

# Age, Growth and Ovarian Histology of *Sardinella aurita* (Valenciennes, 1847) in the South of Atlantic Moroccan Coast

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## Abstract

Otoliths reading and length frequency distribution were used for age determination and growth estimation of *Sardinella aurita* (round sardinella) stock of Southern Atlantic coast of Morocco. Both otoliths' method for age determination and Bhattacharya's method for length frequency analysis showed five age groups. The growth performance index revealed that there is significant accordance among method of length frequency distribution and otoliths reading for stock assessment studies of *Sardinella aurita* stock in the south of Morocco. The microscopic observation of female gonads using histology method was investigated for the first time in our study area and confirms the presence of five principal stages of ovary of *sardinella aurita*: immature, maturing virgin and recovering spent, mature (or pre-spawning phase), spawning, post-spawning or spent. In addition, our results of the fecundity showed that the mean relative fecundity obtained is estimated at  $193 \pm 98$  oocytes/g which is lower compared to those obtained in other areas in the Atlantic coast.

## Introduction

Round sardinella, *Sardinella aurita* Valenciennes, 1847 (Pisces, Clupeidae), is a widely distributed, middle-sized pelagic fish. The distribution of the species is tropical and subtropical often associated with major upwelling systems and sea surface temperature (Durand *et al.*, 1998; Baali *et al.*, 2019). The intermediate position that *S. aurita* occupy in the food web gives them a particular importance compared to other species. In the North West Africa, this species is part of stocks that are not restricted to the territorial waters of a single country, but extend into the territorial waters of two or more neighboring riparian countries (shared stocks). Moreover, some stocks are migrating along the coast: they could be in the exclusive economic zone (EEZ) of a country for part of the year and in other EEZ(s) for the rest of the year. Hence, *S. aurita* is a key species among the ecosystem of the northwest African upwelling

region (Bard and Koranteng, 1995). Although *Sardinella aurita* is still one of the most pelagic species caught in the south of Morocco, its biomass has known fluctuations these last years (Baali *et al.*, 2017). The study of reproduction and growth of the round sardinella has been thoroughly studied in the Mediterranean Sea (Wassef *et al.*, 1985; Bensahla Talet *et al.*, 1988; Stergiou *et al.*, 1997; Bouaziz *et al.*, 2001; Gaamour *et al.*, 2001; Dahel *et al.*, 2016) and the Atlantic Ocean (Fontana, 1969; Pham-Thuoc and Szypula, 1973; Cury and Fontana, 1988; Roy *et al.*, 1989; Bakun and Parrish, 1990; Fréon *et al.*, 1997; Quatey and Maravelias, 1999; Samba, 2011; Baali *et al.*, 2015, 2017). However, little is known about the growth of *Sardinella aurita* and no study has been done about its fecundity and ovarian histology in our study area. In the present investigation, data on the age and growth of *S. aurita* for the southern Moroccan coast are evaluated. In addition, we are going to estimate the *Sardinella aurita* fecundity

for the first time in the south of Moroccan coast as well the microscopic stages of the female gonad using ovarian histological sections. The knowledge of spawning stages and spawning frequency based on growth parameter is important for determining potential annual fecundity as a function of fish size and understanding the dynamic of this species, thus providing appropriate statistical models for rational exploitation (Farley *et al.*, 2015; Baali *et al.*, 2017).

## Materials and Methods

### Sampling

The study was conducted upon 838 specimens of *Sardinella aurita* collected monthly from February 2015 to January 2017 throughout the South of Moroccan Atlantic coast in the area between Cape Boujdor and Cape Blanc from commercial fishermen, who generally use purse seine as fishing gear. Sampling frequency depends on the abundance of sardinella because it is not always available in our fishing areas. In the laboratory, the caught fishes are sorted, identified, inventoried and weighted. The total length (TL) and the total weight (TW) of the fish sampled were measured to the nearest 0.1 cm and 0.1 g respectively. Gonads were removed from each individual to identify the sex and then weighted (GW) at the nearest 0.01 g.

### Growth Study

The length-weight relationship (LWR) was estimated according to Froese (2006) power equation:

$$W = a \times TL^b$$

Where W is the weight (g), TL is the total length (cm), a (intercept) and b (slope). These last two parameters were estimated by regression curve analyses based on the logarithmic transformed equation (Beverton and Holt, 1957):

$$\text{Log}_{10}W = b \times \text{Log}_{10} TL + \text{Log}_{10} a$$

The Pearson's correlation ( $r^2$ ) and the growth exponent (b) were used respectively as an indicator of significance of regression and to determine the type of growth (isometric if  $b=3$ , allometric if  $b \neq 3$ ). The test used for checking the isometry ( $b=3$ ) or allometry ( $b \neq 3$ ) of length-weight relationships is the t test of student after linearization of the relationship by logarithmic transformation (Pajuelo and Lorenzo 2000; Arneri *et al.* 2001).

This test consists of comparing the slope of the regression line (b) with a theoretical slope equal to 3. The main hypothesis proposes that there is no significant difference between the slope (b) and the value 3. The alternative hypothesis assumes that there is a significant difference between the slope (b) and the value 3.

So:

If  $t_{\text{obs}} < t_{0.05, n}$ , the main hypothesis is accepted; and if  $t_{\text{obs}} > t_{0.05, n}$ , the main hypothesis is rejected.

Otoliths were removed from each specimen. We chose the sagittae which have a large size allowing a better readability of the marks. Their extraction was done using a fine tweezer after a transverse head section, inclined at  $45^\circ$  between the eyes and the operculum. The age of *Sardinella aurita* was estimated using two methods: otoliths and Bhattacharya's methods. In the first one (direct method using otoliths); annual rings on otoliths were counted using optical system consisting of Nikon Zoom - Stereomicroscope. From these counts, the age classes were assigned with additional sets of the time of the formation of growth marks and the month the fish were sampled. The date of birth for *S. aurita* were set at April, which approximately corresponds to the peak spawning period based on information on maturity stages and gonadosomatic index occurred in our study area (Baali *et al.*, 2017). On other note, the Bhattacharya method (1967), which is based on length frequency, was also used as second method to estimate length-at-age key through FiSAT II program (version 1.2.2) (Gayanilo *et al.*, 1995). Thereafter, in order to estimate growth parameters K and  $L_\infty$ , FiSAT II (version 1.2.2) (Gayanilo *et al.*, 1995) program was run using Analysis of length-at-age data that calculated from the two methods cited previously. This is a subroutine in FiSAT II to estimate growth parameters K and  $L_\infty$ . This subroutine allows non-linear estimation of growth parameters from length-at-age data. The Von Bertalanffy plot is used to estimate  $t_0$  from the known age/length data and estimated  $L_\infty$  and K, the method is based on the regression analysis of the following formula:  $-\ln(1-L_t/L_\infty) = -K * t_0 + K * t$  Where: the age t is the independent variable (x) and  $-\ln(1-L(t)/L_\infty)$  is the dependent variable (y) of the linear regression (El-haweet *et al.*, 2005). The Growth performance ( $\phi'$ ) was estimated using the empirical equation of (Pauly and Munro, 1984):  $(\phi') = 2 \log_{10} L_\infty + \log_{10} k$  (Munro and Pauly, 1983) where: k and  $L_\infty$  are the Von Bertalanffy's growth parameters.

