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Reproductive biology of *Pagellus acarne* (Risso, 1827) (Teleostei: Sparidae) off western Algerian waters (Western Mediterranean)

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Abstract: The reproduction of *Pagellus acarne* caught in Oran Bay was studied. The samples used were sorted monthly from commercial catches of coastal trawlers operating in this area from April 2008 to July 2009. The overall sex ratio was in favor of females 1:1.27 and length frequency distribution according to sex revealed that the females were highly representative beyond 20.5 cm of total length presuming a sexual inversion already described for this sparidae. The estimated lengths at maturity (L_m) were 12.8 cm for females and 16.0 cm for males. Two spawning periods were made out by the follow-up of the gonado and hepato somatic indexes: a spring period from April to June with a peak in May and an autumnal period, between November and January with a peak in December. The closed season in Oran Bay extends from 1st May to 31th August, which is to our opinion insufficient to safeguard the renewal of the resource and its spawning stock.

Résumé : *Biologie de la reproduction chez* Pagellus acarne *(Risso, 1827) (Teleostei : Sparidae) au large des côtes ouest algériennes (Méditerranée occidentale).* L'étude a cerné le cycle reproducteur du pageot argenté *Pagellus acarne* pêché dans la Baie d'Oran. Les spécimens provenaient mensuellement de la pêche chalutière commerciale entre avril 2008 et juillet 2009. Le sex ratio était en faveur des femelles 1:1,27 et la distribution des fréquences de taille par rapport aux tailles des individus a révélé que les femelles étaient majoritaires au-delà de 20,5 cm de longueur totale reflétant sans doute le phénomène d'inversion sexuelle déjà décrit pour ce sparidé. La taille de première maturité sexuelle a été estimée à 12,8 cm pour les femelles et 16,0 cm pour les mâles. Le suivi mensuel des indices gonado- et hépato-somatiques nous a permis de mettre en évidence deux périodes de ponte : l'une printanière d'avril à mai et la deuxième automnale entre novembre et décembre. La fermeture de la pêche au chalut s'étale du 1er mai au 31 août, ce qui est de notre point de vue insuffisant pour préserver le renouvellement du stock reproducteur ainsi que celui de la ressource.

Keywords: Pagellus acarne ● Oran Bay ● Reproductive cycle ● Sex-ratio ● Spawning season ● Size at first maturity

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Introduction

The quantity of fish caught annually in Oran Bay makes that this port provides almost cities in western Algeria in fish and seafood. To preserve this natural wealth, we found it useful to focus on the axillary sea bream *Pagellus acarne* (Risso, 1827) one of the main sparidae species caught by trawl in the Algerian waters and representing an important part of fish landed in Oran fishery (Bensahla Talet & Boutiba., 2000). The axillary seabream is a species of commercial importance representing 24.28% of total demersal fish landed annually. From an ecological point of view, *Pagellus acarne* is one of the most important species in typifying the demersal fish community of coastal shelf (Colloca et al., 2003; Busalacchi et al., 2010). Fished at depth ranging between 100 and 350 m (pers. obs.) it has been captured by gill, trammel nets and longline but the trawl stay the most appropriate gear for this species in Oran Bay.

Several studies have been carried out on this species in the Mediterranean Sea focusing on its biology and population dynamics (Le Trong-Phan & Kompowsky,1972; Andaloro, 1982; Lamrini, 1986; Santos et al., 1995; Arculeo et al., 2000; Dominguez, 2000; Pajuelo & Lorenzo, 2000; Coelho et al., 2005; Recasens et al., 2007; Velasco et al., 2011). However, biological studies of this species in Algerian coasts are very scarce (Bensahla Talet et al., 2010 & 2013; Boufersaoui & Harchouche, 2015). Therefore, the present study aims to estimate reproduction parameters of this species for the first time, to help comparative studies in the W. Mediterranean, allow renewal of the resources and management implications.

Material and Methods

Specimens of *P. acarne* (n = 892) were caught by trawlers operating in Oran Bay located on the North West of Algerian coasts (Western Mediterranean Sea) situated between "Pointe de l'Aiguille" and "Cap Falcon" (35.95°N-0.65°E) (Fig. 1) at depths ranging between 100 and 350 m between April 2008 and July 2009.

At the laboratory and for each individual, were recorded the total length (TL), measured to nearest millimeter with an ichthyometer (± 1 mm), the total weight (TW), and gonad weight (GW) using a digital precision balance (± 0.01g).

