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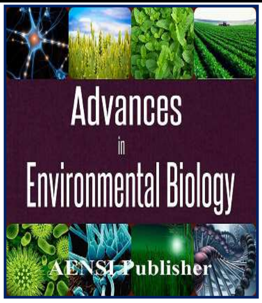

Evaluation the quality of the oil waste Histological Changes in the Ovaries Of the whipfin fish, *Gerres filamentosus* (Cuvier, 1829) during the reproductive cycle in the Hurghada Red Sea, Egypt

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ABSTRACT

In the present study, the results are supported by two methods which are macroscopic (ovarian maturity stages and gonado-somatic index) and microscopic (ovarian histology and egg diameter) criteria. Macroscopic examinations of the ovaries of whip fin silver-biddy, *Gerres filamentosus* (Cuvier, 1829) in the Hurghada Red Sea, Egypt, were carried out during January 2010 to December 2010. No signs of sex reversal were observed. The Female size at the first sexual maturity was equal to 16.36 centimeters. The maturity stages for 412 of female fishes were distinguished into six stages which are immature, maturing(recovering), developed, gravid, ripe (ovulating) and spent. The sequence of appearance of different maturity stages at the same time and extended high peak of gonado-somatic index indicated that, this species is partially spawner with extended spawning season from July to October. From histological investigations, oocyte growth follows a similar general pattern in most teleosts, with general characteristics in yolk composition, yolk deposition, rapidity of growth and surrounding membranes. The main stages of oocyte developments are primary (immaturation and maturation) oocytes and secondary (vacuolization, yolk deposition and ripening) oocytes. One of distinct features of egg development was reported in *Gerres filamentosus*. Oocytes may be attributed to asynchronous and continuous secondary growth development of oocytes which causes *Gerres filamentosus* (Cuvier, 1829) to spawn more than one time in a year.

KEYWORDS: *Gerres filamentosus*, length first maturity, maturity stages, oogenesis.

INTRODUCTION

A good historical review on identifications of fishes from the Red Sea was given by Botros, [1]. Family Gerreidae comprises some 40 species belonging to seven genera [2]. Fishes of the family Gerreidae, mainly species of the genera *Diapterus* and *Eugerres*, have high potential or cultivation, because of their saline tolerance [3]. The success or failure of a species in any environment largely depends on its spawning potential in view of the paucity of works on gonads of marine teleosts. The environment, climate and range of *Gerres filamentosus* are marine; fresh water; brackish; demersal; amphidromous; depth range 1-50 m.; tropical; 26**C**-29**C** and 32**N** -12**S,** respectively [4]. Some biological studies have been conducted on *Gerres* species for developing effective management measures, inspite of their worldwide distribution and their great importance in the fishery and as a delicious food or marine ornamental fish in many countries [5]; Cyrus and Blaber, [6]; El-Agamy, [7]; Iwatsuki, *et al.*, [8,9,10]. Iqbal, *et al.*, [11,12] and Sajeevan and Semvanshi, [13]. First record of three species namely *Gerres filamentosus* ,*Gerres acinaces* and *Gerres lucidus* of Gerreids (Pisces: Perciformes) from the Jaffina, Lagoon, Sri Lanka were discussed by Shutharsan and Sivashanthini, [14]. Histological studies offer the scope to understanding the cellular kinetics of gonad, recruitment, development and reabsorption of gonad cells and finally in staging the maturity state of gonads. However, Divakaran and Kuttyamma, [15] stated that the histological studies of *Gerres filamentosus* are still unknown. This work of histological studies on the ovaries of *Gerres filamentosus (*Cuvier, 1829) in Hurghada area of the Red Sea shows light on the ovarian cycle and different stages of oocytes to introduce additional information to evaluate its growth rate and productivities.

MATERIALS AND METHODS

The fish specimens of the whip fin, silver-biddy, *Gerres filamentosus* (Cuvier, 1829) were collected monthlies from the commercial-trawler catches off Hurghada Red Sea coast of Egypt, during the period from January 2010 to December 2010. During that time, “412” female fish were taken to laboratory directly after catching on ice, then it were dissected and the ovaries were detached.

The fishes were through ally cleaned and the data on total length (cm), total weight (g), gutted weight (g), ovary weight (mg), sex, shape, size, colour and stage of maturity, were recorded. Maturity stages of ovaries were studied by examining dissected fresh specimens. The ovaries were classified into six stages which taking into account the four generalized scales used by Hjort, [16], Yamamoto, [17], Kesteven, [18] and Nikolsky, [19]. The gonado-somatic index (G.S.I.) was computed as the percentage weight of the fish gonads to the total weight of the fish [20] and it was used as an indication of maturity. It was calculated from the following formula:

G.S.I. = (Gonad weight / Gutted weight of fish) x 100

The gutted weight is the weight of fish without the general viscera.

Typically, the data for the reproductive studies are based on an assessment of gonadal development which is the most accurately evaluated with histology which is stated from Lowerre- Barbieri, *et al.*, [21] to improve data and methods which used in reproductive studies. For studying the length at which 50% of fish reach sexual maturity, it was considered to be the length of onset of its sexual maturity. The total length against the percentage of mature fish was graphed according to the method of Pitt, [22].

Only specimens larger than minimum size at sexual maturity were used to examine the seasonal cycle in ovary conditions. Three subsamples of the ovary which taken from three positions were preserved in Bouin’s fluid. Ova diameter measurements were made following the procedure of Clark, [23]. Twenty five of oocytes were measured of both stage III and stage IV in a total of fifteen slides which obtained from the ovaries of *Gerres filamentosus* at spawning season. Egg-diameter was determined monthly by using a dissecting binocular zoom-stereo-microscope at a magnification (16X) fitted with a calibrated ocular micrometer. This method precluded the need to measure oocytes from various sites within the ovary to determine spatial homogeneity of development.

Sequence of oocyte maturation was histologically determined. The ovaries of fishes were preserved in Bouin’s solution for fixation to the subsequent histological analysis, then dehydrated, embedded in paraffin and sectioned with a routine microtome to a thickness of 5:10 µm following Hinton’s conventional processing [24]. The prepared paraffin sections were stained by Heidenhain iron Haematoxylin, counter stained by eosin technique [25] and then photographed under light microscope.

