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Investigations on Some Aspects of Reproductive Biology in *Oreochromis niloticus* (Linnaeus, 1757) Inhabited Abu-zabal Lake, Egypt

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Abstract: The present study was carried out to investigate some aspects of the reproductive biology of *Oreochromis niloticus* inhabited Abu-Zabal Lake to mange this species in this newly man made lake. Size at first maturity, gonado-somatic index (GSI), hepato-somatic index (HSI), egg diameter and fecundity were studied. Moreover, histological examination of the ovary was performed. At the first sexual maturity (L_{50}), the body length of females *O.niloticus* was smaller than males (10.5 and 11.5 cm, respectively). The breeding period (G.S.I.) of females extended from March to September. Fecundity variation was better correlated with length (r = 0.9236) and weight (r = 0.9399) than with gonadal weight(r = 0.7651). Fecundity ranged between 289-1456 eggs corresponding to fish total length of 10-23 cm with an egg diameter ranged from 1.99-2.45 mm. The histological findings support the biological results which elucidated that the spawning season of *O.niloticus* extended from March to September. The development of oocyte during spring go with the secondary growth phase (vitellogenic oocyte), while the development during summer and autumn were found in the primary growth stage (early and late perinucleolus and yolk nucleus stages). In winter, development of oocytes in the secondary growth phase (tertiary yolk stage) was in the artetic form. It was concluded that, it is essential to increase the mesh size used in Abu-Zabal lakes to catch *O.niloticus* to lengths greater than 12.0 cm to protect this species from exploitation.

Key words: Reproductive biology · *Oreochromis niloticus* · Gonadosomatic index · Hepatosomatic index · Histological examination · Fecundity

INTRODUCTION

Descriptions of reproductive strategies and the assessment of fecundity are fundamental topics in the study of the biology and population dynamics of fish species and also for evaluation of the reproductive potential of individual fish species. Of course this will increase our knowledge about the state of a stock and improves standard assessments of many commercially valuable fish species [1, 2]. Moreover, the availability of data based on reproductive parameters and environmental variation leads to a better understanding of observed fluctuations in reproductive output and enhances our ability to estimate recruitment [3].

The Nile tilapia,*Oreochromis niloticus* (Pisces: Cichlidae), is an important fish in the ecology of tropical and sub-tropical region including Egypt. It is the most popular species of the bony fish in Africa [4-6]. This is attributed to many positive qualities including tolerance to poor water quality, wide range of food, plasticity in growth, firm flesh and good taste [7, 8] and the ability to efficiently convert organic and domestic wastes into high quality protein [9].Other advantages are its herbivorous nature and its mouth – brooding habits [10]; extended breeding seasons and their reproductive biology is characterized by short generation time [11, 12]. Moreover, [13] observed that, *O. niloticus* is the most dominant species in the newly formed man mad, Abu-Zabal lake, Egypt, whereas it constituting more than 70.0% of the total cichlid species.

Studying of H.S.I. pointed to the importance of liver for storage of fat and proteins to be used during spawning period [14], also, liver is used as an indicator to record the level of water contamination [15].

The present study was carried out to investigate some aspects of the reproductive biology of

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Oreochromis niloticus inhabited Abu-Zabal Lake, as a trial to mange this species in this lake with emphasis on size at first maturity, gonado-somatic index (GSI), hepato-somatic index (HSI), egg diameter and fecundity. Moreover, histological examination of the ovary was performed.

MATERIALS AND METHODS

Biological Studies: This study was based on samples obtained from boat gill nets and trammel nets operating in Abu-Zabal Lake in the period from March 2005 till February 2006. Abu-Zabal lakes are three newly man-made lakes lying in the north of Qaluobia governorate, 30 Km away from Cairo, Egypt.

