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REPRODUCTIVE BIOLOGY OF FEMALE FRIGATE TUNA *AUXIS THAZARD* (LACEPÈDE, 1800) CAUGHT IN COASTAL MARINE WATERS OF CÔTE D'IVOIRE

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ABSTRACT
Some biological features (length-weight relationship, size at first sexual maturity, eggs variation in diameter, gonado-somatic index, hepato-somatic index, somatic condition and fecundity) and histological characteristics of frigate tuna *Auxis thazard* were studied. The study was carried out on 496 female fish caught in gillnets and measuring in size between 25 and 48 cm FL (centimetre Fork Length) collected from January to December 2004. The results indicated that female frigate tuna reach maturity at 29.88 cm FL. Spawning started in June while females at stage IV numbered 36.36% but it lessened in November. The peak value of gonado-somatic index, GSI (3.21 ± 0.93%), was attained in August and GSI thereafter decreased gradually from September to December. There is a direct correlation between GSI and hepato-somatic index (HSI), and an inverse correlation of these factors to the somatic condition (K_c). Absolute fecundity has linear relationship with the weights of specimens (AF = -9.3792 + 0.5485xW) as well as with the weights of ovaries (AF = 136.11 + 13.416xOW). Eggs-frequency distribution within the maturity stages III (maturing or developing), IV (spawning), and V (post spawning) were also shown in the study.

KEYWORDS: Absolute fecundity, Gillnets, Gonado-somatic index, Hepato-somatic index, Somatic condition.

INTRODUCTION

Frigate tuna (**A***uxis thazard*) are epipelagic and neritic fish (Collette and Nauen, 1983), yet scarcely migratory species (Cayré *et al.*, 1988). They are amongst the most important commercial fishes targeted by a small-scale fishery operating with canoes in continental shelf waters of Côte d'Ivoire, a Western African country (Bahou, 2001; Bahou *et al*., 2007). Several studies regarding the reproductive biology of frigate tuna have been carried out in West Africa (Caverivière *et al*., 1976; Alekseev and Alekseeva, 1980; Rudomiotkina, 1984). Long ago, previous studies attributed to reproduction in frigate tuna the possibility to occur in some West African marine waters with temperature above 24°C (Frade and Postel, 1955; Conand, 1970). By implication, frigate tuna could mainly reproduce in suchlike waters. However, observation of the gonadal status of females and males frigate tuna along with juveniles *Auxis spp*. ingested as prey by large tunas, especially during the cool season when the Sea surface temperature (SST) are usually the lowest between June and October (Varlet, 1958; Verstraète, 1970; Morlière and Rébert, 1972), led us to conclude otherwise. The overall objective of the current study was therefore to investigate the reproduction in frigate tuna in relation with temperatures other than 24°C (between 22 and 29°C), depending on marine-water-season and regardless of cooling. A specific goal was to propose a maturity scale based on histological observations of the female gonads, in addition to macroscopic description.

MATERIALS & METHODS

Study area, Sample collection and SST measure

Specimens of the fish *Auxis thazard* were obtained from a commercial small-scale fishery, in the manner described by Bahou et al. (2007). Tuna were caught with gillnets of 25 and 35 mm mesh by a fishery that operated at night with canoes powered by 40 hp motors, at the edge of the continental shelf of Côte d'Ivoire (Fig. 1). In addition, the Sea surface temperature (SST) were measured daily at 7 am, 11 am and 15 pm for their important role in the distribution of species within a given area. For that purpose, a thermometer was immersed for 10 mn in seawater collected in a 5 to 10-liter capacity container, at a suitable place. The procedure permitted using of an average SST-value as a result of three thermometer-readings each day.

