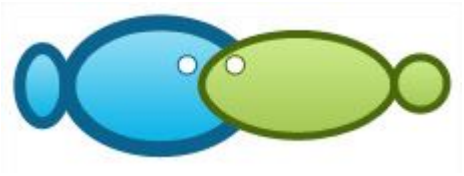

Analysis of hermaphroditism in *Pagellus erythrinus* (Linnaeus, 1758) fished in the Gulf of Annaba, Algeria, Mediterranean Sea

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Abstract. The analysis of the reproduction of *Pagellus erythrinus* samples from Annaba, Algeria Mediterranean Sea between September 2012 and August 2013 shows that the evolution of the gonado somatic index and the condition coefficient K differs especially during the period of sexual maturity from December to April in both sexes of P. erythrinus. In males the coefficient of condition evolves constantly throughout the remaining months of the year of study.
Key Words: *Pagellus erythrinus*, gonadosomatic report, reproduction cycle, Mediterranean Sea.

Introduction. In the Mediterranean Sea there are 23 species belonging to the Sparidae family characterized by their elongated form (Fischer et al 1987). In Algeria, *Pagellus erythrinus* is considered an economic source due to its high market & nutritional values, and it was also chosen due to its aquaculture potential (Manouski et al 2015) and it is classed in the 7th place in exported fishery products in Europe countries (MFFR 2006) such as Italy and Spain.

Academic studies of hermaphroditism of *P. erythrinus* are limited only to the macroscopic and numerical aspects, therefore our objective of this study is to determine the spawning period and the sexual inversion of *P. erythrinus*.

Material and Method. Gulf of Annaba is located in the east of Algeria (Mohdeb & Kara 2015) between 2 provinces "Annaba and El Tarf (Figure 1) lengthening of Cape Guard in West (7°16'E) to Cape Rosa in East (8°15'N), extending 40 km from cape grand.

We used freshly caught *P. erythrinus*. A total of 600 fishes were randomly sampled, 60 of those being used for the analysis of the reproduction parameters. The fish were collected from landings made between September 2012 and August 2013 at the fishing port in the Gulf of Annaba (Figure 1); the frequency was weekly, depending on the availability of the fish.

For each sample, the total length (TL), fork length (FL), standard length (SL) (Figure 2), the total weight (TW), gutt weight (GW) and gonad weights (GOW) were weighed to the tenth of a gram; sex and sexual maturity were macroscopically identified.

For lengths measurement we used fish meter, precision scale, lab binocular, tape measure and Bouin or 10% formaldehyde solution.

For the microscopy analysis we used tube, label, Canada Balm, the immersion oil, dyes (hematoxylin + eosin), automatic microtome knife, paraffin, microscope slides, lamellae, optical microscope equipped with a digital camera.

