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Microscopic study of the gonads in males and females of *Euthynnus alletteratus* (Rafinesque, 1810) and *Sarda sarda* (Bloch, 1793), two species of coastal affinity from the gulf of Guinea, Côte d'Ivoire

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Abstract
The object of this work is to provide more complete information on the microscopic stages of the gonads in males and females of *Euthynnus alletteratus* and *Sarda sarda* from the gulf of Guinea, through a histological study.
This study was carried out from January 2016 to December 2016 on specimens from artisanal marine fisheries. Monthly samples of the two fish species were taken at the Zimbabwe and Abobo-doumé landing stages. A total of 1,221 individuals, including 713 *Euthynnus alletteratus* (362 males and 351 females) and 508 *Sarda sarda* (168 males and 340 females) were examined. The microscopic maturity stages led to the determination of pre-reproductive, reproductive and post-reproductive stages, used as an indicator of reproductive accuracy. The post-reproductive stage was not observed in males. The pre-reproduction stage showed the presence of spermatogonia, spermatocytes and spermatids. As for the reproductive stage, in addition to these cells, it was marked by an abundance of spermatozoa. In females, the pre-reproduction stage revealed oocytes in the primary growth and cortical alveolus stages. The reproductive stage showed oocytes in the vitellogenesis (primary, secondary and tertiary), late migration and hydration stages. The rate of oocyte atresia was 23.55±7.21% in *Euthynnus alletteratus* compared with 10.12±3.53% in *Sarda sarda*. The post-ovulatory follicles observed at the post-breeding stage show that spawning takes place in Ivorian waters.

Keywords: Microscopic, gonads, atresia, *Euthynnus alletteratus, Sarda sarda*

1. Introduction
In Côte d'Ivoire, artisanal marine fishing accounts for 48% of national production [1]. The majority of this fishery's production, which is entirely destined for the local population, is made up of small tunas [2]. Indeed, minor tunas are actively sought after by fishermen because of their easy accessibility (moving around in shoals) and their high commercial and nutritional value [3, 4]. Among these tuna species, *Euthynnus alletteratus* (Rafinesque, 1810) and *Sarda sarda* (Bloch, 1793) are regularly landed by artisanal marine fishermen. These species help to satisfy the population's increased need for animal protein. They therefore represent an important food and economic resource for local populations [2, 5, 6]. What's more, these species represent an important link in the trophic chain, as they are predators in the marine food web [7, 8]. They should be managed rationally, which is not the case. A profitable exploitation strategy is therefore essential. However, the development of sound and efficient stock management requires a prior knowledge of biology. Studies have been carried out worldwide on the reproduction of both species. Most investigations are fragmentary and limited and have only been carried out in females [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]. Yet reproduction cannot take place without males, whose role is to release spermatozoa, which will then fertilize the oocytes released by females. The aim of this work is to study the microscopic stages of the gonads in males and females of these species caught by artisanal fishermen operating in the Gulf of Guinea.

2. Materials and Methods

2.1 Study area

The Gulf of Guinea can be seen as a large maritime area and a hotspot for marine biodiversity [23]. This oceanic zone belongs to the Central Eastern portion of the Atlantic Ocean, which covers West Africa from Morocco to Congo [24]. Côte d'Ivoire's maritime zone, which belongs to the Atlantic Ocean, extends from Cap des Palmes (8°W) in the west to Cap des Trois-Pointes (2°30'W) in the east, over a length of around 600 km [25]. Both species are caught in this fishing zone (Fig 1).