### Histology and Fecundity

After removing ovarian tissue from the body cavity of round sardinella, a maturity stages were attributed to each fish using the macroscopic maturity scale adopted by Holden and Raitt (1974) (Table 1).

Histological study was conducted on the ovarian tissue to establish the microscopic characteristics of each defined macroscopic stage and to highlight the successive stages of vitellogenesis in oocytes. A fraction of the middle part of the ovarian tissue representing different macroscopic maturity stages was sampled and fixed in Bouin solution for two to three days depending on the size and density of the sample. Tissue dehydration with a graduated ethanol solution of

**Table 1.** Maturity scale for “partial spawners” (Holden and Raitt, 1974).

Stages of maturity	State	Features ovarian
Stage I	Immature	Ovary and testis about 1/3rd length of body cavity. Ovaries pinkish, translucent; testis whitish. Ova not visible to naked eye.
Stage II	Maturing virgin and recovering spent	Ovary and testis about ½ length of body cavity. Ovary pinkish, translucent; testis whitish, more or less symmetrical. Ova not visible to naked eye.
Stage III	Mature/pre-spawning phase	Ovary and testis about 2/3rds length of body cavity. Ovary pinkish yellow colour with granular appearance, testis whitish to creamy. No transparent or translucent ova visible.
Stage IV	Ripe/spawning phase	Ovary and testis from 2/3rds to full length of body cavity. Ovary orange-pink in colour with conspicuous superficial blood vessels. Large transparent, ripe ova visible. Testis whitish-creamy, soft.
Stage V	Spent/ post-spawning phase	Ovary and testis shrunken to about ½ length of body cavity. Walls loose. Ovary may contain remnants of disintegrating opaque and ripe ova, darkened or translucent. Testis bloodshot and flabby

increasing concentrations ranging from 70% to 100%, xylene clarification and paraffin infiltration were performed by an automated tissue processor (Leica ASP300). The paraffin-coated tissues were cut with a rotating microtome (Leica RM2255) to obtain 2 µm thick tissue sections. After mounting on glass slides, the sections were dried in an oven for 12 h at 60°C and stained with Harris's hematoxylin and eosin (H&E) (Martoja, 1967) using Sakura Tissue-Tek DRS 2000 Slide Stainer. The histological descriptions were achieved with a light microscope OLYMPUS BX 53 equipped with a camera at magnifications ranging from 40x to 400x.

The fecundity study was conducted on samples of ovaries in stage IV (spawning phase) that were kept in Gilson's liquid (Simpson, 1951) for one month. Then, the oocytes are freed and can be counted under a binocular magnifying glass after removal of the remaining ovarian tissue and rinsing. Absolute fecundity (FA) was assessed by gravimetric method (Bagenal and Braum, 1978). The relationship between FA, length and weight of fish was described using the exponential equation:  $FA = ax^b$ , which after a logarithmic transformation takes the form  $\log FA = \log a + b(\log x)$ , where  $x$  is either length or weight and  $a$ ,  $b$  are the regression constants. In this study, Relative fecundity (FR) was considered as the number of eggs per unit of body weight (Nikolskii, 1963). The round sardinella is a multiple spawner that lays several eggs during the same breeding season (Mustać and Sinovčić, 2012; Baali *et al.*, 2017). The development of the oocytes occurs asynchronously, that is not all the eggs reach maturity simultaneously. Oocytes of different stages and sizes are found throughout the reproductive period. In the present study we used mature oocytes varying in size between 0.3 and 0.8 mm (mean  $0.54 \pm 0.13$  mm).

## Results

### Growth Study

#### Length-Weight Relationship

The analysis of total catch data of *S. aurita* in the southern Moroccan coast shows that the TL ranged from

12 cm to 35.5 cm and the TW varied between 13.12 g and 508 g. The exponential expression  $b$ , of the length-weight relationship was 3.27 for sexes combined that suggests a positive allometric growth and the equation would be  $W = 0.004 TL^{3.27}$  ( $R^2 = 0.98$ ) (Figure 1).

#### Estimation of Age and Growth Parameters

Using the Bhattacharya (1967) method, the population of *S. aurita* in the south of Morocco is composed of five groups with mean lengths of 14.68, 18.07, 26.55, 29.26 and 32.20 cm respectively (Figure 2).

The results of the analysis of length-at-age data (subroutine in FISAT II software) of *S. aurita* allowed us to estimate:  $L_{\infty} = 36.24$  cm (Figure 3),  $K = 0.42 \text{ year}^{-1}$  and  $t_0 = -0.38$  year, hence, the linear growth model for the LT estimation according to Von Bertalanffy's would be  $LT = 36.24 (1 - e^{-0.42(t-0.38)})$ . Therefore, the growth performance index of  $\phi'$  was 2.74.

The results of length-at-age data (Table 2) carried out with otoliths reading method (Figure 4) show that *Sardinella aurita* attain a maximum age of about five years old with predominance of class 3 years old in the samples whereas class 0-year-old was the less caught classes (Table 2). The asymptotic length ( $L_{\infty}$ ), the growth coefficient ( $K$ ) and the theoretical age at length 0 ( $t_0$ ) were 34.74 cm,  $0.42 \text{ year}^{-1}$  and  $-1.03$  year respectively. These results allowed us to determine the equation:  $LT = 34.74 (1 - e^{-0.42(t-1.03)})$  with growth performance index of  $\phi' = 2.70$ .

#### Ovarian Histology

The histological analysis of ovaries was made with reference to the size of the oocytes, the cytoplasmic homogeneity, the amount and the distribution of lipid and protein inclusions (yolk globules), and it showed five stages. Immature phases (stage I and II), mature or pre-spawning phase (stage III), ripe/spawning phase (stage IV), post-spawning phase (stage V).

Immature stage: Only oocytes in primary growth stages are present namely: oogonia ( $\phi < 10 \mu\text{m}$ ), nuclear chromatin oocytes ( $10 < \phi < 50 \mu\text{m}$ ), early and late perinucleolar oocytes ( $50 < \phi < 100 \mu\text{m}$ ) (Figure 5a). Nests

of oogonia were attached to the surface of ovarian lamellae; they were characterized by small cytoplasm and an intensely basophilic rounded central nucleolus (Figure 5b). Nuclear chromatin oocytes had ring of chromatin surrounding the nucleolus and threadlike nuclear chromatin. Like oogonia, they had a small cytoplasm but were more basophilic, irregularly shaped and had a larger nucleus (Figure 5c). The early perinucleolar oocytes were larger, rounded in shape and characterized by cytoplasm and nucleoli of different sizes, both strongly basophilic (Figure 5d). In the late perinucleolar stage oocytes, the cytoplasm became less basophilic with a granular structure and multiple nucleoli that are usually peripheral. Moreover, the cytoplasm of oocytes in advanced stage differentiates into a clear outer layer and a denser inner layer (Figure 5e).