Sex-ratio

The sex-ratio was calculated monthly during the study period in relation to individual sizes (1 cm class interval) and seasons with the following formula:

$$SR = \frac{nF}{nM + nF} \times 100 \qquad (1)$$

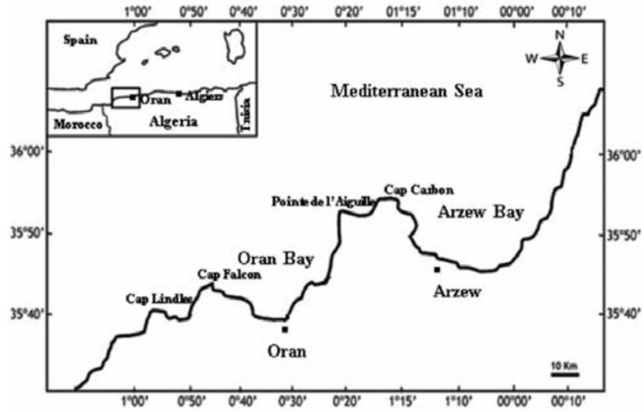


Figure 1. Map of the study area (Oran Bay).

where nF is the number of females and nM the number of males.

In order to detect statistically significant deviations from the expected 1:1 ratio, the chi-square (χ^2, $P < 0.05$) test was used (Sokal & Rohlf, 1987).

Spawning period

For the description of the sexual cycle of *P. acarne*, several approaches were used:

- The maturation scale was established following Holden & Raitt (1975) macroscopic scale, while microscopic description was based on (Aboussouan & Lahaye, 1979) with slight modification to fit *P. acarne* gonad development. Both being based on the macroscopic aspect, relative dimensions and microscopic characteristics of the gonads (I: immature, II: resting, III: ripe, IV: ripe and running, V: spent).
- Between 10 and 30 specimens of *P. acarne* were selected each month for histological examination of sexual organs that was dropped in Bouin's fixative solution for 48 h, dehydration was done with increasing concentrations of acetone/toluene then fragments were embedded in paraffin wax. Gonads were sectioned with microtome to 5-6 µm sections and stained with hematoxylin-eosin.
- Monthly evolution of the gonado-somatic (GSI) and hepato-somatic (HSI) indices were calculated as follows:

$$GSI = \frac{GW}{TW} \times 100 \qquad (2)$$

where GSI is gonado-somatic index, GW is gonad weight and TW is total weight.

$$HIS = \frac{LW}{TW} \times 100 \qquad (3)$$

where HSI is hepato-somatic index, LW is liver weight and TW is total weight.

The nucleoplasmic ratio is considered to be closely related to the mitotic cellular cycle and to the functional phase of cells. It is also a good indicator of dynamic changes in cells. The nucleoplasmic ratio (NPR) is expressed mathematically as:

$$NPR = \frac{Nd}{Cd - Nd} \quad (4)$$

where Nd is the nucleus diameter and Cd is the total cell diameter

Fulton's condition factor (k) was calculated using the equations given by Htun-Han (1978) as:

$$k = \frac{W}{L^3} \times 100 \quad (5)$$

where W is the body weight in g, L is the total length (TL) in cm. According to Froese (2006), the factor 100 is used to bring k a value close to unity

Size at first sexual maturity

Size at first sexual maturity (L_m) was defined as the size class at which 50% of individuals are mature. Specimens were grouped into 1cm size classes and the proportion of mature and immature individuals was recorded. The total length at which 50% of the specimens were mature was estimated by a logistic non-linear least-squares regression (King, 1995):

$$P = 1/[1 + e^{-a(L-L_m)}] \quad (6)$$

where P is the percentage of mature individuals, a is the slope of the curve or rate of increase in maturity, L_m is length at 50% maturity and L is the 1 cm length class.

Results

In total, 892 specimens of *P. acarne* were collected, 272 males (30.49%), 347 females (38.90%), 65 hermaphrodites (7.29%) and 208 unsexed (23.31%). The length frequency distribution of the entire population is shown in figure 2, male length range was 13.0 to 23.0 cm, female length range was 13.0 to 26.3 cm while hermaphrodite length range was 17.5 to 25.7 cm.

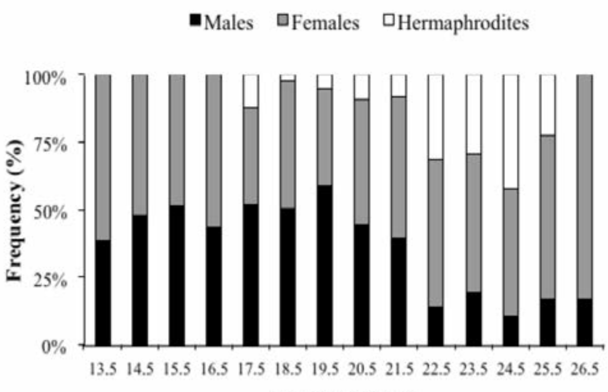


Figure 2. *Pagellus acarne*. Length frequency distribution of males and females caught in Oran Bay.