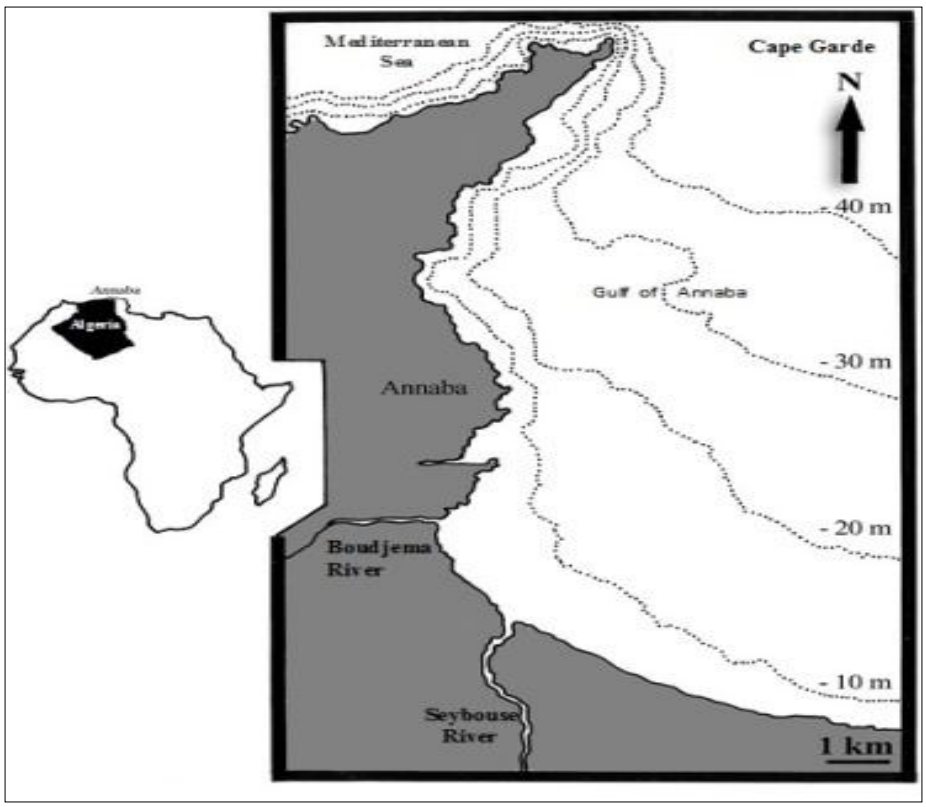

Figure1. Gulf of Annaba (Frehi et al 2007).

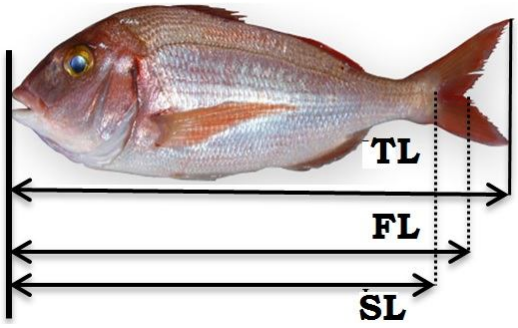

Figure 2. Measurements benchmark made in *P. erythrinus* (TL - total length; FL - fork length; SL - standard length).

Tracking methods of the evolution of sexual maturity. The spawning period of *P. erythrinus* is determined using two approaches: a qualitative approach based on the monitoring of monthly fluctuations in the percentage of different stages of gonad development, and a quantitative approach based on the monitoring of monthly changes in the gonadosomatic index (GSI) and condition factor (K) (Fehri-Bedoui & Gharbi 2008):

$$GSI = \frac{G}{W} \times 100$$

where: G = the gonad weight;
W = the total weight of the fish.

$$K = \frac{W}{L^3} \times 100$$

where: W = the total weight;
L = the total length of the fish.

Macroscopical analysis of the gonads. The scale of sexual maturity is macroscopic (Gonçalves & Erzini 2000) and includes five stages of sexual maturity known in females (Table 1) and males fish (Table 2). The materials used were a binocular microscope (Zeiss), a microscope Carl Zeiss model (Axiostar plus) equipped with a digital camera (uEye32) to determine the morphology and to classify macroscopic gonad stages of sexual maturity (Shapiro 1984).

Table 1

The different stages of sexual maturity in female *P. erythrinus* fished in the Gulf of Annaba (Fehri-Bedoui & Gharbi 2008)

	Stages	*Females*
I	Immature	In immature ovarian oocytes polyhedral shape are arranged regularly along the ovarian lamellae. Thin filament, transparent, oocytes characteristics of stage A oocytes represent the general stock, invisible oocytes.
II	Sexual resting	Close to the stage I, larger volume, light pink colour.
III	Gonadal maturation	Is characterized above all by the beginning of vitellogenesis, gonade large, light orange to dark, ovarian granular, oocytes easily visible through the membrane.
IV	Spawning	Oocytes are round, very thin ovarian membrane, oocytes easily visible and expelled at the slightest pressure; it can occur a start of water absorption by the oocytes.
V	Post-spawning	Pink salmon color, ovary flaccid, vascularized and oocytes smaller with presence of hyaline spaces.

Table 2

The different stages of sexual maturity in the male *P. erythrinus* fished in the Gulf of Annaba (Fehri-Bedoui & Gharbi 2008)

	Stages	*Males*
I	Immature	White or slightly translucent, testes thin, and very fine gonad in the form of knife blade.
II	Sexual resting	White gonad; no liquid flows if there is an incision, large volume.
III	Gonadal maturation	Gonad flaccid and white - white liquid elapses when an incision is practiced.
IV	Spawning	Gonad big and soft, occupy the total abdominal cavity, fperm flowing any pressure exerted on the abdomen.
V	Post-spawning	Gonad flaccid and having a very fine vasculature particularly in the posterior portion. At the end of spawning, gonad very flabby, and highly vascularized exhausted.