Results:

Although, the histological studies on fish-gonads provide the authors with important information which may reflect actually the rhythm of spawning, there is no literature cited on histological characters of gonads of the whip fin, silver-biddy, mojarra, *Gerres filamentosus* (Cuvier, 1829) in the Red Sea. It was against this background that the present study was undertaken to describe the oogenesis and female’s seasonal changes.

Size at first sexual maturity:

Fishes were considered to be mature when the female ovaries contained large yolky eggs. A total of 153 females were considered for determining the minimum size at first maturity stage according to methods of Pitt, [22], Pauly, [26] and King, [27] where (L_{50}) is considered to be the length of onset of its sexual maturity. Three methods were employed to detect the length of female *Gerres filamentosus* at its first sexual maturity. In the first one, the different length groups of fishes were classified during the spawning season to either immature or mature ones. The percentage of mature females is recorded in table (1).

Table 1: The percentage of frequency distribution of mature females of the whip fin silver-biddy, *Gerres filamentosus* (Cuvier, 1829); in the Hurghada Red Sea during the period from January 2010 to December 2010.

Total length group (cm)	Total number of female fishes(210)	No. of Mature female fishes(153)	Percentages (%)
11.5-12.4	1	0	0
12.5-13.4	2	0	0
13.5-14.4	6	0	0
14.5-15.4	7	2	28.57

15.5-16.4	20	9	45.00
16.5-17.4	31	18	58.06
17.5-18.4	38	27	71.05
18.5-19.4	35	28	80.00
19.5-20.4	35	34	97.14
20.5-21.4	12	12	100.00
21.5-22.4	23	23	100.00

The second method was by polling a maturation curve passing as closely as possible to the observed points of mid-class intervals (fish length groups) and the percentage maturity of fish in each length group as in fig. (1). The third method was based on the fact that, the maturation curve can be approximated to a straight line. The maturation curve at mid-points of length groups between 14.5-15.4cm and 21.5-22.4 is represented in fig. (1). Hence, a linear regression is performed between the two variables, where the mid-point of length groups as X and maturity percentage as Y, in the regression formula of:

$Y = a + b X$ (a & b are constants)

Then, the length corresponding to the 50% maturation is deduced from the following equation after the calculation of ‘a’ and ‘b’:

$Y = -125.389 + 10.723 X$ ($r.$ =0.976)

In this way, the length at the first sexual maturity for females *Gerres filamentosus*, (Cuvier, 1829) in the Hurghada Red Sea were statistically estimated and they were found to be 16.36 cm.

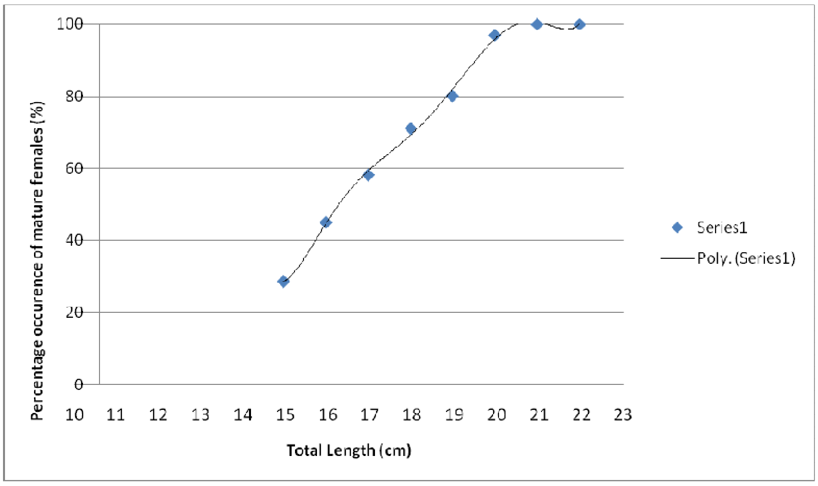

Fig. 1: Percentage occurrence of mature females of the whip fin, silver-biddy, Gerres filamentosus (Cuvier,1829) at different length groups in the Hurghada Red Sea during maturation (January 2010-December 2010).

Macroscopic examination of the ovaries of Gerres filamentosus (Cuvier, 1829):

The reproductive cycle of the whip fin, silver-biddy, *Gerres filamentosus* (Cuvier,1829) in Hurghada Red Sea exhibits remarkable variations in shape , size, colour and texture during the successive developmental stages (gonadal cycle) of the fish. Sexual dimorphism was absent in *Gerres filamentosus*,(Cuvier,1829) which posses a pair of spindle shaped gonads. The ovaries were attached to the dorsal median line of the coelom by a short mesovarium. The length, width and the weight of the gonads were in relation to the body cavity, and more particularly, their colour indicated the degree of maturity during different months of the year.

It can be classified morphologically into six stages which were recorded around the year taking in consideration the four generalized scales used by Hjort [16]; Yammaoto [17]; Kesteven [18] and Nikolsky [19].

Ovarian maturity stages:

Ovarian maturity stages of *Gerres filamentosus*, (Cuvier, 1829) in the Hurghada Red Sea can be explained by using macroscopic and microscopic descriptions of the phases in the female reproductive cycle as shown in table (2) and are summarized as follows:

1-Stage I (Immature or virgin): Never spawned stage ovary which was present throughout the whole year with a peak in January. Ovary was small, thin, cylindrical, often clear, blood vessels indistinct, pinkish translucent and measuring about a quarter of the body cavity length. The ova were not visible to the naked eye. Thin ovarian wall and little space between oocytes were observed. The only oogonia and primary growth oocytes were present .The atresia or muscle bundles were absent (Fig.2, a&b).

Table 2: The percentage of monthly and seasonally distribution of different maturity stages of the female whip fin mojarra, *Gerres filamentosus* (Cuvier, 1829), in the Red Sea at Hurghada area, during the period from January 2010 to December 2010.