The overall ratio of males to females was 1:1.27 and χ^2 analysis showed this to be significant (χ^2 = 9.08, $\chi^2_{1,0.05}$ = 3.84). Sex-ratios of males and females by size intervals departed significantly from 1:1 ratio from 22 cm of total length were females predominate beyond this length, while for smallest length classes the χ^2 test didn't reveal any statistical difference.

Maturity stages

Females with ripe gonads (Stage III) were observed (Fig. 3) in two periods of the year, the first one between April and June and the second in autumn, ripe and running gonads (stage IV) were also met in the same interval and are dominant in May of each cycle, spent fish (stage V) were recorded in late spring with high values in June while the remaining period of the year gonads in stage II were dominant marking the sexual rest period of this sparidae.

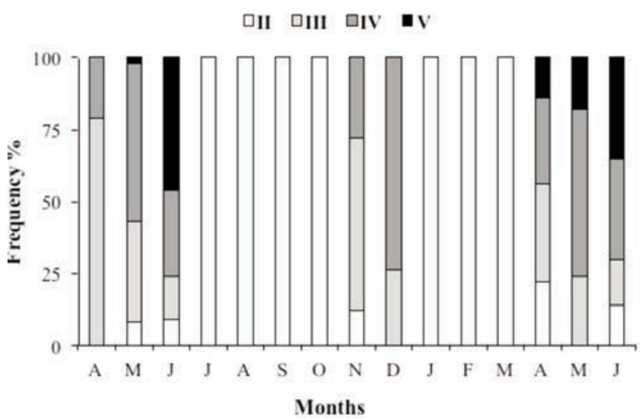


Figure 3. *Pagellus acarne*. Maturity stages of females from Oran Bay. (II: resting, III: maturing, IV: ripe and running, V: spent).

Microscopic analysis

Microscopic analysis of *Pagellus acarne* gonads allowed us to describe the following stages.

<u>Ovaries</u>

- Immature period: oogonia constituting the reserve batch are small round cells not exceeding 40 µm with a clear nucleus (Fig. 4A), some of them have already began their first cellular division.
- Resting period: During this stage (Fig. 4B-D) the oocytes are spherical (about 60 µm) cells arranged as ovarian lamellae with a homogenous and transparent cytoplasm having numerous nucleoli mixed to a large nucleus containing dense chromatin (NPR, nucleoplasmic ratio at its maximum 0.6).