- The total fish lengths to the nearest cm and fish weight to the nearest g were recorded.
- Fish were dissected, sexed and maturity state was determined [16].The liver and gonads were removed, weighed to the nearest 0.01g and placed in 5% formalin.
- Hepato-somatic index (HSI) was calculated as the ratio between weight of liver and gutted weight [17], while, gonado-somatic index (GSI) was calculated as the weight of the gonads relative to the total body weight, expressed as a percentage [18].
- According to [19], the length at which 50 % of a fish population reaches sexual maturity (L_{50}) is considered to be the length of the onset of sexual maturity.
- To estimate the length at first maturity, the total body length was plotted against the frequency percentage of mature individuals during the spawning season and then the length at which 50% of the total individual female's number was consider as the length at first maturity [20].
- Fecundity is estimated from the gonads in the final maturation stage, by the number of oocytes having the largest diameter [21].
- To obtain representative samples of the whole gonads, small portions were taken from the posterior, middle and anterior regions of both lobes of the ovary. These samples were weighed and the numbers of ripe eggs were counted. The total number of ripe eggs in the ovary was estimated by multiplying the number of ripe eggs in the sample by the ratio of the ovary weight to the sample weight.
- These above mentioned data were used to determine regressions for fecundity/length, fecundity/weight, fecundity/ovary weight and fecundity/egg diameter. The relation between the different studied parameters were statistically analyzed by computing the correlation coefficients(r) using Microsoft Excel (2003).
- The egg size was determined by measuring the diameter of 30-40 randomly selected eggs per female along two axes using a calibrated eyepiece micrometer.

Histological Examination: Samples from ovaries of *O. niloticus* were quickly fixed in formalin 10% for 24 h, transferred to ascending series of alcohol, cleared with xylene and embedded in wax then sectioned at 4-5 µm and stained with Haematoxylin and Eosin [22].

RESULTS

Sex Ratio: It was seen from sex distribution in Table 1 that the two sexes did not occur in the same proportion during different seasons of the year, whereas, females predominated during spring (71.2%) and winter (56.0%), while males predominated during summer (55.6%) and autumn (59.3%). The overall sex-ratio (M: F) was 1: 1.37, i.e. the existed number of females was relatively higher than that of males.

Length at First Maturity: From Fig. 1 and 2, it is obvious that L_{50} for females and males of *O. niloticus* were attained at total length of 10.5 and 11.5 cm, respectively. Females were sexually mature above 14 cm, while males after 15 cm.

Table 1: Seasonal variations in sex ratio of *O.niloticus* at Abu-Zabal Lake

	Male		Female		
Season	No.	%	No.	%	Sex ratio (M:F)
Spring	44	28.8	109	71.2	1: 2.48
Summer	40	55.6	32	44.4	1: 0.80
Autumn	35	59.3	24	40.7	1: 0.69
Winter	22	44.0	28	56.0	1: 1.27
Total	141	42.2	193	57.8	1: 1.37

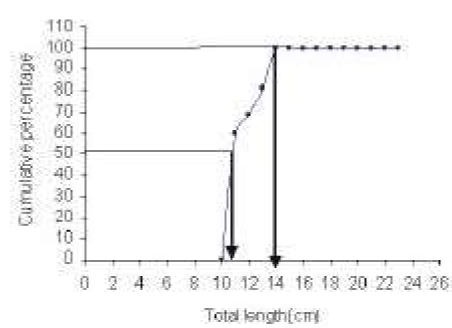


Fig. 1: Length at first sexual maturity of female *O.niloticus* in Abu-Zabal lake

Hepatosomatic Index (HSI): Seasonal variations in values of H.S.I. of female *O. niloticus* inhabited Abu-Zabal lake was shown in Fig. 3. It is noted that, the highest value of H.S.I. was recorded during spring (1.63) and the lowest value during winter (0.74).

Gonadosomatic Index (G.S.I.): Monthly variations in G.S.I. revealed that both sexes followed nearly the same trend. In females, several peaks of G.S.I. values were observed during March, April, June and September (Fig. 4). This means that females could breed more than once in the season and the period from March-September represented the spawning (breeding) period of *O. niloticus* in this region. It is also clear that, females have higher values GSI than males.

Gonadal maturation: Figure 5 Illustrates seasonal variations in the incidence of different stages of gonad maturity. The spent stage (V) represented the common maturity stage throughout the year, especially in winter (100%), autumn (76.9%) then summer (71.15%), followed by maturing stage (II), in spring (31.6%) and summer (28.8%) then, mature (III) in spring (14.6%) and autumn (23.7%). The stage (IV) was recorded during spring only (33.4%). Therefore, the percentage of reproductive process could be expressed by the sum of % of stage III & IV), in spring (48%) and in autumn (23.7%). The highest value of reproductive process was recorded during spring then autumn.