Pre-spawning stage: During this stage, primary growth oocytes are still present in the ovarian follicles concomitantly with early and late developing oocytes. The diameter of early developing oocytes ranged from

100 to 250  $\mu\text{m}$ . They had a large, spherical, and central nucleus that contains at least one small nucleolus (Figure 6a). They are mainly characterized by the appearance of cortical yolk globules along the peripheral region of the homogeneous cytoplasm (Figure 6a) and also by a thin acellular membrane of eosinophilic material, the zona radiata surrounding these oocytes. In the late developing oocytes, the size increased reaching 250 to 350  $\mu\text{m}$  in diameter with the progressive accumulation of small vesicles around the nucleus that merge into larger vesicles. The yolk globules and vesicles invaded gradually the cytoplasm and gave an irregular appearance to the nuclear membrane. Simultaneously, the thickness of zona radiata increase and the follicular envelope became visible (Figure 6b). In both types of oocytes, fine chromatic granules are dispersed in the nucleoplasm.

Ripe/Spawning stage: At this stage, the early stages of oocytes can be observed in the ovarian stroma with oocytes in advanced vitellogenesis which reached a size of between 350 and 450  $\mu\text{m}$ . However, oocytes in

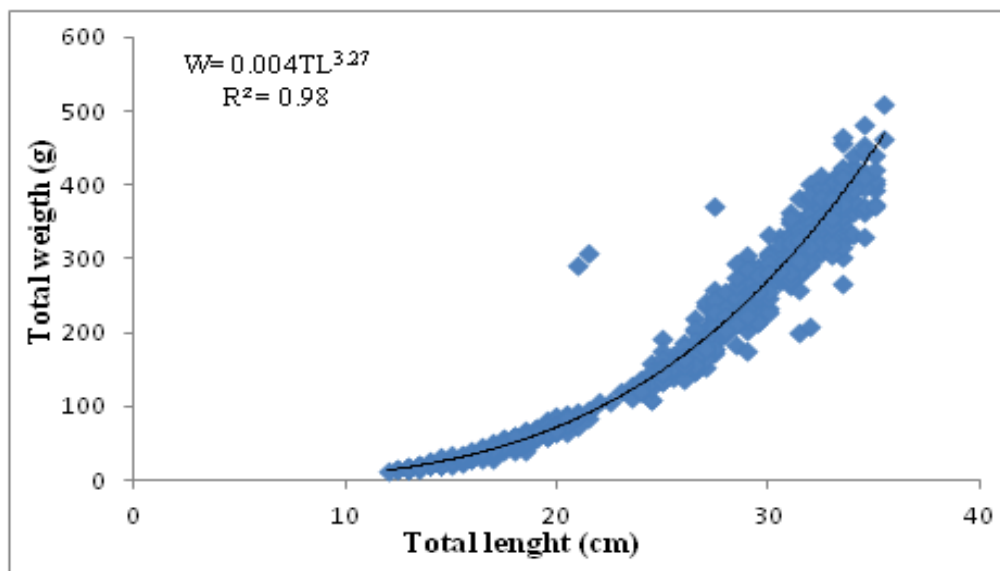


Figure 1. Length-weight relationship of *S. aurita* from the south of Moroccan Atlantic coast.

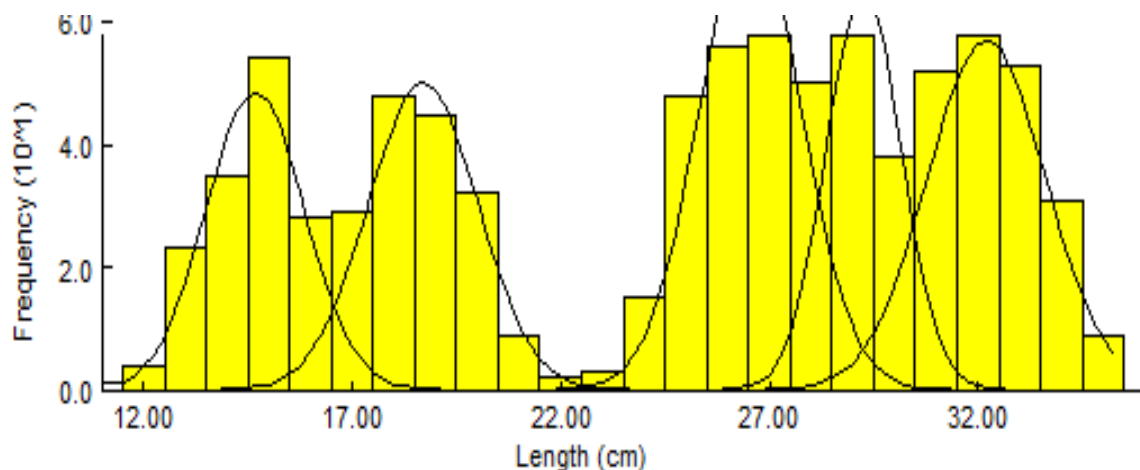
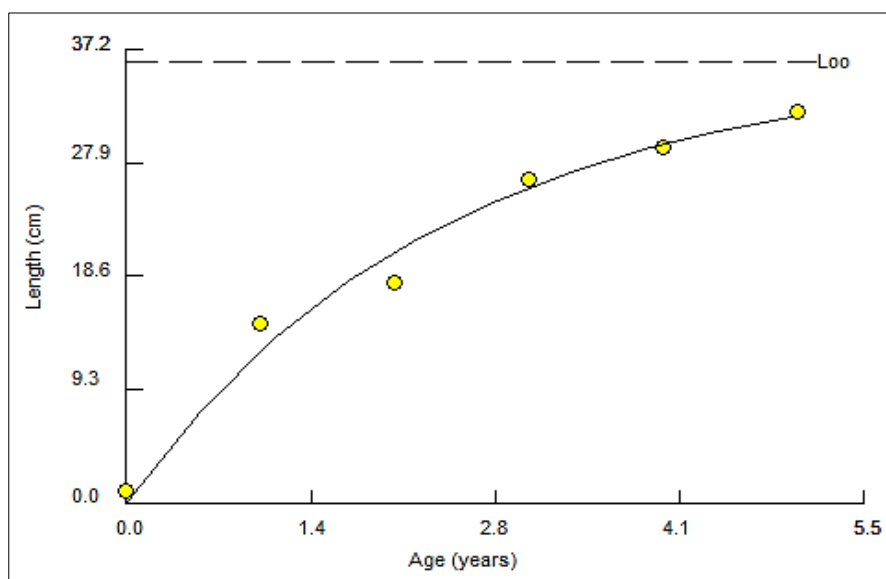


