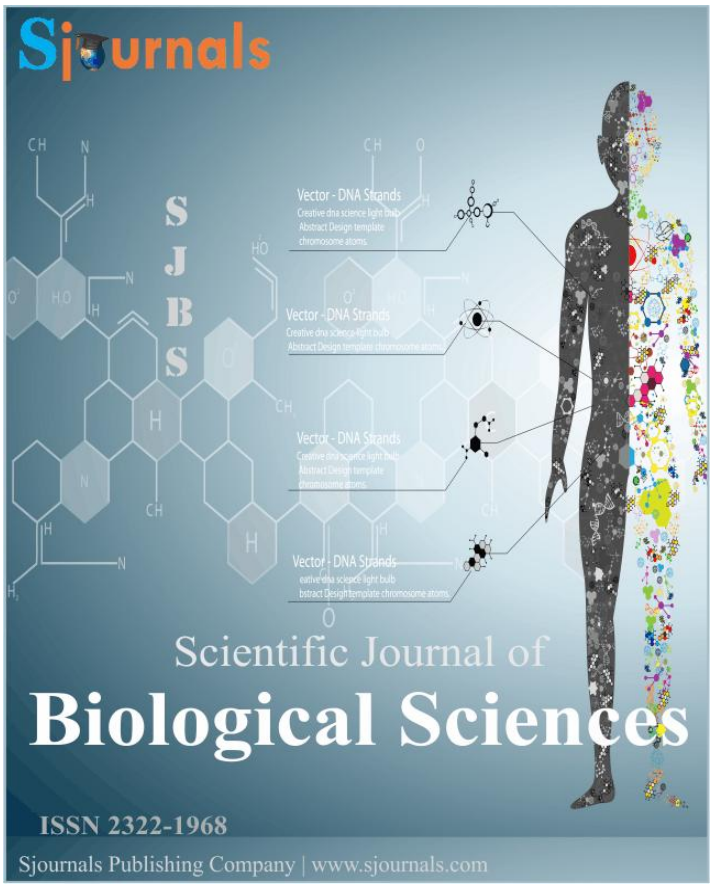

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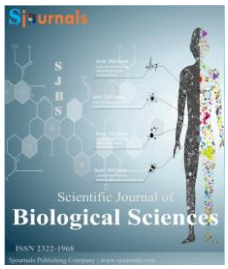

Original article

Spermatogenesis in little tunny *Euthynnus alletteratus* (Rafinesque, 1810) fished on continental shelf of Côte d'Ivoire

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ABSTRACT

Testes of 406 males little tunny (*Euthynnus alletteratus*), collected over a year period from an artisanal commercial fishery, were used to morphologically and histologically assess patterns of the reproductive biology in this small tuna. Most of the specimens examined ranged in size from 35 to 82 cm FL (centimetre fork length). Observation of slides under a light microscope revealed that males investigated have lobular testicular structure. These lobules arrange in a branching system of tubules. Tubules join side by side to one another and yet inter-tubular-spaces separate them. Gametes' developmental stages occur within such a structure. Histological characteristics were consistent with the description of the six-maturity stages of males little tunny determined on the basis of the macroscopic observation.

1. Introduction

Little tunny (*Euthynnus alletteratus*) belong to the family Scombridae and occur in tropical and subtropical seas worldwide (Collette and Nauen, 1983). According to Séret (1986), this species shows a tendency to dwell in

inshore waters with high current as well as deep waters and islet surroundings. Additionally, little tunny tend to migrate, but their movements are rather less extensive than the ones of other tuna species (Séret, 1986). Actually a species may occur in a given area, but the accurate way to deal with its reproductive biology is to study the gonadal status. It is common practice and belief that fish gonad maturation be measured through stages pertaining to applied classification systems. However, due to their using of macroscopic criteria (colour, consistency, resistance of gonads to pressing, external appearance, etc ...) as bases, these classification systems may lack accuracy, unless they are coupled with histological studies. In this respect, various authors (Pavlov et al., 2011; McBride et al., 2013) have emphasized the inaccuracy of macroscopic observation of gonads, while others (Assem et al., 2016) view histological examination of gonads as an important mean to verify visual observations. This paper deals with the reproduction in male little tunny, with an emphasis placed on the histological characteristics in order to enhance data on the reproductive biology in this species sometimes limited to macroscopic description of the male gonads.