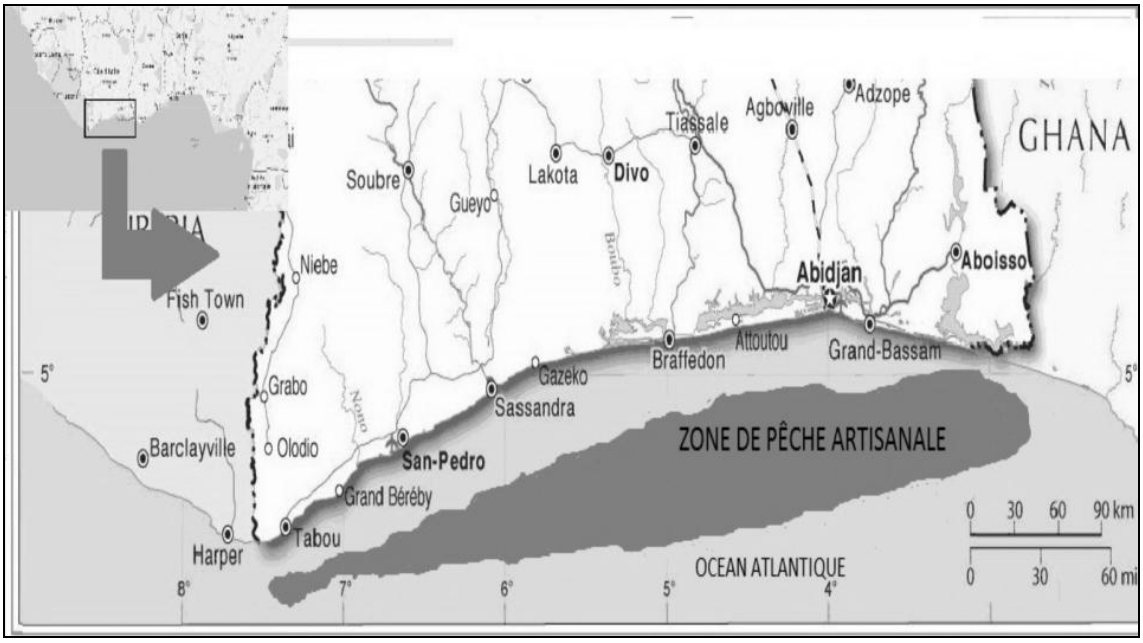


Fig 1: Fishing area for artisanal fishermen in Côte d'Ivoire

2.2 Samples

The *Euthynnus alletteratus* (tuna) and *Sarda sarda* (skipjack) specimens used were collected during weekly landings by artisanal fishermen at the landing stages of Zimbabwe (fishing village, located in Port-Bouët) and Abobo-doumé (Ebrié village, located in Attécoubé) from January 2016 to December 2016. The collected fish were immediately transferred under dry ice in the laboratory of the Department of Living Aquatic Resources of the Oceanological Research Center. For each fish, the fork length was measured to the nearest cm using a caliper, and the total mass was determined using a Scout Pro scale with a precision of 0.1 g and a capacity of 4,000 g. After dissection of each fish, sex was then identified by macroscopic examination of the gonad. A small portion of gonad (4-5 cm) was removed and fixed in 10% formalin for histological study.

2.3 Reproduction parameters

The gonad condition of both species was studied in the following stages:

2.4 Production of sections

The technique used to produce the sections is based on classical histology according to Martoja & Martoja-Pierson (1967) [26]. The various stages are:

2.5 Blocking: organs were first passed through 1 bath of 70° alcohol, 2 baths of 90° alcohol and 2 baths of 100° alcohol for 45 minutes, 25 minutes, 20 minutes, 2 hours (2 times) respectively. Next, the organs were passed through 2 successive xylene baths of one hour each, before passing through 2 kerosene baths for 2 hours each in an oven (60 °C). Finally, the organs were removed from the last kerosene bath and transported to a capsule mold filled with new, filtered, melted kerosene.

2.6 Sections and mounting: The blocks obtained were mounted on the microtome and 6-micron-thick thin sections were made. These were placed in a bath float to extend them and enable perfect mounting between slide and coverslip.

2.7 Slide staining: Sections were stained with Harris hematoxylin and eosin according to Martoja & Martoja-Pierson (1967) [26]. In this method, slides are successively immersed in two alcohol baths (2 min/bath), one hemalun bath (2 min), two running water baths (2 min/bath), one eosin bath (2 min), two alcohol baths (100°) (3 min/bath) and finally three xylene baths (5 min/bath). Once the slide has been removed from the last xylene bath, a drop of Eukitt is added to the preparation. A coverslip is then placed on the slide, which is then air-dried.

2.8 Determination of microscopic stages

Histological sections of testes and ovaries were observed using the microscopic scale of Brown-Peterson *et al*. (2011) [27]. Depending on the largest stage of sex cells present, 2 developmental stages (consisting of 4 stages) were considered in males (Table 1) versus 8 stages in females (Table 2). This made it possible to determine the stage of development reached by the fish. The sections were also observed for post-ovulatory and atretic follicles. The sections were read under a microscope.

2.9 Determination of post-ovulatory follicles (POF)

Post-ovulatory follicles (POFs) were determined in ovaries with oocytes in the germinal vesicle migration and hydration stages after reading histological slides. Taking morphological characteristics into account, 4 types of OPF were described

according to their age (Table 3). The presence of post-ovulatory follicles was used to determine whether or not oviposition had taken place recently.

