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Gonadal Maturation and Histological Observations of *Epinephelus areolatus* and *Lethrinus nebulosus* in Halaieb/Shalatien Area "Red Sea", Egypt

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Abstract: *Epinephelus areolatus* and *Lethrinus nebulosus* are the highly abundant species in the two dominant families (Serranidae and Lethrinidae) in Halaieb/Shalatien area. The objective of the present study is to describe the phases of gonadal development and determine the spawning season of the two mentioned species. Results showed that *E. areolatus* attains first sexual maturity at 22.8 cm (Total Length) for males and 23.5 cm for females while *L. nebulosus* attains it at 25.1 cm for males and 26.2 cm for females both after their second year of life. The gonadosomatic index indicated that, the spawning season of *E. areolatus* was in May and June and it was in June and July for *L. nebulosus.* The different stages of oogenetic development were examined microscopically. It was concluded that, six stages for oocyte developments of the two species under study were identified.

Key words: Red Sea · Halaieb · Shalatien · Oogenesis · Gonadosomatic Index · *E. areolatus* · *L. nebulosus*

INTRODUCTION

Halaieb/Shalatien area is situated in the Southern region of the Egyptian Red Sea coast extending from latitude 22° to 23°8' N (Fig. 1). The dominant fish families in the area of study are Serranidae and Lethrinidae [1]. It is known that developing eggs pass through a series of cytological stages collectively referred to as "Oogenesis" starting from the mother cells or oogonia until the degree of ripeness. A thorough study of gonad morphology, anatomy and histology (which are essential to understand the features of the reproductive biology) is required for proper management of the fishery. In the present study, oogensis of the most dominant economic species *Epinephelus areolatus* and *Lethrinus nebulosus* among the mentioned families were described.

Fig. 1: The study area (Halaieb / Shalatien area)

MATERIALS AND METHODS

Fish samples were collected monthly from the city of Shalatien landing center which is considered as the main landing center in the area of study. Gutted weights to the nearest gram and gonads weights to the nearest milligram of both sexes of the two species under study were recorded for a total of 432 fish. The gonadosomatic index was calculated for all the specimens as a percentage of gonad weight to the gutted fish weight as shown in following equation:

$$\text{Gonadosomatic index} = [\text{gonad weight (gm)} / \text{gutted weight (gm)}] \times 100$$

Gonads were prepared for the histological examination by fixing the gonads in buffered formaldehyde. They were dehydrated in graded alcohol series, exposed to Xylol and embedded in paraffin wax. Sections from 4 to 7 µm thick were prepared from the middle parts of the gonads. The sections were stained with Hematoxilin and Eosin [2], then mounted in Canada balsam and photographed with an Olympus CX41 digital

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camera. Six stages for oocyte development of the two species under study were identified by microscopic examination according to Hilge [3].

RESULTS

Length and Age at First Sexual Maturity: The age at first sexual maturity is the age at which 50% of all fish at this age are mature. For *E. areolatus,* it was found that, fish lengths smaller than 20.2 cm are always immature and all fish over 24.7 cm are mature. Fish belonging to this species attains their first sexual maturity at 22.8 cm for males and 23.5 cm for females. Referring this length to the corresponding age; it was found that *E. areolatus* attains the first sexual maturity after the second year of life for males and females. While *L. nebulosus* were found to be immature at lengths smaller than 22.5 cm and they are always mature at lengths above 26.8 cm. Fishes of *L. nebulosus* attains their first sexual maturity at 25.1 cm for males and 26.2 cm for females, these lengths correspond to age 2.1 years and 2.2 years for males and females, respectively.

Gonadosomatic Index: Gonadosomatic index (G.S.I.) is used to define the gonadal development of *E. areolatus* and *L. nebulosus*. There were considerable differences between the G.S.I. values of males and females of both species. The index value of females was mostly higher than males, especially during and by the end of the spawning periods of both species (Fig. 2, a and b).

By studying the G.S.I. value of *E. areolatus,* it was found that, the minimum values of G.S.I were detected during December (0.08 for males and 0.14 for females). These values represent the quiescent period of this species. The high values of G.S.I. were extending from late March to July for males and from April to August for females, the spawning season was in May and June for both sexes of *E. areolatus* with a peak in May (1.86 for male and 2.43 for female).

In *L. nebulosus* the high values of G.S.I. appeared from April to August for males and from May to September for females. The spawning season was in June and July for both sexes of *L. nebulosus* with a peak in June (2.95 for male and 4.04 for female).

