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# Histological Assessment of Seasonal Gonad Maturation of Common Pandora *Pagellus Erythrinus* From Benghazi Coast, Libya

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## ABSTRACT

The red coral *Pagellus erythrinus* (Linnaeus, 1785) is one of the commercially important fish species in the Libyan fisheries production, from the family of Sparidae, with a wide distribution in the Mediterranean. The study was applied to 225 samples of the red coral *Pagellus erythrinus*, which were collected monthly from the coast of the city of Benghazi, Libya during the period from December 2011 to November 2012 using a commercial fishing boat. The study aimed to use tissues to describe and classify the stages of maturity, to determine the breeding season of *Pagellus erythrinus* in the coast of the city of Benghazi, Libya. Depending on the monthly variations of the external appearance and histological description of the maturity of the gonads may Stages -IV, late developing-III, early developing-II, immature-I (maturity was classified into six stages namely mature, V-ovulation and VI-survival stage). The total length of samples varied between 18 and 32.5 cm. Present results indicated males of common pandora larger than females. Protogynous herma-phroditism (some fish change sex from female to male) characterized the species. The samples were composed of 65% females, 22% males, 13% hermaphrodites (unfunction sex), and small fish (Juveniles) was absent. Sex ratio male to female was1:2.3, females more numerous than males.

**التقييم النسيجي لنضوج الغدد التناسلية الموسمية لسمك المرجان/الأحمر *Pagellus erythrinus* في ساحل بنغازي، ليبيا**

أمينة المجيدب . نادية الرواب

المرجان الأحمر *Pagellus erythrinus* (Linnaeus, 1785) هو أحد أنواع الأسماك ذات الأهمية التجارية في المزارع السمكية الليبية، وهو منتشر على نطاق واسع في البحر الأبيض المتوسط. تم تطبيق الدراسة على 225 عينة من المرجان **الأحمر** *Pagellus erythrinus*، والتي تم جمعها شهرياً من سواحل مدينة بنغازي الليبية خلال الفترة من ديسمبر 2011 إلى نوفمبر 2012 باستخدام قارب صيد تجاري. هدفت الدراسة إلى استخدام الأنسجة لوصف وتصنيف مراحل النضج، لتحديد موسم تكاثر المرجان الأحمر في ساحل مدينة بنغازي بليبيا. اعتمادًا على التغيرات الشهرية للمظهر الخارجي والوصف النسيجي لنضج الغدد التناسلية، تم تصنيف النضج إلى ست مراحل وهي ( غير الناضجة, المبكرة النامية, النامية في وقت متأخر, النضج، الإباضة و مرحلة البقاء). ويتراوح الطول الإجمالي للعينات بين 18 و32.5 سم. أشارت النتائج الحالية إلى أن ذكور المرجان الأحمر الشائعة أكبر حجمًا من الإناث. تتميز هذه الأنواع بالخنوثة المبكرة الأنوثة (بعض الأسماك تغير جنسها من أنثى إلى ذكر). تكونت العينات من 65% إناث، 22% ذكور، 13% خناث (جنس غير وظيفي)، وكانت الأسماك الصغيرة (اليافعات) غائبة. وكانت نسبة الجنس بين الذكور والإناث 1:.3، والإناث أكثر عدداً من الذكور.

## INTRODUCTION

In the Mediterranean Sea there are 23 species belonging to the Sparidae family characterized by their elongated form (Fischer *et al* 2012). The common pandora (*Pagellus erythrinus*) is a fish of the sea bream family, Sparidae. It is a popular food fish in Mediterranean countries, with delicate white flesh. Understanding the reproductive biology of fish is the most important feature to provide a scientific suggestion for fisheries management and fish culture. Common Pandora Pagellus erythrinus is one of the most important commercial fish species in Libyan fishery production. It is an omnivorous species ,but feed mostly as carnivorous. It is usually distributed in the Mediterranean Sea and along the European and African coasts of the Atlantic Ocean ( Klimogianni A2004) . There are, however, no studies that histologically examine the gonadal development of Common pandora, Pagellus erythrinus in libya. Histological studies on reproductive biology always provide more accurate and precise results. One of the main problems encountered is lack of information regarding their histological gonadal development and spawning season of the common pandora and development of organs of those species has also been one of the most discussed topics in recent years.

