World Journal of Fish and Marine Sciences 1 (3): 190-198, 2009
ISSN 1992-0083

Some Aspects of Reproductive Biology with Emphasis on the Effect of Pollution on the Histopathological Structure of Gonads in *Oreochromis niloticus* from Rosetta Branch, Nile River, Egypt

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Abstract: Fish samples of *Oreochromis niloticus* were collected from two main fishing sites on the Rosetta Nile Branch (Kafr El-Zayat and Desok areas), the first area is considered to be more polluted than the later one due to the highly industrial activities. The present work aimed to study some aspect of the reproductive biology of the dominant species (*O. niloticus*) with emphasis on histopathological changes in the gonads. Fish attain the first sexual maturity at length 13.8 cm. The monthly distribution of maturity stages was studied. The gonadosomatic index and the gonads histological examination indicated that, the fish has prolonged spawning activities with a peak value in June. Six stages of gonadal maturation for both sexes were identified. The histopathological changes in gonads due to exposure to different pollutants have been studied. It was concluded that fish exposed to higher concentrations of pollutants showed higher incidence of gonadal abnormalities in the form of deformed oocyte and spermatocyte with reduction in their numbers and lack of active oogensis and spermatogenesis.

Keywords: Nile River % Rosetta branch % *Orecohromis niloticus* % Gonadosomatic Index % Reproduction % Gonads maturation % Histopathology

INTRODUCTION

Rosetta branch is one of the two main branches of the Nile River (Fig. 1). It is about 220 km in length with an average width of 180 m. and depth varying between 2 and 4 m [1]. Rosetta branch water serves for a wide range of functions including agricultural, industrial and domestic water supply, fisheries and recreation. Industrial activities have been accused as being the major source of water pollution in this area [2]. Water pollution affects the ecological conditions which usually affect the biological and physiological conditions of the fish, especially reproduction, which is reported to be affected seriously [3-5].

The metal industry contributes almost 50% of the total wastewater discharges [6]. Other industrial plants constructed at Kafr El-Zayat city on the banks of the branch directly pour their effluents into the branch without any treatment where high values of water turbidity and also high values of Biochemical oxygen demand (BOD), phosphates and total dissolved solids (TDS) were recorded [7]. Daifullah e*t al.* [8] stated that, the concentrations of some metals (Iron, manganese, zinc, copper, lead and cadmium) are higher than the permissible levels in this area. Cichlid species are the most common fish group (96.99%) in the commercial catch of the Rosetta branch of the Nile River during the year 2006.

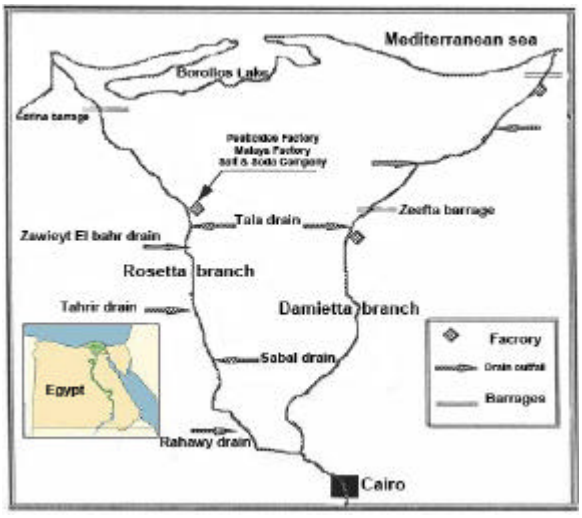

Fig. 1: Rosetta branch and its drain locations (Source of polluted water)

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The landed catch was represented mainly by *Oreochromis niloticus* (51.29%).

The present work aimed to study some aspect of the reproductive biology of the dominant species (*O. niloticus*) with emphasis on histopathological changes in the gonads.