Microscopic Characteristics of Gonads: There were several stages of oogenetic development based on specific cytological changes as follows:

Oogonia and Chromatin Nucleolar Stage: The ovary is composed of clusters of oogonia, oocytes in chromatin nucleolus stage which were considered as a stock for further formation of ovarian sexual cells and perinucleolus oocytes (Plates 1 and 13). Oogonia are the smallest germ cells with no distinct boundaries, they found solitary or in clusters of lamellae close to the germinal epithelium and they have a large light nucleus and weakly basophilic cytoplasm (Plates 2 and 14). The diameter of the oogonium measures from 8 to 14µm in the two species under study.

The chromatin nucleolus stage oocytes are small spherical cells with a thin indistinct peripheral zone "Primordial follicle", the cytoplasm is strongly basophilic which accepts all the given stains, the nucleus is spherical and large, which occupies the greater part of the cell with one nucleolus and encapsulated by a delicate fibrous

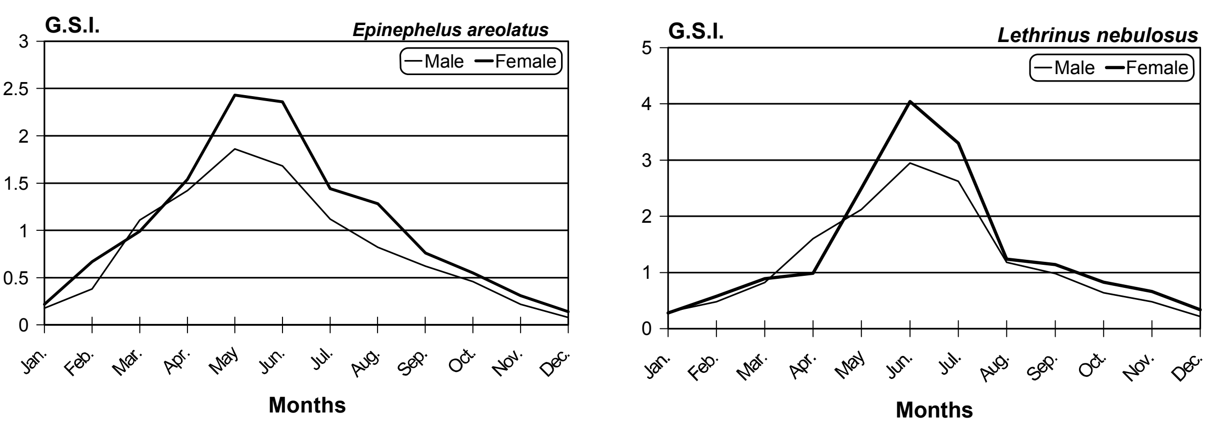

Fig. 2: Gonadosomatic index of *E. areolatus* and *L. nebulosus* in Halaieb/Shalatien area Red Sea

connective tissue (Plates 2 and 14). The average diameters of the oocytes in this stage of maturation measures about 36 µm in *E. areolatus* and 27µm in *L. nebulosus*.

In further development, more than one strongly basophilic nucleolies are frequently present; some of them can be seen to cross the nuclear envelope (Plate. 14). These nucleoli extrusions finally disintegrate and disappear in the ooplasm.

Perinucleolar Stage: In perinucleolar stage, the ovary is composed of nests of oocytes of chromatin nucleolus stage and perinucleolar stage of different sizes which are mostly polygonal and increases in cytoplasmic mass and nuclear volume together. The nucleoli appear smaller, increase in number and closer to the nucleus membrane and the cytoplasm became less basophilic.

The outer layer of follicular epithelial cells surrounding the perinucleolar stage oocyte is thickened but still undifferentiated. Oocytes attained a higher growth of protoplasm (with averages diameter of about 92µm. in *E. areolatus* and 70µm. in *L. nebulosus*). It is noticed that, *E. areolatus* has a larger average oocytes diameter than *L. nebulosus* and it has a larger average nucleus diameter too which may reach 60µm, while it is 31µm. in *L. nebulosus.* The late perinucleolus oocytes are characterized by the appearance of one or a few small vacuoles in the cytoplasmic mass (Plates 3 and 15), these vacuoles start to arrange themselves around the nucleus in more advanced stages of maturity.

Yolk Vesicle (Cortical Alveoli) Stage: The size of ovaries in this period is enlarged owing to the deposition of trophic substance (yolk and fat). This stage was found from March to the biginning of April for *E. areolatus* and from March to the beginning of May in *L. nebulosus.*

The cytoplasm becomes weekly basophilic nature and the nucleus still occupies a central position and it contains a lot of nucleoli attached to the nuclear membrane.

Yolk vesicles (Cortical alveoli) are increased in number and size and arranged in two distinct layers, one of them is close to the nuclear membrane and the other is near the oocyte membrane (Plates 4 and 16). Yolk globules formed in this stage were mostly oval rounded and intensively stained.

Oil or lipid globules begin to accumulate in the cytoplasm of oocytes, the content of these lipid globules or droplets are dissolved during dehydration with different solvents used and appear empty with conventional staining.