Fecundity: The total number of ripe eggs in the ovary (fecundity) in this study ranged between 289-1456 eggs corresponding to fish total length of 10-23 cm and an egg diameter of 1.99-2.45 mm. Fecundity was better correlated with length ($r = 0.9236$) and weight ($r = 0.9399$) than with gonadal weight ($r = 0.7651$). No correlation was existed between absolute fecundity and egg diameter, (Fig. 6-9).

Histological Examination: Most of different developmental stages of oocytes were observed as in Fig. 10-13, which included, first growth phase, consisted of Chromatin nucleolus stage (Cn), where the oocyte had weakly basophilic cytoplasm and more basophilic nucleus with darkly staining nucleoli. Early and late perinucleolus stages (ep, lp) whereas the first stage is characterized by light stain, large nucleus with one or more nucleoli and it had a dark cytoplasm, but the second stage have large size and distinguished by the less basophilic cytoplasm, nucleus became more round shape, in addition to the numerous observed nucleoli at the peripheral of nucleus membrane. Yolk nucleus oocytes (n), Fig. 12 Showed more development, whereas the nucleus had irregular shape (in), lipid droplets (unstained vacuoles) (ld) scattered at the peripheral of cytoplasm and there were number of nucleoli at the margin of nucleular membrane. The second growth phase which contained many stages. In the yolk vesicle stage (yv), the oocyte increased in the size, cytoplasm became more faintly stained with lipid vesicles forming number of irregular rows around the nucleus (Fig.13). Primary and secondary yolk stages were distinguished by acidophilic yolk granules found at the periphery of cytoplasm (Primary yolk stage) which migrate to the middle (Secondary yolk stage) and it had irregular nucleus shape (in). Tertiary yolk stage in which oocyres have no limited membrane, it had amoeboid nucleus, yolk granules united together to form mass of protein granules (Fig.10). Third growth phase (Ripe stage) showed the nucleus(n) migrate toward the animal pole.

Histological examination indicated four stages of maturation summarized as follow:

Maturing Stage (II): observed during spring and summer seasons, distinguish by all stages of oocytes(Fig. 11, 12) in the first growth phase with chromatin nucleus, early and late perinucleolus, which represent the dominant oocyte, also there were scanty of yolk vesicle stage.

Mature Stage (III): found during spring and autumn, it is characterized by predominance of yolk vesicle stage than other oocyte stages (Fig. 12).

Ripping oocyte: Were found during spring (Fig. 10), where the yolk vesicles and granules increased in their size to form yolk mass inside the cytoplasm with irregular shape nucleus.

Spent Stage (V): It was the common stage during the whole year, especially in winter (100%), followed by autumn then summer (Fig. 14, 15 and 16), whereas, all oocytes were observed in the third growth phase but in atretic state (Ao), which is characterized by liquefied cytoplasm (L), disintegrated membrane (dm), fibrosis (f), vacuoles (v) and the granulose cells attacked to yolk matter to become degenerated gradually until the oocyte disappeared.

Also, hypertrophoied (hy) cells surround the oocyte, vacuole (v) instead of spawned ripping oocyte were observed (Fig. 10). In addition, erosion of oocyte membrane which gave abnormal shape of oocyte was noted (Fig.11, 12 and 13).

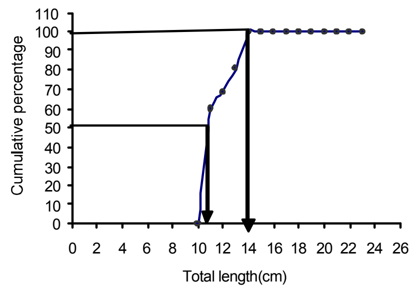


Fig. 2: Length at first sexual maturity of male *O.niloticus* in Abu-Zabal lake

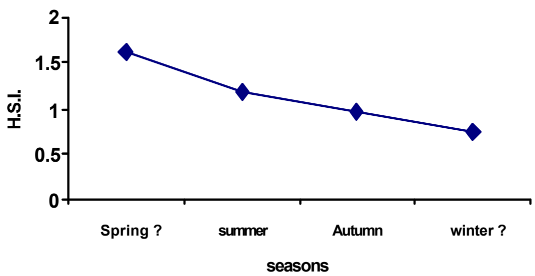


Fig. 3: Seasonal variations in values of HSI of *O. niloticus* in Abu- Zabal Lake