MATERIALS AND METHODS

In order to study the effects of pollution on fish reproduction, samples were collected from two main fishing sites on Rosetta branch namely Kafr El-Zayat and Desok areas during the period from January 2006 to January 2007, the first area is considered to be more polluted than the later one [8].

O. niloticus were identified and sexed for morphological evaluation of gonadal maturation. The Hjort [9] scale which was further developed by Sakun and Butskaya [10] was used in this respect. A total of 575 gonads of both sexes of *O. niloticus* were weighed to the nearest milligram. The gonadosomatic index was calculated for all the specimens as the percentage of gonad weight to the gutted fish weight. Gonadosomatic index = [gonad weight (g) / gutted weight (g)] X 100.

Gonads were prepared for the histological examination by fixing in 10% neutralized formalin solution. Paraffin embedding was carried out after dehydration in ascending series of ethyl alcohol, sections ranging from 3 to 8 µ thickness were stained with eosin and iron alum hematoxylin then mounted with Canada balsam. They were then examined by a light microscope (Leitz), Microphotographs with an Olympus CX41 digital camera were taken. Oogenesis was identified according to Hilge [11].

RESULTS

The results of this study indicate that, there is no difference in the length at first sexual maturity and monthly distribution of maturity stages of *O. niloticus* in the two areas under study.

Length and Age at First Sexual Maturity: In the present study, it was found that fish with lengths smaller than 11.9 cm were always immature. The frequency of mature individuals increased with increasing fish lengths where all fish over 15.2 cm were mature. Results showed also that fishes attained their first sexual maturity at 13.8 cm. When referring this length to the corresponding age; it was found that, *O. niloticus* should pass their first year of life before attaining sexual maturity.

Monthly Distribution of Maturity Stages: The monthly variations of maturity stages of *O. niloticus* were represented in Table 1. It is obvious that, immature stages (I and II) are present throughout all months of the year with a peak in January (65.85%). This species reaches stage III in December and reaches the maximum percentage of this stage in February (64.71%). The spawning period (stage V) extends from May to September with a peak in June (54.84%), while spent stage was found through the months from July to November with a peak in August (47.46%).

Gonadosomatic Index (G.S.I.): The monthly variations of gonadosomatic index of *O. niloticus* are shown in Figure 2. It is clear that, the higher values of G.S.I. appear during the period from May to November with two peaks, the highest one in June (3.16) and the smallest one in October (2.19).

Table 1: The percentage distribution of different maturity stages of *O. niloticus* in Rosetta Branch, Nile River

Month	No. of fish	immature	III	IV	V	VI
Jan.	41	27 (65.85)	14 (34.15)	-	-	-
Feb.	51	18 (35.29)	33 (64.71)	-	-	-
Mar.	52	16 (30.77)	24 (46.15)	12 (23.08)	-	-
Apr.	49	15 (30.61)	14 (28.57)	20 (40.82)	-	-
May	65	11 (16.92)	9 (13.85)	32 (49.23)	13 (20.00)	-
Jun.	62	10 (16.13)	-	18 (29.03)	34 (54.84)	-
Jul.	56	11 (19.64)	-	13 (23.21)	22 (39.29)	10 (17.86)
Aug.	59	10 (16.95)	-	8 (13.56)	13 (22.03)	28 (47.46)
Sep.	43	16 (37.21)	-	-	9 (20.93)	18 (41.86)
Oct.	34	20 (58.82)	-	-	-	14 (41.18)
Nov.	34	22 (64.71)	-	-	-	12 (35.29)
Dec.	29	19 (65.52)	10 (34.48)	-	-	-
Tot.	575	195	104	103	91	82