The outer membrane of the oocytes becomes thicker (an average of 11µm. for *E. areolatus* and 10µm. of *L. nebulosus*) in this stage and composed of a noncellular inner layer called zona radiate, surrounded by zona granuloza (Cuboidal epithelial cells) and a thin external layer (Follicular layer or thecal cells) as shown in plates 5 and 17.

At the end of this stage the oocytes almost reached their definite size with average about 300µm. in *E. areolatus* and 262µm. in *L. nebulosus*. Vacuoles reach a bigger size too; they fill the whole cytoplasm together with the growing yolk particles (Plates 6 and 18).

Vitellogenic (Yolk) Stage: During this stage, the ovary is filled mainly with previtellogenic and vitellogenic oocytes in different stages of yolk deposition. Most of the vacuoles in this stage were connected to each other and formed spaces between the yolk granules in the cytoplasm. The deposition of yolk granules that contain lipoprotein appear at the marginal regions of the maturing oocytes and then spread until the entire central cytoplasm of the oocytes.

Cortical alveoli began to be liquefied, with a concomitant increase in size. As a result of this, the yolk homogeneity started in the periphery of the oocytes. The nucleus start to migrate to the animal pole (Plates 7 and 19).

With continuous growth the oocyte membrane became well developed with average diameter of 12.5µm in the two species under study, zona radiata was found of two different layers, an internal spongy thick layer followed by a very thin external noncellular homogeneous layer. A thick layer of well-developed epithelial follicle with very prominent large cubic cells surrounds these two layers externally. The most external layer of connective tissues still a thin layer and can be detected (Plates 8 and 20).

Ripe Stage: This stage is recognized by the nucleus movement which migrates toward the animal pole (Plates 9 and 21). Nucleoli migrate towards the center of the nucleus away from the nuclear membrane which loses its circularity and stiffness being a winding weak membrane in the way of disintegration completely. During the migration of nucleus it began to liberate it's substances into the cytoplasm. The average of oocytes diameters in this stage is about 344µm. in *E. areolatus* and 320µm. in *L. nebulosus*. The ripe ova appeared in an oval and frequently pearl like shape. As ovulation progresses, the

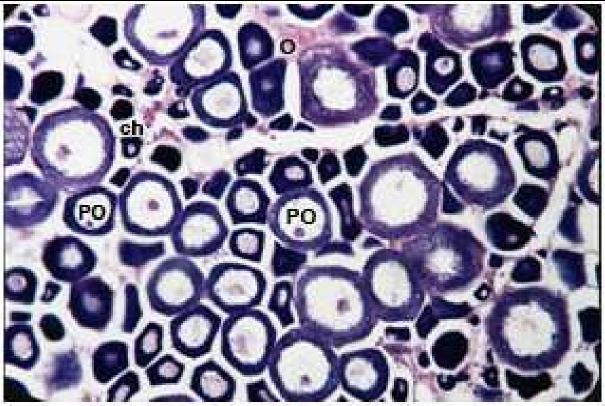

Plate 1: Transverse section of the ovary of *E.areolatus* showing Oogonia (O), chromatin nucleolus (ch) and perinucleolus oocytes (PO) (X200).

Plate 2: Small groups of Oogonia (O) and chromatin nucleolus oocytes (ch) in the ovary of *E. areolatus* (X1000).

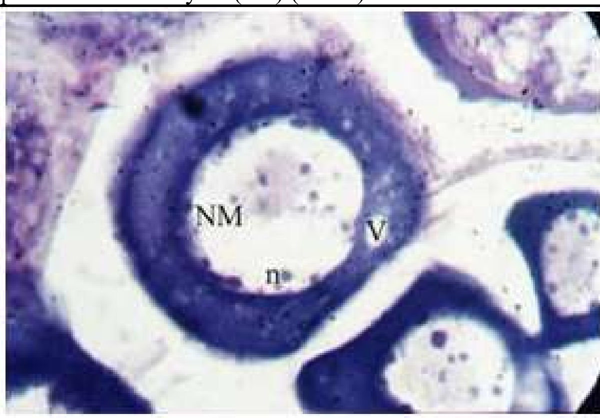

Plate 3: Small nucleolus (n) near to the nucleus membrane (NM) with appearance of vacuoles (V) in the perinucleolus oocytes of *E. areolatus* (X1000).