Microscopical analysis of the gonads. Microscopic examination is performed following the histology of all collected gonads. The ovaries and testes samples were analyzed histologically in the histopathology laboratory "NIHA" located in the province of Annaba. The right lobe of the gonads were sectioned at 5 µm, included in aqueous Bouin in a small volume, due to its rapid penetration into the tissues of fish (Gabe 1968) and colored with hematoxylin and eosin (McDonough et al 2005). Then samples were included in paraffin blocks. The transverse sections are formed using a microtome.

Results

Morphometric results. The results of measurements performed during the twelve-month study shows homogenous changes in different length averages, minimum and maximum values correspond to the months of April and July of 2013 (Table 3).

Table 3

Results of morphometric analysis performed on *P. erythrinus* during the months of study

Months	*TL (cm) average*	*FL (cm) average*	*SL (cm) average*	*TW(g) average*	*GOW (g) average*	*GW (g) average*
September 2012	19.26	17.30	16.06	105.56	0.65	98.56
October 2012	19.20	17.04	15.76	103.24	0.57	95.80
November 2012	21.48	19.58	18.18	146.58	0.77	138.62
December 2012	20.74	18.56	17.34	117.52	0.74	110.34
January 2013	21.32	19.06	17.90	121.54	0.72	114.26
February 2013	22.06	20.24	18.74	161.9	1.15	152.62
March 2013	19.26	17.82	16	127.64	0.83	119.98
April 2013	18.48	16.10	15.14	135.08	0.58	129.34
May 2013	20.36	18.86	17.82	121.32	0.69	115.86
June 2013	20.10	18.34	17.24	126.7	0.53	120.04
July 2013	22.26	20.26	19.10	156.80	0.61	148.52
August 2013	21.48	19.42	18.10	141.68	0.70	134.40

TL = total length; SL = standard length; FL - fork length; TW = total weight; GOW = gonad weight; GW = gutt weight.

Macroscopic results. Among the fish analyzed 360 were females compared to 220 males. Figure 3 shows a dominance of females from the period of October and December 2012, then from the period of February to August 2013.

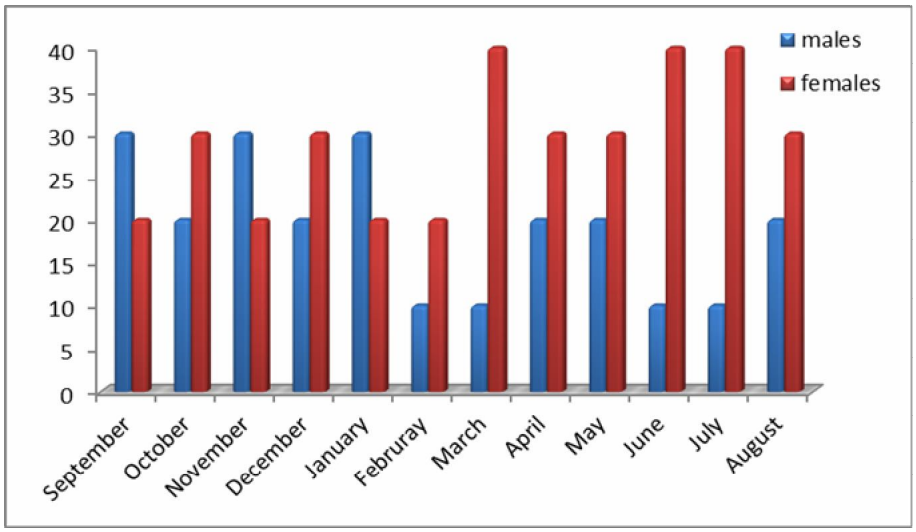

Figure 3. Monthly distribution of genders of *P. erythrinus* (September 2012 - August 2013).

The 60 samples of *P. erythrinus* shows the following results (Table 4).

