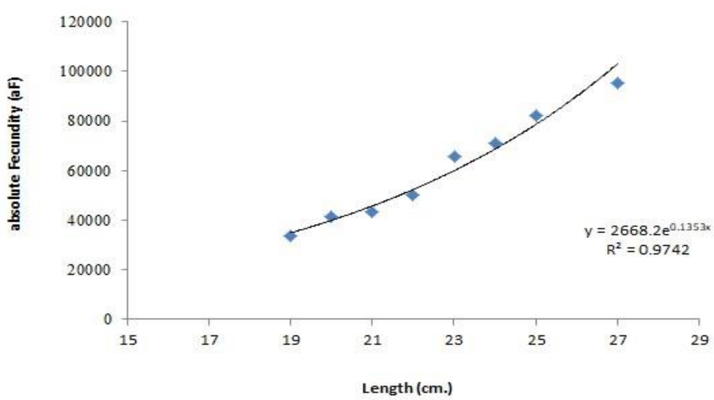

Fig. 3. The relationship between absolute fecundity (aF) and length of *S. aegyptiaca* fish in Lower Lake of Wadi El-Rayan during October 2022 to September 2023

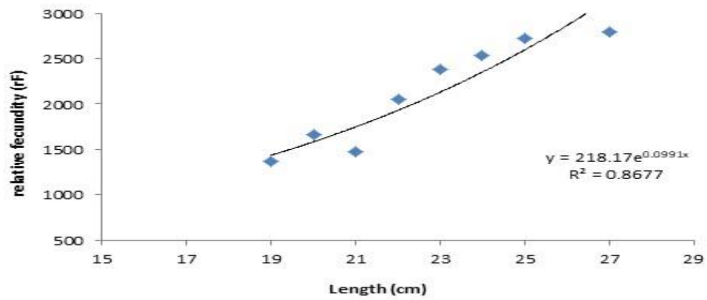

Fig. 4. The relationship between relative fecundity (rF) and length of *S. aegyptiaca* fish in Lower Lake of Wadi El-Rayan during October 2022 to September 2023

b- Relationship of fecundity-weight

The relationship between absolute fecundity and gutted weight is presented in Table (1). The growth regression equation is expressed as follows: Ln AF = 10.246 + 0.007W, with an adjusted $R^2 = 0.892$, where (W) is the gutted weight in grams, and the correlation coefficient is 0.95. The relative fecundity increased from about 440 eggs per gram of gutted weight in individuals weighing 55.5g to approximately 650 eggs per gram in heavier individuals weighing 180 g. The relationship between gutted weight (g) and relative fecundity is shown in Fig. (5). The regression equation for this relationship is: Ln RF/W = 5.889 + 0.003W, with an adjusted $R^2 = 0.825$, where W is the gutted weight in grams.

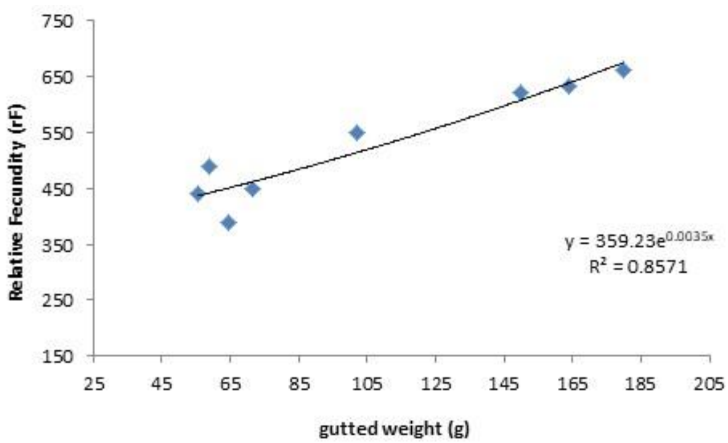

Fig. 5. The relationship between relative fecundity/gutted weight (rF/gutt.Wt) with gutted weight of *S. aegyptiaca* fish in Lower Lake of Wadi El-Rayan during October 2022 to September 2023

The correlation matrix indicates a strong correlation, as all correlation coefficients are greater than 0.9, showing a direct relationship between the variables at a 99% confidence level. This means that a 1% change in the independent variable results in a significant change in the same direction in the dependent variable.

1.4 Egg diameter and spawning

From Figs. (6a- f), it can be observed that small and transparent ova, with diameters ranging from 0.1 to 0.30mm, and yolky ova, with diameters greater than 0.40 mm, are present throughout the spawning season in varying percentages.