The aim of this study was using histological techniques to describe seasonal gonad development and to confirm spawning season. This study will provide preliminary histological data on the species, and thus, pave the way for further research.

## MATERIALS AND METHODS

A total of 225 samples of *Pagellus erythrinus* (Mean total length 21.73 ± 2.57 cm and mean body weight 138.75 ± 54.30 g) were monthly collected from December 2011 until November 2012 off the coast of Benghazi, Libya using gill nets with 40mm stretched mesh size. The total body length and body weight were measured. Water temperature was between 12.5-27±2 $^{o}$C, during the months of sampling

The specimens were directly kept in ice and transferred to the Laboratory, Zoology Department, Faculty of Science, Benghazi University.

When the fish arrived to the laboratory washed by normal saline solution (0.9%) and examined externally to ensure that fish do not have any infection then ordered and numbered on dissection plates

The total length (TL, in cm), standard length (SL, in cm) and weight of fish (W, in g), determined for each specimen using fish measurements ruler and digital balance, respectively.

Fish were dissected to removing gonads then weighted and kept in buffered neutral formaldehyde solution (Formaldehyd,37% solution 100ml, Distilled water 900ml, Sodium phosphate monobasic 4g and Sodium phosphate dibasic 6.5g). sex was determined by gonads morphology.

Gonads histology:

Histological methods followed the paraffin methods of Preece (1972).

From the center of gonads, the samples taken were cut (approximately 10 mm thick), placed in labeled tissue tube, stored in buffered neutral formaldehyde solution for up to 20 days for processing. The tissues were dehydrated in ascending grades of alcohol, cleared in xylene and infiltrated with paraffin (Table 2). after infiltration, the tissues were embedded and 3 μm sections using microtome. Slides were stained using hematoxylin and eosin stains and covers lipped. Each slide was viewed using light microscope to determine the maturity stages of female (Table 4) and hermaphrodite, testis tissue structure (Bucholtz et al. ,2008) after that, digital-camera associated to light microscope for taken photos.

**Statistical analysis:**

Most data were subjected to one-way (ANOVA) at significant level 0.05, and differences among means were calculated with Post Hoc test. All Statistical analysis and calculation were performed using SPSS statistical package and Microsoft office excel 2007.

## RESULTS AND DISCUSSION

The result of macroscopic (morphology) examined of the gonads of common pandora confirmed that gonads were paired, longitudinally flattened, located in dorso-posterior region, attached to coellomic wall cover by the mesorectum and mesovariun, the gonads joined each other at the caudal region, forming a common duct, opening at the uro-genital papillae, this similar to that found in most fish (Duarte et al., 2007 and Bucholtz et al., 2008).

And indicated there three sexes female (ovaries), male (testes) and unfunction sex" hermaphrodite" (ovarian and testes tissue in the same organ) "ovotestes", confirmed with Valdes et al., (2004) worked on common pandora Pagellus erythrinus, from Mazarron Bay (Murcia, southeast coast of Spain).

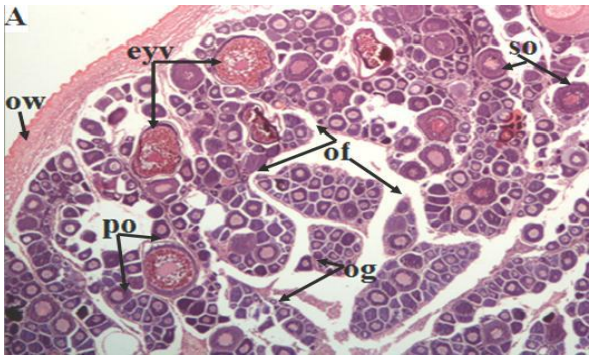
**1. Histological structure of ovary and maturity stages**

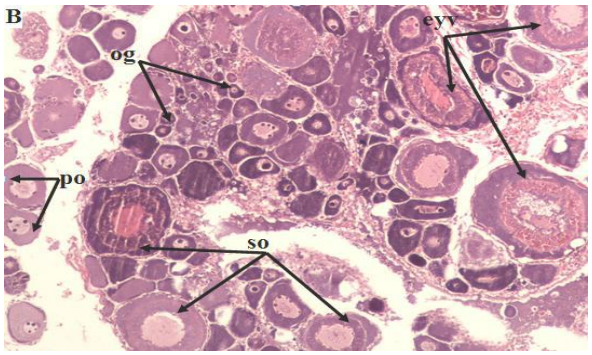
The results of microscopic examination of female maturity stages identified sex maturity stages.