Body measurements, macroscopically and histological observations of gonads

Fish Fork Length were measured to the nearest centimetre. Specimens were weighed to the nearest gram and their ovaries and liver were weighed to the nearest milligram. Sex and maturity stage were determined through macroscopic observation of the gonads of dissected specimens. Maturity stages were based on external features (morphological appearance, colour, consistency, resistance of gonads to pressing) and histological examination. Ovaries are consisted of either symmetrical or dissymmetrical sacs (lobes) joined to each other by intermixed membranes and by a posterior ovarian end that

seems to taper off. Ovaries removed from those females' abdominal cavity were subdivided into two main groups. The former, made up with ovaries whose eggs were visible with the naked eyes (ovaries at stages III, IV, and V), were used for the measurement of eggs' diameters. Two sections, each of 1 cm in length (the former from the central part and the later from the upper extremity of gonads), were taken from one single ovary and stored in 10% formalin. Intra-ovarian eggs diameter was measured using an ocular micrometer. Ovaries that constituted the second group were fixed in Bouin's fixative for two weeks, underwent a series of different ethanol concentration baths (in order to rinse off the picric acid) and then were embedded in paraffin. They were used for histological examination. Sectioning of the gonads was carried out. Sections were cleared in Xylene, impregnated in liquid paraffin wax (melted at 52-60°C) and poured into metal rectangular Lucas moulds. The moulds were placed under running tap water to harden and positioned in the microtome for trimming. The samples were sectioned at approximately 7 µm width with a microtome. Hardened wax trimmings housing the gonads were stretched out properly. With the help of a clean slide, the stretched films of gonads were picked and placed on hot plate for about 30 minutes to dry up so as to properly attach to the slide. Each slide was passed through Xylene (for about 10 minutes) and through descending concentrations of ethanol (95, 90, and 70%). Then, the preparations were stained with hematoxylin-eosin and mounted using Distrene Platicizal Xylene (DPX). Sections were observed under a binocular microscope at various magnifications and photographed. The internal structure of the ovary and the developmental stages of eggs were classified depending on the observations.

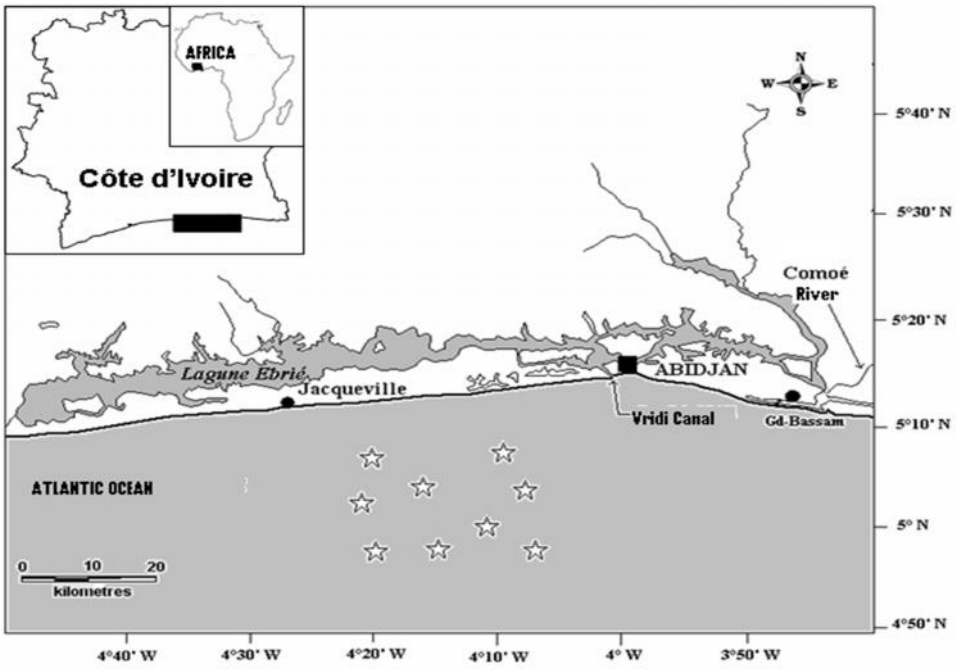

FIGURE 1. Map of the fishing area (the centre of the fishing area is approximately 4°80'N - 5°10'N and 4°30'W – 4°W) (BAHOU *et al*., 2007)