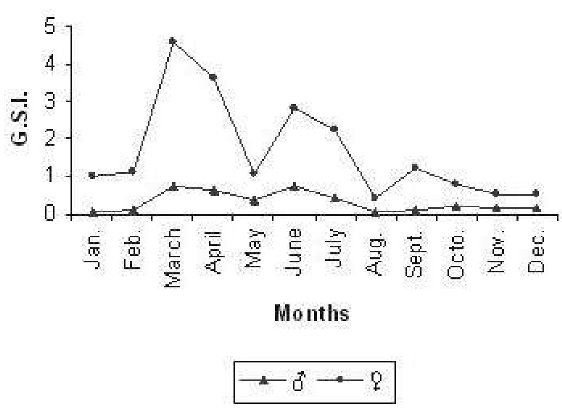


Fig. 4: Monthly variations in G.S.I. for females and males of *O.niloticus* in Abu-Zabal lake

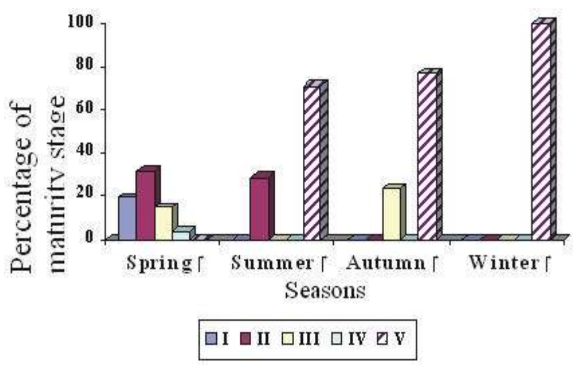


Fig. 5: Seasonal distribution of different stages of maturity in the ovary of *O. niloticus*