2. Materials and methods

Fishing operation and the way males little tunny were processed are fully described in previous studies (Bahou et al., 2007; Bahou, 2013; Bahou et al., 2016). Briefly, fish were collected weekly from commercial catches taken in coastal waters. Specimens (n = 406) were caught between January and December 2004 with drifting gillnets deployed over the continental shelf of Côte d'Ivoire. Prior to histological processing, specimens were dissected. Testes were carefully removed from the body cavity. Photographs were taken of the fish while dissecting them and each individual fish was assigned a maturity stage, following Chur (1972) and Diouf (1980), as enhanced by our own observation of the gonads of dissected specimens. Testes were closely observed with the naked eye (Macroscopic observation), focussing on criteria such as the colour, consistency, resistance to pressing, and vascularization of testes. Standard histological techniques (Martoja and Martoja-Pierson, 1967) were adopted while preparing for the micro slides of testes. All testes were preserved in Bouin's fluid, embedded in paraffin, and thin-sectioned at 7 µm. Staining of slides was carried out with Hematoxilin and Eosin (H & E). The slides were finally mounted and observed under a light microscope. Photographs of the testes corresponding to each maturity stage were taken using a digital camera.

3. Results and discussion

The six maturity stages in male little tunny we identified were related to testes' size, the size of germ cells, the occurrence of lobules or tubules and the size of the main sperm duct as well as the emptiness of the medullo-cortical zone and the fullness of the medulla. These stages are the immature stage, the onset of maturation, the maturation stage III, the ripening stage, the spent stage, and the resting stage VI, respectively and consistently described, as follows:

Testes were recognizable to their thin-plate-shape (Fig. 1A). They were not vascularised, measuring from 10.6 to 14.3 cm (12.8 ± 1.0 cm) and weighing roughly 0.21 to 0.50 g (0.31 ± 0.07 g) in specimens of 35 to 41 cm FL (37.8 ± 1.9 cm FL). On histological level, the testis is characterized by the presence of long and branching tubules joined side by side to one another and yet separate in some areas by inter-tubular-spaces that are an extension of the sperm duct (Fig. 1B). The current section of the zone closer to the anterior extremity of the testis shows the overall setting in elongated tubules. The differentiation process of germ cells occurs within the tubules. Those tubules join together within the central testicular zone known as the medulla, where they form the main sperm duct.

Testes appear as two large lobes joined to each other, showing slight vascularization (i.e. a few blood vessels) on their ventral sides (Fig. 2A). Testes got obviously bigger than the ones in the preceding stage. Observation of slides showed that the inner part of the tubules is constituted by several lobules (Fig. 2B and 2C). The current histological section actually shows numerous lobules within which the differentiation of germ cells (such as spermatogonia and spermatocytes derived from mitotic and meiotic divisions, respectively) occurs. These germ cells appear to be like dark-coloured or purple-coloured dots.

Testes were more vascularised on their ventral sides. They were white-coloured (Fig. 3A). A close observation of slides made it possible to note that the differentiation of germ cells (spermatocytes-I, spermatocytes-II and spermatids) took place from the medullo-cortical zone (i.e. the transition zone between the medulla and the cortex) toward the medulla (Fig. 3B). Hence, the spermatids migrated from the medullo-cortical zone and they got

to the medulla (i.e. central zone of the testis) and moved straight ahead to the main sperm duct (Fig. 3B). Histological sections of testes show the tubules convergence zone (TCZ) as the place for the antero-posterior draining of spermatids. As we can see, spermatids completely withdrew from the cortical zone and headed for the main sperm duct. The main sperm duct gathers spermatids and spermatozoa fluxes together before the spermatozoa are transported from the testes to outside the body.