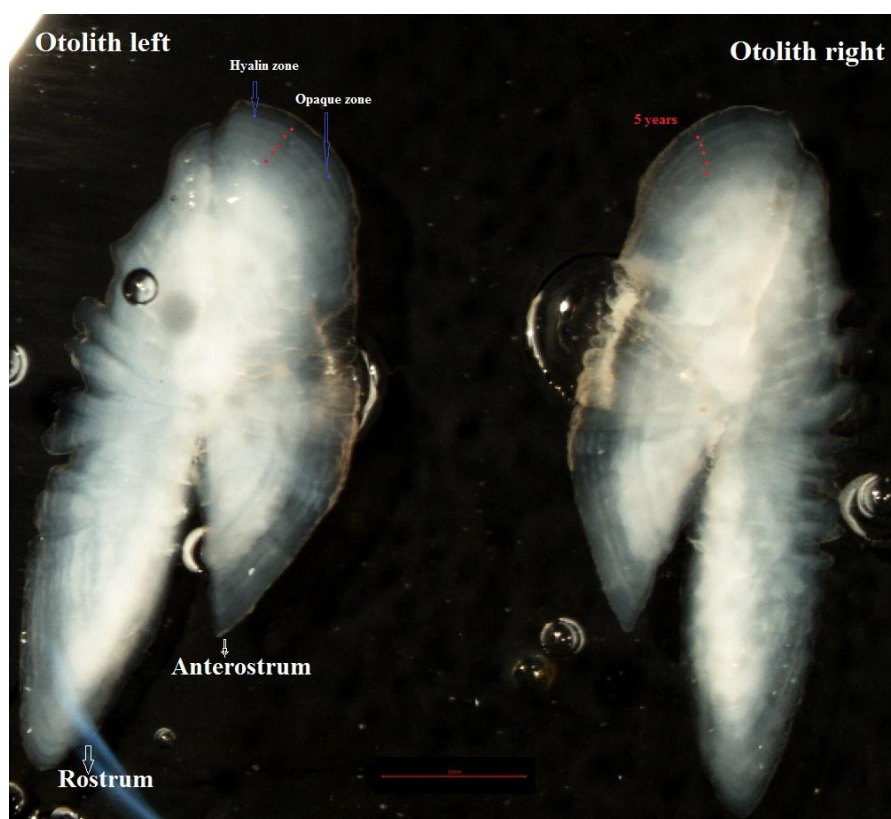
Figure 2. Length distribution of *S. aurita* separated by Bhattacharya method from the Atlantic Moroccan coast (FISAT II 1.2.2).



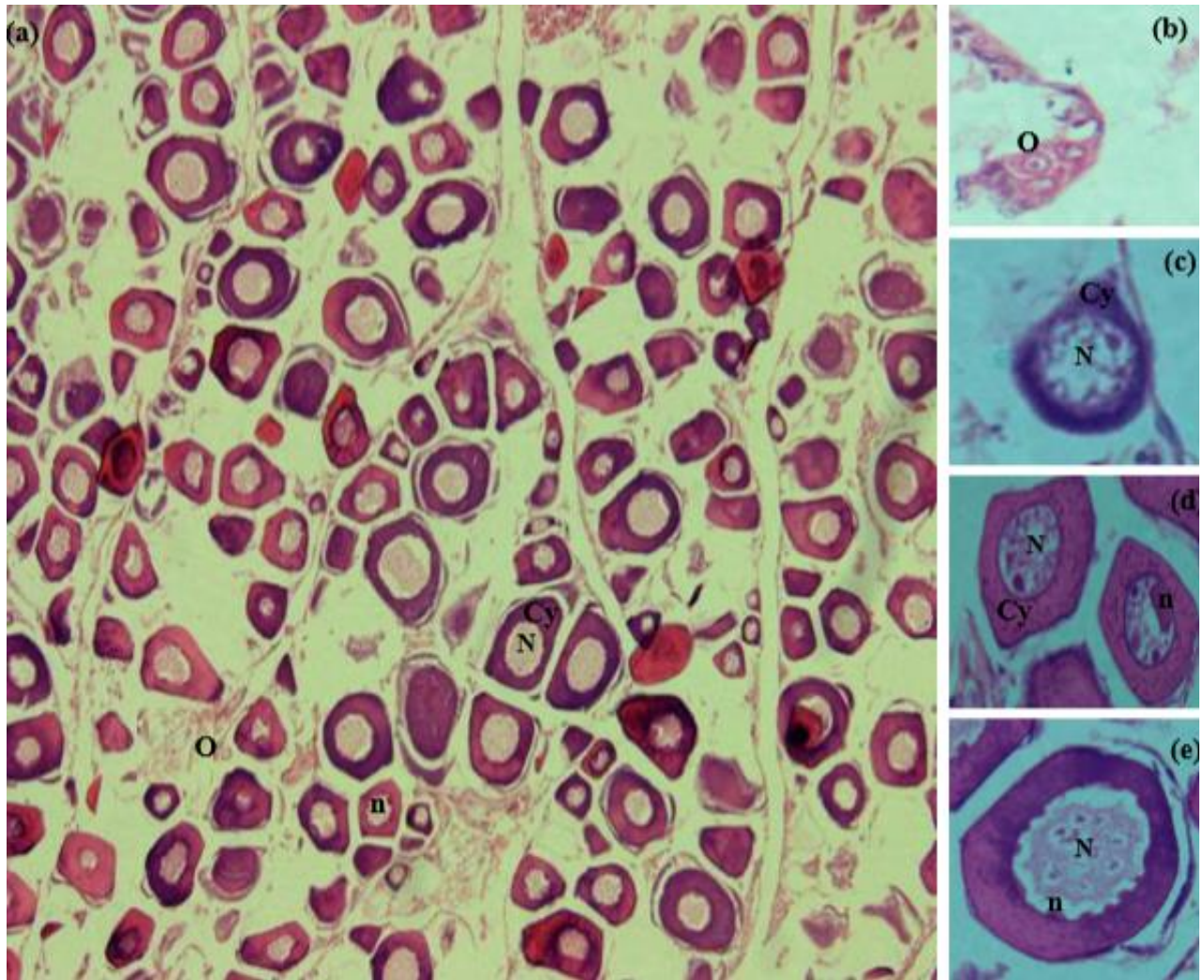
**Figure 3.** Asymptotic lengths  $L_{\infty}$  of *S. aurita* from the Atlantic Moroccan coast (FISAT II 1.2.2).