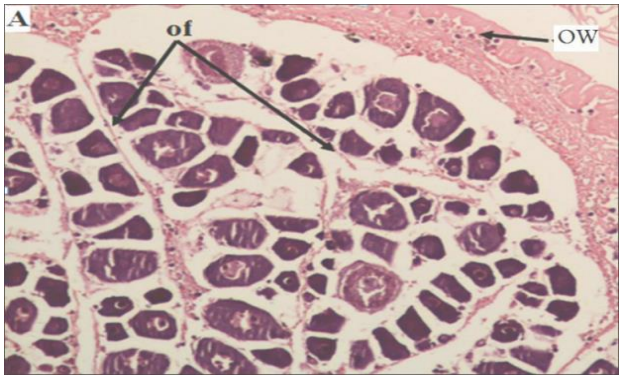
**I-Immature stage:**

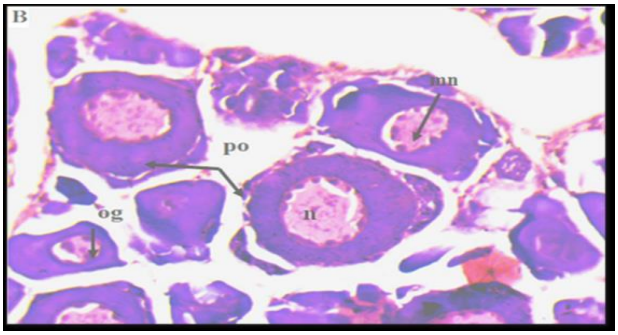
Noticed from December to March, in July, October and November, the highest percentage was in December with numerous ovigerous folds of projected into the ovarian cavity. Oogonia (og) and primary oocytes (po) which stained deeply purple with large pale pink central nucleus, having several nucleoli (strongly basophilic cytoplasm and an acidophilic nucleus, the ratio of nucleus to cytoplasm volume was high). Oogonia and primary oocytes irregular or spherical in shape and embedded in finger-like ovigerous folds, ovary wall (ow) thick and blood vessels unclear through the immature stage (Figures 1. A, B).

Note: although in the mammals the nucleus is basophilic which different from nucleus of fish that is acidophilic.




**Figures. 1(A & B). Photomicrographs showing transverse section (TS) of stage I ovary of Pagellus erythrinus showing numerous of oogonia (og) and primary oocytes (po), nucleus (n), multi nucleolus (mn), ovigerous folds (of), ovary wall (ow). (Hematoxylin and eosin stained (H &E), A: X 100 and B: X 400) High power view of (po) and (og) showen in Fig. A.**

**II-Early developing stage:**

Noticed from December to February, highest percentage was in January and the oocyte of this period characterized by the appearance of marginal vacuoles in secondary oocytes (so), enlarged and the ratio nucleus (n) to oocyte area has decreased. Oogonia generally occur in nest, while primary, secondary oocytes and early yolk vesicles oocytes (eyv) arranged in well-organized ovigerous folds (of), ovigerous folds oriented towards the center. The vacuoles at first appeared few in numbers, small in size and scattered in the cytoplasm, vitellogenic oocytes were very few with small yolk droplets (Figures 2 A, B).

**Figures 2(A & B). Photomicrographs showing transverse section (TS) of stage II ovary of Pagellus erythrinus: secondary oocyte (so), ovigerous folds (of), early yolked vesicle oocyte (eyv), oogonia (og), primary oocytes (po), ovary wall (ow). ( H &E, A: X 100 and B: X 400) together with a detail at high power in B.**

**III-Late developing stage:**

The late developing stage observed from January to March, July and August, we noticed that the highest percentages were in February and March with clear appearance of vitellogenic oocytes (vo) characterized by the presence of numerous small yolk protein granules in the cytoplasm; these granules stain very intense pink (acidophilic), first appear in the outer cortex and gradually increase in size and numbers as they move towards inner cortex. vitellogenic oocytes were arranged in well-organized ovigerous folds with few of post-vitellogenic oocytes interspersed with primary and secondary oocytes, early yolk vesicle oocytes and

oogonia that present little quantities and the wall of ovary became thin (Figure 3 A,B).