Fig. (6a) shows that in October, fish with a gonadosomatic index (GSI) of 3.9 had transparent ova with egg diameters ranging from 5 to 7%, and yolky ova with diameters ranging from 3 to 35%. In November (Fig. 6b), fish with a GSI of 4.45 had small ova with diameters ranging from 10 to 12%, and yolky ova ranging from 5 to 20%. In December (Fig. 6c), fish with a GSI of 8.2 had small ova accounting for about 5%, and yolky ova ranging from 5 to 43%. In January (Fig. 6d), fish with a GSI of 10.6 had small ova accounting for 2%, and yolky ova ranging from 3 to 50%. In February (Fig. 6e), fish with a GSI of 6.9 had small ova varying between 2% and 8%, and yolky ova ranging from 2 to 30%. In March (Fig. 6f), fish with a GSI of 5.38 had small ova ranging from 5 to 10%, and yolky ova ranging from 2 to 20%.

The percentage frequency distribution of ova diameters and the presence of more than two modes of ova sizes for the fish under study, spanning six months from November to March, indicate that the fish spawns its ripe ova through fractional spawning in batches during the spawning period.

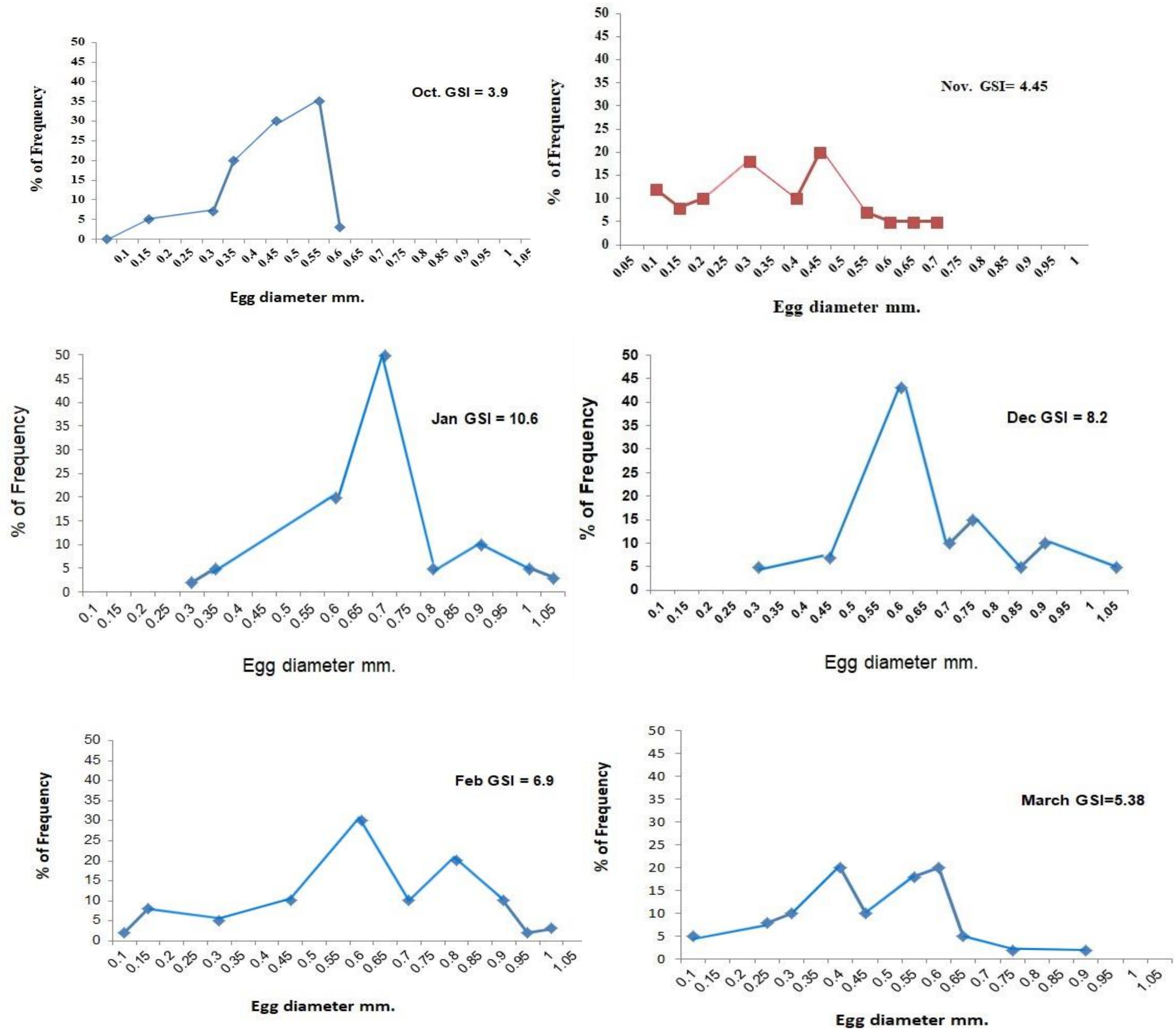

Fig. 6a-f. Frequency distribution of oocyte diameters in *Solea aegyptiaca* from October 2022 to September 2023.

2. Histological characteristics of *Solea aegyptiaca* ovaries

The histology of *Solea aegyptiaca* in the Lower Lake of Wadi El-Rayan during the reproductive cycle is divided into two phases based on yolk accumulation: Previtellogenesis and Vitellogenesis. These phases are further divided into the following periods: immaturation, maturation, vacuolization, yolk deposition, ripening, and the ripe (spawning) period.

2.1 Previtellogenesis phase

A. Immaturation period

a.1- Oogonia (chromatin-nucleolus stage)

The youngest oocytes are called oogonia. An oogonium consists of a large nucleus surrounded by a thin layer of cytoplasm, which is encased by follicular cells, as observed in Fig. (7a). Oogonia are very small and spherical, measuring approximately 31 ± 8.4µm in diameter. The nucleus is large and centrally located, with a diameter of about 22.5 ± 6.4µm. It contains a single nucleolus and several chromatin threads. The nucleus is surrounded by a narrow zone of basophilic, homogeneous cytoplasm. A thin layer of follicular cells surrounds the oogonia. These cells are sparsely distributed in the ovarian cavity and embedded in the ovarian lamella. Oogonia are present throughout the year, being particularly abundant during the resting season, and less numerous during the spawning period.

B. Maturation period