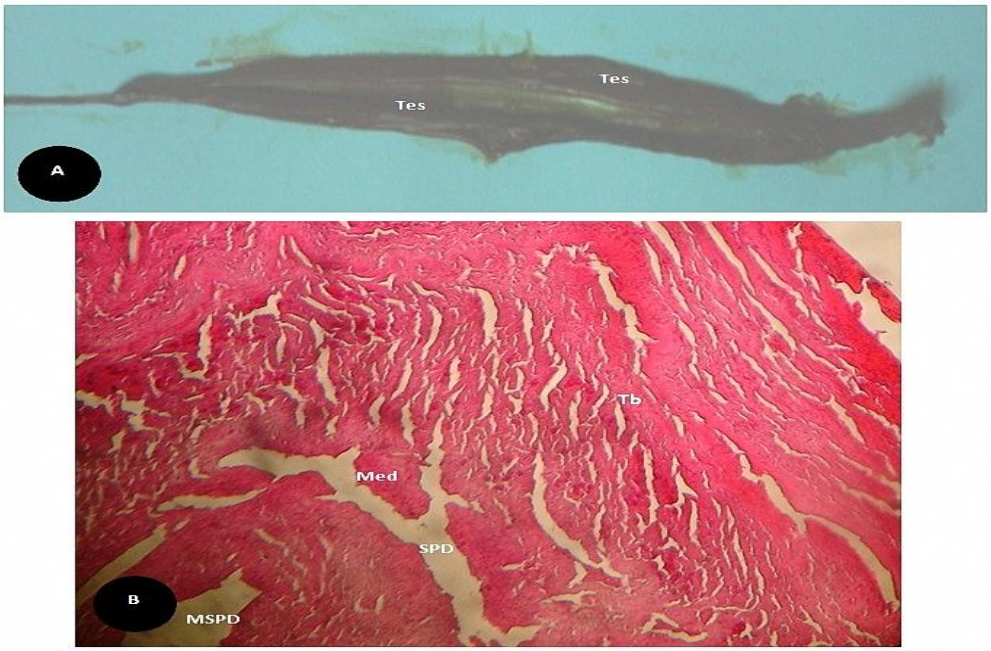

Fig. 1. Overall appearance of the testis of an immature (Stage I) male *Euthynnus alletteratus.* (A)**:** Morphology of the immature testes of a specimen of little tunny. (B)**:** Cross section within the testis of an immature specimen of *Euthynnus alletteratus* (×200) (Hematoxylin and Eosin). Med = Medulla; SPD = Sperm Duct; MSPD = Main Sperm Duct; Tes = Testes; Tb = Tubule.