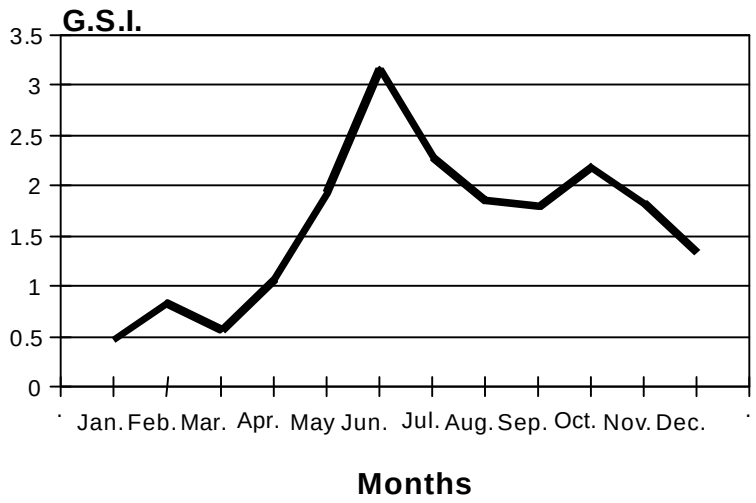

Fig. 2: Gonadosomatic index of *O. niloticus* in Desok and Kafr El Zayat areas

Histological Studies: Histological examinations were made for gonads of both sexes of *O. niloticus* from both areas (Desok and Kafr El Zayat). It appeared that, there are several spermatogenic stages, that could be identified in the testis; namely spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The relative abundance of each kind characterizes each stage of maturity.

Testis of *O. niloticus* consists of seminiferous lobules, interspersed in the connective stroma, radiating from the longitudinal main sperm duct towards the testicular periphery (Fig. 3). Spermatogonia are single cells distributed all along the germinal epithelium and showed a roundish nucleus with an eccentric basophilic nucleolus and a slightly acidophilic cytoplasm.

Primary spermatocytes and secondary spermatocytes characterized by a heterochromatic nucleus whose appearance varied according to the different phases of the meiosis. Spermatids are characterized by having a strongly basophilic nucleus, which condenses progressively and became indented. The appearance of spermatozoa indicates the accomplishment of the period of spermatogensis (Fig. 4). Spermatozoa are flagellated cells with an oval head intensely stained with haematoxylin. Mature spermatozoa are then released into the tubule lumen after the breakdown of cysts (Fig. 5). Spent stage testis reduces its volume dramatically, residual spermatozoa occupy a high percentage of the testis and a few spermatogonia are observed (Fig. 6).

The histological examination of the developing ovaries of *O. niloticus* showed that, in early stage of maturation the ovary is mainly composed of clusters of small cells (oogonia). The oogonium has a thin indistinct peripheral zone of mild basophilic cytoplasm and a central large nucleolus. Oogonia are increased in size and number with a basophilic cytoplasm, the nucleus enlarged in size and having one or two nucleolii (Fig. 7). This stage is called the chromatin nucleolus stage.

The perinucleolar oocytes are enlarged in size with undifferentiated membrane, the nucleoli increased in number and become close to the nuclear membrane. Few small vacuoles appear in the cytoplasmic mass and arranged near the periphery of the oocyte (Fig. 8).

On further maturation, the ovaries are entering the yolk vesicle stage; this stage is characterized by the deposition of yolk vesicles and fat globules which increase the size of maturing egg. The oocyte membrane at this stage of maturation became well developed. The chorion is composed of a thin outer layer (Theca layer), followed by a cellular layer of epithelial follicle (Granulosa) and the most internal non cellular layer (Zona radiata) (Fig. 9). The cytoplasm loses its basophilic nature and became fully occupied with yolk granules. Besides, the yolk vesicles (Cortical alveoli) are noticed in the periphery of oocyte and around the nucleus (Fig. 10).

At the vitellogenic stage the vacuoles become connected to each other and the yolk globules become hydrated and homogenate. The nucleus began to liberate its substances into the cytoplasm, the membrane becomes obviously well developed and the nucleus starts to migrate to the animal pole (Fig. 11).