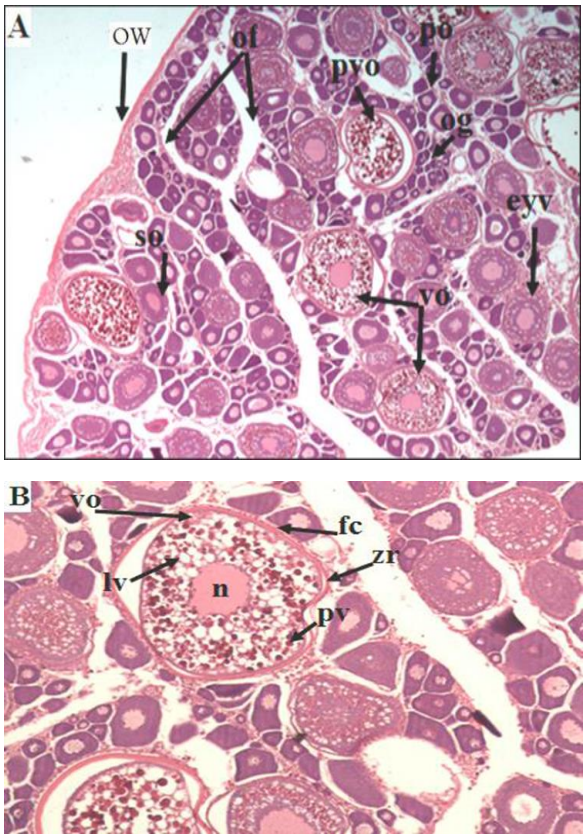
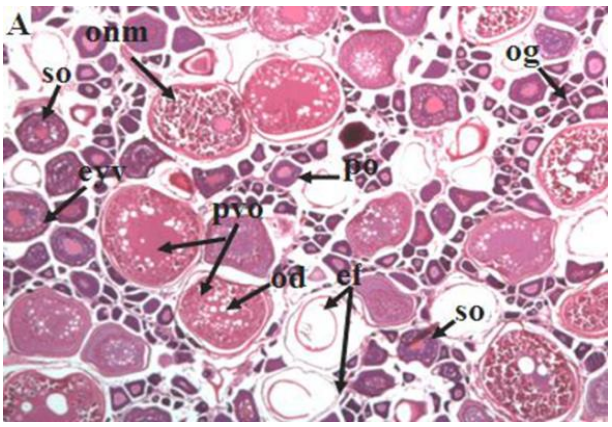



**Figure 3 (A & B). Photomicrographs showing transverse section (TS) of stage III ovary of Pagellus erythrinus vitellogenic oocytes (vo), post-vitellogenic oocytes (pvo), secondary oocyte (so), ovigerous folds (of), early yolked vesicle oocyte(eyv), oogonia (og) and primary oocytes (po), nucleus (n), wall of ovary (ow) lipid vesicle (lv), protein vesicle(pv), zona radiate (zr), and follicular cells (fc) ( H &E, A: X 100 and B: X 400).**