**Table 2.** Age-length key of *S. aurita* sampled in the south of Atlantic Moroccan coast.

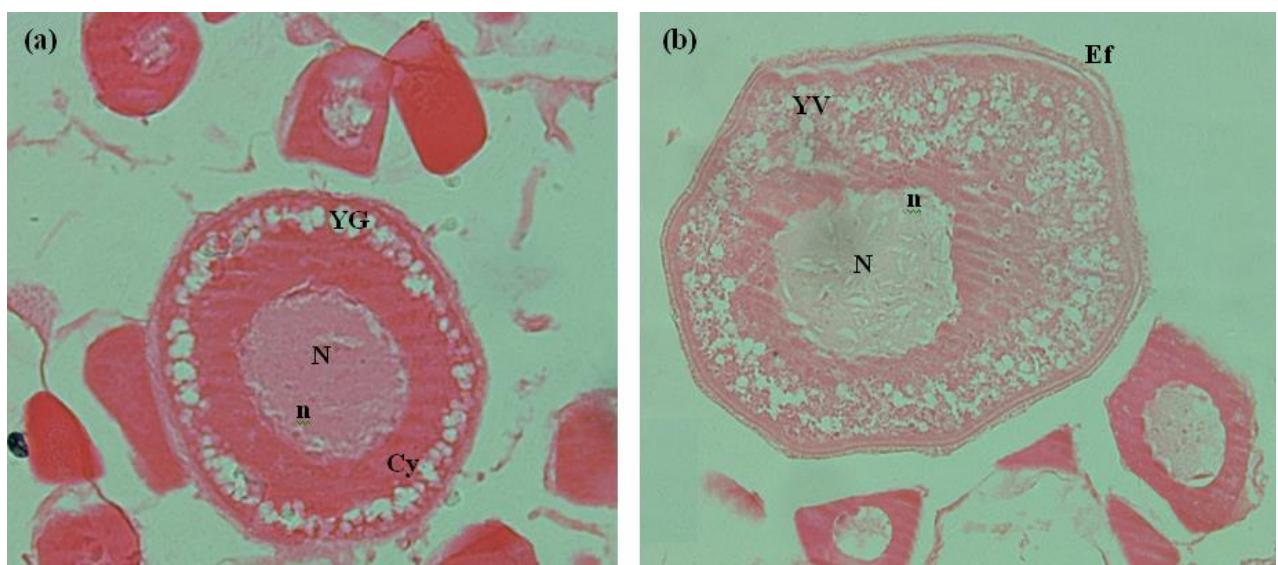
Age group (year)	0	1	2	3	4	5
Frequency	4	153	102	233	89	27
Mean TL (cm) (otoliths' method)	14.62	18.05	23.87	28.86	30.72	32
Mean TL (cm) (length frequency method)		14.68	18.07	26.55	29.26	32.20



**Figure 4.** Otoliths (sagittae) of *Sardinella aurita* observed under a binocular magnifying glass in reflected light.



**Figure 5.** Histological characteristics of female gonad of *S. aurita* at the immature stage. a) cross section through an immature ovary visualizing oocytes at different stages of maturity (100x), b) Oogonia (400x), c) Nuclear chromatin stage oocyte (400x), d) and e) Early and late perinucleolar stage oocyte (400x). Cy: Cytoplasm, N: nucleus, n: nucleolus, O: oogonia.



**Figure 6.** Histological characteristics of female gonad of *S. aurita* at the pre-spawning stage. a) Early development oocyte (200x), b) Late development oocyte (200x); Cy: cytoplasm, Ef: follicular envelope, N: nucleus, n: nucleolus, YG: yolk globules, YV: yolk vesicles.

primary growth are less evident. The oocytes in advanced vitellogenesis seem to be liquefied with fused vitelline inclusions giving it a hyaline appearance with an irregular nuclear envelope. The large cytoplasm was slowly replaced with yolk platelets and the zona radiata increases in thickness. Just before laying, nucleus begins to leave central position and migrates toward periphery (Figure 7a). It is generally difficult to observe on histological sections. Ripe oocytes are detached from their follicular envelop and are released into the ovarian lumen to be expelled (Figure 7b). The appearance of follicular lights devoid of matures oocytes reveal the initiation of egg laying process (Figure 7b).

Post-spawning: Empty post-ovulatory follicles and oocytes with a loose, atretic structure characterize the ovaries at this stage (Figure 8). Also, oocytes at different stages of development are encountered but atretic oocytes are more common. Furthermore, yolk inclusions are dispersed into the lumen of the ovarian lamellae after rupture of the zona radiata of vitellogenic atretic oocytes.

Spent/Resting stage: The ovarian lamellae appeared longer and narrower, and the interstitial space between them was wider. Only some atretic oocytes and previtellogenic oocytes were discernible among the ovarian lamellae. The spaces freed by degeneration of vitellogenic oocytes are recolonized by these resting previtellogenic oocytes containing a voluminous central nucleus with numerous nucleoli (Figure 9).

### Fecundity

The absolute fecundity (AF) was highly varied from 9,397 oocytes for females having 27 cm in TL to 176,712 oocytes for those with 33.5 cm in TL, although the average absolute fecundity recorded was at 71,858 oocytes per female. As for the average of the relative fecundity, it was evaluated at 193 oocytes/g of female and ranged between 41 and 418 oocytes/g of female

(Table 3). For matured females of round sardinella, the evolution of the absolute fecundity as a function of size (TL) and total body weight (TW) showed a positive correlation estimated at 0.59 and 0.63, respectively and an exponential relationship expressed by the following equations (Figure 10):

$$FA=5 \times 10^{-8} \times TL^{8.02} (n=36; R^2=0.59)$$

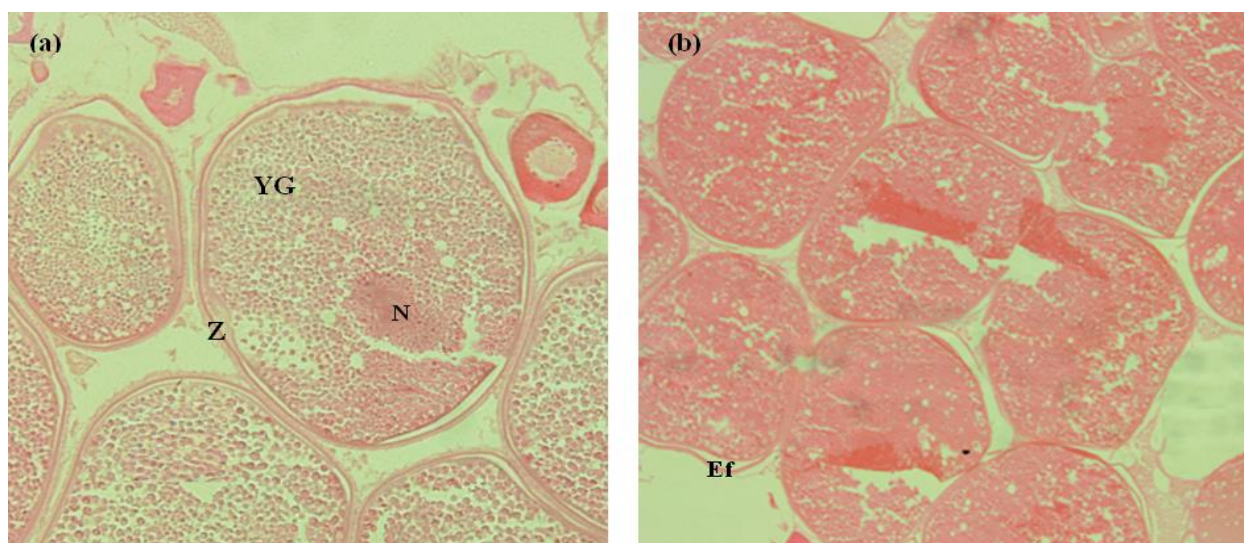
$$FA=0.012 \times TW^{2.64} (n=36; R^2=0.63)$$