Fecundity

Fecundity was estimated using portions of ovaries, 1 g each, taken from the central part of the ripe ovaries and immersed in Gilson fluid for 2 weeks to harden (which caused eggs to disaggregate). Those ripe ovaries were taken from thirty-one gravid females harvested from June to October. When the Gilson fluid had been filled up as to reach 50 ml, it was shaken vigorously for a while to make it homogeneous. That resulted in separated connective ovarian tissues. Then 1 ml sample was taken with a Pasteur pipette and put in a tinny receptacle. Thereafter, eggs were manually enumerated and recorded. That procedure was repeated three times to enable choosing of mean values. Absolute fecundity (AF) was taken as the number of ripe oocytes in the female gonad prior to spawning. Relative fecundity (RF) was the number of oocytes per kilogram of fish body weight (Wootton, 1979). Absolute fecundity was calculated as follows:

AF = Number of oocytes (No) × 50 × Total weight of ovaries (TW)

Reproduction

Gonado-somatic index, Hepato-somatic index and Somatic condition were calculated using the formulae:

$$GSI = \frac{Ovary\ weight\ (OW) \times 100}{Total\ weight\ (TW) - Ovary\ weight\ (OW)},$$

Gibson and Ezzi (1980)

$$HSI = \frac{Liver\ weight\ (LW) \times 100}{Total\ weight\ (TW) - Ovary\ weight\ (OW)}$$

$$K_C = \frac{[Total\ weight\ (TW) - Ovary\ weight\ (OW)] \times 100}{Fork\ length\ (FL)^3},$$

Htun-Han (1978).

The relationship between the absolute fecundity and fork length was estimated. The Length-weight relationship was

determined according to Ricker (1980). Female fish at stages IV and V were chosen to determine the size at first sexual maturity (LF_{50}), ranging them into 1 cm size classes and calculating their proportions. The Statistica 7.1 software (Statsoft, Inc.) made it possible to estimate the mean size at first sexual maturity by fitting the logistic function to the proportion of these mature fish. Such a function fits in with logistic regression and the probability associated with it satisfies the following equation:

$$\log it\,[p(X)] = \log\left[\frac{p(X)}{1-p(X)}\right] = \sim + \quad X$$

Another option to the logistic regression was found through the utilization of the exponential function as expressed by the equation:

$p(X) = \dfrac{e^{(\sim + \quad X)}}{1+e^{(\sim + \quad X)}}$, where α and β are two constants.

In such a case, the probability for p (X) was attributed to mature females and the values in middle of size classes referred to X. Mature females counted 50% as LF_{50} = -α/β. Relationships between any pair of variables were determined. Statistical analyses were performed using Statistical 7.1 software while 0.05 was adopted as level for significance.

RESULTS

Biological characteristics

The length-weight relationship determined for female frigate tuna was $W = 0.723\times10^{-5}$ $(FL)^{3,206}$. (r^2 = 0.98), suggesting allometric growth, the value of "b" (3.206) being superior to the commonly referred value "3" ("t" statistics, F = 3.24; t = 12.173). First sexual maturity was attained at 29.88 cm FL (LF_{50} = 73.939/2.47428), referring to the equation for the curve that led to its calculation (y = exp (-73.939 + (2.47428)*x)/(1 + exp(-73.939 + (2.47428)*x), where "y" represents the percentage for mature individuals and "x" the size of these individuals. Therefore, all females larger than 30 cm in length were mature.