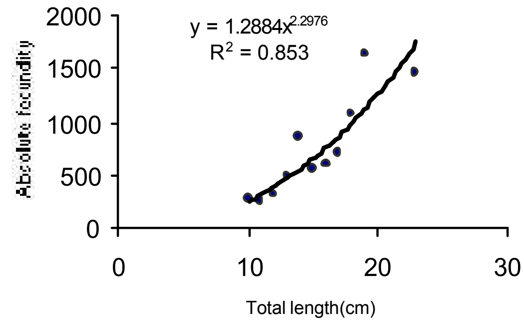


Fig. 6: Relation between fecundity and total length of *O.niloticus*

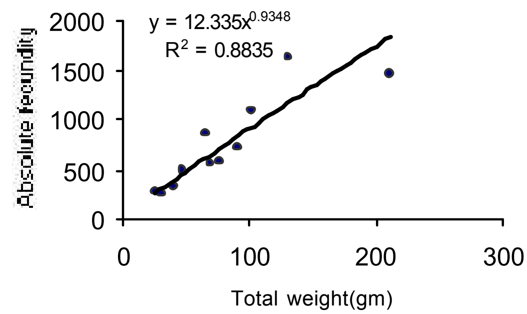


Fig. 7: Relation between fecundity and total weight of *O.niloticus*

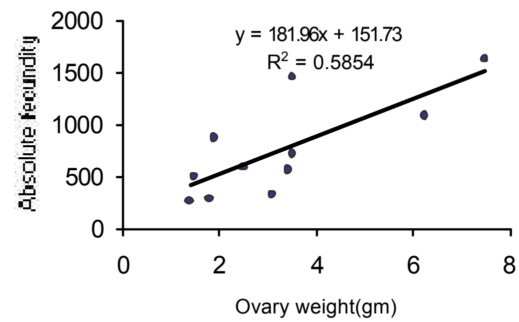


Fig. 8: Relation between fecundity and gonad weight of *O.niloticus*

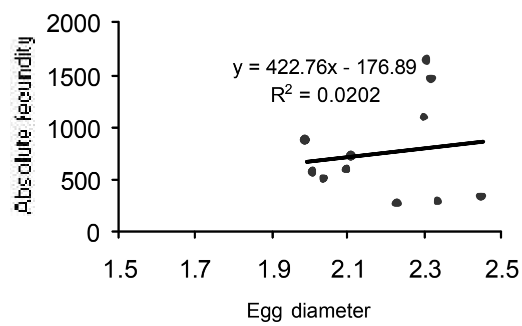
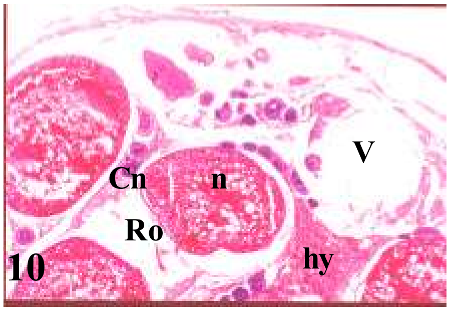
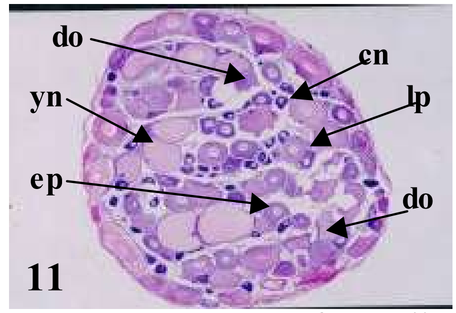


Fig. 9: Relation between fecundity and egg diameter of *O. niloticus*



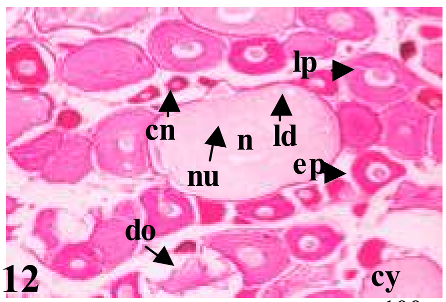
H & E x40

T.S of ovary of *O. niloticus* during spring season showing tertiary yolk stage yolk granules (yg), vacuole (v), hypertrophoied follicular tissue (hy) irregular nucles (in),basophilic wall (w).



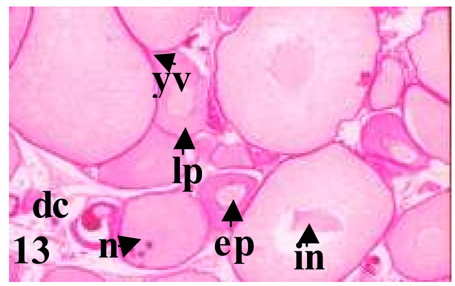
H & E x40

T.S of ovary of *O. niloticus* during summer of Abu Zabal lake showing oocytes in early and late perinucleolus stage (ep&lp) – chromatin nucleus(cn)-some of degenerated oocytes(do).



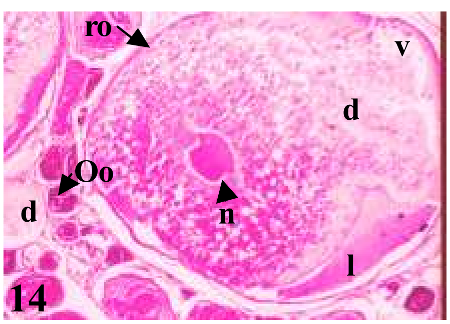
H & E x100

T.S of ovary of *O. niloticu* s of Abu-Zabal lake showing most of oocytes stages (cn), (ep&lp), nucleus (n) , nucluleos (nu), lipid drop (Ld) and degrnerated oocytes (do).



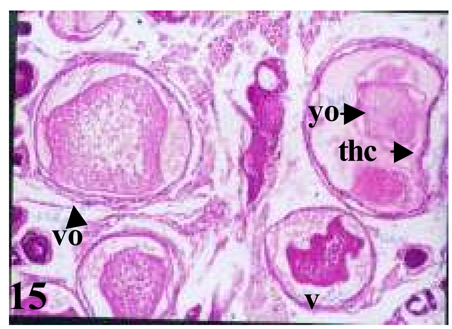
H & E x100

T.S of ovary of *O. niloticus* during Autumn showing : yolk vesicle oocytes (yv) (e&lp) nucleus (n), nucleolus (nu)and degenerated chromatin nucleus (dc). Irregular nucleus (in).