## Discussion

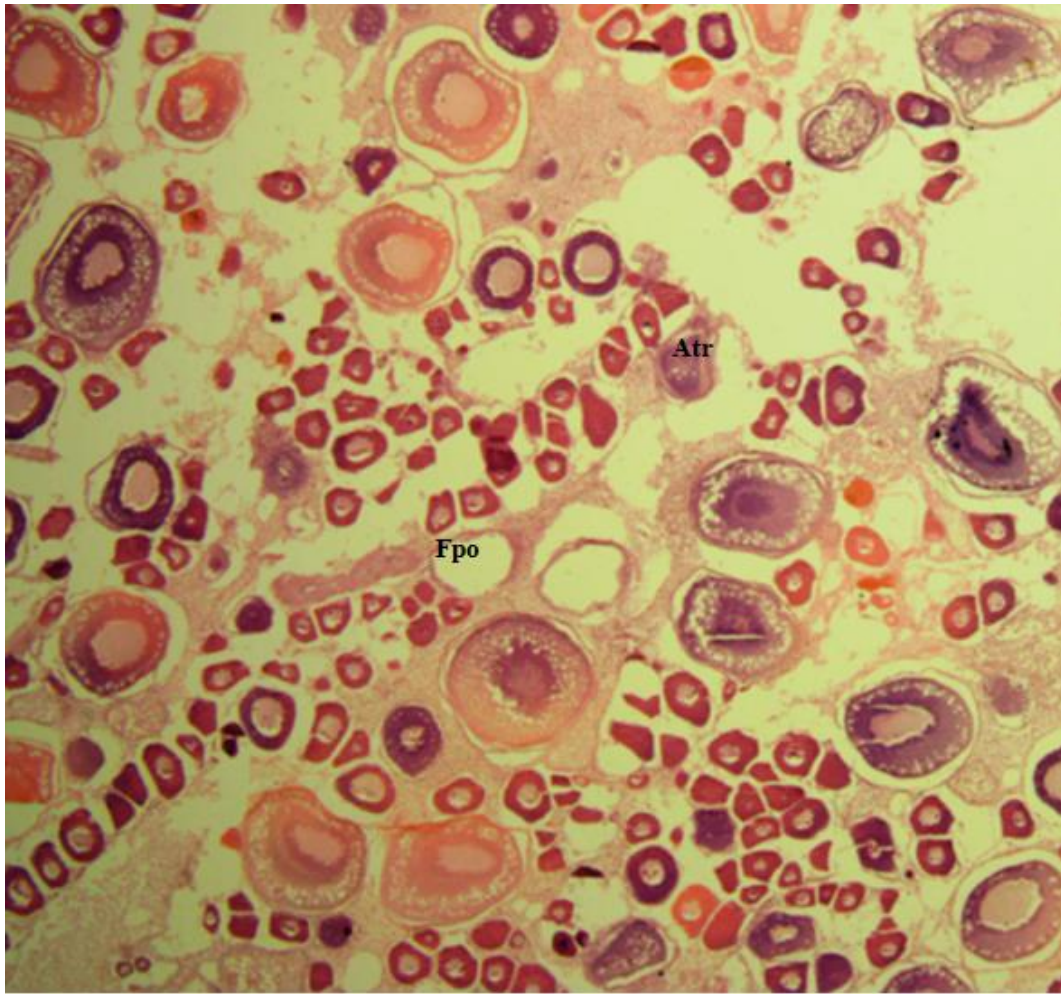
### Growth Study

In our study, the correlation between length and weight was high with a positive allometry, i.e. weight grows faster than length. Such results were reported from Senegal (Boëly, 1979; Camarena-Lurhs, 1986; Fréon, 1988; Diouf *et al.*, 2010; Samba, 2011), Congo (Ghéno and Fontana, 1981), Mauritania (Holzlöhner *et al.*, 1983; Wague and Mboji, 2002; Pascual-Alayon *et al.*, 2008), Algeria (Bouaziz, 2007; Dahel *et al.*, 2016) and Morocco (Baali *et al.*, 2015) whereas Kartas (1981) and Merella *et al.* (1997) revealed that weight increases proportionally with length respectively in Tunis and the Balearic Islands (Spain) respectively (Table 4). It is important to note that the authors used different size ranges and units of measurement (fork length or total length, eviscerate weight), which may explain the difference between the results.

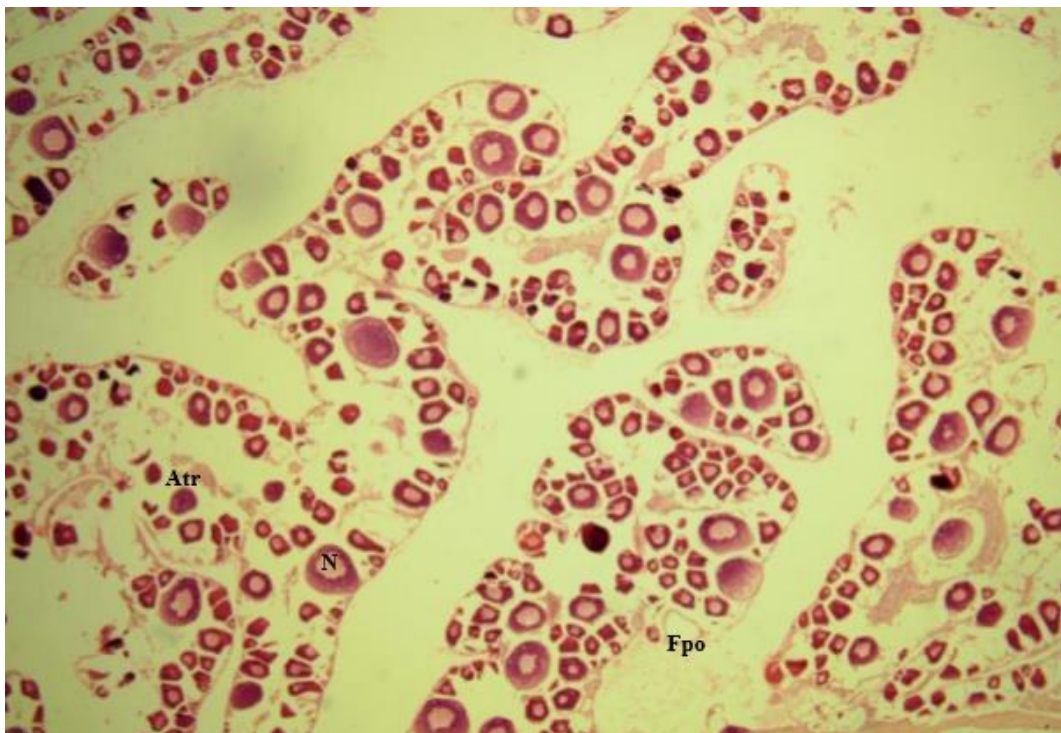
In the present study length-at-age was determined by otolith reading and Bhattacharya method. The corroboration between the two methods was satisfactory and showed that we have five age groups, which apparently agreed well with previous studies in the same area (Baali *et al.*, 2015; Amenzoui and Baali, 2018). Growth rates of South of Moroccan Atlantic Coast specimens seem to be similar to other specimens from neighboring waters. Thus, in Mauritanian coast,



**Figure 7.** Histological characteristics of female gonad of *S. aurita* at the spawning stage. a) Nucleus Migration (100x); b) Oocytes in the spawning stage (100x); Ef: follicular envelope; YG: Yolk globules; N: nucleus; Z: zona radiata.