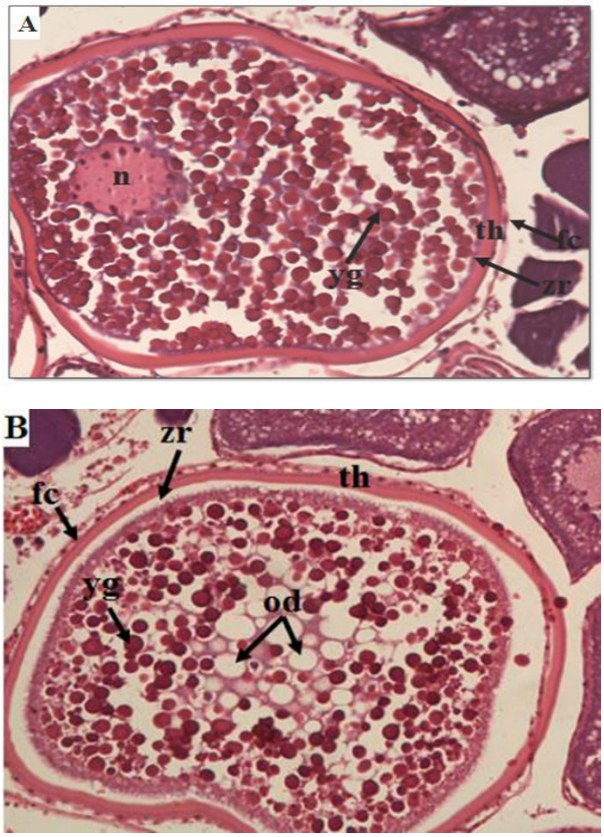
**IV- Mature stage:**

Mature stage dominated by post-vitellogenic oocytes, the oocyte membrane (theca) consisted of zona radiate (zr), coated with a follicular cells layer (fc). Primary and secondary oocytes, oogonia, vitellogenic oocytes, early yolk vesicle oocytes and empty follicle (ef) few between post-vitellogenic oocytes and ovigerous lamellae unclear because increase oocyte size. The ovarian wall thin due to distension of the ovary by the oocytes, with clear blood vessels in this stage (Figure 4).




**FFigure 4. Photomicrographs showing transverse section (TS) of stage IV ovary of Pagellus erythrinus: oocytes in the nuclear migration stage (onm), post-vitellogenic oocytes (pvo), secondary oocyte (so), early yolked vesicle oocyte (eyv), oogonia (og) and primary oocytes (po), oil droplets (od) empty follicles (ef), ( H&E, X 100).**

in January, March, from April to June and October, highest percentage in May, final maturation oocytes containing yolk granules and nucleus migrated to micropyle (Figure 5 A), in the end of stage IV oocytes completely filled with yolk granules and oil droplets, in addition to cortical alveoli present against the theca (th), named post-vitellogenic oocyte (Figure 5 B).




**Figures 5 (A & B).A: Photomicrographs showing oocytes in the nuclear migration stage and B. Shows post-vitellogenic oocyte: oil drops (od),**

**yolked granules, (yg) theca (th), zona radiate (zr), and follicular cells (fc) ( H&E, X 400).**

**V-Spawning stage:**

Start from April to July and from September to November and the highest percentage was in May.

Early and late hydrated oocytes which appeared transparent with oil drop present in this stage, and empty follicles (previously containing ripe eggs) with contents extruded in the spawning. Oogonia and primary, secondary oocytes reduced in numbers, with little of post-vitellogenic oocytes (pvo) present, the ovary wall was thinner than previous stages, with clear blood vessels, through the tissues (Figures 6 A, B).

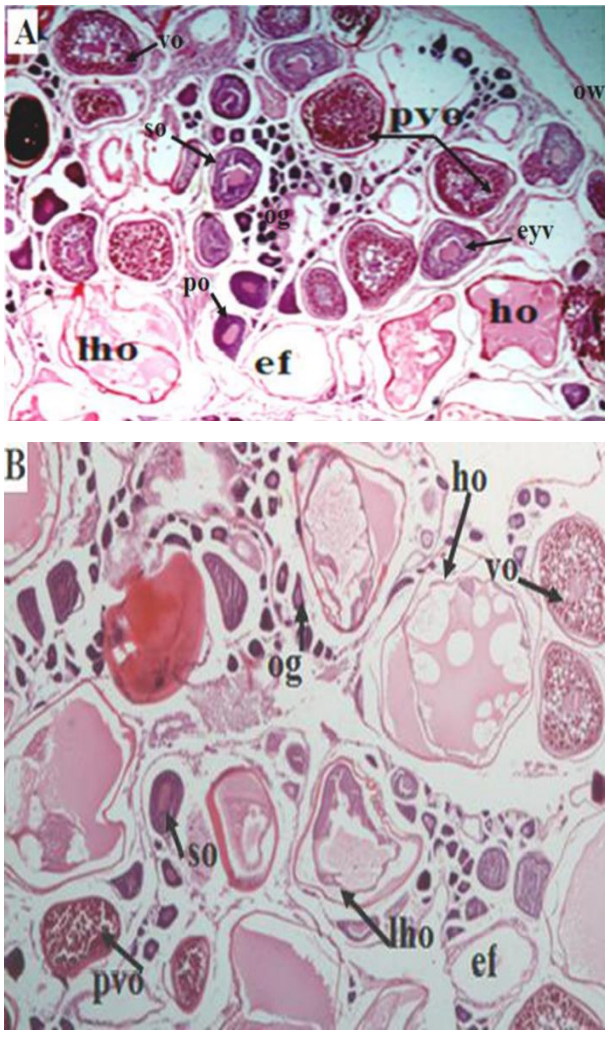



**Figure 6 (A & B). Photomicrographs showing transverse section (TS) of stage V ovary of Pagellus erythrinus showing hydrated oocytes (ho), oogonia (og) and primary oocytes (po), secondary oocyte (so), post-vitellogenic oocytes (pvo), vitellogenic oocytes (vo), empty follicle (ef), late hydrated oocytes (lho), ovary wall (ow) ( H &E, A: X 40 and B: X 100).**