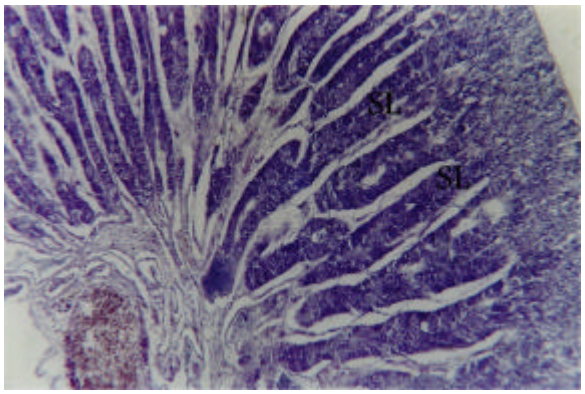

Fig. 3: Seminiferous lobules (SL) radiating from the main sperm duct towards the testicular periphery (40X)

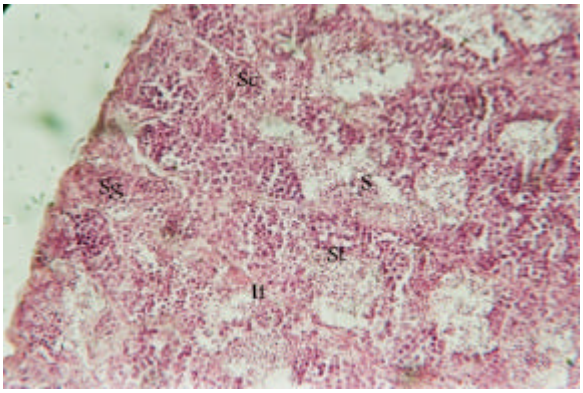

Fig. 4: S. lobules filled with spermatogonia (Sg), spermatocytes (Sc), spermatids (St), spermatozoa (S) and thin interstitial tissue (It) (40X)

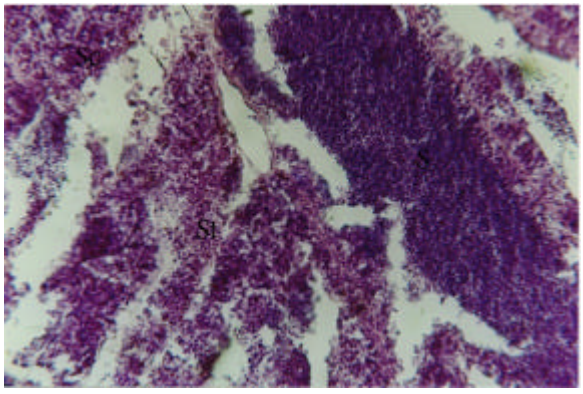

Fig. 5: Ripe testis showing spermatocytes (Sc), spermatids (St) and spermatozoa (S) which released into the tubule lumen (40X)

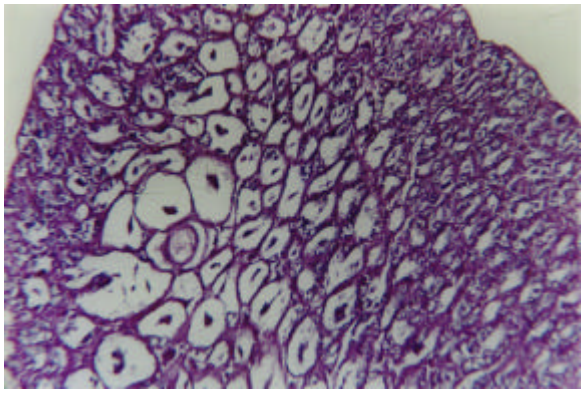

Fig. 6: Spent stage showing the residual spermatozoa and a few spermatogonia (20X)