**Figure 8.** Histological characteristics of female gonad of *S. aurita* at the post-spawning stage. Atr: atretic oocytes, Fpo: post-ovulatory follicles (40x).

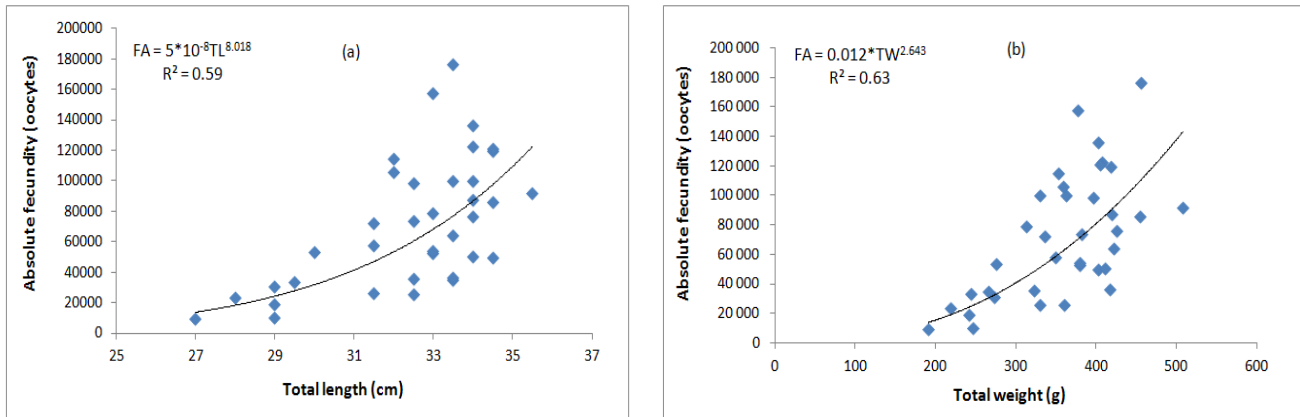


**Figure 9.** Histological characteristics of female gonad of *S. aurita* at the resting stage. Atr: atretic oocytes, Fpo: post-ovulatory follicles, N: nucleus (40x).



**Table 3.** Estimated mean of absolute and relative fecundity for mature females of *S. aurita*.

	Mean	Standard deviation	Minimum	Maximum
Absolute fecundity (oocytes)	71 858	42224	9 397	176 712
Relative fecundity (oocytes/g)	193	98	41	418

**Figure 10.** Absolute fecundity (FA) of round sardinella as a function of (a) total length (TL, cm) and (b) body weight (W, g).**Table 4.** Length-weight relationship parameters of *S. aurita* sampled in different areas.

Study area	Size range (cm)	a	b	Authors
Senegal	5-32 (FL)	0.006	3.290	Camarena-Luhrs (1986)
Senegal	-	1.00E-06	3.388	Boëly (1979)
Senegal	4-32 (FL)	0,006	3.274	Fréon (1988)
Senegal	7.7-40 (TL)	3.00E-05	3.162	Diouf <i>et al.</i> (2010)
Senegal	8-36.5 (TL)	7.00E-06	3.04	Samba (2011)
Mauritania	22-37 (TL)	0.002	3.375	Holzlohner <i>et al.</i> (1983)
Mauritania	15-32 (FL)	0.008	3.227	Lawal and Mylnikov (1988)
Mauritania	23.6-37.5 (TL)	0.007	3.126	Pascual-Alayón <i>et al.</i> (2008)
Tunisia	-	0.007	3.01	Kartas (1981)
Spain	-	0.006	2.99	Merella <i>et al.</i> (1997)
Algeria	-	0.003	3.28	Bouaziz (2007)
Algeria	8-25.5 (TL)	0.005 (M)	3.13 (M)	Dahel <i>et al.</i> (2016)
		0.004 (F)	3.16 (F)	
Morocco	14.5-34.5 (TL)	0.004 (M)	3.25 (M)	Baali <i>et al.</i> (2015)
		0.004 (F)	3.24 (F)	
Morocco	12-35.5 (TL)	0.004	3.27	Present work

*Sardinella aurita* are growing rapidly: 1.5 cm per month for the sizes ranging from 20 to 25 cm and 1.2 cm per month for sizes between 25 and 30 cm (Ould Sidina *et al.*, 2006). Also, the study of Boëly (1982) in Senegal and Samba (2011) in the Cape Verde peninsula showed a very rapid growth with a maximum age observed at 4 years. Instead, a recent study conducted by Dahel *et al.* (2016) in the Mediterranean Sea (Algeria) showed that the round sardinella have a slow growth with a maximum age of 7 years. With regards to our study area, the values of von Bertalanffy's equation found for *Sardinella aurita* were not comparable with those reported in other literature (Table 5). The interpretation of the results is complex because of fish migrations and sampling method used by the authors. Indeed, we suggest that may be a problem of representativeness of one of the size classes in the samples according to the

authors (a fringe of the stock may be absent or undersampled). In addition, reading otolith method tends to overestimate the K growth rate due to reading difficulties and the presence of false marks as indicated by Santamaria *et al.* (2008). Similarly, contrasts with the findings of several authors were reported for the method of reading scales (Pham-Thuoc and Szypula, 1973; Krzeptowski, 1981; Fréon, 1986; Maxim and Maxim, 1987; Chesheva, 1998). These differences can be explained by the varying of interpretation of the scales. Otherwise, whatever the method used in the present work, the  $L_{\infty}$  obtained in our study area was higher than that recorded for the Mediterranean Sea by Dahel *et al.* (2016) where females reach a final size  $L_{\infty}=32.26$  cm and males  $L_{\infty}=27.3$  cm. The variability in growth may result from several factors such as difference in mortality rates, environmental conditions

or genetic variations (Dutka-Gianelli and Murie, 2001). However, the value of growth performance ( $\phi'$ ) estimated using the otolith method for age determination was similar to that of Bhattacharya's method for length frequency analysis, which supports the validity and reliability of the growth parameters calculated from both methods (Pauly, 1979). The high values obtained from this index in the Atlantic Ocean could be associated with the upwelling that takes place in the study area, while lower values could be due to low productivity waters in the Mediterranean Sea (Table 5).