**VI-Spent stage:**

Noticed from August to November, the highest percentage was in September. In this period the unovulated (residual) eggs undergo atresia at the end of breeding season. Irregular, convoluted ovigerous folds containing large numbers of ruptured follicles, postovulatory follicles, as well as atretic oocytes interspersed with few oogonia.

The ovary decreased in size and accompanied with decrease in the numbers of ripe ova. The ovary wall (ow) returned thick from the contraction of the ovary after spawning (Figures 7 A,B).

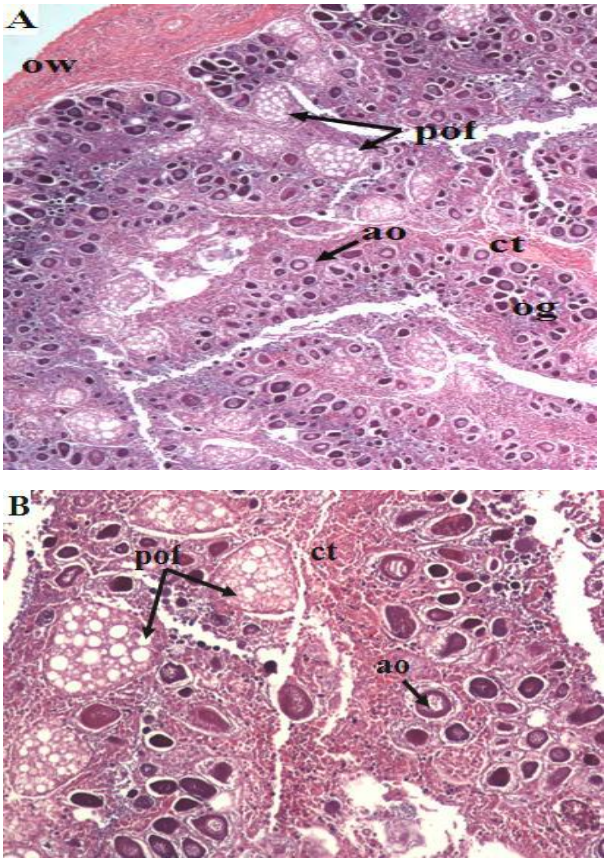



**Figure 7(A & B). Photomicrographs showing transverse section (TS) of stage VI ovary of Pagellus erythrinus: atretic follicles (ao), postovulatory follicles (pof), ovary wall (ow) and connective tissue (ct), ( H &E, A: X 100 and B: X 400).**

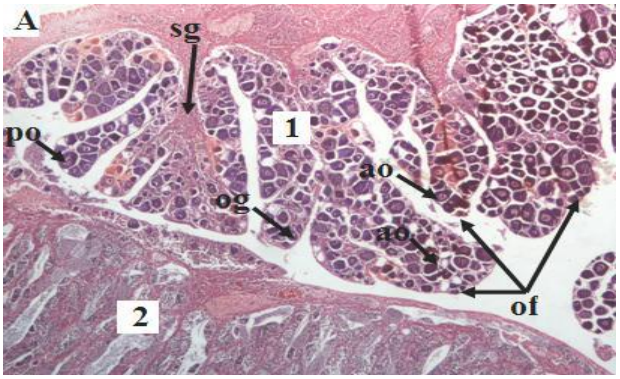
In the present research noticed sex maturity stages (I-Immature, II- Early developing, III- Late developing, IV- Mature, V- Spawn and VI- Spent stage) indicated reproductive cycle of common pandora, this similar to (Valdes et al., 2004; Solomon & Ramnarine, 2007; Sweelem, 2008-2009; AL-Absawy, 2010 and Saeed et al., 2010). In present work resulted that the highest percentages of mature and spawn stages were from April to July for both sex, this confermed with results of Sweelem, (2008-2009) worked on P. erythrinus from the Libyan coast at AL-Khoms.