The maturation period was characterized by the appearance of two stages as follow:

b.1- Early perinucleolus oocytes stage

These oocytes measure approximately 75.3 ± 14.6µm in diameter, with the nuclear diameter ranging from 45.18 ± 8.76 µm. The cytoplasm is weakly basophilic and homogeneous, although the innermost zone near the nuclear membrane occasionally appears strongly basophilic. The nucleus is large, round, and centrally located, with several smaller nucleoli, approximately 5.0 ± 1.5 in number, positioned at the periphery close to the nuclear membrane, as shown in Fig. (7b). The diameter of the nucleoli varies from a minimum of 1.0µm to a maximum of 6.0µm.

The early perinucleolus oocytes are still surrounded by a thin layer of follicular epithelial cells. Occasionally, a few scattered connective tissue fibers are observed surrounding the follicular epithelial cells. These oocytes are present throughout the year, but their numbers increase during spring and summer (from March to September). They are the dominant oocyte type during the post-spawning and resting periods.

b.2 - Late perinucleolus oocytes stage

The diameter of the oocytes at this stage measures approximately 103.4 ± 14.7µm. The nucleus increases in size, with a diameter of about 51.7 ± 7.35µm. The cytoplasm is mostly homogeneous, except for the innermost zone, which appears more compact and intensely stained. The nucleoli increase in number and become more peripheral, positioned near the nuclear membrane. They range in number from 5 to 8 and measure approximately 8.5 ± 2.12µm in diameter. In the early stage, the yolk nucleus lies close to the nuclear membrane, but it gradually migrates to the periphery of the cytoplasm as the oocytes grow, as shown in Fig. (7c). By the end of this stage, the yolk nucleus disappears. The oocytes are surrounded by a very thin follicular layer. Oocytes at this stage are present year-round but are most numerous during the resting season (from June to September).

During the spring and summer seasons, all stages of oocytes—such as chromatin nucleolus, early and late perinucleolus—are observed, representing the dominant oocyte types in the first growth phase, as shown in Fig. (8e).

C- Vacuolization period

This stage is characterized by the formation of cytoplasmic vesicles, which represent vacuoles. Most of the oocytes increase in size to 130.0 ± 10.0µm in diameter, while the nuclear diameter measures 58.5 ± 4.5µm. The nucleoli are embedded within the oocyte, numbering between 10 and 15, with a range in diameter from 3 to 9µm, as shown in Fig. (7d).