### Histology

Since the cellular and tissular changes in ovaries due to gametogenesis are related to the external morphochromatic features, the gonadal development phases can be identified by morphochromatic features. Nevertheless, degree of ovary's maturity, spawning's sub-phases and the difference between ovarian immature and resting phases can only be noted by the histological diagnosis. Thus, microscopic features become essential to determine with any accuracy the maturation of the gametes and confirm the macroscopic analysis.

The round sardinella is a multiple spawner that lays several times during the same breeding season. The development of oocytes takes place asynchronously, that is to say that all the oocytes do not reach maturity simultaneously. Oocytes of different stages are found throughout the reproductive period (Baali *et al.*, 2017). The multiple breeding has been described in majority of the teleosts (Cortés and Aron, 2011).

In the present study, based on microscopic features, five stages of ovarian development were described: immature phase, mature or pre-spawning

phase, spawning phase, post-spawning phase and spent phase. These same stages were found by Bouhali *et al.* (2015) for *Sardina pilchardus* from the Gulf of Annaba (Algeria), and by Chakrabarti and Barun (2017) for *Gudusia chapra*. The study of sardinella conducted by Fontana (1969) in the Pointe Noire region (Congo) showed the existence of six sexual stages. These same stages have been found in *Sardina pilchardus* (Pinto and Andreu, 1957; Amenzoui, 2010; Bedairia *et al.*, 2016) while in *Sardinella brasiliensis*, seven maturity stages are established (Isaac-Nahum *et al.*, 1983). Although the number of maturity stages differs between authors, the general pattern of oocyte development was similar in all *Sardinella* species. This was also reported by Le Duff (1997) in other teleost fish (*Solea vulgaris*, *Scomber scombrus*, *Trachurus trachurus*).

Histological comparisons between immature and resting stages revealed striking morphological resemblances such as diffused chromatin and small cytoplasm, with oocytes of stage I and II. Thus, in order to differentiate between the two stages, size at first maturity was used to identify fish in the first breeding season and fish in the resting stage after spawning period.

### Fecundity

Despite the fact that round sardinella is a highly fertile species but its fecundity varies through time even if in similar environmental conditions; besides, it is dependent on the weight and size of individuals (Fontana and Pianet, 1973; Conand, 1977). The absolute fecundity (AF) of *S. aurita* showed a close interrelationship with size and weight of fish, which has been reported by other authors (Figuera, 1967; Reyes, 1981; Ramírez and Huq, 1986; Guzmán *et al.*, 1999;

**Table 5.** Parameters of von Bertalanffy's equation of *S. aurita* from different areas.

Study area	Authors	Methods	$L_{\infty}$ (TL, cm)	$t_0$ (an)	$k$ (an <sup>-1</sup> )	$\phi'$
Mauritania	Pham-Thuoc and Szyplula, (1973)	Scales	40.70	0.33	-0.63	2.73
Mauritania	Chesheva (1998)	Scales	41.63	0.26	-0.87	2.66
Mauritania	Pascual-Alayón <i>et al.</i> (2008)	Otoliths	36.6	0.39	1.46	2.72
Mauritania	Santamaria <i>et al.</i> (2008)	Scales and otoliths	38.97	0.32	-1.78	2.69
Senegal and Mauritania	Maxim and Maxim, 1987	Scales	45.22	0.36	-0.69	2.72
Senegal	Boëly (1979)	Length frequency and scales	36.92	0.97	0.21	2.97
Senegal	Krzepkowski (1981)	Scales	43	0.3	-0.95	2.75
			40.69	0.27	-0.99	2.66
			42.12	0.28	-0.99	2.7
Senegal	Boëly <i>et al.</i> (1982)	Length frequency and scales	36.2	1.21	-0.06	3.05
Senegal	Camarena-Luhrus (1986)	Scales	37.77	0.61	-0.75	2.94
Senegal	Fréon (1986)	Scales	36.2	1.21	-0.06	3.03
Senegal	Samba (2011)	Otoliths	31.45	1.79	0.55	3.6
Algeria	Dahel <i>et al.</i> (2016)	Otoliths	32.26	-1.99	0.13	2.13
			27.3	-1.9	0.18	2.12
Morocco	Baali <i>et al.</i> (2015)	Otoliths	33.72	-0.34	0.83	2.97
			33.66	-0.02	0.97	3.04
Morocco	Present study	Otoliths	34.74	-1.03	0.42	2.74
		Length frequency	36.24	-0.38	0.42	2.70

Tsikliras and Antonopoulou, 2006). While a positive correlation has only been reported between AF and length for round sardinella populations in the Mediterranean (Bensahla Talet *et al.*, 1988; Gaamour *et al.*, 2001) and the eastern Atlantic (Pham-Thuoc and Szyplula, 1973). Our data analysis showed that the mean relative fecundity was at  $193 \pm 98$  oocytes/g, which was low compared to those obtained by Samba (2011) in the Senegalese zone (644 oocytes/g), Conand (1977) and FAO (1979) in the same area (Senegal) (400 oocytes/g), Ramírez and Huq (1986) (222 oocytes/g) in the Gulf of Cariaco, Tsikliras and Antonopoulou (2006) (462 oocytes/g) in the north-eastern Mediterranean Sea, Bensahla Talet *et al.* (1988) (246-933 oocytes/g) in Algerian waters and Gaamour *et al.* (2001) (240-480 oocytes/g) in Tunisian coast. This high variability of results could be due to either genetic differences among the round sardinella populations, or environmental conditions or a combination of both (Stearns, 1992; Wootton, 1998).

## Conclusion

The data on age of *Sardinella aurita* (round sardinella) produced by this study suggest that age can be identified by the length frequency analysis (LFA) even if the age determination is possible from otoliths. Thus, the LFA method gives possibility to define ages only with measurement, this being beneficial since it is quick, feasible to use and less hard, especially if we overcome its disadvantages such as sampling bias (a small number of younger and/or older fish), gear and site selectivity, that makes modal separation difficult (Gallucci *et al.*, 1996). However, the direct reading of otoliths remains the most precise method, because it gives more reliable results in years instead of age group. In addition, the current study contributes to better understand the ovarian changes of *Sardinella aurita* by histological diagnosis. This information also helps in understanding the histology cell pattern of the round sardinella reproduction in Moroccan waters.

## Ethical Statements

The care and use of experimental animals, sampling and analysis techniques used in this work are consistent with the Moroccan Legislation Article 14, Section 3, Bill 122.22 and the Cartagena Protocol on Biosafety to the Convention on Biological Diversity promulgated by the royal decree "1.09.123".

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## Authors Contributions

All authors are responsible for the general design of the manuscript. AB conducted the research and the collect of sample and wrote the first draft of the manuscript. OB wrote the first draft of the manuscript and conducted the histological study. KCO wrote the first draft of the manuscript and conducted statistical analyses. KA and AY conduct and supervise the research project. All authors contributed on specific aspects.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported on the paper.

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