In present work the microscopic (histology) examined of the ovaries indicated sex maturity stages: I-immature stage determined by oogonia and primary oocytes irregular or spherical in shape and embedded in finger-like ovigerous folds, II-early developing stage characterized by present oogonia in nest, while primary, secondary oocytes and early yolk vesicles oocytes arranged in well-organized ovigerous folds , ovigerous folds oriented towards the centre, III-Late developing stage noticed by vitellogenic oocytes characterized by the presence of numerous small yolk protein granules in the cytoplasm; these granules stain very intense pink (acidophilic), first appear in the outer cortex and gradually increase in size and numbers as they move towards inner cortex. vitellogenic oocytes were arranged in well-organized ovigerous folds with few of post-vitellogenic oocytes, IV-mature stage indicated by final maturation oocytes containing yolk granules and nucleus migrated to micropyle in the end of stage IV oocytes completely filled with yolk granules and oil droplets, in addition to cortical alveoli present against the theca, named post-vitellogenic oocyte. However, dominated oocytes were post-vitellogenic oocytes, the oocyte membrane (theca) consisted of zona radiate (zr), coated with a follicular cells layer, confirmed with that reported by Assem et al., (2008) worked on Mugil cephalus.

V-spawn stage defined by present of hydrated oocytes which appeared transparent with oil drop, VI-spent stage determined by noticed unovaluated (residual) eggs undergo atresia which indicated the end of reproductive season Present result similar to results which indicated by Abou-seedo et al., (2003) studied on Acanthopagrus latus from Kuwait Bay, Assem et al., (2008) worked on Mugil cephalus, Esmaeili et al., (2010) studied on Iranocichla hormuzensis, from Mehran River, southern Iran, and Olele, (2010) worked on Sarotherodon galilaeus from Onah Lake, Nigeria.

**2. Histological structure of hermaphrodite gonad:**

The present result indicated hermaphrodite appeared in the whole of year excepted December, April, May and July, gonads hermaphrodite combined of two different tissues, 1- ovarian tissue interspersed and coated with testis tissue and oogonia, atretic oocyte and primary oocyte present in well-organized ovigerous folds which interspersed with spermatogonia. 2- well developing of testis tissue spermatogonia observed inside seminifrous tubules which present inside lobules of testis that joined together by connective tissue and clear blood vessels through the tissues (Figure 8 A,B).



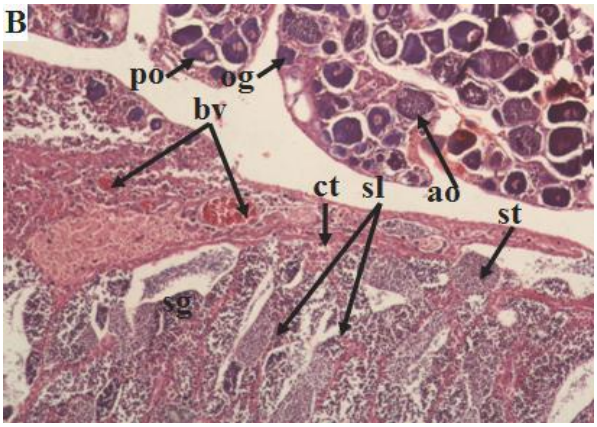




**Figure 8.A. Photomicrographs showing transverse section (TS) of hermaphrodites "ovotestes" Pagellus erythrinus showing two tissues (1) ovary tissue and (2) testis tissues, spermatogonia (sg), atretic oocyte (ao), primary oocyte (po) and seminifrous lobules (sl) ( H &E, X 40 ).**