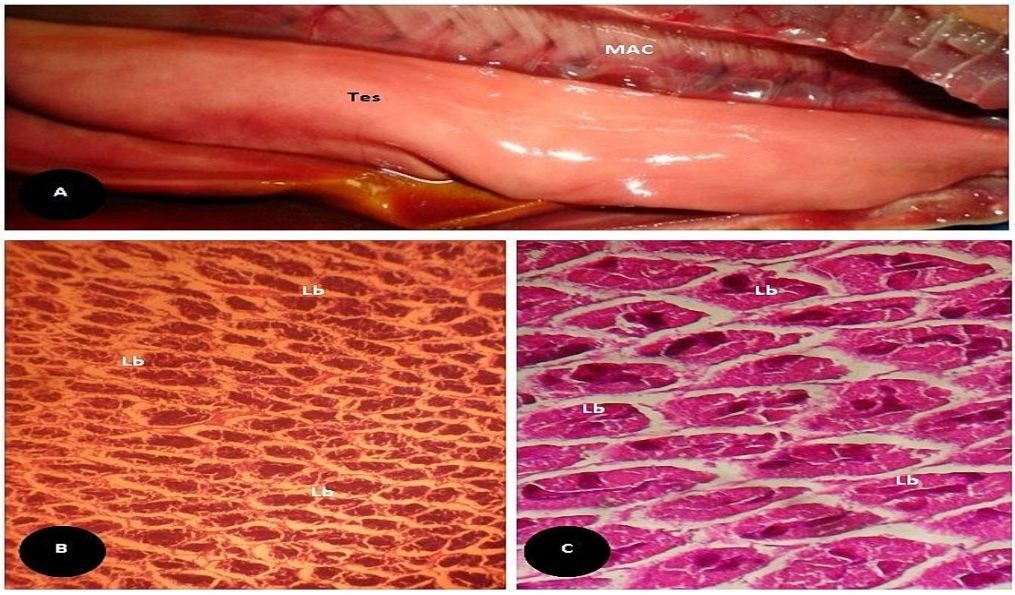

Fig. 2. Testes of a specimen of *Euthynnus alletteratus* at stage II (onset of maturation). (A)**:** Overall appearance of the testes at the onset of maturation. (B): Cross section within the medulla of the testis of a young male little tunny at stage II observed under low magnification (×32) (Hematoxylin and Eosin). (C): Cross section within the medulla of the testis of a young male little tunny at stage II observed under higher magnification (×200) (Hematoxylin and Eosin). Lb = Lobules; MAC = Muscles of Abdominal Cavity; Tes = Testes.

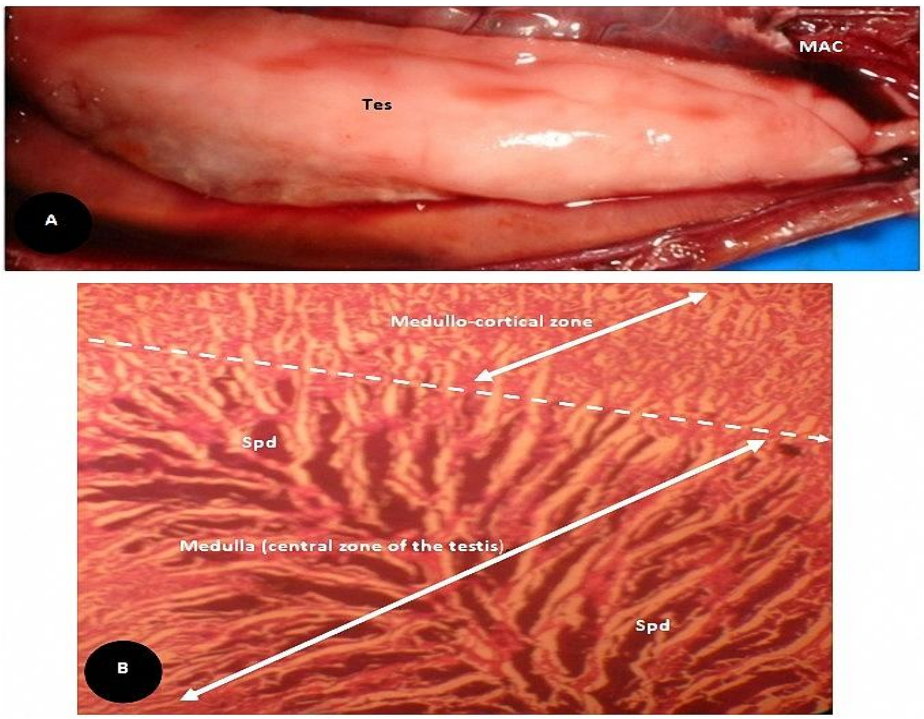

Fig. 3. Male gonads of a specimen of little tunny *Euthynnus alletteratus* at stage III (maturation). (A): Overall appearance of the testes of a specimen of little tunny at maturation stage. (B)**:** Cross section within the medulla of the testis of an adult fish little tunny at maturation stage (×100) (Hematoxylin and Eosin). MAC = Muscles of Abdominal Cavity; Spd = Spermatid; Tes = Testes.