2.10 Determination of oocyte atresia rate: Oocyte atresia is the degeneration of oocytes during growth or maturation. The rate of oocyte atresia is defined as the percentage of oocytes in the ovary that fail to mature. Determination of this parameter in this study followed the protocol of Murua & Motos (2006) [28]. Using histological slides from mature fish gonads (stages III, IV and V), atretic oocytes were counted. Subsequently, all oocytes in the ovary were totaled and the atresia rate was defined according to the following formula:

$$\%AO = \frac{NAO}{TNO} \times 100$$

With % AO: Percentage of atretic oocytes; NAO: number of atretic oocytes; TNO: total number of oocytes.

Table 1: Developmental stages based on microscopic examination of fish testicles [27]

Stages of testicles development	Internal characteristics
Pre-reproduction	Presence of spermatogonia, spermatocytes and spermatids. Spermatogonia are larger than spermatocytes, and spermatocytes are larger than spermatids. Absence of spermatozoa (smallest cells of the germ line).
Spawning or recently spawned (breeding)	Presence of spermatocytes, spermatids and spermatozoa (abundant).

Table 2: Developmental stages based on microscopic examination of fish ovaries [27]

Ovarian development stages	Internal characteristics
Primary growth	The oocyte is surrounded by a few follicular cells. The nucleus is large and centrally located, surrounded by a thin layer of cytoplasm and containing a single, large nucleolus.
Cortical alveoli	Spherical vesicles begin to appear at the periphery of the cytoplasm. They increase in size and number, forming rows and giving rise to cortical alveoli. Lipid droplets begin to accumulate in the cytoplasm. Chorion and follicle layers are evident at this stage.
Vitellogenesis 1	Lipid droplets occupy more cytoplasmic surface area than vitellus.
Vitellogenesis 2	Lipid droplets occupy a cytoplasmic surface similar to that of vitellus.
Vitellogenesis 3	Lipid droplets occupy less cytoplasmic surface area than vitellus.
Start of core migration	The nucleus begins to emigrate to the animal pole and the lipid droplets coalesce into an oily globule.
End of core migration	The nucleus migrates to the animal pole and the lipid droplet is clearly visible in the central part of the oocyte.
Hydration	Yolk and oil globules fuse to form a homogeneous mixture. The nucleus has disintegrated. Cortical alveoli and cytoplasm form a thin peripheral layer. The oocyte increases in size and has a translucent appearance.

Table 3: Method for determining post-ovulatory follicles (POF) in fish [29]

POF types	Morphological characteristics
POF 0	POF less than 24 h old. The POF is irregularly shaped, the granule cells are aligned and a number of folds and the lumen are clearly visible.
POF 1	POF aged between 24 and 48 h. The POF shows degeneration, with a linear appearance; the granular cells are still identifiable, but the lumen is reduced in size.
POF 2	POF aged between 48 and 72 h. Fragmentation of the basement membrane separates the granulosa from the theca, and pycnotic cells and vacuoles appear in the inner zone of the follicle's granulosa wall.
POF >2	POF more than 72 h old. The lumen is minimal or absent, and the granule cell walls are indistinguishable. There is no difference between theca and connective tissue. At this age, POF can be confused with the β-atresia stage.

3. Results

3.1 Description of microscopic stages in males

The cells observed are shown in figure 2. In the pre-reproduction stage, only cells of more or less spherical shape (spermatogonia, spermatocytes and spermatids) were identified. Spermatogonia are larger than spermatocytes, which are larger than spermatids. The estimated rate of pre-replication in *E. alletteratus* and *S. sarda* is 52.3% and 56.8% respectively. However, this rate is higher than that determined in *E. alletteratus* (47.7%) and *S. sarda* (43.2%) at the reproductive stage. At this stage, all cells were observed. In addition to more or less spherical cells, flagellated cells (spermatozoa) appear. Spermatogonia evolve into spermatocytes, which transform into spermatids and finally into spermatozoa.