Month	No. of Fish	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	G.S.I.
January	51	25 (50.98)	17 (33.33)	2 (3.92)	1 (1.96)	-	5 (9.80)	5.00 ± 0.12
February	34	12 (35.29)	11 (32.35)	5 (14.71)	3 (8.82)	-	3 (8.82)	5.10 ± 0.55
March	47	15 (31.91)	17 (36.17)	7 (14.89)	4 (8.51)	2 (4.26)	2 (4.26)	5.50 ± 0.92
April	40	5 (12.50)	8 (20.00)	13 (32.50)	9 (22.50)	2 (5.00)	3 (7.50)	8.90 ± 0.82
May	90	15 (16.67)	16 (17.78)	23 (25.56)	16 (17.78)	12 (13.33)	8 (8.89)	10.50 ± 0.90
June	31	5 (16.13)	4 (12.90)	5 (16.13)	9 (29.03)	7 (22.58)	1 (3.23)	10.81 ± 1.20
July	15	1 (6.67)	1 (6.67)	2 (13.33)	4 (26.67)	7 (46.67)	-	13.69 ±0.88
August	17	1 (5.88)	1 (5.88)	2 (11.76)	2 (11.76)	5 (29.41)	6 (35.29)	12.05 ±1.02
September	17	2 (11.76)	1 (5.88)	1 (5.88)	3 (17.65)	6 (35.29)	4 (23.53)	13.26 ±0.91
October	23	2 (8.70)	5 (21.74)	7 (30.43)	2 (8.70)	2 (8.70)	5 (21.74)	8.56 ±0.08
November	21	5 (23.81)	2 (9.52)	2 (9.52)	2 (9.52)	3 (14.29)	7 (33.33)	7.99 ±0.07
December	26	6 (23.08)	4 (15.38)	2 (7.69)	3 (11.54)	2 (7.69)	9 (34.62)	7.66 ±0.06
Winter	132	52 (39.39)	45 (34.09)	14 (10.61)	8 (6.06)	2 (1.52)	10 (7.58)	5.20 ±0.53
Spring	161	25 (15.53)	28 (17.39)	41 (25.47)	34 (21.12)	21 (13.04)	12 (7.45)	10.07 ±0.91
Summer	49	4 (8.16)	3 (6.12)	5 (10.20)	9 (18.37)	18 (36.73)	10 (20.41)	13.00 ±0.55
Autumn	70	13 (18.57)	11 (15.71)	11 (15.71)	7 (10.00)	7 (10.00)	21 (30.00)	8.07 ±1.29

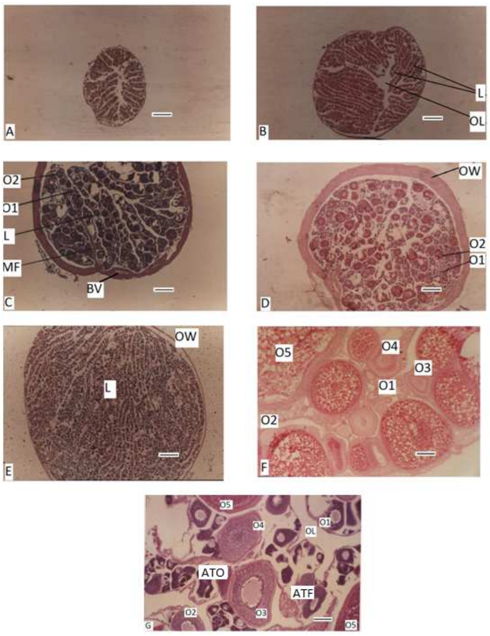

Fig. 2: Photomicrographs showing transverse histological sections in the ovaries of whip fin,silver-biddy, *Gerres filamentosus* (Cuvier, 1829) in various stages of development. Scale bars represent (1 mm) for figure (A) to figure (E) except figure F = (0.2mm) and figure (G) = (7μm). Stain: Heidenhain iron haematoxylin and eosin.**(A) and (B):** Stage I, immature ovaries; ovarian lumen (OL) & lamellae (L).**(C):** Stage II, maturing or recovering ovary; primary oocyte (O1), secondary oocyte (O2), muscle bundle (MB), and blood vessel (BV).**(D):** Stage III, developing ovary; ovarian wall (OW). **(E):** Stage IV, gravid ovary.**(F):** Stage V, ripe ovary, primary sub-stage of yolk deposition oocyte (O4a), secondary sub-stage of yolk deposition oocyte (O4b), tertiary sub-stage of yolk deposition oocyte (O4c) and stage V oocyte (O5). **(G):** Stage VI, spent ovary, atretic oocyte (ATO) and atretic follicle (ATF).

2-Stage II (Maturing or Recovering): It was observed at February, March and April. Ovary was small, dull reddish-brown in colour and translucent. The two lobes were unequal in size and the right lobe was slight larger than the left one which occupied about one-third of the body cavity. The ova were not visible to the naked eye. Blood vessels were more distinct. Muscle bundles, thick ovarian wall and/or atretic oocyte or old, degenerating postovulatory follicles might be present (Fig. 2, c).

3-Stage III (Developing): Mid-maturation ovary was detected throughout April and May. Ovary became larger than the above stages and occupied about one-half of the body cavity length. It had reddish yellow colour and the dark granular eggs were visible to naked eye. Primary growth oocytes , cortical alveolar oocytes, Primary vitellogenic oocytes, and secondary vitellogenic oocytes were present. No evidence of postovulatory oocytes or tertiary vitellogenic oocytes was observed. Some atresia could be present (Fig.2, d).

4-Stage IV (Gravid): Nearly ripe ovary was encountered from May and July with a peak in June. Ovary was larger creamy yellow or orange in colour. The eggs became larger, yellow and easily extruded by stripping. Ovary measure was about two-thirds of the body cavity length. Early stages of oocyte maturation could be present. Blood vessels were prominent and individual oocytes were visible macroscopically (Fig.2, e).

5- Stage V (Ripe / running / spawning): Total maturation ovary was observed at July, August, September and October. Ovary was orange to pink in colour, long, board and turgid, which was filling all the body cavity. Ripe ova were visible, large spherical, transparent, besides eggs running freely on slight pressure and producing a speckled appearance. Some ova were visible to the exterior at the vent region. Oocytes undergo to the late germinal vesicle migration ova, germinal vesicle breakdown ova, hydration, and ovulation ova (Fig.2, f).

6-Stage VI (Spent): It was extended about two months; November and December. It had a deep red colour with unhealthy and un-spawned mature or ripe ova. Ovary was flaccid and much reduced in size (occupying almost half of the body cavity). Some ova were seen in the ovarian sac in Fig. (2, g). Table (2) showed the maturity stages of 412 female specimens during the examined period of this work.