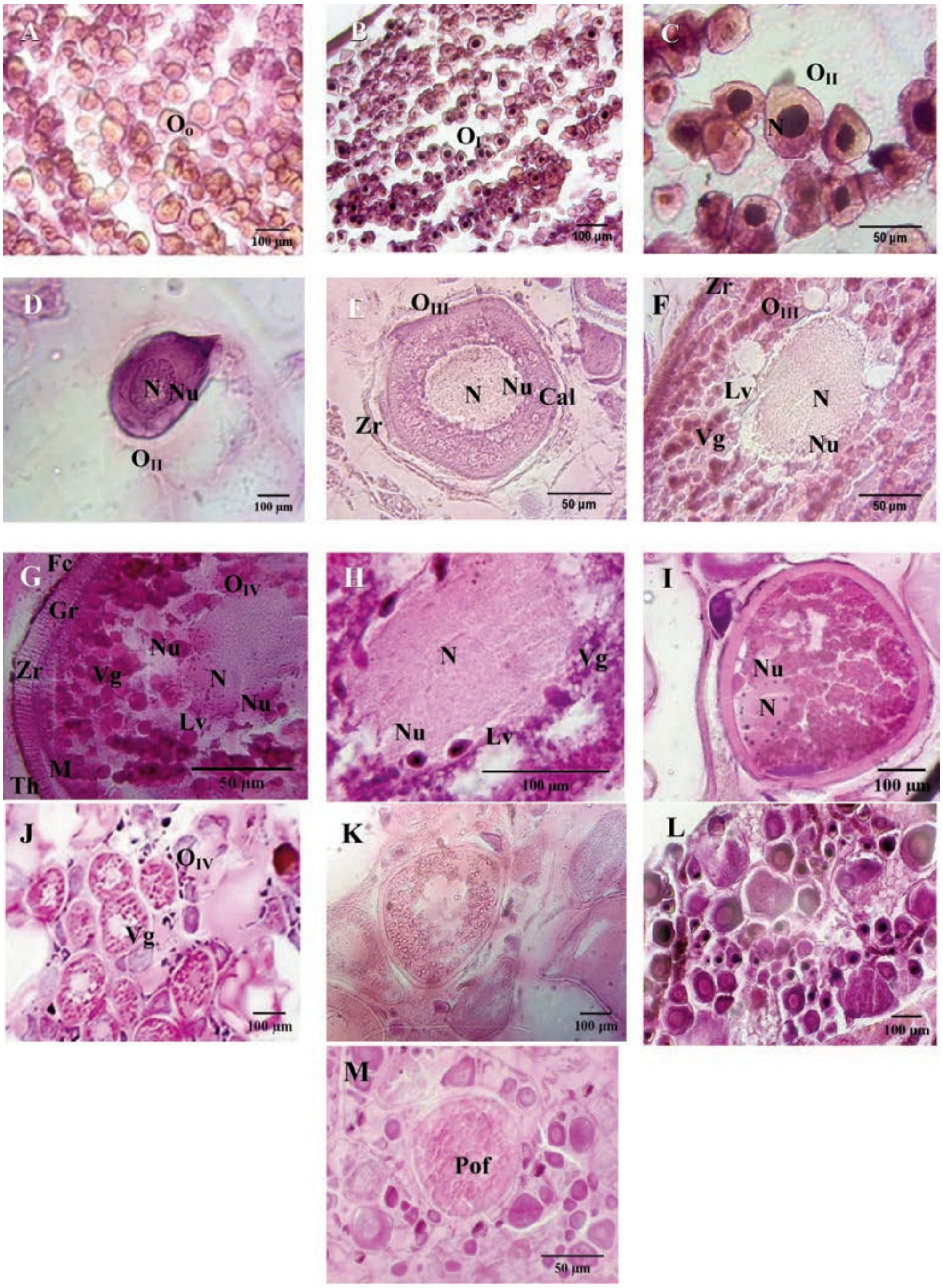


Figure 4. *Pagellus acarne*. Ovarian histological slides of females gonads (A-M) stained by haematoxylin-eosin. Resting ovary with: **A.** Initial peri nucleolar oocytes (O_I) x100. **B.** Advanced peri nucleolar oocytes (O_{II}) with large nucleus and homogenous cytoplasm x400. **C.** Advanced peri nucleolar stage with a homogenous cytoplasm and several nucleoli peripherally arranged x400. **D.** Maturing ovary in previtellogenic stage (O_{III}) presenting large nucleus surrounded by several lipoprotein vesicles x100. **E.** Vitellogenic oocytes with large translucent vitelline vesicles and thin zona radiata (O_{III}) x400. **F.** Early maturing large O_{III} with important nucleus, nucleoli, thick zona radiata and apparition of granulosa x400. **G.** Nucleus surrounded by nucleoli x1000. **H.** Hydrated large oocytes (mature) O_{IV} ready to be released with numerous important lipid vesicles fused with the nucleus x40. **I.** Hydrated large oocytes (mature) x100. **J.** Hydrated large oocytes (mature) OIV ready to be released with numerous important lipid vesicles fused with the nucleus x40. **K.** Hydrated large oocytes (mature)x100. **L.** Beginning of atrisea x100. **M.** Post-ovulatory follicle x400. **Cal**: Cortical alveoli; **Fc**: Follicular cells; **Gr**:granulosa ; **Gv**: Germinal vesicle; **Lv**: Lipid vesicle; **M**: oocyte membrane; **N**: nucleus; **Nu**: Nucleoli; **O**:oocyte; **O**:oogonia; **Pof**: Post-ovulatory follicle; **Th:** theca; **Vg**: Viteline globules; **Zr**: Zona *radiata*.

- Maturing period: during previtellogenesis, ovogonia undergo progressive increase in size reaching (Fig. 4E-F) of up to 217.6 µm with an average NPR equal to 0.41. The period is mainly characterized by a clearer central nucleus containing some supernumerary nucleoli distributed at the periphery; the appearance of thousands of cortical alveoli that marks the beginning of the endogenous lipidic vitellogenesis; granulosa and zona radiata becomes visible.
- Vitellogenesis period (pre-spawning): this phase is characterized by a rapid increase in the size of the oocytes (422 µm), resulting from a massive entry of the proteinic yolk, also the vitelline vesicles are rounded and colored in red marking the exogenous vitellogenesis. (Fig. 4G & H). Lipid inclusions (colorless) move massively near the nucleus and fuse with it (Fig. 4H). The central cytoplasmic zone is filled and repels the cytoplasm at the periphery. The NPR decreases to attain 0.36.At this stage, the oocyte membrane is enveloped by a protective thick membrane zona radiata, measuring 7.26 µm, the whole is enveloped by one layer of ovarian cells forming the granulosa, and the fibrous theca (Fig. 4G).
- Ripening and spawning period: The nucleus moves to the animal pole to initiate the first meiotic division (Fig. 4I). The ovary is full of mature oocytes characterized by fusion of the yolk vesicles into a single hyaline vacuole, hence the name of hyaline mature oocytes (Fig. 4J & K), their diameter is at most 500 µm.
- Post spawning: Some un-emitted mature oocytes enter atresia; the cytoplasm becomes disorganized, the nucleus enters into pycnosis and the yolk disintegrates into a compact cluster. Remains in the ovary only the oogonia reserve that will be activated during the next spawning season (Fig. 4L & M). At the end of the laying period, the fish enters the resting phase. The ovary gets back to its initial appearance, recognized by the presence of the ovigerous lamellae (Fig. 4B). The presence of the atretic oocytes marks the end of the spawning season.