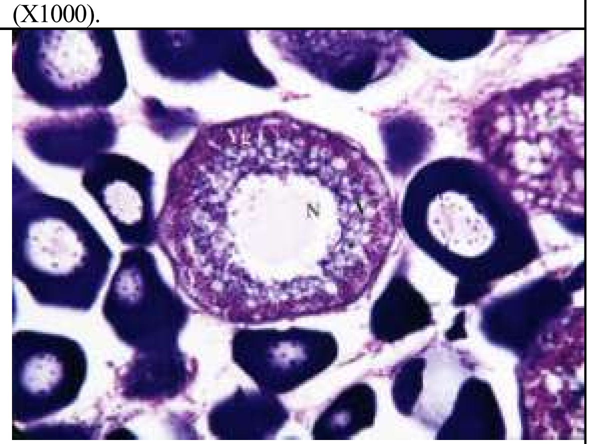

Plate 4: Yolk vesicle oocyte with central nucleus (N), rings of vacuoles (V) and a periphery ring of yolk granules (Yg) in *E. areolatus* (X400).

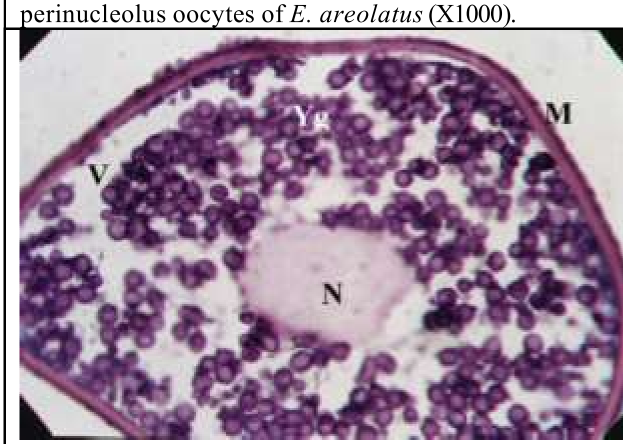

Plate 5: Yolk vesicle oocyte of *E. areolatus* showing the well developed membrane (M), oval rounded yolk globules (Yg), large connected vacuoles (V) and central nucleus (N) (X400).

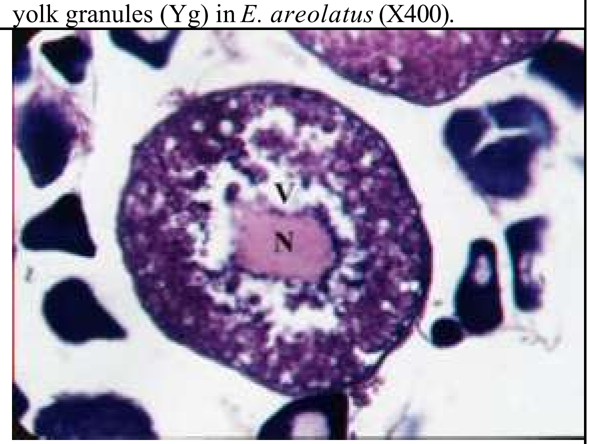

Plate 6: A ring of large connected vacuoles (V) around the nucleus (N) in a Yolk vesicle oocyte of *E. areolatus* (X400).

Plates (1-6): Light micrographs in the ovaries of females of *Epinephelus areolatus* and *Lethrinus nebulosus* in Halaieb / Shalatien area

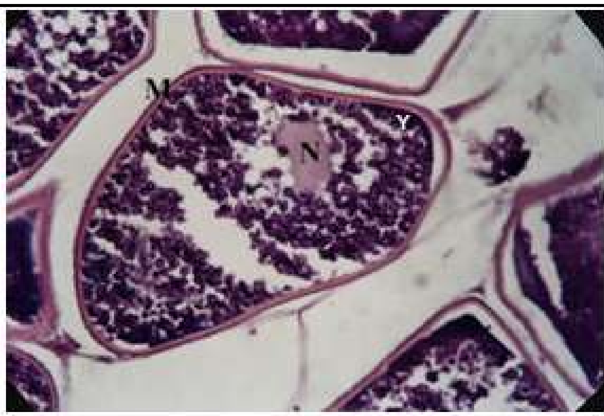

Plate 7: Start of nucleus (N) migration and start of yolk liquefaction (Y) in a vitellogenic oocyte with developed membrane (M) of *E. areolatus* (X200).

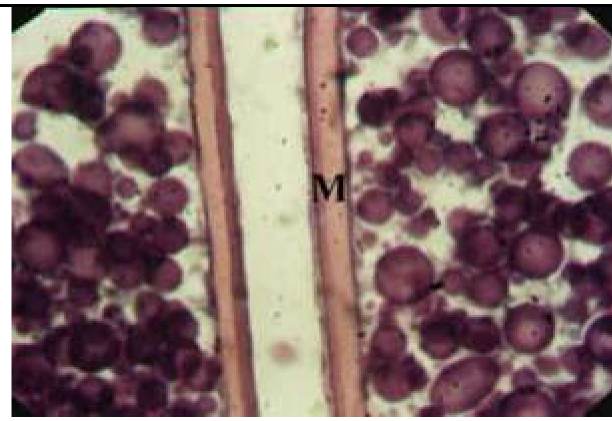

Plate 8: Developed vitellogenic oocyte membrane (M) in *E. areolatus* (X1000).