Egg-diameter investigation:

Microscopically, the ovary found to be contain different generations of eggs, and their diameter varies in the different periods of the year. Towards the spawning season, the eggs progressively increased in size during the pre-spawning period. The different generations were of varying levels of vitellogenesis. The ovaries of mature female, *Gerres filamentosus* (Cuvier, 1829), that approach spawning, contained three sizes of ova as will be seen in fig. (3,b). Fig.(3) shows that the first size involves ova having diameters less than 0.2mm. These ova are minute, rounded grey-colored, yolkless and considered to represent the oocyte stock (recruitment stock). The other two sizes of ova were either yolky opaque-colored, about 0.3-0.8mm in diameter or translucent large eggs about 0.9-1.0mm in diameter. Both of these two sizes were involved in the immediate spawning while the first type was being the eggs which would develop for spawning in the following or subsequent seasons. From Histological investigation, table (3) showed oocytes at ovarian stage III obtain three modes in the frequency distribution of ova diameters. These three modes were represented the group (a) of diameter 0.3mm (22.0%), the group (b) of diameter 0.5mm (23.1%) and the group (c) of diameter 0.7mm (25.2%).

Table 3: Ova –diameter frequency distribution in stage III and stage IV of maturation of *Gerres filamentosus* (Cuvier,1829) in Hurghada Red Sea during the spawning season(July2010 to October2010).

Ovary of stage III of maturation									
No. of specimens	Ova-diameter (mm)								
	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
5 x 25	6	25	7	34	18	27	8		
5 x 25	9	26	8	28	12	32	10		
5 x 25	5	32	9	26	20	27	6		
Total	20	83	24	88	50	86	24		
percentage	5.3	22.1	6.4	23.5	13.3	22.9	6.4		
	Ovary of stage IV of maturation								
5 x 25		1	12	6	13	12	51	18	12
5 x 25			15	3	20	13	45	18	11
5 x 25			13	2	22	14	40	20	14
Total		1	40	11	55	39	136	56	37
percentage		0.27	10.7	2.9	14.7	10.4	36.3	14.9	9.9

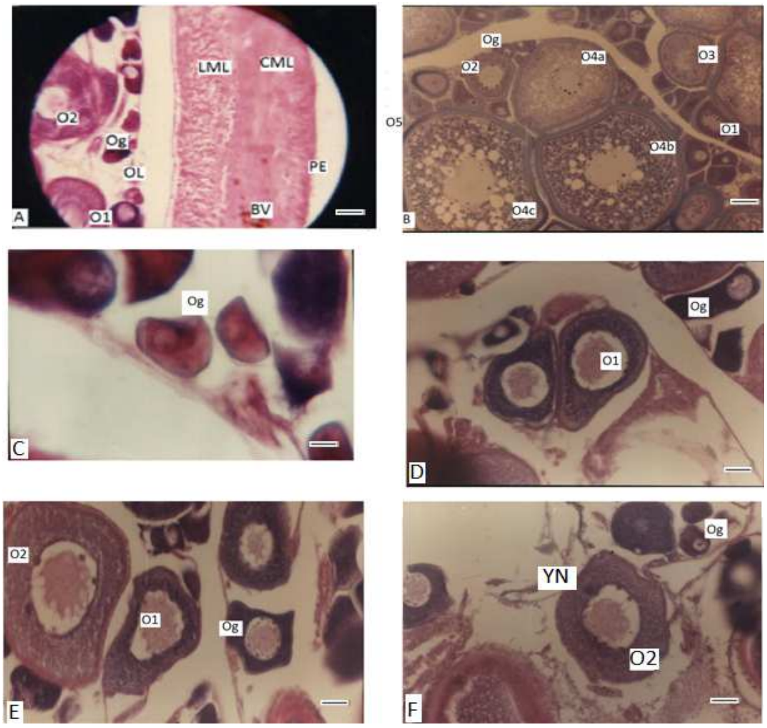

Fig. 3: Photomicrographs showing transverse histological sections in the ovaries of whip fin, silver-biddy, *Gerres filamentosus* (Cuvier, 1829) in various stages of oocyte's development. Scale bars represent : figure A= 30 µm, figure B= 0.1mm and figure C =5µm while figure E, figure D figure and figure F = 6µm. Stain: Heidenhain iron haematoxylin and eosin. **(A):** Mature ovary showed ovarian wall or tunica albuginea , peritoneal epithelium (PE), longitudinal muscle layer (LML), circular muscle layer (CML), blood vessel (BV)and oogonia (Og). **(B):** Oogonia (Og) and oocytes at various stages of development, primary oocytes (O1& O2), secondary oocytes (O3,O4a,O4b and O4c) which embedded in the lamellae without any definite pattern of arrangement. **(C):** Magnified oogonia. **(D):** Only a single eccentrally located nucleolus { characteristic for the oogonium of *Gerres filamentous* (Cuvier,1829)}. **(E):** Chromatin mass was present in the nucleus, more than one similar nucleoli often contain vesicle were present at the periphery of the nucleus of stage I oocyte (O1). A layer of cells (Theca folliculi) first was appeared with spindle-shaped nucleoli. **(F):** A small round darkly stained yolk nucleus was appeared in the ooplasm near the nucleus of stage II oocyte (O2).

At the following of maturity stage IV, three modes were shown the progression of the ova-diameter. It showed mode at diameter 0.4mm (10.7%) representing group (a), mode at diameter 0.6mm (14.7%) representing group (b) and mode at diameter 0.8mm (36.3%) representing group (c).

Thus, it is evident that *Gerres filamentosus* (Cuvier, 1829) is a fractional spawner, releasing its ripe eggs in three batches during the spawning season that extends from July to October. Table (4) shows the largest eggs which was a wide range-diameter dealing with months. From January to July, there is a progressive increase in the size of the oldest eggs, after that the egg diameter falls down. So the ovaries may have large eggs in different stages or degree of vitellogenesis.

Table 4: Monthly variation of egg-diameter of *Gerres filamentosus* (Cuvier,1829) in Hurghada Red Sea, Egypt .