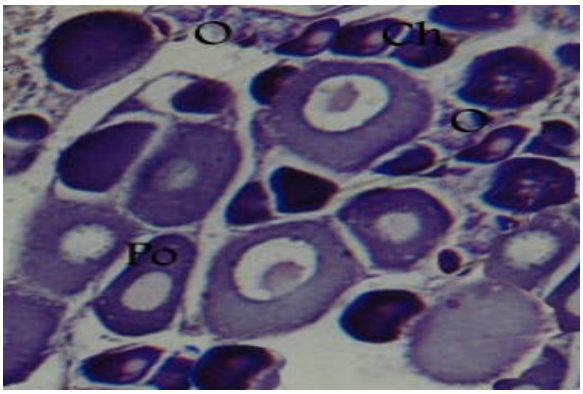

Fig. 7: Small groups of oogonia (O), chromatin nucleolar oocytes (Ch) and perinucleolar oocytes (Po) (40X)

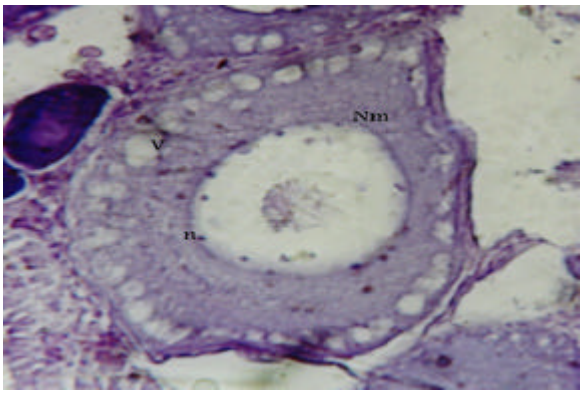

Fig. 8: Few nucleolii (n) close to the nuclear membrane (Nm) and a ring of vacuoles (V) near the oocyte periphery (40X)

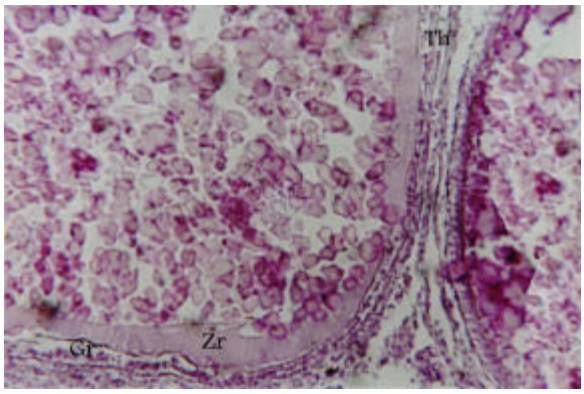

Fig. 9: Well developed oocyte membrane thecal cell (Th), granulose (Gn) and zona radiata (Zn) (40X)

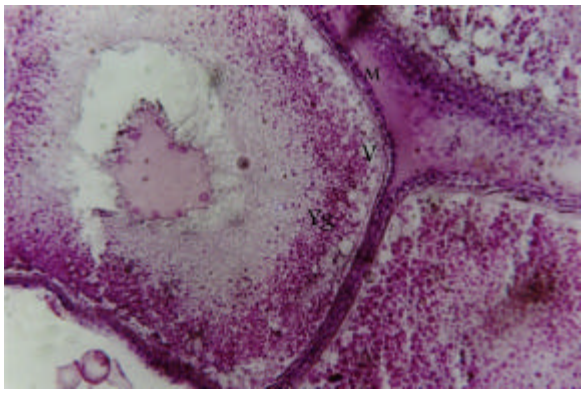

Fig. 10: Yolk vesicle oocyte showing a ring of yolk granules (Yg) and vacuoles (V) near the oocyte membrane (M) (40X)

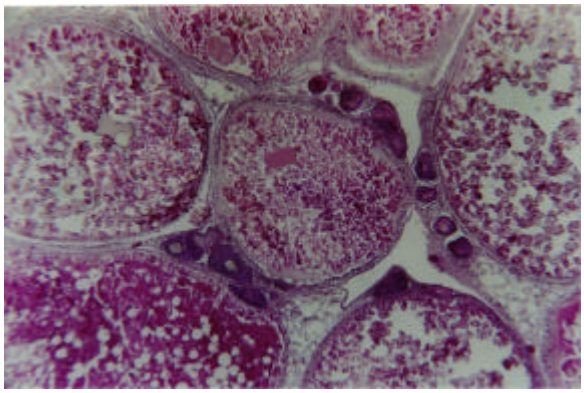

Fig. 11: Vitellogenic oocyte showing the start of the nucleus migration to the animal pole (20X)