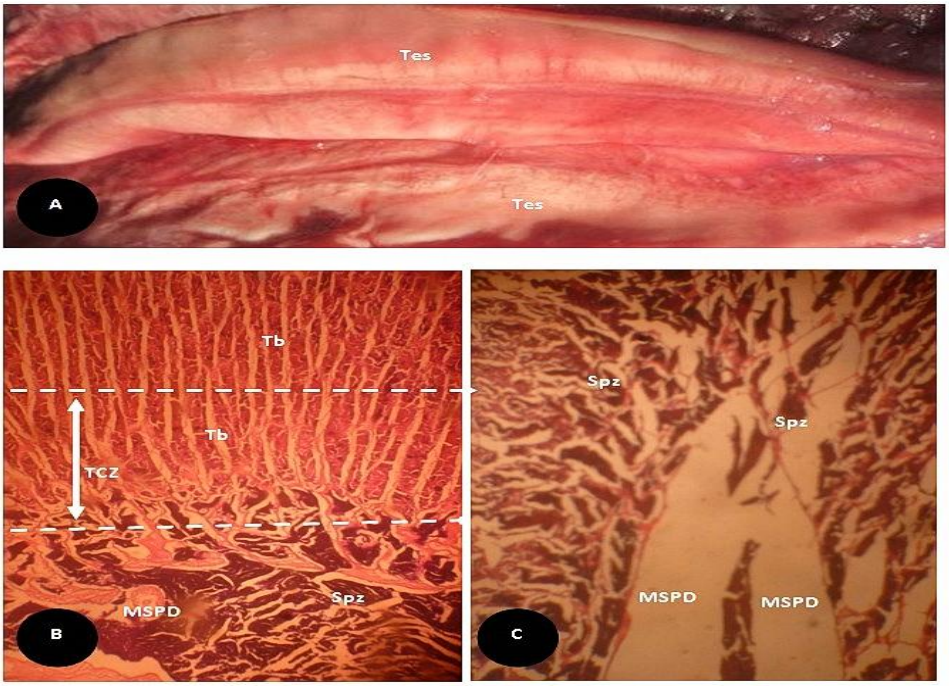

Fig. 4. Testes of a little tunny *Euthynnus alletteratus* specimen at stage IV (running male, ripe fish). (A): Overall appearance of testes in a running male. (B): Observation of the portion of the testis of a running specimen of *Euthynnus alletteratus*. (×100) (Hematoxylin and Eosin). (C): Cross-section within the medulla of the testes of a ripe male fish (×100) (Hematoxylin and Eosin). MSPD = Main Sperm Duct; Spz = Spermatozoa; Tb = Tubule; TCZ = Tubules Convergence Zone; Tes = Testes.