No. of specimens	Months	Egg-diameter (mm) Mean±D
10	January 2010	0.401±0.02
13	February	0.422±0.51
9	March	0.510±0.63
7	April	0.551±0.66
6	May	0.600±0.70
8	June	0.620±0.73
9	July	0.822±1.01
14	August	0.735±o.80
11	September	0.636±0.60
13	October	0.568±0.52
8	November	0.360±0.40
7	December2010	0.351±0.53

Total number of specimens =115

Microscopic examinations of the ovaries of Gerres filamentosus (Cuvier, 1829):

From microscopic examinations, each ovary was enveloped within a thick vascular connective tissue layer; the tunica albuginea which was composed of smooth muscle fibers and was covered externally by a single layer of a simple squamous epithelium or the peritoneal epithelium. The tunica albuginea was subdivided into two layers. The first one was a thick external layer with fibers extending circularly while the second internal layer had the fibers which mostly arranged themselves longitudinally along the ovary. Blood vessels were found in between both layers (Fig.3, a). The tunica albuginea together with the germinal-epithelium (A single layer of epithelial cells with indistinct boundaries which lined the ovarian cavity) projected at several places into the ovocoel to form the characteristic ovigerous lamellae or folds. These folds contained oogonia and oocytes at various stages of development which embedded in the lamellae without any definite pattern of arrangement (Fig.3, b).The central cavity had lateral extensions between the lamellae forming wide or narrow spaces. These spaces may become difficult to locate and hardly distinct during the spawning season due to the distension of the ovigerous folds at maturation of the ovary (Fig.2, e). On the other hand, these folds were distorted during the spent condition (Fig.2, g).

Histological sections of all examined ovaries showed no any signs of sex reversal. The oocytes of *Gerres filamentosus* (Cuvier, 1829) during its development showed many changes such as, the increase in size and the appearance of primary and secondary oocytes. i.e. different stages of oocytes simultaneously found in the ovary (Fig.3, b).

The main stages of oocyte development (oogenesis):

The oogenesis passes through successive degrees of development which are multiplication, growth, maturation and ripening. Many changes occur throughout two main periods of growth; protoplasmic and trophoplasmic. The oocyte development in this study can be divided into five progressive stages (immaturation stage, maturation stage, vacuolization stage, yolk deposition stage and ripening stage), representing the two main periods of growth.

A-Protoplasmic growth period (Primary oocytes):

1-Stage I (Immaturation stage):

The newly divided oogonia or primary germ cells are usually the smallest sex cells of the female series, with 15.0µm to 30µm in diameter and have a lightly basophilic staining affinity. They are usually oval-shaped with a well defined cell membrane, occur singly or in groups and form the precursor of eggs as in fig. (3, b & c). Oogonium contains a relatively large nucleus (8.0µm) with a conspicuous nuclear membrane and characterized by only a single eccentrally located nucleolus as shown in Fig.(3, b, d & f|). In adult *Gerres filamentosus* (Cuvier, 1829), these cells are always visible during all months and are most abundant in January when they undergo proliferation as evidenced by the various mitotic figures observed in them.

Oogonia grow up giving rise the chromatin-nucleolus oocytes (28.0µm to 29.0 µm) which are mostly polygonal or hexagonal. Each one is characterized by a large spherical nucleus (15.0µm), with two or more nucleoli occupy the greater part of the cell and dark homogenous cytoplasm. The chromosomes are highly compact together forming network of staining mass inside the nucleus (Fig.3, d). The chromatin-nucleolus oocyte was most abundant from January to March. Due to the growth of the cytoplasm and the nucleus, the latter oocyte transformed into the perinucleolus oocyte (35.0µm), measuring with nuclear size of 20.0µm. Chromatin mass is present in the nucleus, more than one similar nucleoli often containing vesicle are present at the periphery of the nucleus, (Fig.3, d). An increase in the basophilia of the cytoplasm was observed. A layer of cells (Theca folliculi) was first appearing with spindle-shaped nucleoli and was enveloped of each oocyte (Fig.3, e). Cell and nuclear sizes increased to 50.0µm and 25.0µm, respectively. Then, the monolayer oocyte was stained slightly and might be round or oval. Small vacuoles were present in the perinuclear area in the large oocyte(Fig.3, e). The perinucleolus oocyte and monolayer were the most abundant in March and April.

2-Stage II (The maturation stage):

Oocytes were continued to increase in mean size to 0.1 mm for the cells and 50.0µm for the nucleus. Two distinct features were characterized the oocytes at this stage. At the beginning of maturation, isolated follicular epithelial cells were appeared around the oocyte. Also, a small round or oval darkly stained yolk nucleus was appeared in the ooplasm near the nucleus. Later on, this yolk nucleus was moved towards the oocyte periphery (Fig.3, f) where it gradually disintegrated and finally disappeared. The small nucleoli were aligned at the periphery of the nucleus. Granulosa cells were appeared between the theca and the plasma membrane of the oocyte. At the end of this stage, many small vacuoles were appeared in the cortical region of the cytoplasm. Large numbers of primary oocytes were presented in immature and inactive states of ovaries which showed no evidence of development towards the next stage. This stage was become evident in April and was become abundant during May.

B- Trophoplasmic growth period (Secondary oocytes):

1-Stage III (The vacuolization stage):

The most characteristic feature of this stage was the comprehensive deposition of trophic elements in the cytoplasm of oocytes, as a result of which, the oocyte was increased in size to reach about 0.2mm in diameter and 0.08mm in nuclear size. In many cases, the nucleus showed an irregular outline and nucleoli were still visible adherent to the periphery of the nucleus, and their number varied from 5 to 8 (Fig.4, a).