The vesicles begin as small and few, located at the periphery of the cytoplasm. Over time, they increase in size (from 7 to 32µm) and number, becoming scattered throughout the cytoplasm. During this stage, a new glycoprotein structure is formed between the oocyte membrane and the follicular cell layer, resulting in the cortical alveolar layer. Additionally, the vitelline membrane (zona radiata) is formed during vitellogenesis. These stages are typically observed during the autumn and winter months.

2.2 Vitellogenesis phase

It is characterized by the occurrence of yolk granules, and vitellogenic oocytes.

A.Yolk deposition period

a.1. Primary yolk deposition stage

Yolk granules are scattered in the peripheral cytoplasm and are spread centripetally throughout the central cytoplasm, with diameters ranging from 4 to 12µm. The oocyte at the primary yolk stage is characterized by the appearance of yolk globules in the cytoplasm, located between lipid droplets and yolk vesicles (Fig. 7d). These yolk globules increase in number and size as the oocyte grows, eventually filling the entire oocyte, except for a peripheral ring of cytoplasm near the nucleus. Oocytes at this stage measure approximately 312.0µm in diameter. The nucleus appears granulated with an irregular boundary and measures around 73.0µm in diameter. The nucleoli are distributed around the periphery of the nucleus. The yolk globules, which are eosinophilic, measure about 11.0µm. Additionally, fat vacuoles increase in size, reaching approximately 18.0µm. The thickness of the zona radiata measures about 10.0µm.

a.2. Secondary yolk deposition stage

Yolk accumulation proceeded rapidly, resulting in a noticeable increase in the oocyte diameter and the amount of yolk globules, as shown in Fig. (7e). The diameter of the oocyte increased from 390 to 455µm, with an average of approximately 422.5µm. Both yolk and lipid globules increased in size and number, occupying more or less the outer half of the cytoplasm. At the beginning of this stage, the nucleus became oval in shape due to the increase in yolk globules. The lipid vesicles increased in size to about 20.0µm in diameter and were distributed within the cytoplasm alongside the yolk globules. The nucleoli reduced in size and arranged close to the nuclear membrane. The zona radiata increased in thickness, measuring about 14.0µm.

a.3. Tertiary yolk deposition stage

This stage is characterized by the accumulation of yolk globules in the cytoplasm of the oocyte, as shown in Fig. (7e). The average diameter of the oocyte at this stage is approximately 446µm. The nucleus remains oval, with a wavy boundary and a granulated appearance. The lipid droplets increase in size and number during this stage. The yolk globules grow in diameter to 18.0µm, and the zona radiata increases in thickness, measuring 12.5µm. This stage was observed during the autumn, which is characterized by the predominance of the yolk vesicle stage.

B. Ripening period

At this stage, the oocytes increased in diameter, reaching 550µm. This period is characterized by the migration of the nucleus toward the animal pole. The diameter of the nucleus reached 60µm, as shown in Fig. (7f, 8A). The nucleoli were scattered throughout the nucleus, with their number ranging from 16 to 18, and their diameter ranged from 2.0 to 5.0µm. The yolk globules began to merge, with an average diameter of about 23.2µm. Lipid droplets also increased in size, measuring approximately 37.7µm in diameter. The zona radiata thickened to about 16.3µm, as shown in Fig. (8B).

C. Spawning period

Spawning extends from late December to early March (winter). During this period, the ovary contains hydrated oocytes, measuring between 650 and 850µm in diameter (Fig. 8C). Numerous empty follicles are formed after the shedding of ova, as shown in Fig. (8D). At this stage, the ovary displays all stages of oocyte development (oogonia, perinucleolus, and maturation stages) in varying ratios, depending on the maturity state of the ovary. The wall of the ovary is very thin. During the post-spawning period, atretic follicles (corpora atretica) appear within the ovary, along with non-ovulated follicles. Atretic follicles are characterized by abnormal oocyte shapes. These follicles are derived from vitellogenic oocytes that cease to grow and are reabsorbed, leaving empty follicles, as shown in Fig. (8D, E, F).

c.1. Spent and recovery stages

After the ovulation period and the shedding of ova, the ovaries enter the spent stage, which lasts from late March to September. During this stage, the ovarian wall becomes very thick, measuring approximately 120µm, as shown in Fig. (8F). The ovarian lamellae are narrow and contain a high percentage of oocytes at both the early and late perinucleolus stages (about 80%), as well as at the yolk vesicle stage (about 20%). This spent stage is observed throughout the year, with a peak activity occurring in the summer, reaching 100%.