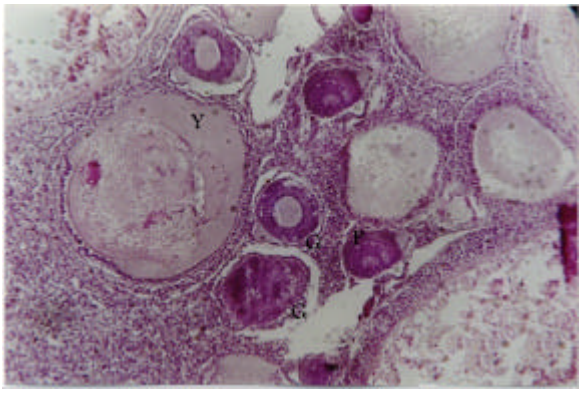

Fig. 12: Liquefied yolk (Y) in ripe oocyte, degeneration changes of perinucleolar stage (P), corrigation of their membranes (G) (40X)

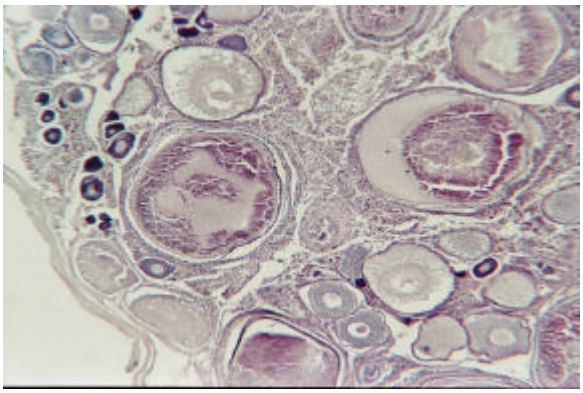

Fig. 13: Oocyte in spent stage showing different forms of atretic oocytes with breaking down of zona radiata (20X)

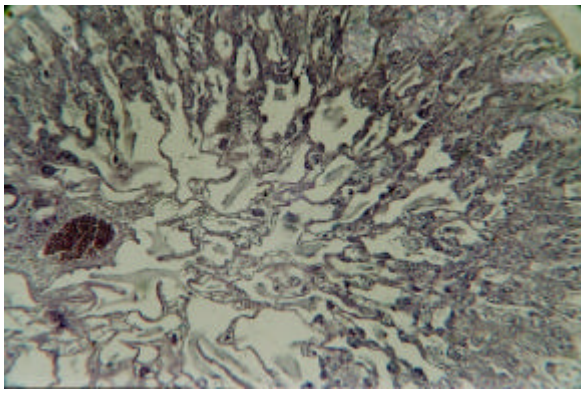

Fig. 14: Degenerative and necrotic changes in the cellular elements of S. lobules and focal areas of necrosis (20X)

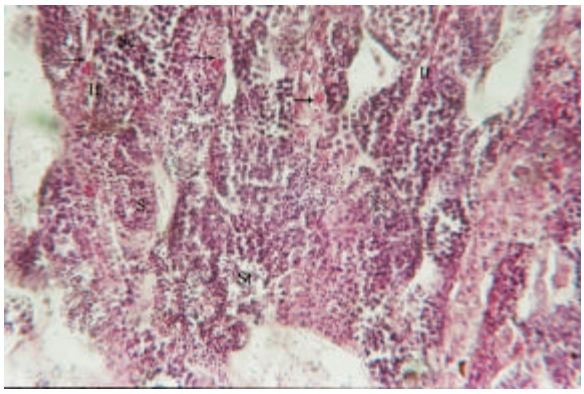

Fig. 15: Spermatocytes (Sc), spermatids (St), spermatozoa (S), disrupted testicular tissue with fibrous interstitial connective tissue (It) and vacuolated leyding cell (6) (40X)