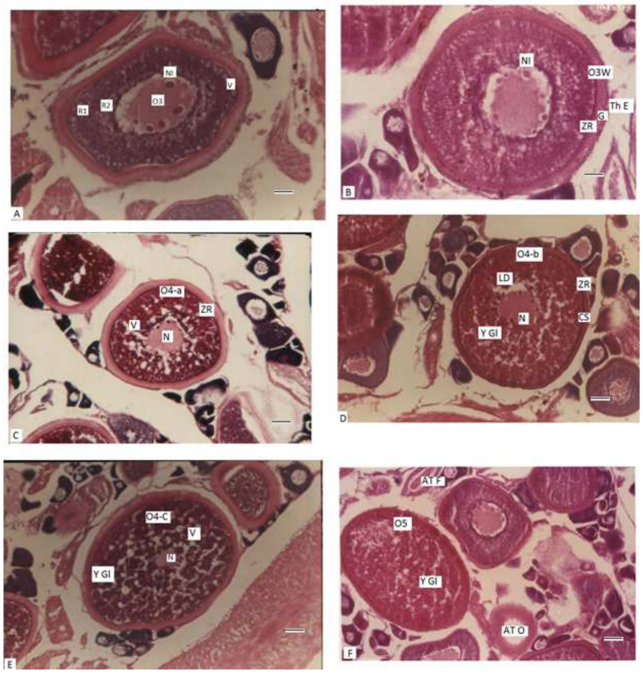

Fig. 4: Photomicrographs showing transverse histological sections in the ovaries of whip fin, silver-biddy, *Gerres filamentosus* (Cuvier, 1829) in various stages of oocyte's development. Scale bars represent: 0.02 mm of figures (A&B); (figure C=0.07mm); (figure B=0.09mm); (figure E=0.08mm) and (figure F=0.1mm). Stain: Heidenhain iron haematoxylin and eosin. (**A**): Stage III oocyte (O3), nucleoli are still visible adherent to the periphery of the nucleus, and their number equals five. Two distinct rings, one near the periphery of the oocyte (R1) while the other around and near the nucleus (R2). (**B**): The oocyte wall(OW) consists of an outer covering, theca external (ThE), median layer of granulosa (G) and the inner layer of zona radiata (ZR)(Non-cellular). (**C**): The primary sub-stage of stage IV oocyte (O4a), in which the cytoplasm contains true yolk granules. The variable size oil-droplets(LD) increases in number and it forms a circular ring around the nucleus. Zona radiata (ZR) becomes visible adjacent the follicular layers; theca and granulose. (**D**): The secondary sub-stage of stage IV oocyte (O4b) is characterized by lipid vesicles which appear in the cytoplasm as unstained empty vacuoles. Cross striations or pores (CS) are on zona radiata. (**E**): The tertiary sub-stage of stage IV oocyte (O4c) where the oocyte contains large red-staining prominent yolk globules(YGl) which invade the space occupied by the lipoid droplets (LD). (**F**): Stage V oocyte (O5), No distinct nucleus is observed. The oocyte contents show a remarkable degree of homogeneity of clear vacuoles among the yolk spheres. Atretic oocyte and follicle are present.

Minute yolk vesicles firstly were appeared at the periphery of the cytoplasm and spread towards the center. Vacuoles were dispersed in the ooplasm forming two distinct rings, one around and near the nucleus while the other near the periphery of the oocyte. Chromosome threads in the nucleoplasm were appeared condensed. The oocyte wall was consisted of an outer covering, theca externa, median layer of granulosa and the inner layer of zona radiata (Non-cellular) (Fig.4, b).This stage was observed in April.

2-Stage IV (The yolk deposition stage):

The oocytes in this stage considerably were increased in size with dense deposition of yolk granules. Egg membrane was highly developed (very thick) and more prominent. The follicular layer was consisted of an outer theca externa and an inner granulose. The zona radiata was become thicker than in the preceding stage. Later, cross striations or pore canals were visible on the zona radiata. This stage can be comprised to three sub-stages of yolk deposition as follows:

a- The primary sub-stage, in which the cytoplasm was contained true yolk granules. The membrane was become progressively thicker, where zona radiata (Non-cellular) was become visible adjacent the follicular layers i.e. theca and granulosa . The variable size oil-droplets was increased in number and it was formed a circular ring around the nucleus. The elaboration of the vitellogenic materials was resulted in further increase in size of the cell to 0.35mm, with an increase in nuclear size to 0.07mm (Fig.4, c).

b- The secondary sub-stage was characterized by yolk globules accumulation in the inner cytoplasmic part. Lipid vesicles were appeared in the cytoplasm as unstained empty vacuoles. Considerable increase in the size of the oocyte was to 0.54mm and 0.09mm. In the nucleus, as more yolk spherules were elaborated in the cytoplasm and the yolk vesicles were spread. (Fig.4, d).

c- The tertiary sub-stage, where the oocytes were contained large red-staining prominent yolk globules which invade the space occupied by the lipoid droplets. Oocytes were continued to increase in size to 0.75mm and 0.08mm in the nucleus. At the onset, the nucleus was still occupied a central position (Fig.4, e). It was started to acquire an eccentric position. Accumulation of yolk and trophic substances in the oocyte were completed and the egg case growth was oval in shape (Fig.4, e).

3- Stage V (Ripening stage):

As the oocytes ripen, they were gone many changes, first of all the appearance of clear vacuoles among the yolk spheres. The nucleus was shifted towards the animal pole. At this stage, the envelope layers were clearly observed. The theca interna had small blood vessels to each oocyte. The vitellogenic granules were found very conspicuous and well distributed. No distinct nucleus was observed. The oocyte contents were showed a remarkable degree of homogeneity (Fig.4, f). The ripe stage oocyte was the most abundant during from July to October. Although final oocyte maturation was restricted to a limited period, all other phases did not occur at a fixed moment in the annual cycle. Oocyte by this period was distinctly pear-shaped, reaching maximum size of 0.75mm to 0.95mm or 1.00mm in diameter. Unfortunately, the final stage of ripening oocyte was seldom observed during histological analysis. It was evidently occurred during a short period of time immediately before ovulation. Especially, in fish species which live in the natural habitat, it was difficult to get completely ripe eggs. The discharge of ripe ova during the spawning season was accompanied by the appearance of empty follicles as shown in Fig. (5, a).