Testes

Parallel to the study of oogenesis, we followed spermatogenesis. The testes were mature at the same time as the ovaries. The testicles are formed of a multitude of oval ampoules separated by a connective tissue (Fig. 5). Seminiferous tubules are composed of isogenic cysts at different stages of maturation (Fig. 5B & C). At maturity, spermatids are transformed to spermatozoids that are dumped in the central lumen then in the deferent ducts.

Hermaphrodite gonads

Pagellus acarne exhibits a protandric hermhroditism already described (Andaloro, 1982; Lamrini, 1986; Pajuelo & Lorenzo, 2000; Arculeo et al., 2000; Coelho et al., 2005) and during our sampling period 65 individuals were hermaphrodites presenting the two sexes in the same gonad, the ovotestis. The histological analysis of ovotestis allowed us to examine germinal tissues for males and females which co-exist. The female part is not yet functional and is represented in each case by oogonous cells and few ovocytes I (O_I) arranged in ovigerous lamellae. The male part is active and is made of seminiferous tubes containing isogenic cysts at different stages of spermatogenesis (Fig. 6).

Sexual cycle and spawning period

The gonado-somatic index (GSI) was used to determine the reproductive period, which was calculated from samples taken monthly. For females, the maximum GSI value was 3.36 in May and 3.29 in December while for males the highest values were 3.57 and 1.90 in the same period. The rest of the year and for the both sexes, the GSI values decreased considerably in summer and February-March period confirming the sexual rest period (Fig. 7) which is in accordance with our macroscopic observation on maturity stages of *P. acarne*.

The monthly changes in hepato-somatic index (HSI) for both sexes followed the same pattern of gonado-somatic index reaching the maximal values in mid spring and autumn respectively 1.99 and 0.79% for males and 1.64 and 1.77% for females. However, the HSI values recorded during these two periods of the year highlight the important metabolic role of the liver in the reproductive cycle of the species. The variations in the condition factor (k) were not very palpable. In fact, k values varied between 0.110 and 0.134 for males and between 0.110 and 0.143 for females all over the sampling period showing that *P. acarne* doesn't undergo important physio-morphological changes during the sexual cycle

Size at first sexual maturity

Proportions of mature specimens analysis (Fig. 8) for each sex showed statistical differences (t-test, $t = 15.35 > t_{0.05,614} = 1.65$), in fact 50% of females were mature before males at 12.75 cm of total length and males were mature at 15.99 cm of total length in Oran Bay.

Discussion

The predominance of males in smaller length classes and females in larger classes is probably due to protandric hermaphroditism already described for this species (Le Trong-Phan & Kompowsky, 1972; Andaloro, 1982; Pajuelo & Lorenzo, 2000; Coelho et al., 2005; Velasco et al., 2011; Bensahla Talet et al., 2010 & 2013; Boufersaoui & Harchouche, 2015), specimens being first males and becoming progressively females.