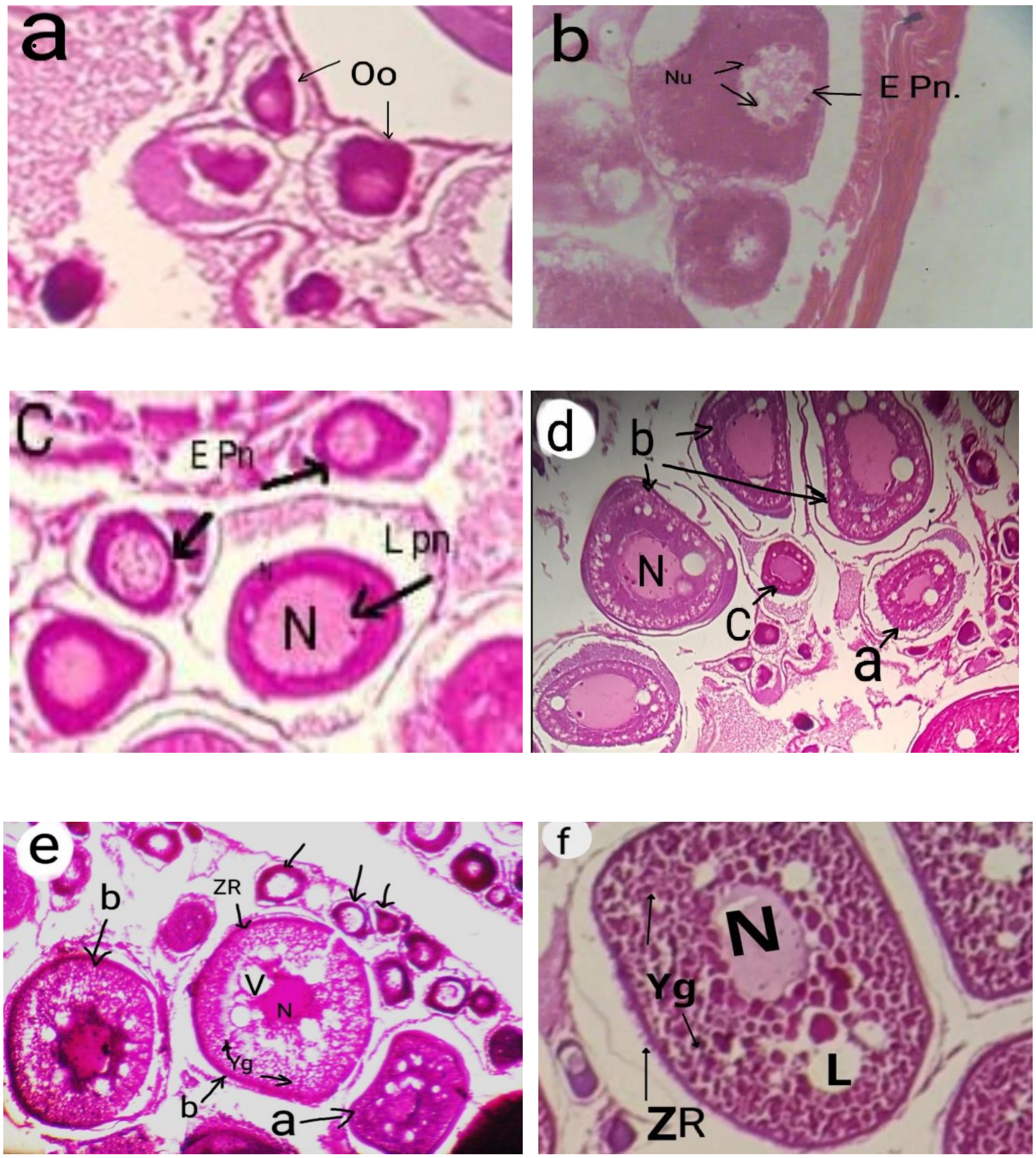

Fig. 7. Cross section of ovary of *S. aegyptiaca* showing: **(a)** An immaturation stage: oogonia (Oo); **(b)** Maturation period, Early perinucleolus stage (EPn); **(c)** Late perinucleolus oocyte (LPn) and Nucleolus (Nu); **(d)** Vacuolization stage **(e)** 1^{st} and 2^{nd} yolk deposition; **(f)** 3^{rd} yolk deposition, yolk vesicles (YV); lipid vesicles (LV), and different stages of oocytes (arrows) (X200)