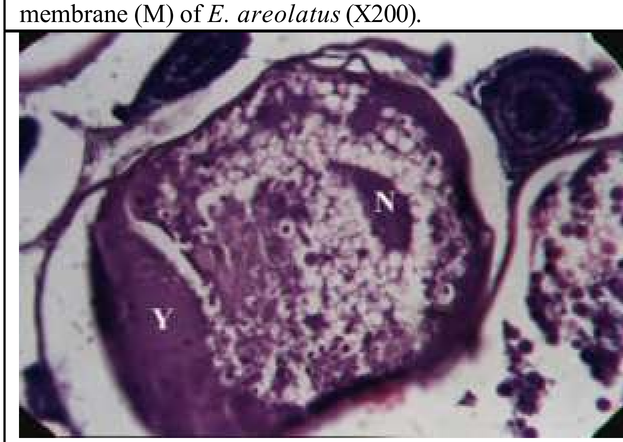

Plate 9: Ripe oocyte of *E. areolatus* shows yolk (Y) liquefaction and the nucleus (N) loses its circularity (X400).

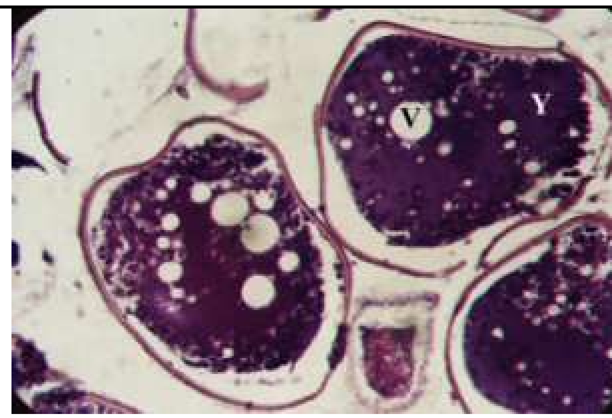

Plate 10: Ripe oocyte shows the liquefaction of the yolk sphere (Y) with large vacuoles (V) of *E. areolatus* (X200).

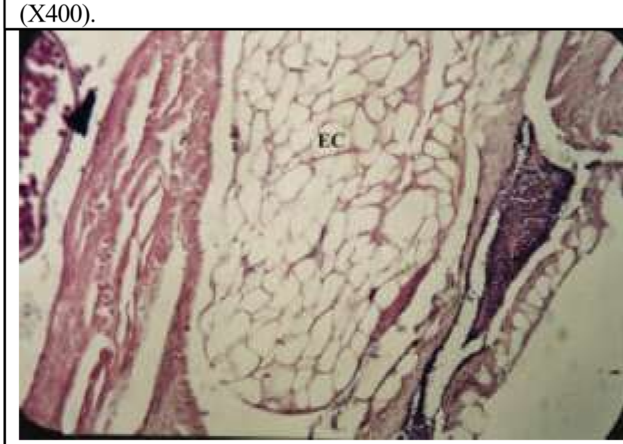

Plate 11: Large numbers of empty nests (EC) in post spawning period of *E. areolatus* (X200).

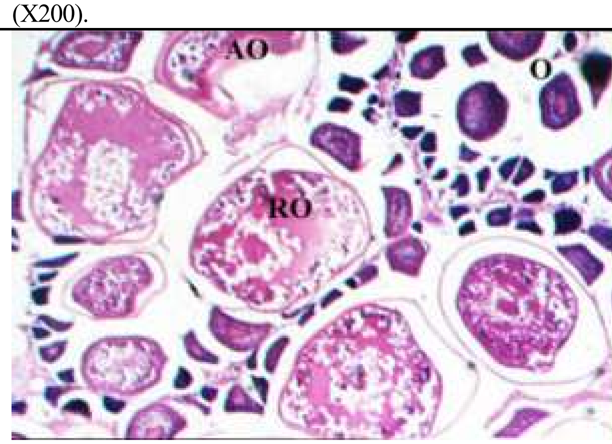

Plate 12: Spent period shows ripe oocytes (RO) atretic oocyte (AO) and few numbers of oocytes in different maturity stages of *E. areolatus* (X200).