H & E x100

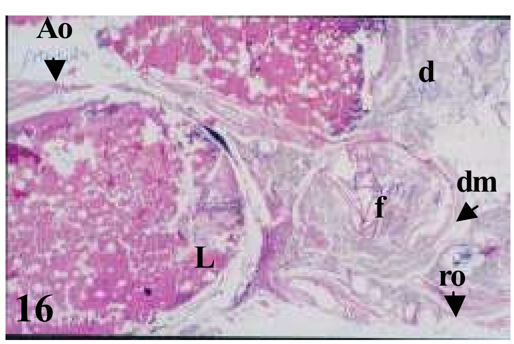
T.S of ovary of *O. niloticus* in winter season showing atretic ova Ao where cytoplasm liquefied (l) vacule (v) degenerated to lipd and protein vesicle (d)



H & E x100

T.S of ovary of *O. niloticus* showing another type of atretic ova in winter season: yolk oocyte (yo) erosion theca (the), vacuole (v).

T.S of ovary of *O. niloticus* Showing type of atresia in winter season.



H & E x40

DISCUSSION

The overall sex-ratio (M: F) for *O. niloticus* in Abu-Zabal lake was 1: 1.37, i.e. females occurrence was relatively higher than males, this means that, males and females are found in different numbers with obvious deviation from the expected ratio (1: 1). This agrees with [10, 23]. The last authors found that sex ratio (male: female) of *O. niloticus* in Coatetelco lake, Mexico was approximately1: 1.02. On the other hand, [24] found that the sex ratio of *Liposarcus multiradiatus* was 2.4 females: 1 male and they attributed this result to less energy input of females towards reproduction and more energy for environmental adaptation. On the other hand, [7] pointed out that, in the African lakes, it is common in the cichlid populations that males dominate because they generally exhibited more growth than females. The observed seasonal variation in sex ratio is probably because once the fertilization of the eggs was completed, males possibly emigrate from spawning areas towards feeding grounds located in shallow part of the lake (where they are captured), while females go towards submerged vegetation and rocky areas to avoid predators (including fishermen) and to carry out the incubation and protection of offspring [6, 10].

Length at first maturity (L_{50}) has a great importance in the determination of optimum mesh size [25].The estimated lengths for females and males of *O. niloticus* in this study were 10.5 and 11.5 cm, respectively, while they are in their first year of life [13].The smallest length recorded in the catch of sample was 10.0 cm, which is slightly less than L_{50}. This means that *O.niloticus* in this region was slightly exploited specially at spawning period, so it is recommended to increase the mesh size used to catch fish for lengths greater than 12.0 cm. In this respect,[23] pointed out that length at maturity was 11.7 cm (males) and 12.0 cm (females) for *O. niloticus* in Coatetelco Lake, Mexico. While, [10] found that these lengthes for *O. niloticus* at Emiliano Zapata dam, Morelos, Mexico were 15.1 and 15.2 cm for females and males, respectively. The size at first maturity in the Nile at Khartoum for males and females *Tilapia nilotica* is 11.4 and 14.3 cm, respectively [26]. In Lake Manzalah, [27] referred that the minimum total lengths of *S. niloticus* were 17.4 and 16.3 cm, for males and females respectively. While, [28] mentioned that the tilapias attain their sexual maturity at three months old with a total length of 8-16 cm. It was cited [9] that the first maturation size for reared Nile tilapia is 30-50 g. These differences arise because the sexual maturity is a function of the size and may be influenced by the abundance and seasonal availability of food, temperature, photoperiod and other environmental factors and different localities. In this respect, [29] reported that fish size at maturity was influenced by the feeding level, which affects the growth.

The highest values of female's HSI of *O. niloticus* in the present study during spring may be due to the favorable levels of hormones relevant to sexual activities, in addition to increased in feeding activity after spawning [30]. In the same time, decreased HSI during other seasons of the year may be due to the poor somatic condition during the spawning season, so that, the somatic growth was limited due to the development of gonad which leads to depletion of body reserves during gonad maturation [31].

The breeding period (G.S.I.) of females of *O. niloticus* in the present study exhibited several peaks in March, April, June and September. This prolonged period indicated that females breed more than once a season i.e. partial spawning character of this species. Spawning of *Tilapia* in Egypt is seasonal owing to the distinct winter period. In the Delta area it may extend during April to November but with a peak during early summer [32].Similarly, [26] pointed out that, there is more than one spawning per year for *O.niloticus*, while, [27] indicated that *S. niloticus* has a relatively restricted spawning season with a peak during April to May. *O.niloticus* can spawn three times a year and therefore three offspring can be produced in the same season [33].