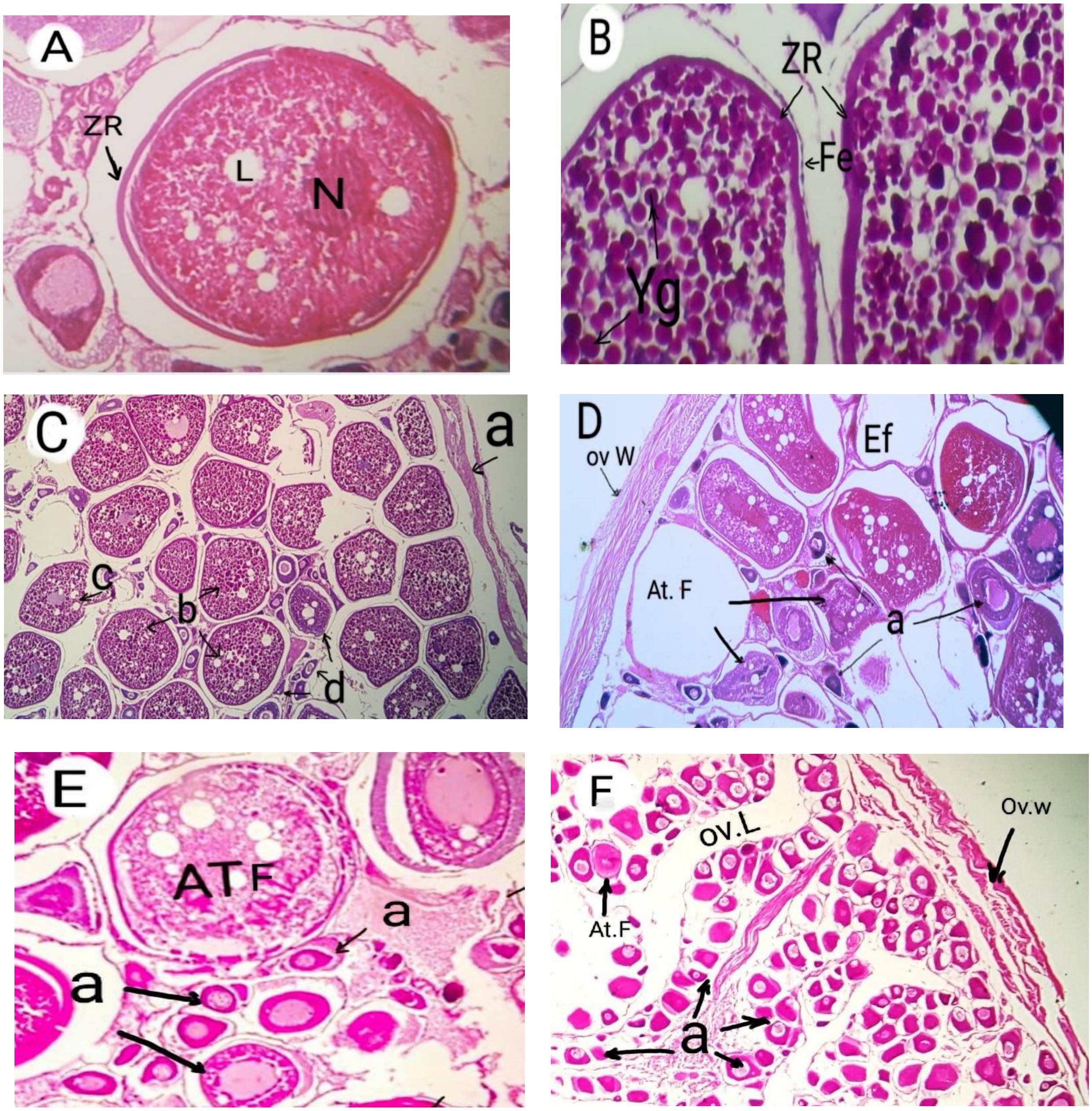

Fig. (8). Cross-sections of *S. aegyptiaca* ovaries showing: **(A)** Ripening period during migration of the nucleus towards the animal pole (EH, X250). **(B)** Magnification of ripe oocyte showing: zona radiate (ZR), follicular epithelium layer (FE) and yolk granules (Yg), (x1000). **(C)** Early Spawning period, **(b)** Ripe oocyte, **(a)** ovarian wall, and **(c)** different stage of cytoplasmic growth (EH) (X40). **(D & E)** Late spawning period showing: (EF) empty follicle (At.F) atriatic follicle oocyte. **(F)** Post-spawning, showing: different stages of oocyte **(a)**, thick ovarian wall (ov W), ovarian lamella (ov L) (EH) (X40)