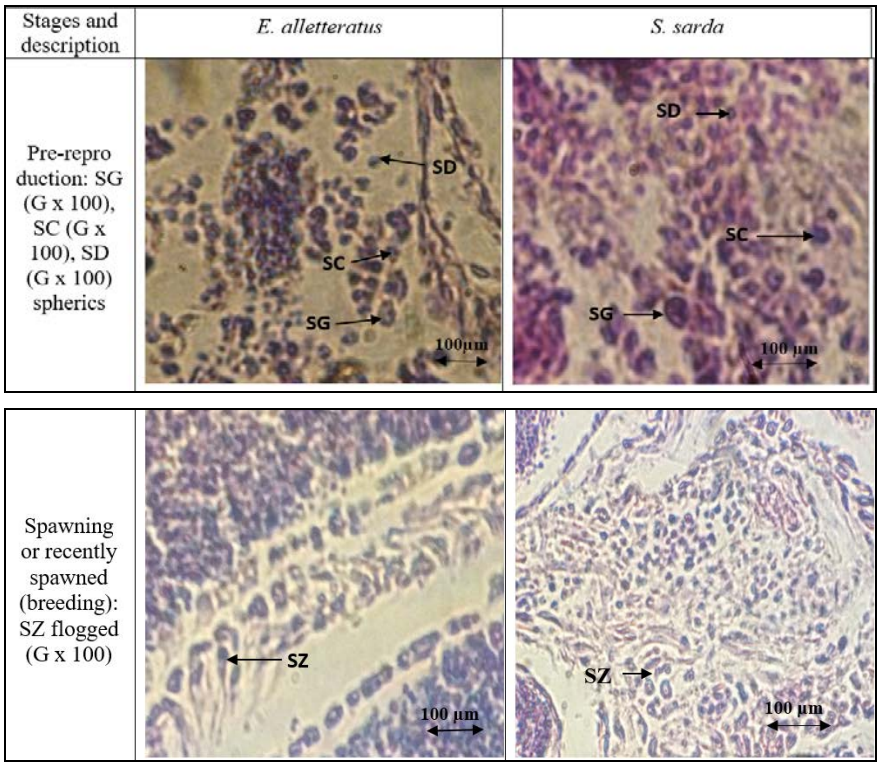
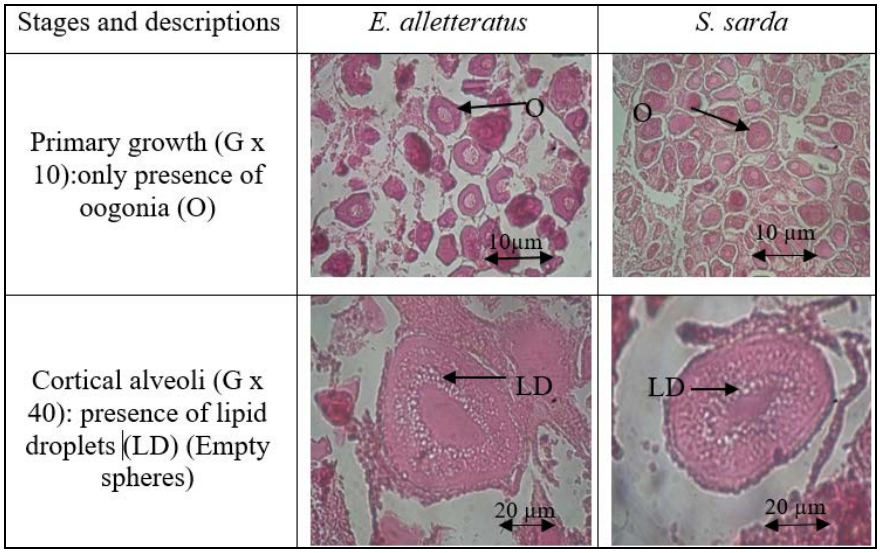


Fig 2: Cross-section of testicles showing the different stages of spermatogenesis in *E. alletteratus* and *S. sarda* from the Exclusive Economic Zone of Côte d'Ivoire from January 2016 to December 2016. (SG: spermatogonia; SC: spermatocyte; SD: spermatide; SZ: spermatozoa)