Table 4

Results of the distribution of the stages of sexual maturity (from stage I to V) according to the months of study

Month	No monthly total individuals	Maturity stages									
		I		II		III		IV		V	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
September 2012	5					3			1	1	
October 2012	5				1	3					1
November 2012	5		1	1	2	1					
December 2012	5			2		1	2				
January 2013	5				2	2	1				
February 2013	5	1		1		1	2				
March 2013	5	1				3	1				
April 2013	5					3	1		1		
May 2013	5					1		1	2	1	
June 2013	5							2	1	2	
July 2013	5							1		3	1
August 2013	5			1					1	2	1

Monthly variation in the percentage of stages of sexual maturity. The monthly variation in the percentage of stages of sexual maturity shows that males and females are at different stages of sexual maturity, but their percentages are variable (Figure 4). The mature stage is highlighted in the month of April and in our study we considered stage (III) as the beginning of maturation stage. We can distinguish 4 categories of activities:

- the rest category (stage I and II);
- the maturation category (stages III);
- the reproduction category (stages IV);
- the post spawning category (stage V).

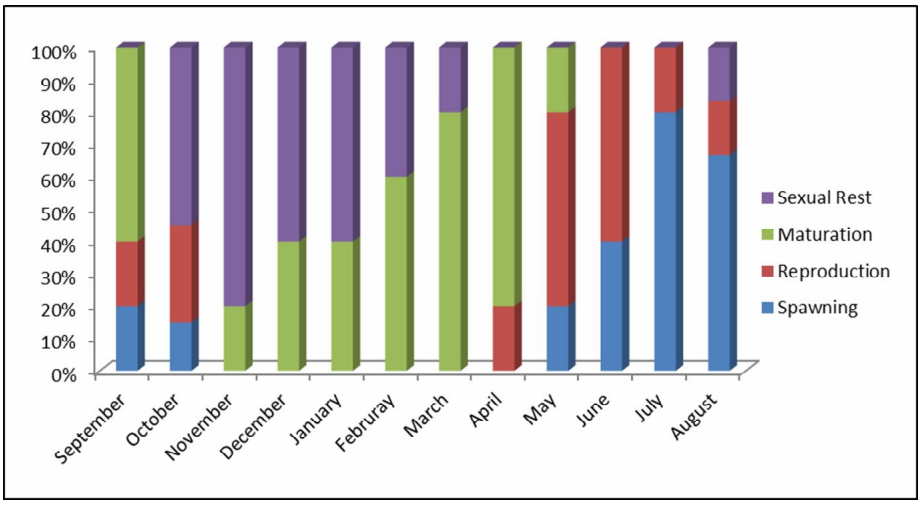

Figure 4. The different stages of sexual maturation in *P. erythrinus* in the Gulf of Annaba.

Results of gonadosomatic index (GSI). It is observed that in males, the average GSI is maximum in March and becomes minimal in June, from June 2012 to May 2013, a similar evolution of the maximum GSI was observed in females (Figure 5). However, in females, we observed that the maximum GSI had higher values than males.

In Annaba, *P. erythrinus* can breed between April through October, while individuals of both sexes are mature during the period from November to May.

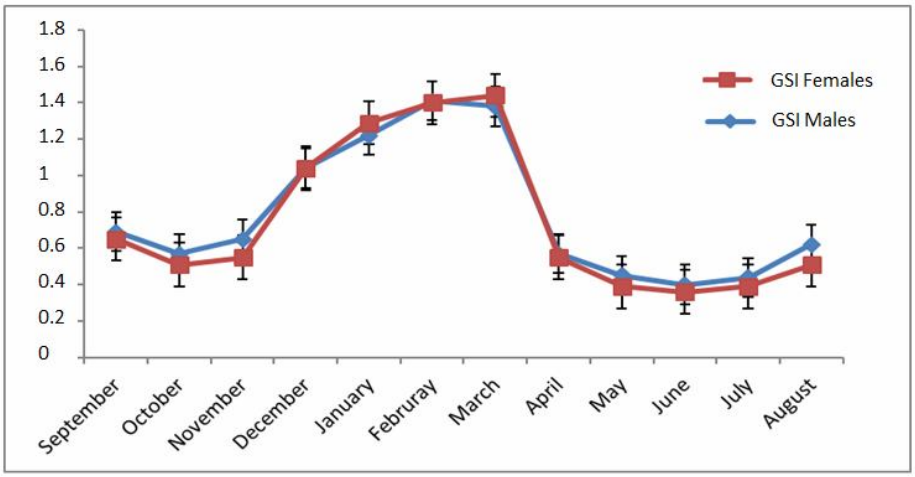

Figure 5. Evolution of GSR% in *P. erythrinus.*

Results of K factor. The graphical representation of the monthly average shows that K has a similar tendency in both sexes and variability between the months of the study period (Figure 6). And the difference is at the peak which is observed in April in both males and females.