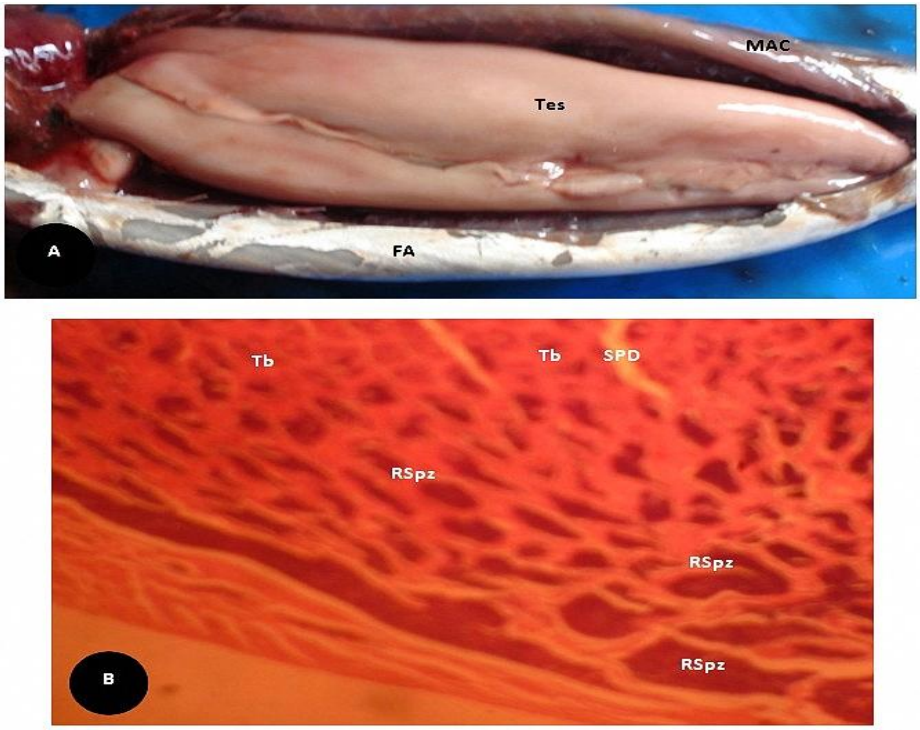

Fig. 5. Testes of a specimen of *Euthynnus alletteratus* at stage V (post-spermiation). (A): Morphology of the testes of a specimen of little tunny at stage post-spermiation. (B): Microscopic observation of the central part of the testis of a specimen of *Euthynnus alletteratus* at stage V. (×100) (Hematoxylin and Eosin). FA = Fish Abdomen; MAC = Muscles of Abdominal Cavity; RSpz = Residual Spermatozoa; SPD = Sperm Duct; Tes = Testes.

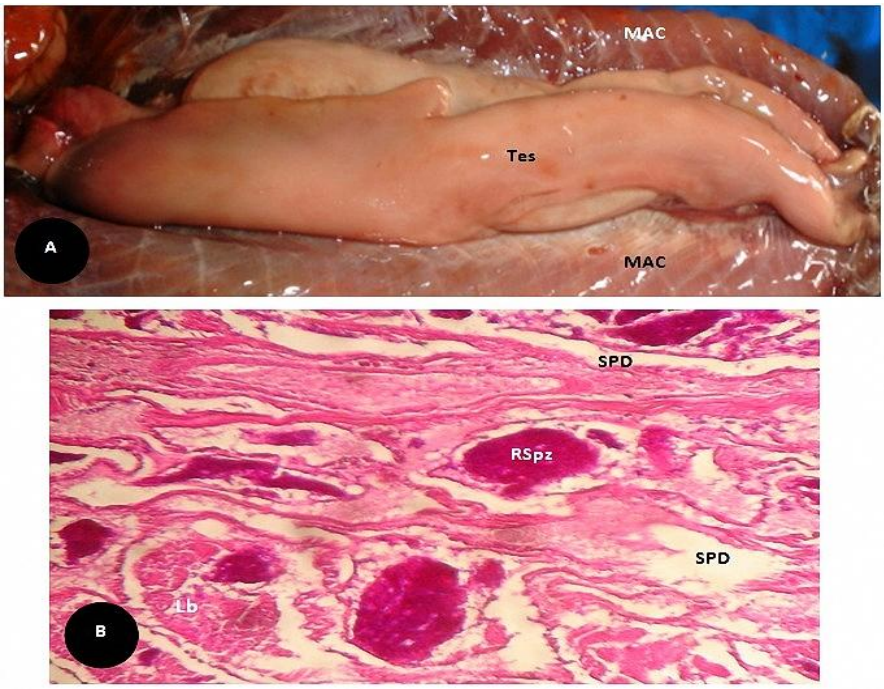

Fig. 6. Testes of a specimen of *Euthynnus alletteratus* at stage VI (resting stage). (A): Overall appearance of the testes of little tunny at resting stage. (B): Cross section within the testis of a specimen of little tunny *Euthynnus alletteratus* at stage VI (×200) (Hematoxylin and Eosin). Lb = Lobules; MAC = Muscles of Abdominal Cavity; RSpz = Residual Spermatozoa; SPD = Sperm Duct; Tes = Testes.

Testes got prominently vascularised on their ventral sides and reached maximum size (Fig. 4A). They measured between 14.6 and 19.6 cm (17.4 ± 1.6 cm) and weighed 47.39 to 79.16 g (65.93 ± 9.29 g) in specimens whose sizes varied between 45 and 52 cm FL (48.9 ± 2.1 cm FL). White-coloured milt flew out when pressing slightly the fish abdomen. On the histological level, the germ cells observed obviously are spermatozoa undoubtedly formed within the tubules convergence zone, an area that leads to the main sperm duct (Fig. 4B and 4C). The section shows accumulation of spermatozoa (purple-coloured dots) within the tubules convergence zone located near the center of the testis. The main sperm duct is located in the center of the testis as well. It also got filled with spermatozoa derived from double meiotic division of spermatids. We note that some spermatozoa are around or are accumulated within the common sperm duct. This facilitates their attempt to make their way through the sperm duct when they are about to be released outside the body.