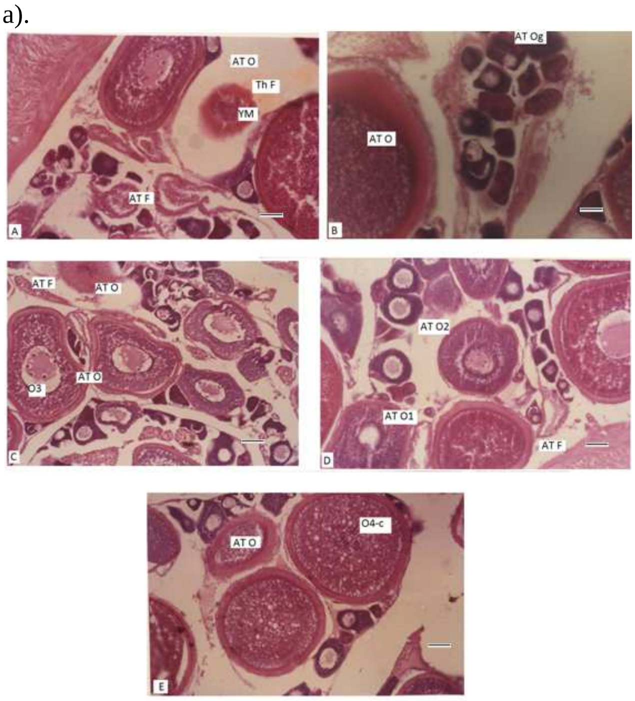

Fig. 5: Photomicrographs showing transverse histological sections in the ovaries of whip fin, silver-biddy, *Gerres filamentosus* (Cuvier, 1829) in various stages of oocyte's development. Scale bars represent: 0.02 mm of figures A, B, D & E and for figure C = 0.08mm. Stain: Heidenhain iron haematoxylin and eosin. **(A):** Corpus luteum was characterized by its stratified follicular epithelium and marked thickness with high vascularity of yolk mass (YM) and of the theca folliculi (ThF). **(B):** Artesian oogonia (ATOg) were found with lipoid structures and atrophy of nucleus and cytoplasm. In addition, complete destruction of chromatin-nucleolus oocytes was present. **(C):** The first form of cystic atresia (ATO) was in vacuolized oocytes which show empty spaces at periphery due to hydrolysis of cytoplasm. The whole yolky mass becomes loose and intermixes with the invading follicular cell. **(D):** Crescent-shape atresia (ATO1) and the first sign of horse-shoe atresia (oocyte of vacuolization stage) (ATO2). **(E):** Oocyte in late yolk deposition stage (O4c) showing the hypertrophic atresia (ATO).

Atretic oocytes:

Examination of histological sections showed multi-form atresia (Oocytes in all stages of oogenesis, suddenly cease growth and become hyperplastic or hypertrophic atretic type) especially in the early spawning and post-spawning seasons. However, atretic oocyte was widespread in the whip fin, silver biddy, *Gerres filamentosus*(Cuvier,1829) not only in the ovaries of young and adult specimens but it was also observed in winter and summer months. Fig.(5, a) showed the hyperplastic atresia in the late vacuolated follicle which leads to the formation of pre-ovulatory corpus luteum that characterized by its stratified follicular epithelium and marked thickness with high vascularity of the theca folliculi. Fig.(5, b) illustrated artesian of oogonia with lipoid structures and atrophy of nucleus and cytoplasm. In addition, complete destruction of chromatin-nucleolus oocytes was observed. Fig.(5, c) clearly showed the first form of cystic atresia in vacuolized oocytes which show empty spaces at the periphery due to hydrolysis of cytoplasm. Moreover, atritic secondary oocyte (yolk deposition stage) in late stage of pre-ovulatory corpora lutea, where the whole yolky mass was become loose and intermixes with the invading follicular cell. In fig. (5, d), atretic secondary oocytes at late stage showed the crescent-shape atresian and the first sign of horse-shoe atresian (vacuolization stage). One of three oocytes in late yolk deposition stage was showed the hypertrophic atresian (Fig.5, e).

Discussion:

In the present study, the examined fish, *Gerres filamentosus*(Cuvier,1829), commonly known as the whip fin, silver-biddy, is a fish native to the coastline of Africa and Madagascar east to Japan, Australia and New Caledonia [28]. This results were supported by two methods which are macroscopic (ovarian maturity stages and gonado-somatic index) and microscopic (ovarian histology and egg diameter) criteria. Each of these methods had its own type of classification scheme as mentioned by West [29] and Murua *et al*. [30]. While, Durham and Wilde [31] showed that the combination of asynchronous and synchronous spawning by using three patterns or methods commonly used to assess reproductive ecology which are oocyte development, gonadosomatic index and oocyte size distribution for pelagic broadcast-spawning cyprinids, smalleye shiner, *Notropis buccula* in North American Great.

Length at first maturity of the fish-species helps in the determination of the minimum legal size which must be avoided in fishing in order to protect an adequate spawning stock and ensure at least one spawning for the mature individuals. The present study showed the smallest mature female of *Gerres filamentosus*(Cuvier,1829) measuring 16.36 cm total length. A reproductive biological studies on other species of family Gerreidae in Okinawa Island of southern Japan showed that the maximum standard length (SL) at sexual maturity was 89.7 mm for females *Gerres oyena* (Kanak and Tachihara, 2008)[32], but a previous study on age and growth of *Gerres equulus* in western Kyushu, Japan (Iqbal *et al*. 2006b)[12], found that the estimated maturity standard length of female was 141 mm SL. While in Natal estuaries the size at sexual maturity of *Gerres rappi, Gerres acinaces, Gerres filamentosus* and *Gerres equulus* were 110, 110 ,70 and 134mm SL, respectively [6]. The mean calculated total length of *Gerres oyena* was 133.3mm at I year of age for pooled sexes in the Arabian Gulf waters [7]. Sivashanthini [33] estimated the length at first capture L_c **(**length at 50% capture) by backward extrapolation of the straight portion of the right descending part of the catch curve was 6.74 and 6.73 cm for males and females *Gerres filamentosus*, respectively. The difference in sizes between the above mentioned species may result from differences in growth characteristics and population density [34]; Shimose and [35]. However, Trindade-Santos and Freire, [36] stated that contrary to some expectations, there is seasonality in the reproduction of tropical fishes. Moreover, size at first maturity is not efficiently used as a tool fisheries management in the ecosystems analyzed of North, East and South Brazil shelves. Sequence of oocyte maturation only by histological studies has been determined by Cyrus and Blaber (1984) on *Gerres sp.* in Natal estuaries where they stated that individuals reached its gonad maturation for the first time in an estuarine area.