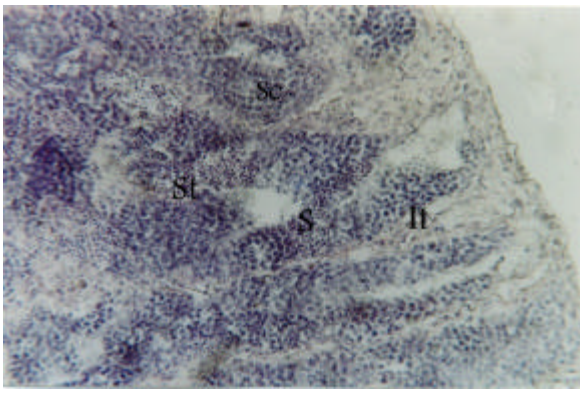

Fig. 16: S. lobules filled with spermatocytes (Sc), spermatids (St), spermatozoa (S) and degeneration in lumen with thin interstitial tissue (It) and edema (40X)

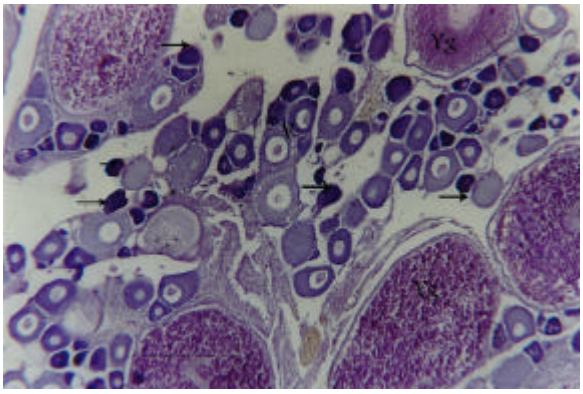

Fig. 17: Oocyte in yolk vesicle stage showing yolk granules (Yg), vacuoles (V) and groups of destroyed oocyte (6) (20X)

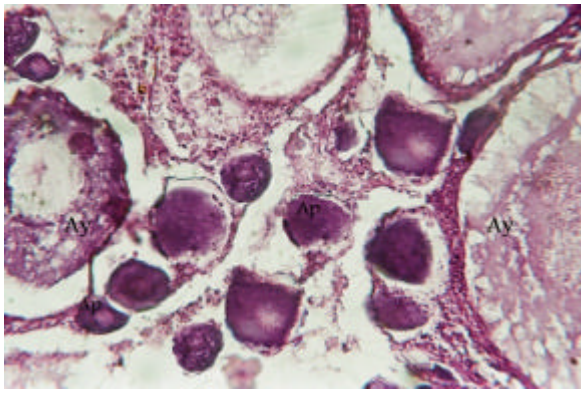

Fig. 18: Atretic perinucleolus oocytes (Ap) as a solid mass and atretic yolk vesicle oocytes (Ay) (40X)

The nucleus in ripe egg consists entirely of the cytoplasmic inclusions and appears near the animal pole, liquefied yolk material is distributed throughout the cytosome and fat globules began to disappear (Fig. 12). In the spent stage a few atretic oocytes of the remaining vitellogenic ova after spawning become present. Atretic oocytes are characterized by breaking down zona radiata and proliferation of granulosa layer which invade the dead ova and vacant spaces in the ovary (Fig. 13).

The Histopathological Examination: From the histopathological point of view, examinations of the testis of *O. niloticus* (Mostly in Kafr El Zayat area) indicated severe degenerative, collapsing and necrotic changes in both the wall and the cellular elements of the seminiferous tubules with focal areas of fibrosis. The seminiferous lobules appear with a lesser number of sperms or lucent indicating lack of active spermatogenesis (Fig. 14).