Testes were flaccid, with milt still present therein. Testes were whitish, containing residual spermatozoa within the main sperm duct. The cross section shows a portion of the tubules convergence zone with some spermatozoa therein (Fig. 5A and 5B).

Testes got softer, shrunken and lighter than those in the preceding stage (Fig. 6A). They were not whitish on their dorsal sides. Cross section of testes showed that testes resembled a structure in reconstitution, with few residual spermatozoa therein (Fig. 6B).

In the current study, we assessed the reproductive biology of male little tunny *Euthynnus alletteratus* identifying six maturity stages on the bases of both external and internal appearances of testes. By taking into account immature and mature (sub-adult and adult) fish, we reached the conclusion that all internal features of testes in male little tunny are present, even in the immature fish. The subsequent changes that follow occur as the fish grow to reach maturity. Externally, no matter how mature the fish may be, the testis is constituted by two parts, namely the dorsal and the ventral sides. The dorsal side is generally fit for showing thickness, colouring, consistency as well as resistance of gonads to pressing and overall external appearance. The ventral side, however, is fit for showing how intense or conspicuous vascularization is. Internally, the testis is constituted by two regions known as the cortex (i.e. the most external part) and the medulla (i.e. the most internal part of the testis). Although the current study on little tunny did not show this specific aspect of the testis, we believe it is worth recalling in order to single out the main difference between the cortex and the medulla because it also applies for little tunny and enhances comprehension of other persons who may not be familiar with fish gonad anatomy. A preceding research by Bahou et al. (2017) on the reproductive biology of frigate tuna *Auxis thazard* showed that depending on the maturity stages of the fish, each region gets different in shade. In immature fish, the cortex is much more shaded than the medulla, probably because it is much thicker. As the fish mature, shade in both sides becomes inverted. As the medulla becomes progressively filled with germ cells at different division stages, the medulla gets thicker, which makes it become more shaded, and the cortex lightens because of its getting thinner (Bahou et al., 2017).

In addition, the main sperm duct is present in immature little tunny testis, even though no spermatozoa are observed yet therein. This characteristic has been noted in other tuna species such as South Pacific Albacore tuna *Thunnus alalunga* (Ratty et al., 1990) and frigate tuna *Auxis thazard* (Bahou et al., 2017). As regard testicular structure arrangement, similarity does exist between *Auxis thazard* and *Euthynnus alletteratus*. As little tunny mature, changes occur within the testis. A branching system of tubules radiates probably from the tubules convergence zone (TCZ) toward the outermost part of the testis (i.e. the cortex). The main sperm duct becomes wider. Both the TCZ and the main sperm duct get located in the central region of the testis. Meister and Pashuk (2005) emphasized that the main testicular ducts of each testis join together to form the common sperm duct through which the male gametes are transported from the testes to outside the body. However, little tunny may differ from other species in this regard. For example, Grier et al. (1980) made it known that in many other teleost fishes, the main duct is located along the dorsal surface of the testis and that it may not be present in immature stages of the testis. Yet little tunny has the same testicular cellular development as the majority of teleosts. According to Weltzien et al. (2002), in the teleost testis, spermatogenesis can be separated into five different stages, based on the presence in the testis of specific germ cell developmental stages (spermatogonia, spermatocytes, spermatids, spermatozoa, and testis in regression). Furthermore, the lobular-type cellular arrangement we observed in little tunny testis is typical of most teleosts (See Billard et al., 1982). Therefore, we classified the germ cells into the following phases of developmental order: spermatogonia, spermatocytes, spermatids, and spermatozoa. Additionally, the current study showed observation of germ cells that appeared as if dots-like, as a result of non-utilization of an electron microscope that enables observation of spermatozoa in their

entirety. That seems to be common to male fishes, regardless of species, as shown by many other studies in which the slides were observed under a light microscope (Bahou et al., 2017).

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