This results of the spawning season for whip fin, silver biddy, *Gerres filamentosus*(Cuvier,1829) showed that it extends throughout the summer and autumn months during from July to October in the Hurghada Red Sea, Egypt as evidenced by the histological studies on the ovaries, the peaks in ova diameters and GSI values during this period. I.e. asynchrounized fish. These results are in agreement with those results of the same species in central west coast of India by Golikatte and Bhat [37]. They stated that *Gerres filamentosus* declined in feeding activity in the months of July to September which coincided with its spawning period. Plots of catch rates for individual species indicated some lunar periodicity in the patterns of abundance for many species such as *Gerres filamentosus* (Cuvier, 1829) which had a distinct peak during the first-quarter moon (October-November), this results made by Salini *et al*. [38] which are to some extent in agreement with the post-spawning months of the same species in this work.

Everson, *et al.* [39] supported the former results by describing the regenerating phase of a batch-spawning species, spotted seatrout that showed a presence of the muscle bundle in transverse section of asynchrounized fish ovary as mentioned in this results of ovarian recovering stage or developing stage of fig. (2,c).

This pattern has been reported in other places for other species including snappers, *Pristipomoides filamentosus* [40]; *Aprion virescens* and *Etelis coruscans* [39]; black breams, *Acanthopagrus butcheri* [41] and sharks, *Acanthopagrus latus* [42] who added a note that once a protandrous hermaphrodite fish has become a functional female, it remains a female throughout the rest of its life. Seasonal variations in the size of eggs are taken as an index of the relative sexual maturity of the ovaries of fishes, and can also give evidence of the duration of the spawning season as shown by Hickling and Rutenberg, [43]. In the present investigation, the diameter of yolked oocytes, which observed monthly, increased progressively during the maturation and vacuolization periods till reached the yolk deposition stage at the maximum mean of egg-diameter in July and October which coincided with the spawning season. The diameter of yolked oocytes gave more than one peak in the egg-diameter analysis, from the ovaries of stage III and stage IV of maturation, indicating the presence of three modes (Table,3). These results indicated that *Gerres filamentosus* (Cuvier,1829) is fractional spawner in the Hurghada Red Sea, Egypt, i.e. stretched spawning season.

Using the composite histograms based on the diameters of all oocytes at successive stages of maturation, this results are converged with the findings of Sivashanthini *et al.* [44] on *Gerres abbreviatus* which spawned around three large batches of eggs during a season. This study of histology on the whip fin, silver-biddy, *Gerres filamentosus* (Cuvier,1829) from the Red Sea, Egypt showed that microscopic investigations on the ovaries had no signs of sex reversal. This result agrees with that of the same species in India by Divakaran and Kuttyamma, [15].

Oogenesis was compared between species by assessing general ovarian and oocyte morphology based on the conventional understanding of oocyte growth in teleosts [45]. In this study, the oocyte development was classified into two main stages; primary stage (immaturation and maturation) oocytes and secondary stage (vacuolization, yolk deposition and ripening) oocytes which are in agreement with that of Albieri *et al.* [46], who mentioned that the two main stages were previtellogenic stage (young germ cells and peri-nucleolus oocytes from reserve stock) and vitellogenic stage (oocytes with lipid vitellogenesis, oocytes with lipid and protein vitellogenesis and post-vitellogenic stage). Garcia-Lopez *et al.*, [47] were interpreted the compensatory ovarian growth as it based on an increase the number of early perinucleolar oocytes and mid-late stage vitellogenic follicles without an apparent recruitment of primary oocytes into the secondary growth phase. On the other hand, Ramadan and El-Halfawy [48] stated that by using the monthly changes in the percentage frequency of ovarian phases, the ovarian cycle of *Upeneus pori* (Mullidae) was divided into three periods which were a long period of early oogenesis, a short period of vitellogenesis and a spawning period. The presence of oogonia of *Gerres filamentosus* (Cuvier, 1829) in this study, are in agreement with that the results of Takashima *et al.*, [49]; Beers and Dekel, [50] and Carrasco, *et al.* [51] on rainbow trout, *Oncorhynchus mykiss*, which showed the most germ cells as in other salmonids and most vertebrate species. The most germ cells enclosed in the oogonial cysts and a few numbers outside the cysts where oogonia organized in clusters connected by intercellular bridges and surrounded by stromal cells . It has been suggested that the intercellular bridges within the genial clusters play an important role in synchronizing oocyte differentiation.

To sum up, these results of *Gerres filamentosus* in the Hurghada Red Sea showed that this fish is one of the batch-spawner fish which may spawn more than one time in a year. These results are also confirmed with results of the Lowerre- Barbieri, *et al.* [21]. So, the batch-spawning species with indeterminate fecundity will have different oocyte developmental patterns depending on how quickly the oocytes are recruited to various stages of vitelogenesis, which drives how asynchronous the oocyte pattern appears. In addition, Morris *et al.* [52] added that this mode of reproduction (asynchronous and indeterminate batch spawner) thus capable of sustained reproduction throughout the year when conditions are suitable.

In agreement with Mayer, *et al.* [53], the yolk deposition stages in this study had the criteria of both the lipid droplet stage and the cortical alveoli (yolk vesicle) stage where, unstained empty vacuoles appeared in the cytoplasm as lipid vesicles and bright pink structures as protein yolk granules This paper showed the most famous forms of atresia which mentioned in the lizardfish, *Synodus saurus* (Linnaeus,1758) in the Mediterranean Sea at Alexandria area by Abu El-Nasr, T.M. [54], where the common forms of different atresia distinguished into eight forms ; lipoid atresia, cystic atresia, crescent-shape atresia, horse-shoe atresia, liquefied atresia, crumpled atresia, complete degeneration atresia and phagocytosied atresia. The abnormalities in morphology of oocytes during maturation, vitellogenesis and (less often) previtellogenesis were seen in females *Parupeneus multifasciatus* which based on that observations , unfavorable environmental conditions in its area induced by anthropogenic factors which suggested by Savvaitova, *et al.* [55] and Belova, *et al.*, [56]. On the contrarily, Sivashanthini [57] mentioned the only normal morphology of oocytes in *Gerres filamentosus* from the Parangipettai Waters of India. New researches on gonads, Caruso *et al.,* [58] stated that the female summer flounder, *Paralichthys dentatus* grow considerably faster and larger than males, and a tremendous increase in performance can therefore be realized through production of monosex female populations. Moreover, Rojo-Bartolome [59] concluded that the estimation of maturity and sex of fish stocks in European waters is a requirement of the European Data Collection Framework as part of the policy to improve fisheries management.

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