The deformed testis showed tissue necrosis, congestion of capillaries and vacuolated leyding cells (Fig.15), it also shows disrupted testicular tissue with spacious fibrous interstitial connective tissue with fibrobalsts and edema (Fig. 16).

Females of *O. niloticus* which were collected from Kafr El Zayat area appeared to have ovaries with vitellogenic and ripe ova. Ovaries which are exposed to higher concentrations of pollutants showed different histological forms (Fig. 17), the tunica albuginea is loose and the oogonia are destroyed. The cytoplasm of early and late perinucleolus oocytes stained deep purple. The perinucleolus oocytes appeared as a solid mass, also late perinucleolus oocytes become atretic (Fig. 18).

Corrigation of the perinuclear ova membranes and vacuolation of the vitellogenic ova was observed (Fig.12); these deformed cells cause damage of membrane layers.

DISCUSION

The length at first sexual maturity means the length at which 50% of all fish at that length are mature [12]. Age and size at first maturity of tilapia are affected by environmental conditions [13, 14], overfishing [15], food supply [16-18] and water body area [19]. This study revealed that, *O. niloticus* fishes in Rosetta branch attained their first sexual maturity at 13.8 cm. On the other hand, Mehanna [20] found that lengths at first sexual maturity of *O. niloticus* in Wadi El Raiyan lakes to be 28.9 cm (2.57 years old) in the first lake which has an area of about 63 Km^2 and 17.8 cm (1.51 years old) in the second lake which has an area of about 110 Km^2.

The study of the gonadosomatic index in different months gives a good indication of the spawning season of the species [21-27]. *O. niloticus* has prolonged spawning activities with a peak in June; these results are in accordance with the results of Payne and Collinson [28], Bayoumi and khalil [29] and El Shazly [26].

Six stages of gonads maturation for both sexes of *O. niloticus* were identified, many authors described these maturity stages such as Mahmoud [30], Mazrouh [31], Coward and Bromage [32], Assem and El Zaeem [33], Morrison *et al.* [34] and Gaber [35, 36].

The different pollutants such as industrial and agriculture wastes, pesticides and also different types of bacteria have histopathological effects on the reproductive tissues of fish gonads [37, 38], these effects may disturb the development of germ cells and may reduce the ability of fish to reproduce, while metal accumulation occurring in the testis, affects the process of spermatogenesis and suppressing sperm production [39-42].

The histopathological effects of different pollutants on gill, liver, blood, brain, gonads and other organs have been studied by many investigators [43, 44], they mentioned that, the perinucleolus oocytes appeared as a solid mass and later on perinucleolus oocytes become atretic. These lesions may be attributed to the direct or indirect effect of high value of pH and high concentrations of nutrients and heavy metals in the effluent. Kumar and Pant [45] found that 2-4 month exposures of an Indian teleost to copper, zinc, or lead caused disappearance of oocytes in the ovaries.

In the present study higher incidence of oocyte atresia was found in the area of Kafr El Zayat more than Desok area. Jobling *et al*. [46] stated that, atresia was recorded in roach living in rivers that receive treated sewage effluents. Johnson *et al*. [47] reported that, atersia is thought to be an uncommon event in healthy females and it has been linked to poor nutrition, environmental stress and starvation.

Abou Shabana *et al.* [48] cited data indicating that, heavy metals and pesticides which are present in industrial waste water are the main reason of reproductive impairment occurring in the entire lakes and fresh water canals exposed to these effluents.

Reduced number of oocytes with large follicular spaces and pycnotic nuclei of perinucleolus stages are observed in many ovaries of *O. niloticus*, these observations coincides with those recorded by Kamel [49]. He attributed these alterations to environmental conditions, especially temperature. Severe damage of gonadal tissue occurred by infiltration of blood tissue and its necrosis.

From this study, it could be concluded that deformed and infected gonads of *O. niloticus* collected from Kafr El Zayat area were found in a higher percentage than those of Desok area. These results come from the highly presence of different types of pollutants and heavy metals in the water of Kafr El Zayat area.

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