3.2 Description of microscopic stages in females

The evolution of microscopic stages in both species is shown in fig 3. Observation of the ovaries of *E. alletteratus* and *S. sarda* revealed 8 stages of development. In stage 1 (primary growth), ovogonia derived from primordial germ cells are ovoid, with an indistinct plasma membrane. They are small with central nucleoli. They evolve to give a larger oocyte. In stage 2 (cortical alveolus), the oocyte increases in volume, and the cytoplasm begins to become heterogeneous with the presence of lipid droplets or cortical alveoli (CA) organized in a corona. Hematoxylin-eosin staining shows the alveoli as empty spheres. This stage progresses to stage 3 (primary vitellogenesis), at which point the oocyte enters the vitellogenesis phase. During this stage, lipid droplets occupy more cytoplasmic surface area than protein granules. This stage evolves into stage 4 (Secondary vitellogenesis). During this stage, lipid droplets occupy the same cytoplasmic surface area as protein granules. The evolution of this stage leads to stage 5 (tertiary vitellogenesis), during which protein granules occupy more cytoplasmic surface area than lipid droplets. The evolution of this stage leads to stage 6 (beginning of migration), during which the lipid droplets move closer together. This stage evolves into stage 7 (end of migration). In this stage, the lipid droplets form a single droplet which moves the nucleus towards the animal pole of the cell. The evolution of this stage leads to stage 8 (hydration). This stage corresponds to the final stage of oocyte maturation. The cytoplasm is homogeneous, with no cellular structure. The pre-reproduction stage is characterized by the presence of stage 1 and 2 individuals. The estimated rate of individuals is 51.9% in *E. alletteratus* and 55.4% in *S. sarda*. This rate is higher than that determined for *E. alletteratus* (48.1%) and *S. sarda* (44.6%) in the reproductive stage (stages 3 to 8).



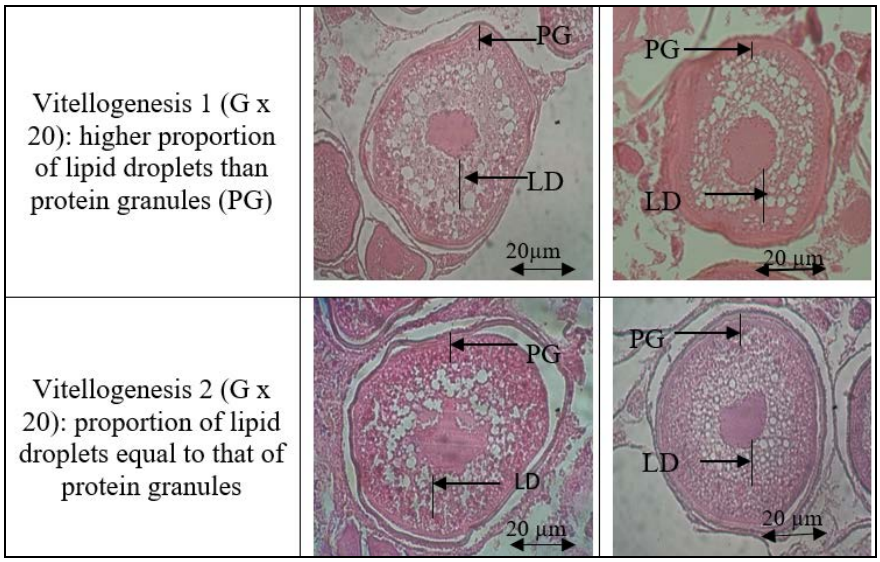


Fig 3: Cross-section of ovaries showing the different stages of ovogenesis in *E. alletteratus* and *S. sarda* from the Exclusive Economic Zone of Côte d'Ivoire from January 2016 to December 2016