Evolution of sex ratio in relation to length classes allow

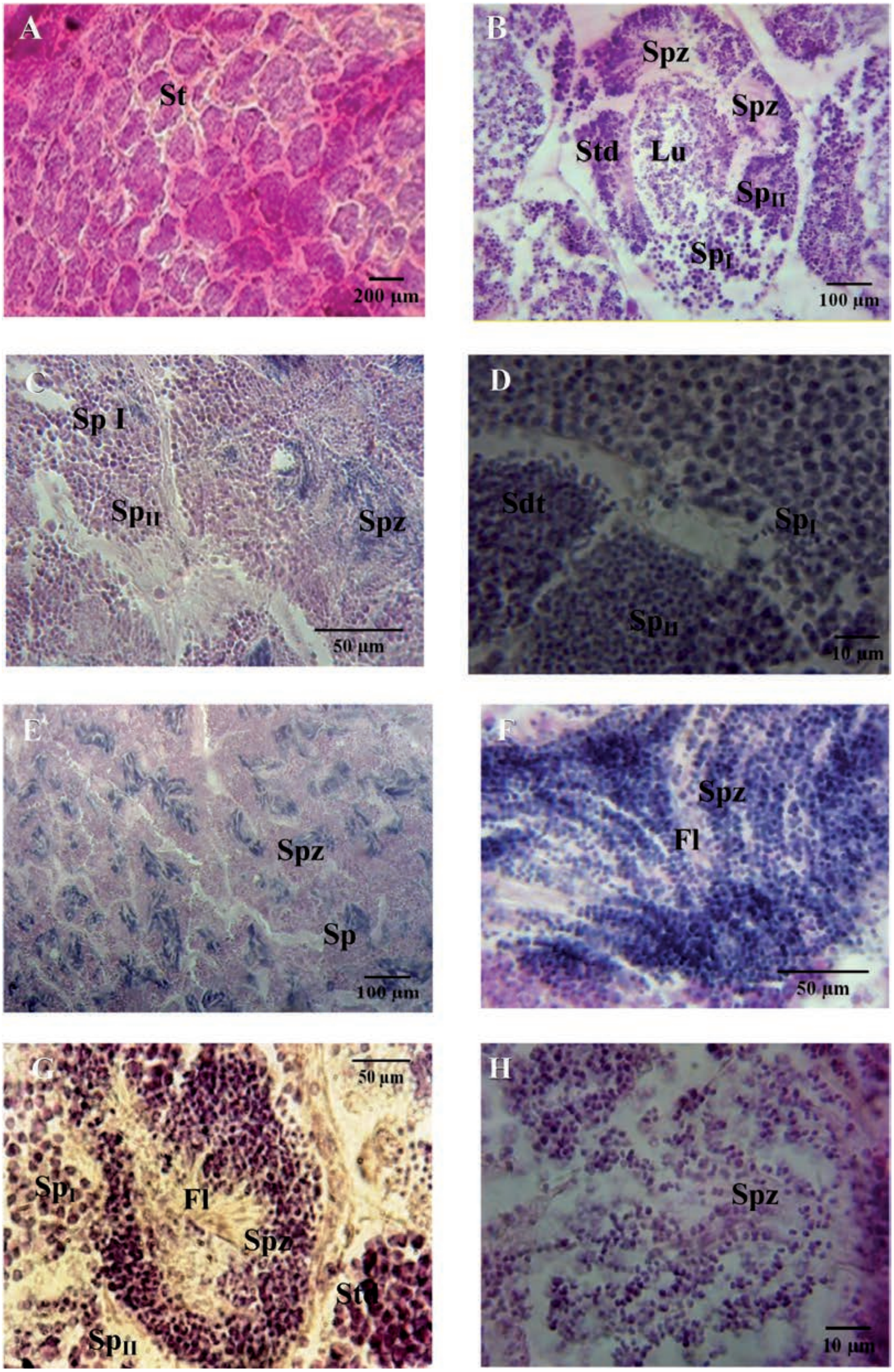


Figure 5. *Pagellus acarne.* Spermatogenesis stages. **A.** Overview of seminiferous tubules x40. **B.** Isogenic cyst at different stages of maturation x100. **C.** x400. **D.** x1000**. E.** x100. **F.** x400: spermiation at the beginning. **G.** x400. **H.** x1000: mature testicle showing seminiferous tubules with lumen full of spermatozoa with their flagella x400. **Fl:** flagella; **Lu**: central lumen; **Sp I**: spermatocyte I; **Sp II**: spermatocyte II; **Spz**: spermatozoa; **St**: seminiferous tubule; **Std**: spermatids.