Plates (7-12): Light micrographs in the ovaries of females of *Epinephelus areolatus* and *Lethrinus nebulosus* in Halaieb / Shalatien area

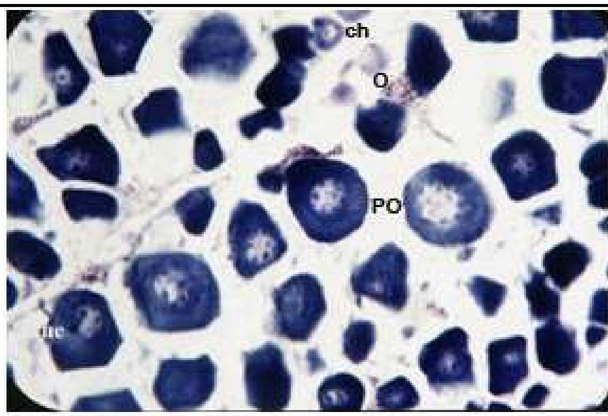

Plate 13: Large number of Oogonia (O), chromatin nucleolus (ch) with nucleoli extrusions (ne) and perinucleolus oocytes (PO) in the ovary of *L. nebulosus* (X400).

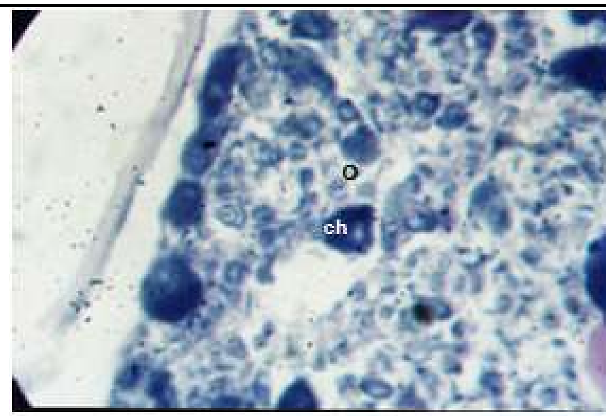

Plate 14: Small groups of Oogonia (O) and chromatin nucleolus oocytes (ch) in the ovary of *L. nebulosus* (X1000).

Plate 15: small nucleolus (n) near to the nucleus membrane (NM) with appearance vacuoles (V) in the perinucleolus oocytes of *L. nebulosus* (X1000).

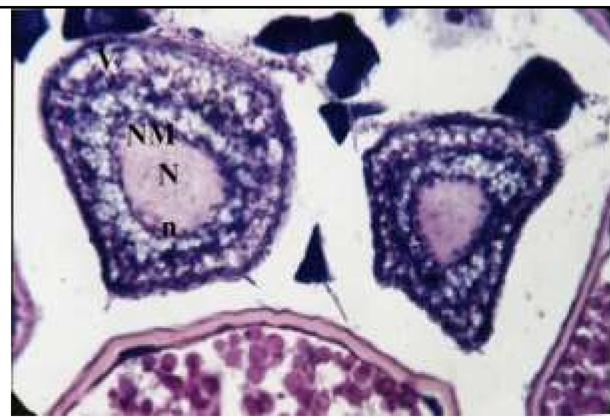

Plate 16: Two rings of vacuoles (V) around the nucleus (N) and a lot of nucleoli (n) attached to the nuclear membrane (NM) in the ovary of *L. nebulosus* (X400).

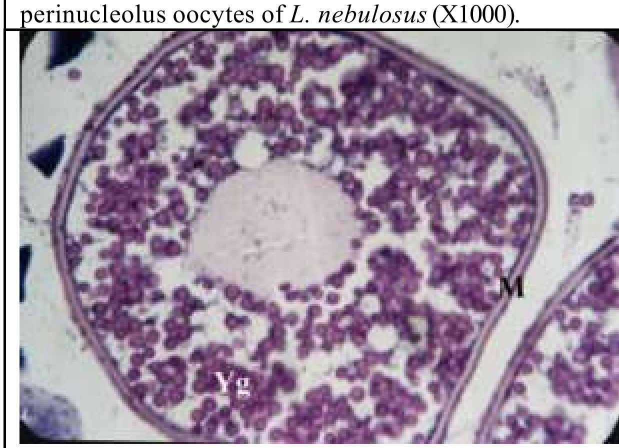

Plate 17: Oval rounded yolk globules (Yg) and well developed yolk vesicle oocyte oocyte membrane (M) in *L. nebulosus* (X400).

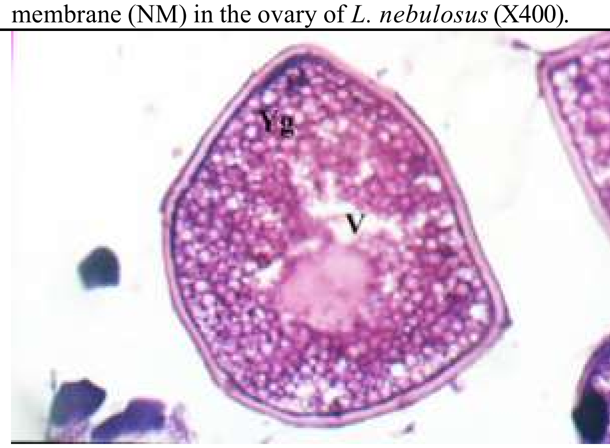

Plate 18: Large vacuoles (V) and yolk globules (Yg) fill the whole cytoplasm in a yolk vesicle oocyte of *L. nebulosus* (X400).