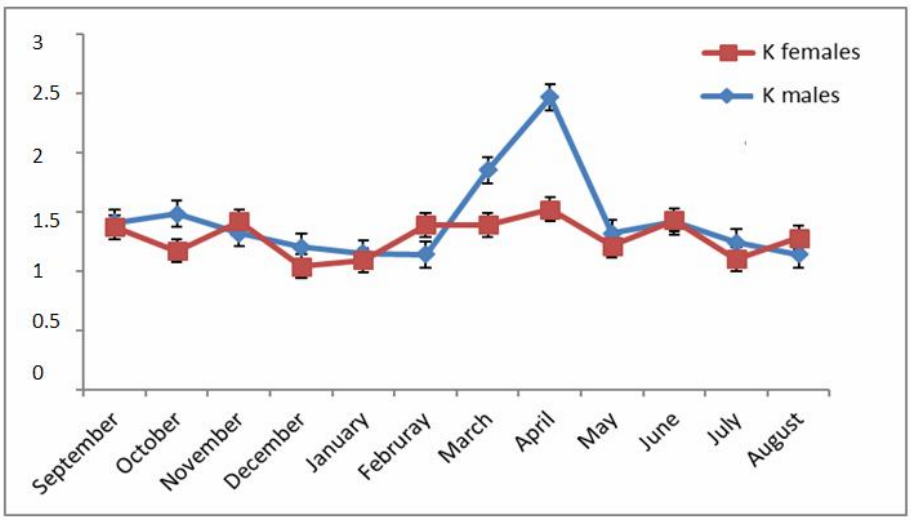

Figure 6. Evolution of K factor.

Microscopic results

In the females. Relying on the histological observations of gonadal development and the monthly evolution of GSI, the ovaries of *P. erythrinus* go through five successive stages. The frequency distribution of the stage of development of the ovary is shown in Figure 7:

- early growing stage: species in early growth phase were observed for the first time in November. The number of oocytes increases with their diameter in the ovaries, and the oocytes are in the oil drop step ranging from 10 to 40 µm (Figure 7a);
- growing stage: the ovary has accumulated oocytes in the phase of the globules of the yolk with a granular globule of egg yolk ranging from 20 to 80 µm in diameter and it occurs at the beginning of January (Figure 7b);
- mature stage: the GSI reaches the peak in February and March. Individuals have mature oocyte stages ranging from 40 to 100 µm in diameter with a large droplet of oil (Figure 7c);
- spawning stage: individual variations during the breeding period occurs inside the gulf of Annaba in proximity of the rocky areas of the Mediterranean Sea between the period of December and March, in order to protect their eggs from tidal waves;

- resting and recovery stage: there was a rapid decrease in GSR in April. The ovaries contained mainly immature oocytes and some ovulatory follicles. Individuals at this stage appeared from May to August (Figure 7d).