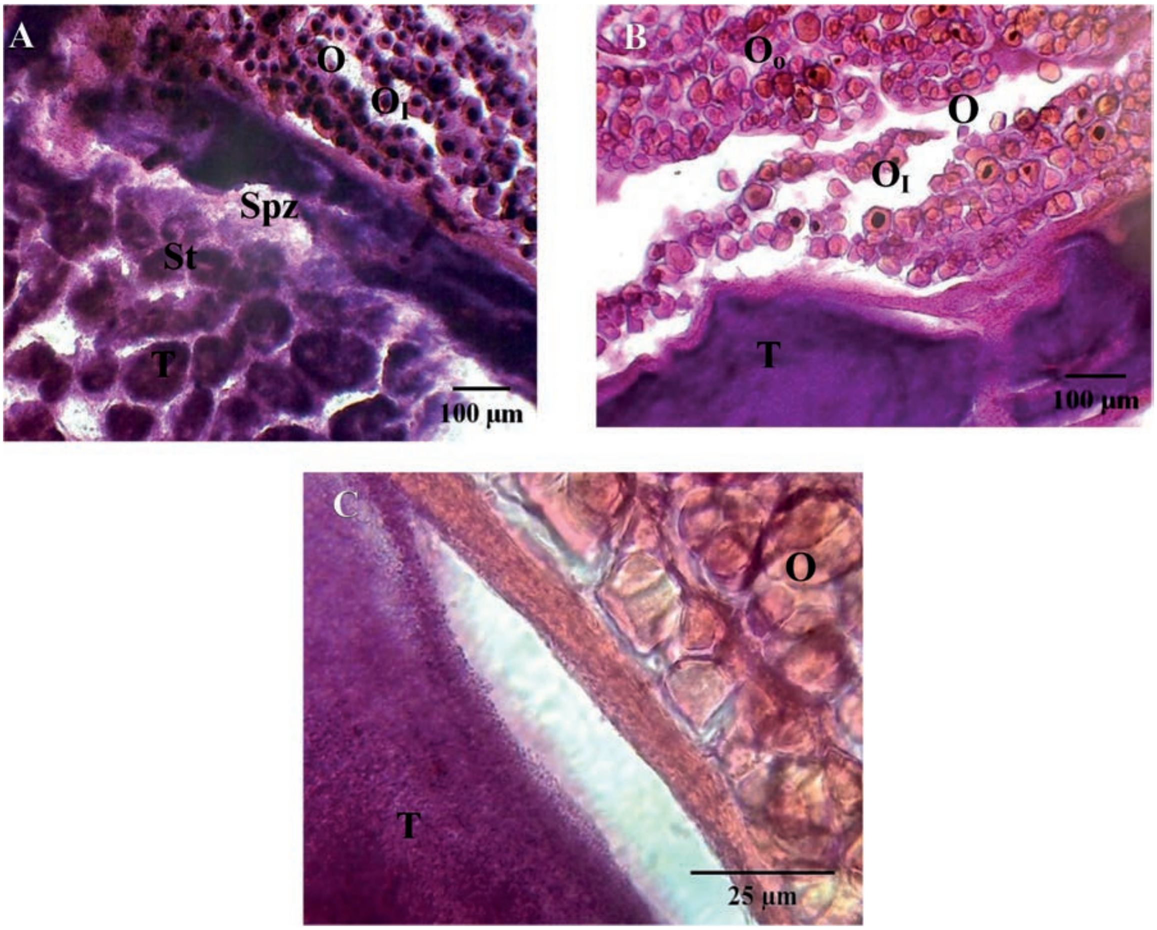
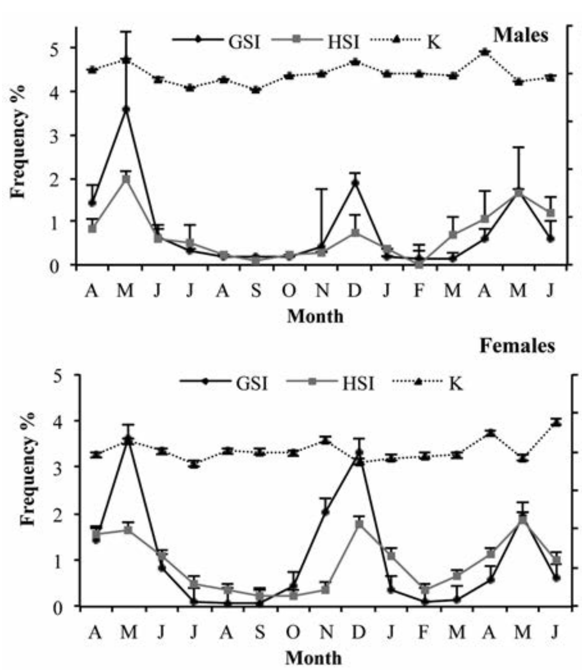


Figure 6. *Pagellus acarne*. Section in hermaphrodite gonad. **A** & **B.** x100. **C.** Mature male gonad with immature female part x1000. **O**: Ovary; **O_I**: ovocyte I; **Oo**: Oogonia; **Spz**: Spermatozoa; **St**: Seminiferous tubule; **T**: Testis.



us to affirm that sexual inversion for this sparidae occurs at 22 cm of total length which is in accordance to observation already made by other authors working on the same species: 20.5-20.9 cm (Dominguez, 2000), 20-24 cm (Coelho et al*.,* 2005), 21.5-23.5 cm (Velasco et al*.,* 2011).

Conducted studies on the reproduction period of *P. acarne* showed that this species had one long spawning season that extends from May to October in south Spain (Dominguez, 2000; Velasco et al*.,* 2011) and Portugal (Santos et al*.,* 1995; Coelho et al*.,* 2005) with an important peak in summer. In western Algerian coasts the monthly evolution of the different indexes showed that this sparidae had two spawning periods the first one between April to June with a peak in May and an autumnal period, between November and January with a peak in December a finding

Figure 7. *Pagellus acarne*. Monthly changes in the gonado-somatic, hepato-somatic indexes and condition factor (GSI, HIS and k) of males and females caught in Oran Bay.

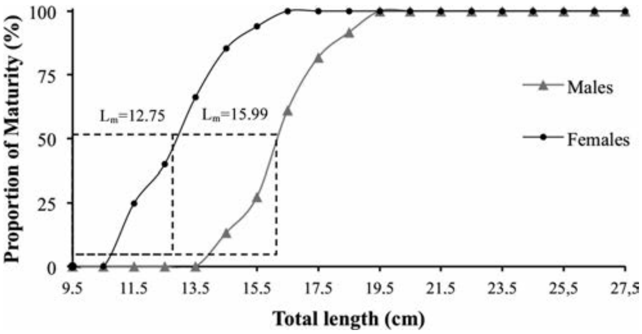


Figure 8. *Pagellus acarne*. Sexual maturity ogives for males and females caught in Oran Bay.

already described in Algiers Bay (Boufersaoui & Harchouche, 2015) for the same species, also in Sarpa genus (Corbera et al, 1996; Mendez-Villamil et al., 2001; Criscoli et al., 2006).