Plates (13-18): Light micrographs in the ovaries of females of *Epinephelus areolatus* and *Lethrinus nebulosus* in Halaieb / Shalatien area

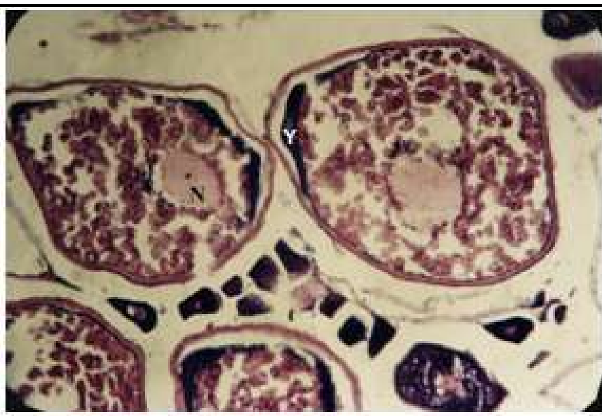

Plate 19: The start of nucleus (N) migration and start of yolk liquefaction (Y) in vitellogenic oocytes of *L. nebulosus* (X200).

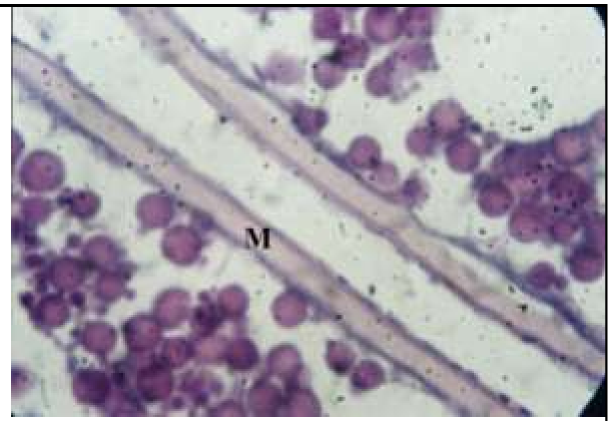

Plate 20: Developed vitellogenic oocyte membrane (M) in *L. nebulosus* (X1000).

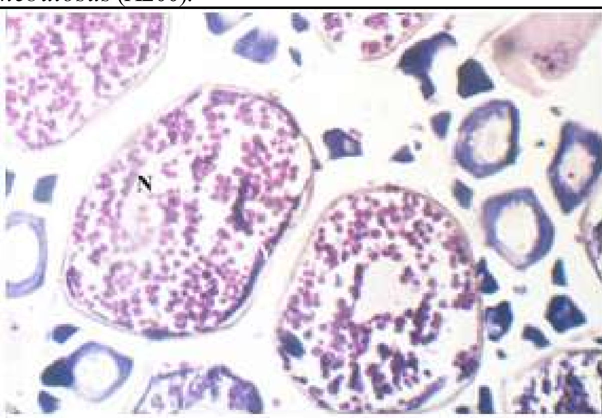

Plate 21: A noncircular migrated nucleus (N) with liquefied yolk globules (Yg) in *L. nebulosus* (X200).

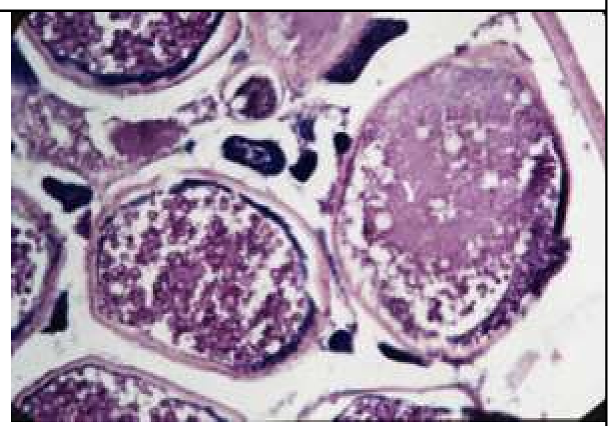

Plate 22: Pearl like shape ripe oocyte shows the yolk liquefaction (Y) in the ovary of *L. nebulosus* (X200).

Plate 23: Cross section shows empty follicles (EC) and unovulated mature yolky egg (UE) undergoing resorption of *L. nebulosus* (X100).