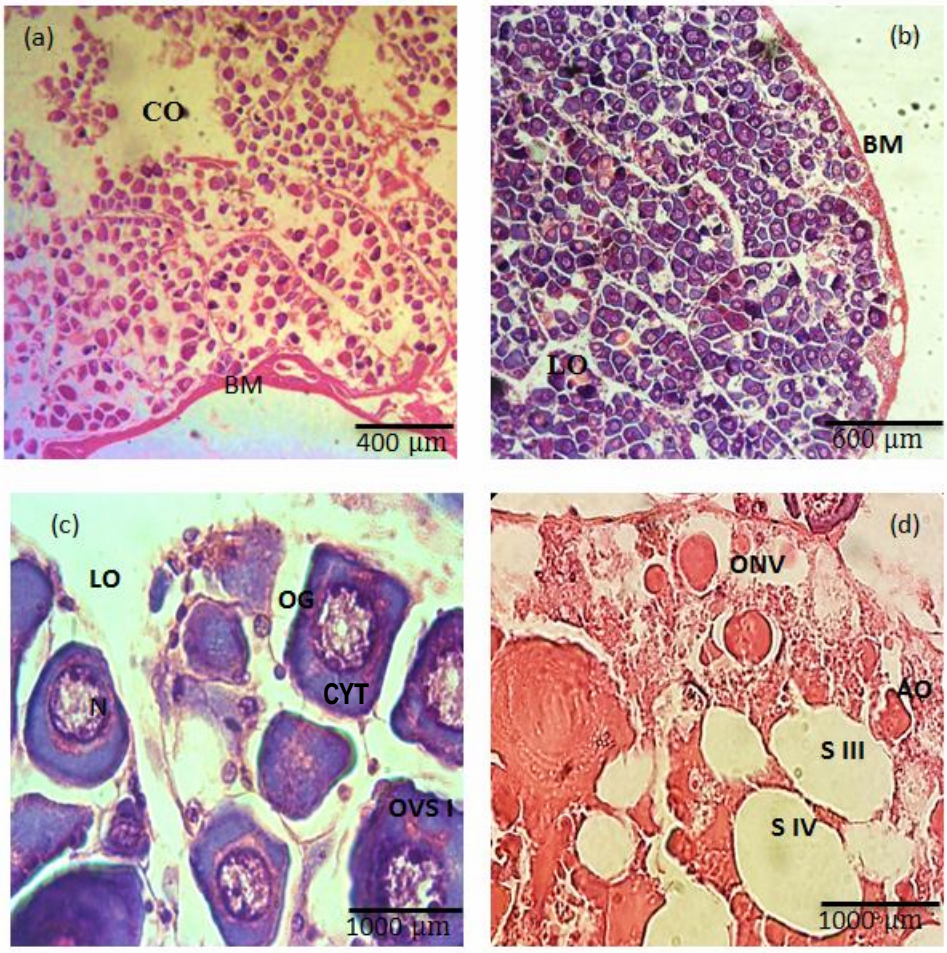

Figure 7. Maturation stage of the ovaries of *P. erythrinus*: (a) pre-vitellogenic yolk stage; (b, c) mature in the late vitellogenic yolk stage; (d) after spawning. CO - ovarian cavity, OG - oogonia, OVS - oocyte vitellogenic (G x 1000), SIII - Stage III oocytes (G x 1000), Onset of maturation of the ovary (stage III), BM - basement membrane (Gx 1000) , SIV- Stage IV oocyte, OVS 1 - oocyte vitellogenic type 1 (G x 1000), CYT - cytoplasm, LO - ovarian lumen (G x 1000), N - nucleus, AO - start of oocyte atresia (G x 1000). ONV - oocyte non vitellogenic (G x 1000) stage 5 post spawning.

In the males. Concerning the evolution of the sexual maturity of the male gonads, we observed a similar evolution with the females and correspond to the period going from November to March which coincides with the spawning period. Figure 8 clearly shows the dominance of the spermatid stage.