Unlike, spawning period of the axillary seabream in the eastern Atlantic begins in autumn and ends in spring, from November to March with a maximum in winter (Le Trong-Phan & Kompowsky, 1972; Lamrini, 1986; Pajuelo & Lorenzo, 2000). While this process occurs in August in the Italian waters (Arculeo et al*.,* 2000) and in autumn along the Egyptian coasts (Faltas, 1995). It appears that, *P. acarne* has a tendency to spawn in winter and mid spring in low latitudes and late during the rest of the year in high latitudes.

In the other hand, some authors (Wootton, 1979; Pajuelo & Lorenzo, 2000; Mouine et al., 2007) link these regional differences in time and duration of the spawning act with biotic and abiotic factors such as hydro dynamism, temperatures, salinity and food availability in the different regions of the Mediterranean basin. In fact, the reproduction period is correspondingly much longer when environmental conditions are favorable (Gonçalves & Erzini, 2000), the upwellings and related zooplankton abundance can also affect many species reproduction (Parrish et al., 1981).

For the length of first sexual maturity (table 1), Dominguez (2000), Pajuelo & Lorenzo (2000), Velasco et al. (2011) reported it between 19 and 21.7 cm in Spanish waters. In Italian coasts Andaloro (1982) recorded it at 16.5 cm while in Portuguese waters Coelho et al. (2005) found it equal to 17.6 cm for females and 18.1 cm for males. In the eastern Atlantic, the size at the first sexual maturity was comprised between 19 and 20.9 cm of TL (Le-Trong Phan & Kompowski, 1972; Lamrini, 1986). In the Egyptian waters, the first sexual maturity is reached for most of the population at sizes between 14 and 19 cm of TL (Faltas, 1995). In Algerian waters, Boufersaoui & Harchouche (2015) found females to be mature at 16.45 cm while males were mature at 16.80 cm while in Oran Bay first sexual maturity was reached at 12.75 cm for females and 15.99 cm for males precociously compared to females in other regions of the Mediterranean Sea.

The richness of Algerian waters is probably due to Algerian current which is considered to be the most energetic in the Mediterranean basin (Taupier-Letage & Millot, 1988; Mortier, 1992; Salas et al., 2001). In fact, the entrance of Atlantic waters via the strait of Gibraltar generates important upwelling's enhancing the development of plankton first link of the aquatic food web. Differences in sizes at first sexual maturity can be attributed to the geographic location of the studied areas and consequently to the different environmental conditions, such as temperature, salinity and food (Gonçalves & Erzini, 2000; Moutopoulos & Stergiou, 2002; Bottari et al., 2014).

Table 1. Length at first sexual maturity of *Pagellus acarne* given by different authors. **M**: Males, **F**: females, **Lm**: length at first sexual maturity (cm).

Region		L_m	Author
Portugal	Atlantic	M 18.10 F 17.60	Coelho et al., 2005
		20.95	Santos et al., 1995
Morocco		19.00	Le-Trong Phan & Kompowski, 1972
		20.90	Lamrini, 1986
Spain	Canary Island	M 15.80 F 19.40	Pajuelo & Lorenzo, 2000
	Atlantic	F21.70	Velasco et al., 2011
	Alboran Sea	F20.10	
	Alboran Sea	19.00	Dominguez, 2000
Algeria	Oran Bay	M 15.99 F 12.75	**Present study**
Italy	Tyrrhenian Sea	16.50	Andaloro, 1982
Egypt	Alexandria	14.00-19.00	Faltas, 1995

The MLS (Minimum Landing Size) set for *P. acarne* at 15 cm of TL according to Algerian legislation (Executive decree n° 2004-86 of 18 March 2004), seems to be insufficient given that it is higher than the length at first sexual maturity found during our study (12.75 cm for females and 15.99 cm for males), it would be recommended to increase this length and set it at 18 cm assuring the renewal of the resource.

Conclusions

The sparidae family constitutes an important portion of daily landed demersal fish in Algerian fisheries and *P. acarne* is of commercial importance to local and national fisheries. According to Algerian legislation, the trawl fisheries closure from the 1st May to the 1st August (Executive decree n° 3-481a of 24 April 2004) aiming to protect renewal of the national resources is to our viewpoint insufficient to insure this objective. We can suggest the increase of the Minimum landing size and the establishment of two closure fishing periods per year to allow renewal of the resources by protecting juvenile and adult spawners from excessive fishing.

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