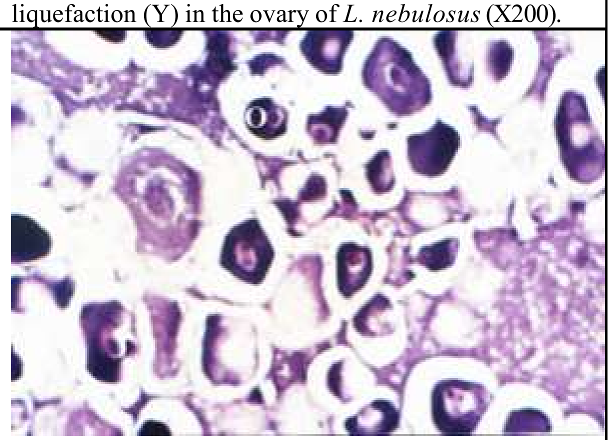

Plate 24: New generation of oogonia (O) in spent period in the ovary of *L. nebulosus* (X400).

Plates (19-24): Light micrographs in the ovaries of females of *Epinephelus areolatus* and *Lethrinus nebulosus* in Halaieb / Shalatien area

nucleus lied behind the animal pole directly, the whole oocyte becomes hydrated. When this occurred the nucleus of the oocyte cannot be identified.

The large yolk granules coalesced to form larger drops toward the center of the oocytes until all the yolk spheres undergo liquefaction (Plates 10 and 22).

With continuation of oocyte development towards the full ripeness a release of oocytes into the ovarian lumen begins, zona radiata (external and internal) showed concurrent diminution of thickness, because of their stretching following the size increase of the cell and the outer layer (Follicular epithelium) became ruptured. These stages of maturation (spawning stage) were observed in May and June for *E. areolatus* and from late May to the middle of July for *L. nebulosus*.

Spent (Rest) Stage: A great part of the completely depleted ovaries contained large numbers of empty nests (Post spawning period) and also contained unovulated mature yolky eggs undergoing resorption (Atresia) as shown in Plates 11 and 23. Ovaries were characterized by the appearance of empty follicle and few numbers of oogonia representing the reserved stock for the further spawning (Plates 12 and 24).

The spent gonads appeared in July and August for *E. areolatus* and in August and September for *L. nebulosus.* In the present study, both the data of G.S.I. and the results of the gonads histological examination gave a clear picture of the spawning seasons of the two species under study.

DISCUSSION

According to the back calculated lengths obtained by Mahmoud *et al*. [1], the age at first maturity was determined for each sex of the two species under study. They attain their first sexual maturity after their second year of life and the females of the two species under study attained their sexual maturity at bigger ages and higher lengths than males. The length at maturity was suggested as the minimum size that should be allowed to be caught in commercial fishing, as it will allow the fish to gain considerable biomass and spawn at least once in its life [4].

Shakeel and Ahmed [5] stated that, the minimum size allowed to be caught for *E.* areolatus in Malé, Maldives is 25 cm. McIlwain *et al.* [6] in their study on *L. nebulosus* in the Arabian Sea, Sultanat of Oman found that, the immature individuals constituted more than 40% of the fish sampled for both sexes. Rathacharen *et al.* [7] claimed that, the age at first maturity of *L. nebulosus* was 1.58 year which corresponds to length 29.5 cm in the coastal areas of Mauritius.

The length at first sexual maturity of *L. nebulosus* in Arab Emirates in front of Abu Dhabi was determined by Grandcourt *et al*. [8], as 28.6 cm for males and 31.3 cm for females. While, Grandcourt *et al*. [9] found that, the length at first sexual maturity of *L. nebulosus* in the Southern Arabian Gulf was 27.6 cm for males and 28.6 cm for females.

Both G.S.I. and gonads histological examinations were used to determine the spawning season of each species [10 - 18]. In this study, it was found during May and June for both sexes of *E. areolatus* and it was during June and July for both sexes of *L. nebulosus.* These results are in agreement with Young and Martin [19] who found that, the spawning time of *L. nebulosus* extends during June and July in the North-West shelf and Gulf of Carpentaria. While Grandcourt *et al*. [9] stated that, the peak of gonadosomatic index of *L. nebulosus* appear during April and May in the Southern Arabian Gulf.

Many authors paid their attention to study the oogensis of different species [20 -30] due to its importance in the decisions of fisheries management.

In conclusion; the available results indicated that males of both species under study begin to be mature before females. The spawning months of the two species under study was during May and June for both sexes of *E. areolatus* and it was during June and July for both sexes of *L. nebulosus* and G.S.I. values had an increasing trend for both sexes during the period of spawning. These values reached their maximum at the spawning time then started to decrease for both species under study tell the spent stage.

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