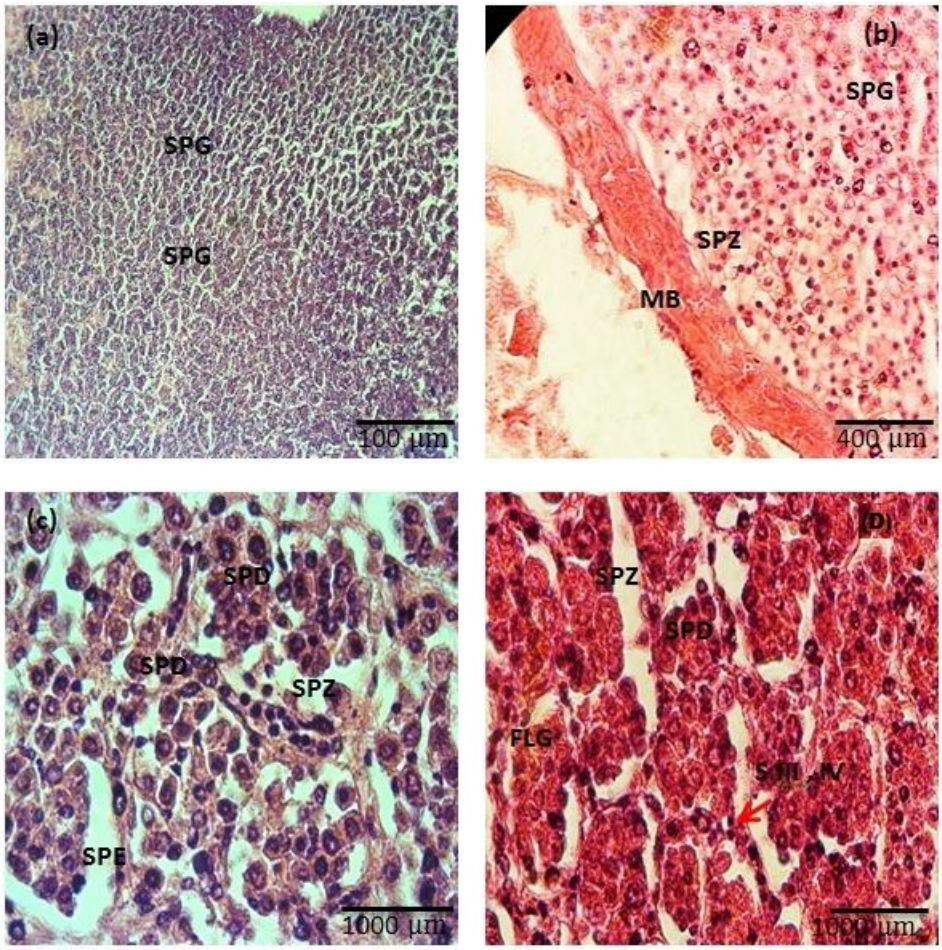

Figure 8. Maturation of testes of *P. erythrinus*: SPG - spermatogonia, SPZ - spermatozoid, SPD - spermatids, FLG - flagellum sperm, S III, IV - stage of sexual maturity (G x 1000), SPE - sperm (G x 1000), MB - basement membrane (G x 1000).

Ovotestis. Figure 9(a) shows dominance of female territories relative to male with ovulatory stages, the inverse is shown in Figure 9(b), and the figure show the dominance of spermatogonia which has not reached sexual maturity.

Figure 9(c) shows differential stages with the presence of well differentiated (mature) spermatozoa. Figure 9(d) shows the oocytes in stage II (no ovulation).

According to the samples collected and observed the sexual inversion and according to the anatomical dominance of the male or female territories given by histological observation of the gonads but also physiological, expressed through advancement in the stages of sexual maturity, it is sexual cells (spermatogonia or oocytes) differentiates and in large numbers.