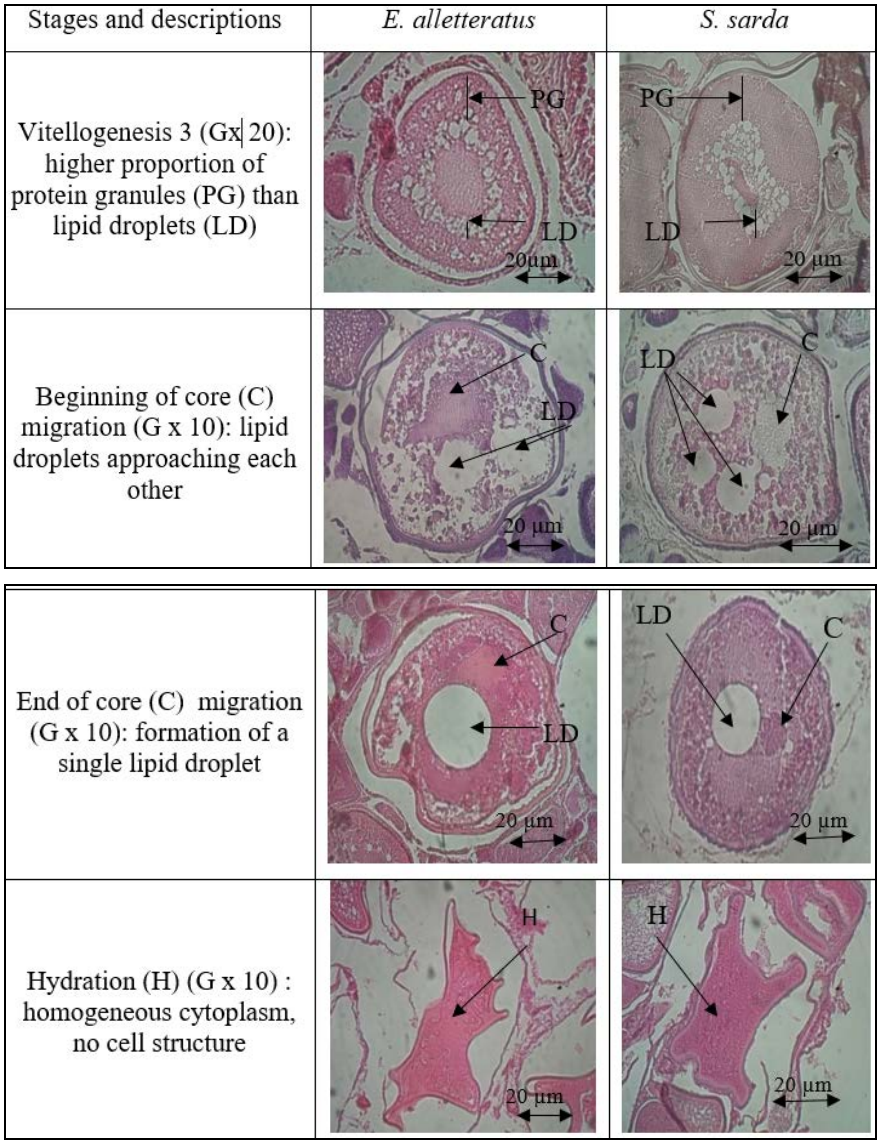


Fig 3: Continuation and end: Cross-section of ovaries showing the different stages of ovogenesis in *E. alletteratus* and *S. sarda* from the Côte d'Ivoire Exclusive Economic Zone from January 2016 to December 2016.

3.3 Post-ovulatory follicle (POF)

A total of 4 types of POF were observed according to age (Fig 4):

- **POF 0:** POF less than 24 h old, irregular in shape, with a clearly visible number of folds and lumen;
- **POF 1:** POF aged between 24 and 48 h, irregular in

shape but with reduced lumen size;

- **POF 2:** POF aged between 48 and 72 h with fragmentation of the basement membrane separating the granulosa from the theca, and the lumen is smaller;
- **POF > 2:** POF older than 72 h with minimal or absent lumen. There is no difference between theca and connective tissue.

Post-ovulatory follicles are evidence of laid oocytes. The rate of individuals having laid eggs before capture is estimated at 30.7% in *E. alletteratus* and 27.6% in *S. sarda*.