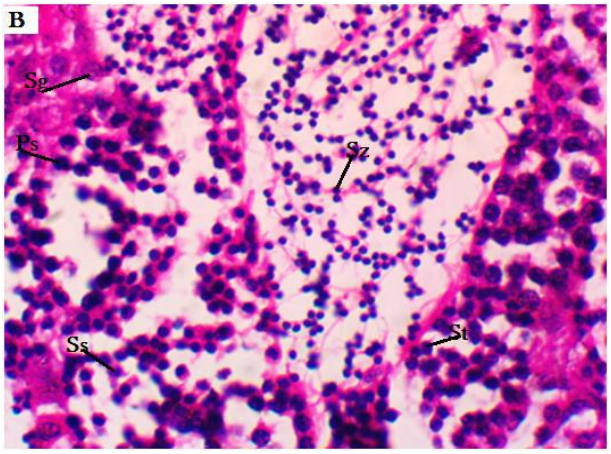



**Figure8.B. showing transverse section (TS) of hermaphrodites Pagellus erythrinus showing spermatogonia (sg), atretic oocyte (ao), primary oocyte (po) and blood vessel (bv), connective tissue (ct), oogonia (og), spermatids (st) ( H &E, X 400 ).**

In present work the microscopic (histology) examined of the hermaphrodites indicated that there two different part in the section, part 1-ovarian tissue interspersed and coated with testis tissue and oogonia, atretic oocyte and primary oocyte present in well-organized ovigerous folds which interspersed with spermatogonia, part 2-

well developing of testis tissue spermatogonia observed inside seminifrous tubules which present inside lobules of testis that joined together by connective tissue, this similar to that reported by Mouine et al., (2012) worked on Diplodus sargus sargus from the Gulf of Tunis.

**.3. Histological structure of testes:**

The results showed testes composed of numerous lobules which are separated from each other by a thin layer of fibrous connective tissue, each lobules contains seminferous tubules, within it spermatogonia divide to produce primary and secondary spermatocytes and spermatozoa formed by the differentiation of the spermatids, showed in (Figure 9 A, B).

Note: spermatogonia are the largest among male germ cells, while in mammals, the primary spermatocyte is the largest.

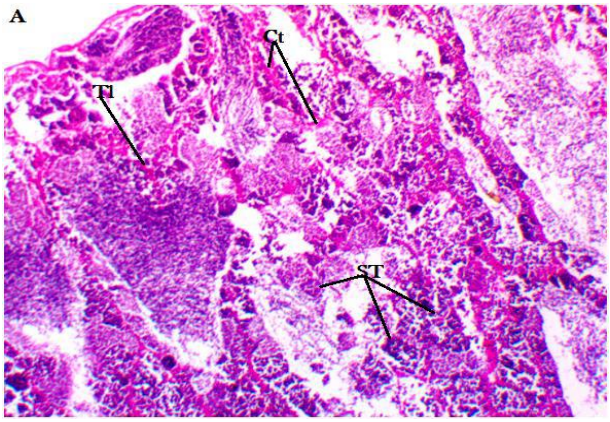



**Figure 9.A. Photomicrographs showing transverse section of testis Pagellus erythrinus, testes lobules (Tl), connective tissue (Ct), seminferous tubules(ST) ( H &E, X 100 ).**

**Figure 9.B. Photomicrographs showing transverse section of testis Pagellus erythrinus, indicated spermatogonia (Sg), primary spermatocytes (Ps), secondary spermatocytes (Ss), spermatids (St), and spermatozoa (Sz) (H &E, X 1000).**

In the present study indicated that testes composed of numerous lobules which are separated from each other by a thin layer of fibrous connective tissue, each lobules contains seminferous tubules, within it spermatogonia divide to produce primary and secondary spermatocytes and spermatozoa formed by the differentiation of the spermatids, Dziewulska and Domagala, (2003) worked on sea trout Salmo trutta from Poland.

The results appeared that spermatogonia was the largest among male germ cells, this similar to that reported by EL-Greisy (2005) worked on male Saurida undosquamis fish in the Egyptian Mediterranean coast at Alexandria, and Dziewulska and Domagala, (2003) worked on sea trout Salmo trutta from Poland.

## . CONCLUSION

nderstanding the reproductive biology of a fish species is the main aspect of providing sound scientific advice for fisheries management and aquaculture. Common pandora Pagellus erythrinus (Linnaeus, 1758) (Sparidae family) is one of the main target species of commercial fisheries and a promising candidate for Mediterranean marine aquaculture due to its high market price and good reproduction rate in captive conditions

During our study, a clear dominance of females relative to males was observed, particularly with stages of sexual maturity superior to stage III (Table 4) that suggests a phenomenon of sexual inversion that begins from the female sex towards the males.

The histology confirms the presence of different stages of sexual maturity into the same gonad which can vary from the stage of spawning to sexual rest.

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