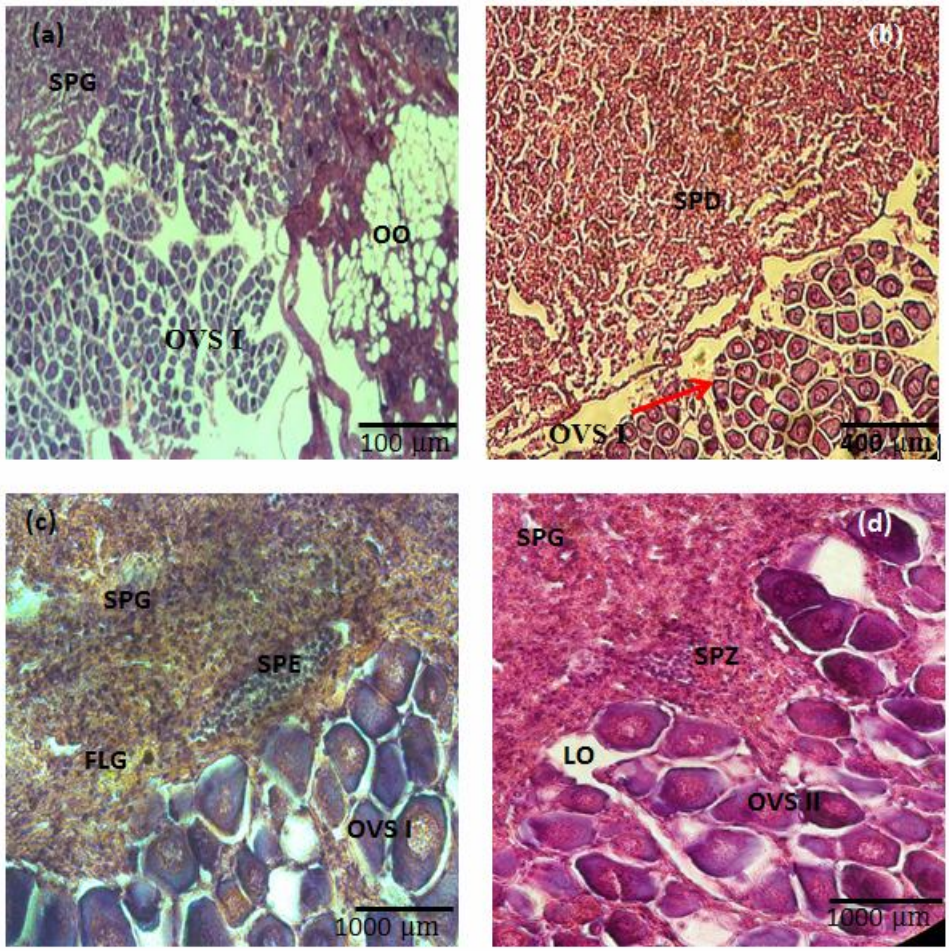

Figure 9. Ovotestis of *P. erythrinus*: SPG - spermatogonia, SPZ - sperm, SPD - spermatids, FLG - flagellum sperm, S: II Stage of sexual maturity (G x 1000), OO - ovarian ovum, LO - ovarian lumen (G x 1000), OVS I - oocyte vitellogenic stage I, OVS II - oocyte vitellogenic stage II (G x 1000).

Discussion. Morphometric parameters TL, FL, SL, TW, GW, GOW, and the stages of sexual maturity recorded during 12 months of sampling for the species *P. erythrinus* clearly show variations on all the parameters studied. These variations are due to the sampling that was (simple and independent strategy) taken randomly. 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 results are confirmed by many studies in the Mediterranean, notably that of Papaconstantinou et al (1988), Mytilinéou (1989), Zarrad et al (2010), carried out at the level of the Gulf of Tunis-Tunisia, Ghorbel & Ktari (1982) in the Gulf of Gabes-Tunisia and in the Atlantic Ocean may include the work of Pajuelo & Lorenzo (1998).

The monthly variation of GSI shows that the breeding season took place from October to April with a peak recorded in March for both males and females. Other periods have been reported by other authors in the Mediterranean (Table 5) notably Ghorbel et al (1996) which notes that in the Gulf of Gabes the reproduction of *P. erythrinus* occurs between May and July. And our study shows a similarity with the study conducted by Zarrad et al (2010). In the Adriatic Sea a summer breeding season is reported by Metin et al (2011), while a breeding period was recorded from March to July in southern Portugal, according to Coelho et al (2010). The same results have been described in other species of fish (Layachi et al 2015; Samy-Kamal et al 2015).

Concerning the annual evolution of the condition coefficient K during a given year, shows a slight variation even during the breeding period. This may be justified by the

availability of food, and elevated temperatures which maintain and sustain normal physiological activity (Brett 1979).

Finally, *P. erythrinus* reaches maturity and reproduces differently according to the geographical zones and the trophic conditions of the aquatic environment in which it lives.

Table 5

Period of reproduction of *Pagellus erythrinus* in different sea of the world

Areas	*Reproduction period*	*Author*
Gulf of Tunisia	April-October	Zarrad et al (2010)
Gulf of Gabes	May-July	Ghorbel et al (1996)
Gulf of Lion	June-August	Girardin (1981); Girardin & Quignard (1985)
South of Portugal	May-August	Santos et al (1995)
Canary islands	April-September	Pajuelo & Lorenzo (1998)
Gulf of Annaba	May-October	This study

Conclusions. This study serves as an information base for *P. erythrinus* in the Mediterranean Sea. The current study shows that in the gulf of Annaba *P. erythrinus* during their life cycle, a period of high gonadal activity (maturation) occurs during the period from December to April, followed by a breeding period from May to August.

According to the histological study of gonads, we conclude that throughout the period of our study, the presence of an impressive number of ovotestis is an evidence of the hermaphrodite character of this species. 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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