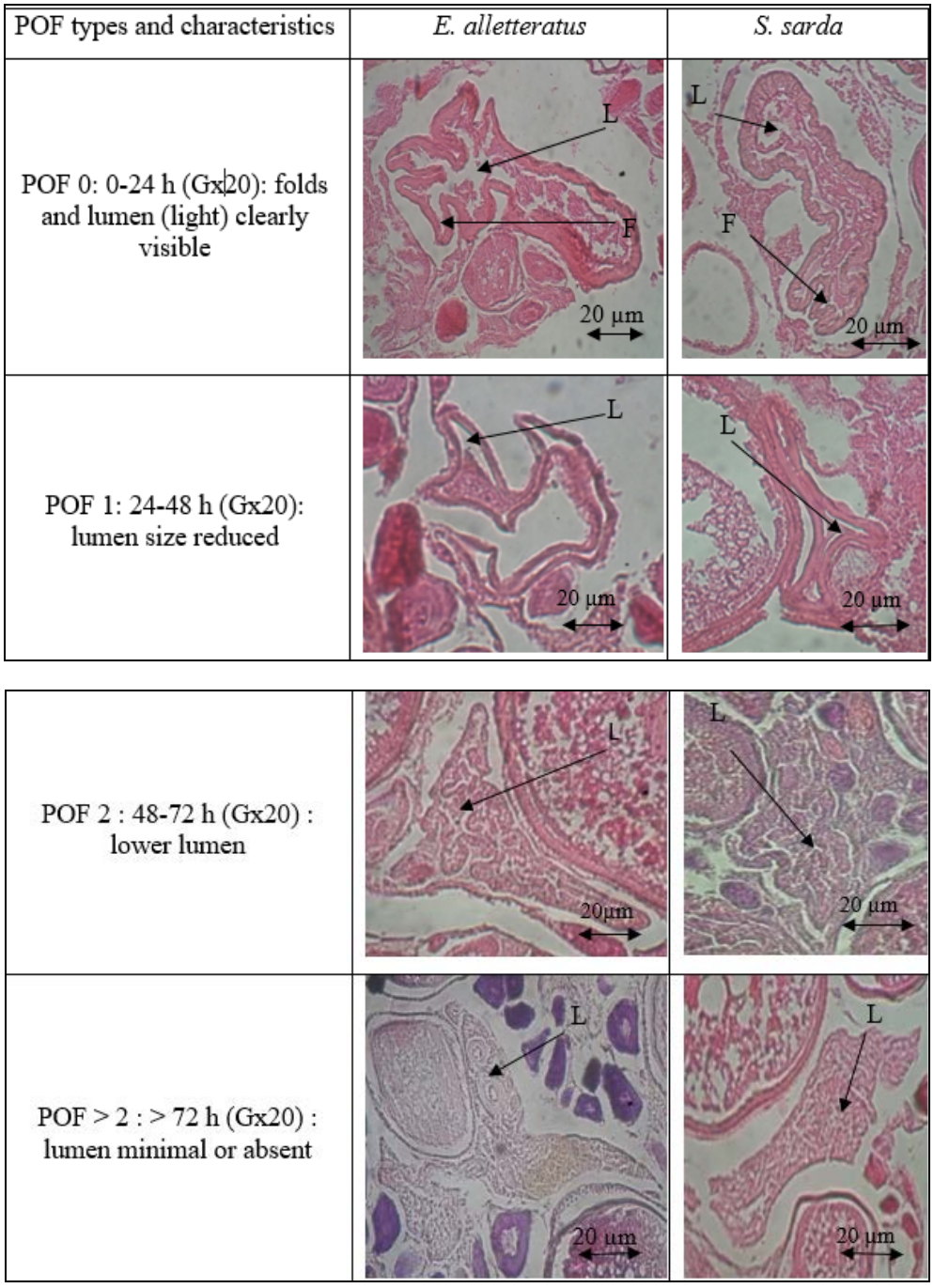


Fig 4: Different types of post-ovulatory follicles (POF) encountered in the ovaries of *E. alletteratus* and *S. sarda* from the Exclusive Economic Zone of Côte d'Ivoire from January 2016 to December 2016.

3.4 Oocyte atresia rates: Atresia (Degeneration) only affects mature oocytes, to know those in vitellogenesis (1, 2 and 3) and in migration (Fig 5). The overall rate of oocyte atresia in *E. alletteratus* and *S. sarda* was estimated at 23.55% (Vitellogenesis 1: 5.87%; vitellogenesis 2: 7.26%; vitellogenesis 3: 8.95% and migrating: 1.47%) and 10.12% (Vitellogenesis 1: 1.34%; vitellogenesis 2: 5.02%; vitellogenesis 3: 3.11% and migrating: 0.65%) respectively.