DISCUSSION

Solea aegyptiaca specimens were introduced to the Lower Lake of Wadi El-Rayan in 2008, where they were acclimatized and successfully bred, adapting to the increasing salinity levels, as reported by **Aboul-ela and Khalil (1988)**, **Saleh *et al.* (1988)** and **Konsowa and Abd Ellah (2002a, 2002b)**. The annual fish reproduction has been reported to increase, as noted in the **Fish Statistics Yearbook (2021)**. Through investigation and identification, *Solea aegyptiaca* was confirmed by the characteristic of the reduced membrane joining the dorsal and anal fins to the caudal peduncle, as described by **Abdel-Missih (1995)** and **Sabatini *et al*. (2018)**.

This study analyzed the annual reproductive cycle of *S. aegyptiaca* from September 2022 to October 2023, including parameters such as gonadosomatic index (GSI), ova diameter, fecundity relative to body length and weight, and oogenesis. These aspects were examined monthly to observe gonadal development in the Lower Lake of the El-Rayan depression.

In this study, the morphological development of gonads in *S. aegyptiaca* was divided into six stages: immature (June to August); maturation (September to October); nearly ripe (late October to November); ripe and spawning (December to late March), and spent (late March to May). This developmental pattern aligns with those observed in other multi-spawning teleost species, such as *Solea vulgaris* **(Boulos & Ashour, 1973)**, *S. solea* **(Assem, 1995)**, *Oblada melanura* **(Zaki *et al.*, 1995)**, *Caranx crysos* **(Assem, 2000)**, and *Solea senegalensis* **(García-López *et al.*, 2006a)**.

The monthly fluctuations in GSI values revealed that the spawning period extended from November to March, with peak values observed in December (9.58±1.5) and January (10.6±1.9). These findings are in agreement with studies by **Zaki and Hamza (1986)** and **Quéro *et al.* (1986)**, who reported that the spawning period for the common sole extended from September to April. The increase in GSI values indicates a higher percentage of ripe fish, marking the onset of the spawning season. This aligns with the results of **Garcia-Lopez *et al.* (2007)** and **Ahmed *et al.* (2010)**. In contrast, **Narimatsu and Hirobumi (2005)** observed that ovulation of *Dexistes rikuzenius* occurred between September and December, peaking in September and October. Similarly, **Attia *et al.* (2019)** recorded that the GSI of *S. solea* peaked in November, December, and January (6.77, 8.72, and 8.48%, respectively), with the lowest value observed in August (1.09%).

In this study, the relative fecundity of *S. aegyptiaca* ranged from 1,367 to 2,695.25 eggs/cm of total body length, showing a positive correlation between fecundity and body length. These results support previous findings by **El-Husseiny (2001)**, who found relative fecundity ranging from 1,745.2 to 2,946 eggs/cm. Similarly, **Ahmed *et al.***

(2010) and **Attia *et al*. (2019)** observed that fecundity gradually increased from 8,092.5 to 36,680.6 eggs/cm (average 20,766.6 eggs/cm). The correlation coefficient in this study between fecundity and length was 0.94, confirming the results of **Horwood and Walker (1990)** for *S. solea* from the Bristol Channel. The study also found that fecundity was positively correlated with gutted weight, with a maximum of 650 eggs/g. The relative fecundity of *S. aegyptiaca* was described by the equation LN rF/W = 4.880 + 0.061 L (R^2 = 0.664), indicating a positive correlation with weight. These findings are consistent with those of **Zaki and Hamza (1986)** on *S. solea*, as well as similar results by **Assem *et al.* (2019)** for *S. solea* in the Mediterranean Sea. Additionally, similar findings were reported by **Assem (1995)** for *S. vulgaris*, and **Horwood *et al.* (1986)** and **Ahmed *et al.* (2010)** for *S. aegyptiaca*.

The study also examined the ova diameter, finding that multiple groups of ova existed within the samples. The first group was small and transparent, while the second consisted of yolky, larger ova. Ova diameter increased throughout the spawning season (from December to March), reaching approximately 1.05µm, with other diameters ranging from 0.15 to 0.32mm during the Previtellogenesis period. These findings are consistent with studies on *S. solea* from Abu Kir **(Zaki & Hamza, 1986)**, *S. aegyptiaca* from Lake Qarun **(El-Husseiny, 2001)**, and *Rikuzen sole* **(Narimatsu & Hirobumi, 2005)**.