Regarding the seasonal variations in gonad maturity stages, in this study mature stage (III) predominated during spring and autumn and ripe stage (IV) during spring only. Therefore, the breeding season of *O. niloticus* in Abu-Zabal Lake takes place in spring and autumn (twice in the year). This finding is in agreement with [23] who said that the periodicity of spawning for *O. niloticus* at Coatetelco lake spawn at least twice in the breeding season.

The total number of ripe eggs in the ovary (fecundity) in this study ranged between 289-1456 eggs with an egg diameter ranged from 1.99-2.45 mm corresponding to fish total length of 10-23 cm. The fecundity ranged between 104-709 eggs corresponding to fish length of 12.5-20.9 cm, with an egg diameter of 1.0-3.0 mm [23]. It was found that a fecundity ranged from 243-847 eggs per fish (*O.niloticus*) in Zapata dam, Mexico with egg diameter from 300-3 700 μm.Within a given species, fecundity may vary as a result of different adaptations to environmental habitats [35]. Even within a stock, fecundity is known to vary annually, undergo long-term changes [36] and has been shown to be proportional to fish size (and hence, age) and condition. Thus, fish size and condition are key parameters to properly assess fecundity at the population level [2]. It was mentioned [37] that in mouth brooding cichlids, the fecundity is considerably low because the parents assure the survival of the offspring and in consequence less mortality. In addition, the variation in fecundity may be attributed to differential abundance of food within the members of the population. Also, [29] pointed out that fecundity increased with increased feeding levels. The maximum numbers of eggs counted in the ripe ovaries of any mouth-brooding cichlid are 4 300 in a specimen of *T.aurea* with 25 cm of total length. Marked differences in fecundity among species often reflect different reproductive strategies [34]. Within a given species, fecundity may vary as a result of different adaptations to environmental habitats [35]. Moreover, Sendecor [20] mentioned that, fecundity correspond better with temperature variations. In the present study, fecundity variation was better correlated with length ($r = 0.9236$) and weight ($r = 0.9399$) than with gonad weight($r = 0.7651$). In this respect [38] found positive correlation between fecundity and mass and length of plaice *(Pleuronectes platessa* L.), whereas, Sendecor [20] found an inverse correlation between size and number of oocytes produced by females, but Smitherman *et al*. [39] did not found any correlation.

Different development stages of oocytes were found in this work as recorded by [26, 40, 41 and 42]. Oocyte is the building unit in ovary of any species of fish. It undergoes sequential changes named Oogenesis. [42-44] divided the development of oocytes into stages. In the present work, three phases were found; first growth phase included three stages (Chromatin nucleolus, Perinucleolus [early and late stages] and yolk nucleus stag). All the previous stages of development of oocytes were found numerously in maturing and mature ovary stage (II, III). Second growth phase consists of four stages (yolk vesicle, [primary and secondary] yolk and tertiary yolk stage) in mature ovary (III). Third growth phase had one stage (ripe oocyte) characteristic for ripe ovary (IV).

Microscopic examination indicated that there were permanent presences of many different stages of oocytes. This indicated that *O. niloticus* is multispawning fish. This finding was similar to many investigators in this field [40,45,46] .The second author concluded that in *Tilapia nilotica*, different generations of eggs of different size groups are distinguished in the ovary during the different periods of the year.

In the present study, degenerative changes were observed in different stages of development of oocytes as erosion in margin of some oocytes and atretic state in ripe oocytes, these observations were in agreement with several authors[37,47,48,49,50, 51] who attributed the changes of oocytes to many causes, such as a mechanism to regulate fecundity [47] or due to starvation occurred during critical period for gonad growth [37,48,49,50,51] attributed the atretic oocytes to environmental stress.

It could be concluded that the spawning season of *O.niloticus* extended during March to September, with a peak during March, April, June and September. It is recommended to increase the mesh size used in Abu-Zabal lakes to catch *O.niloticus* to lengths greater than 12.0 cm to protect this species from exploitation.

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