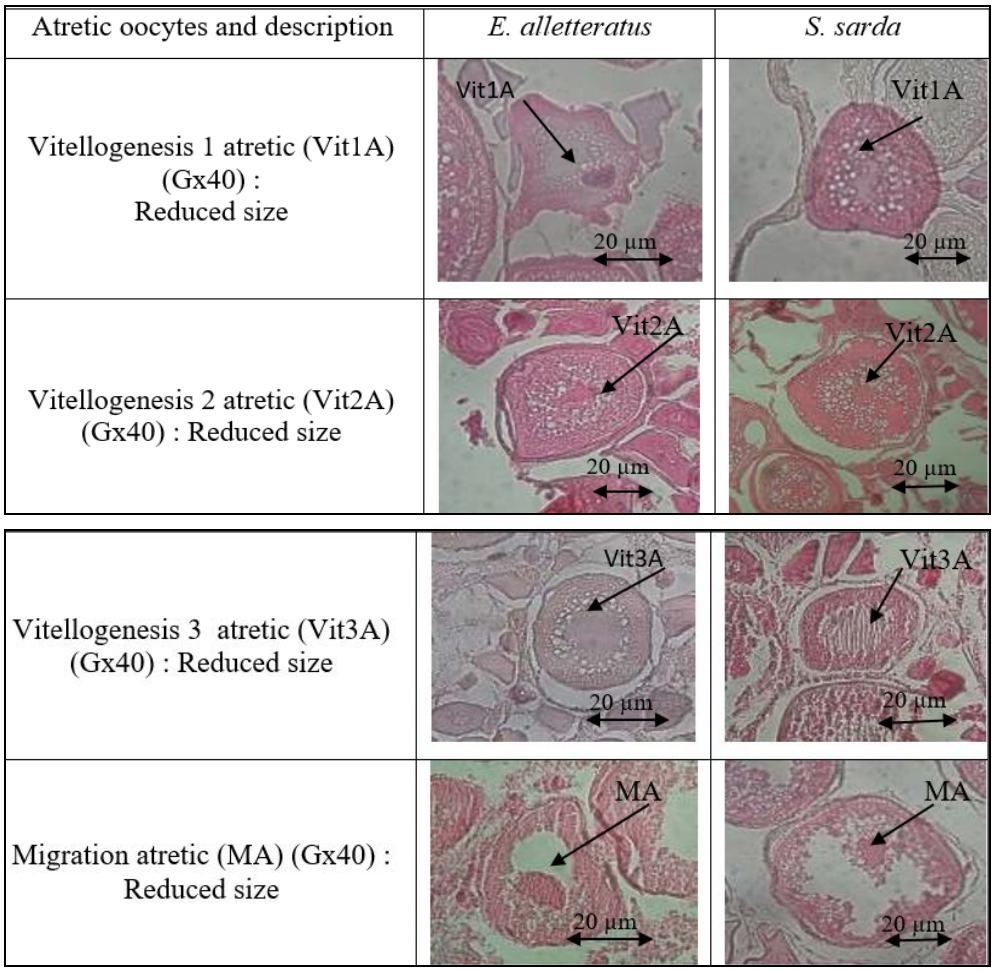


Fig 5: Different oocytes with atresia in *E. alletteratus* and *S. sarda* from the Exclusive Economic Zone of Côte d'Ivoire from January 2016 to December 2016.

4. Discussion

In the Ivorian Exclusive Economic Zone, microscopic study of the gonads of *E. alletteratus* and *S. sarda* has enabled us to identify individuals in the pre-reproduction, reproduction and post-reproduction stages. Individuals in the pre-reproductive stage are immature, while mature individuals are those in the reproductive and post-reproductive stages. In general, this study showed a dominance of immature individuals in the catches. This may be due to the high fishing pressure exerted on these fish [30]. Similarly, the rate of individuals estimated at the pre-reproduction stage is higher than that at the reproduction stage in males and females of *E. alletteratus* and *S. sarda*. The dominance of immature individuals in the capture of these two species indicates that the majority of *E. alletteratus* and *S. sarda* individuals have not had time to reproduce at least once before being captured. The immature individuals that make up more than half the tuna and skipjack populations have not contributed to the sustainability of either species. The sustainability of these fish stocks is therefore threatened.

Post-reproductive individuals have only been observed in females of both species. They are characterized by the presence of post-ovulatory follicles within the ovary. The presence of these follicular structures indicates that recent egg-laying has taken place in the environment. This shows that the environmental characteristics of the habitat are conducive to the species' reproduction [31]. The rate of individuals having laid eggs before capture is estimated at 30.7% for *E. alletteratus* females and 27.6% for *S. sarda*. This rate is considerably lower than that of individuals that did not lay eggs before capture. All in all, these captured fish are a hindrance to the survival of both species.

The rate of oocyte atresia observed in mature individuals of both species increases progressively with the stage of sexual maturity. From the above, we can deduce that as oocytes grow, a significant proportion fail to mature. This degeneration during maturation is linked to a number of factors, including inappropriate environmental conditions, lack of food and the physiological state of the fish [28]. Indeed, the development of the ovaries, intimately linked to the energy provided by food, is affected if food is lacking. Similarly, in unhealthy individuals, gonadal development is subject to disturbance. A high rate of atresia will reduce the number of oocytes laid, and thus the fertility of these species.

5. Conclusion

Microscopic study of the gonads of *E. alletteratus* and *S. sarda* caught in the Gulf of Guinea, Côte d'Ivoire, showed a dominance of immatures in both sexes and the presence of atresic oocytes in females. The sustainability of the *E. alletteratus* and *S. sarda* stocks is threatened by two potential factors: atresia (especially in *E. alletteratus*) and pressure from artisanal fishing. The results of this study will serve as a database for the rational management of both species.

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