Oogenesis in *S. aegyptiaca* was divided into two main phases: Previtellogenesis and Vitellogenesis. Previtellogenesis included the immature period, consisting of oogonia, early perinucleolus oocytes, and late perinucleolus oocytes. Vitellogenesis included maturation, vacuolization, yolk deposition, ripening, and spawning periods. The oogenic development of *S. aegyptiaca* follows patterns similar to those described for other species, including *Solea* sp. **(Assem, 1995)**, *Rikuzen sole* **(Narimatsu & Hirobumi, 2005)**, and *S. senegalensis* **(García-López *et al.*, 2007)**. Moreover, they follow the same pattern of other *teleost* species like *Liza ramada* **(Abd El-Kawi, 2002)**, *Mugil cephalus* **(Assem *et al.*, 2008)**, *M. merluccius* **(Al-Absawy, 2010)**, and *Pagrus caeruleostictus* **(Ismail *et al.*, 2018)**. **Narimatsu and Hirobumi (2005)** described the maturity stages of *Dexistes rikuzenius* based on eight phases of oocyte development, showing that the species has group-synchronous ovaries and is a multiple spawner.

This study also noted that the number and timing of immature and maturing germ cells were present throughout the reproductive season, confirming findings by **Assem (1995)** and **Mehanna (2014)** on *sole* species. The ripening and spawning stages for *S. aegyptiaca* occurred from December to early March, with peak percentages in January. These results align with those of **Assem *et al*. (2019)**, who observed ripening stages of *S. solea* from October to January, with peak percentages in December.

The ovarian development of *S. aegyptiaca* in this study followed nine stages, including oogonia, chromatin nucleolar, early and late perinucleolar, cortical alveolar, early and late vitellogenic, early maturation, and atresial stages, as recorded by **Assem (1995)**, **El-Husseiny (2001)** and **García-López *et al.* (2007)**. In contrast, the ovarian follicular development of the flatfish *Etropus crossotus* was classified into five phases, excluding the atretic and empty follicular phases **(Oliveira & Favaro, 2011)**. During the immature stage, primary and secondary oocytes are formed from the highly basophilic cytoplasm surrounding the nucleus, containing scattered peripheral nucleoli. This is consistent with studies by **Abdel-Kawi (2002)** on *Liza ramada* and by **Assem *et al.* (2008)** on *Mugil cephalus*.

The vacuolization stage was marked by the appearance of vesicles in the cytoplasm, initially small and few at the periphery, which increased in size (from 7 to 32µm) and number, becoming scattered throughout the slightly basophilic cytoplasm. The formation of the vitelline membrane (*zona radiata*) between the follicular epithelial alveolar layer was also observed during vitellogenesis. These observations are in line with studies on *S. senegalensis* **(García López *et al.*, 2007)** and *E. crossotus* **(Oliveira & Favaro, 2011)**.

At the early vitellogenic stage, yolk deposition appeared as a yolk nucleus in the center of the nucleus, spreading toward the cytoplasm. These granules gradually occupied the entire cytoplasm, reaching the ripe and spawning stages, similar to the late vitellogenic and early maturation phases in *S. aegyptiaca* **(El-Husseiny, 2001)**, *Sole* species **(Ali, 1995)**, and *S. senegalensis* **(García-López *et al.*, 2007)**. The vitellogenesis was complete when the nucleus migrated toward the animal pole, and the oocyte diameter increased. The follicular epithelial layer consisted of irregular nuclei with undifferentiated cytoplasm, confirming observations on *Liza ramada* **(Abd El-Kawi, 2002)**, *Mugil cephalus* **(Assem *et al.*, 2008)**, and *M. merluccius* **(Al-Absawy, 2010)**. The atretic and empty follicle phase, present post-ovulation, was also recorded, with residual oocyte cells being resorbed and converted into phagocytic cells that secrete enzymes to digest the remaining content, as reported by **Santos *et al.* (2008)** and **Tingaud-Sequeira *et al.* (2009)**.

CONCLUSION

This study focuses on the oogenesis of the Egyptian sole, *Solea aegyptiaca*, which was transferred in 2008 to the Lower Lake of the Wadi El Rayan depression. It demonstrates that this species has an extended spawning period and exhibits fractional spawning during the winter. Observations indicate that *S. aegyptiaca* has acclimatized and has successfully reproduced in the new environment. These findings are consistent with previous studies conducted on the same species.

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