Figure 2 shows the monthly variation of the maturity stages in female frigate tuna. Though present throughout the year, individuals at immature and maturing stages fluctuated in number each month, especially the former, among which juveniles were numerous, reached higher percentages from June to September. Females at stage III have been observed in varying percentages, 48.00% and 8.82%, between April and October, respectively. Gravid females (stage IV) got numerous from June (36.36%) to November (32.26%). Though fewer in July (9.76%), post spawners (stage V) increased in November (54.84%) and December (55.17%). Individuals at resting stage VI were observed from December (20.69%) to March (36.00%), yet more numerous in January (51.85%).

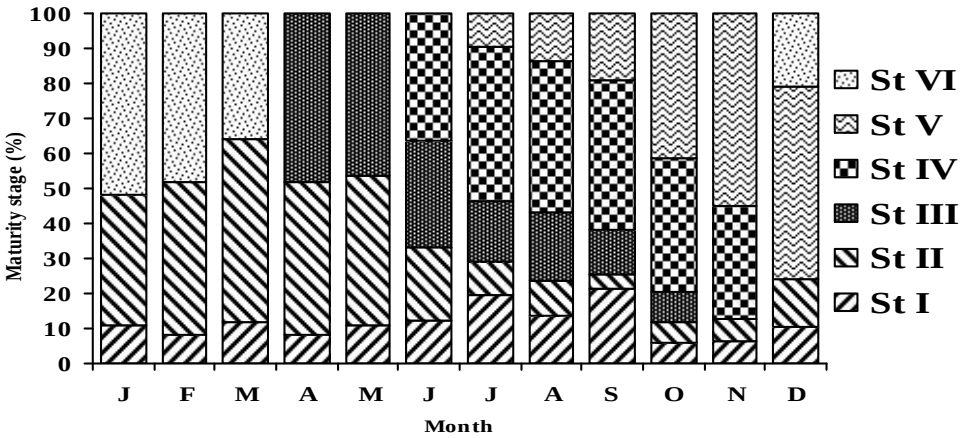

FIGURE 2. Proportion of females frigate tuna *Auxis thazard* by maturity stage and month. St = stage; St I (immature); St II (early maturing); St III (maturing); St IV (spawning); St V (post spawning and spent); St VI (recovery and resting adult)

Variation of the GSI is shown in Figure 3A. Gonado-somatic index increased gradually from 0.91 ± 0.13% in January to 1.25 ± 0.36% in April as ovaries scarcely increased in weight during the recovery and resting adult period. From a relatively higher value in May (GSI = 1.50 ± 0.20%), the GSI reached a peak in August (3.21 ± 0.93%). GSI thereafter decreased untill December (GSI = 0.92 ± 0.19%). Variations in GSI made it possible to determine four main phases in the course of the reproduction of frigate tuna. These are the resting phase, the maturation, the spawning, and the post spawning phases (Fig. 3A). As shown in Figure 3B, the increase in HSI was scarce from January to April (HSI = 0.427 ± 0.003%) but it reached a peak in August (HSI = 0.429 ± 0.003%). Overall, HSI showed the same trend as GSI did. The somatic condition (K_c) showed a tendency to increase from January (K_c = 1.71 ± 0.02%) to April (K_c = 1.72 ± 0.02%) and from July (K_c = 1.69 ± 0.03%) to November (K_c = 1.72 ± 0.03%) (Fig. 3C). Temperature scarcely increased from January (27.44 ± 1.48°C) to May (28.53 ± 1.48°C) yet steadily decreased untill August (22.14 ± 1.49°C) (Fig. 4). Following a decrease due to cooling of water masses, an increase in temperature occurred from September (23.06 ± 1.51°C) to November (27.71 ± 1.46°C). Temperature thereafter scarcely decreased in December (27.49 ± 1.52°C). Sea surface temperature variation resulted in four hydroclimatic periods, of which the minor cool season (mCS, from January to February), the main warm-water season (MHS, from March to June), the main upwelling season or main cool season (MUS, from July to October), and the minor warm-water season (mHS, from November to December).

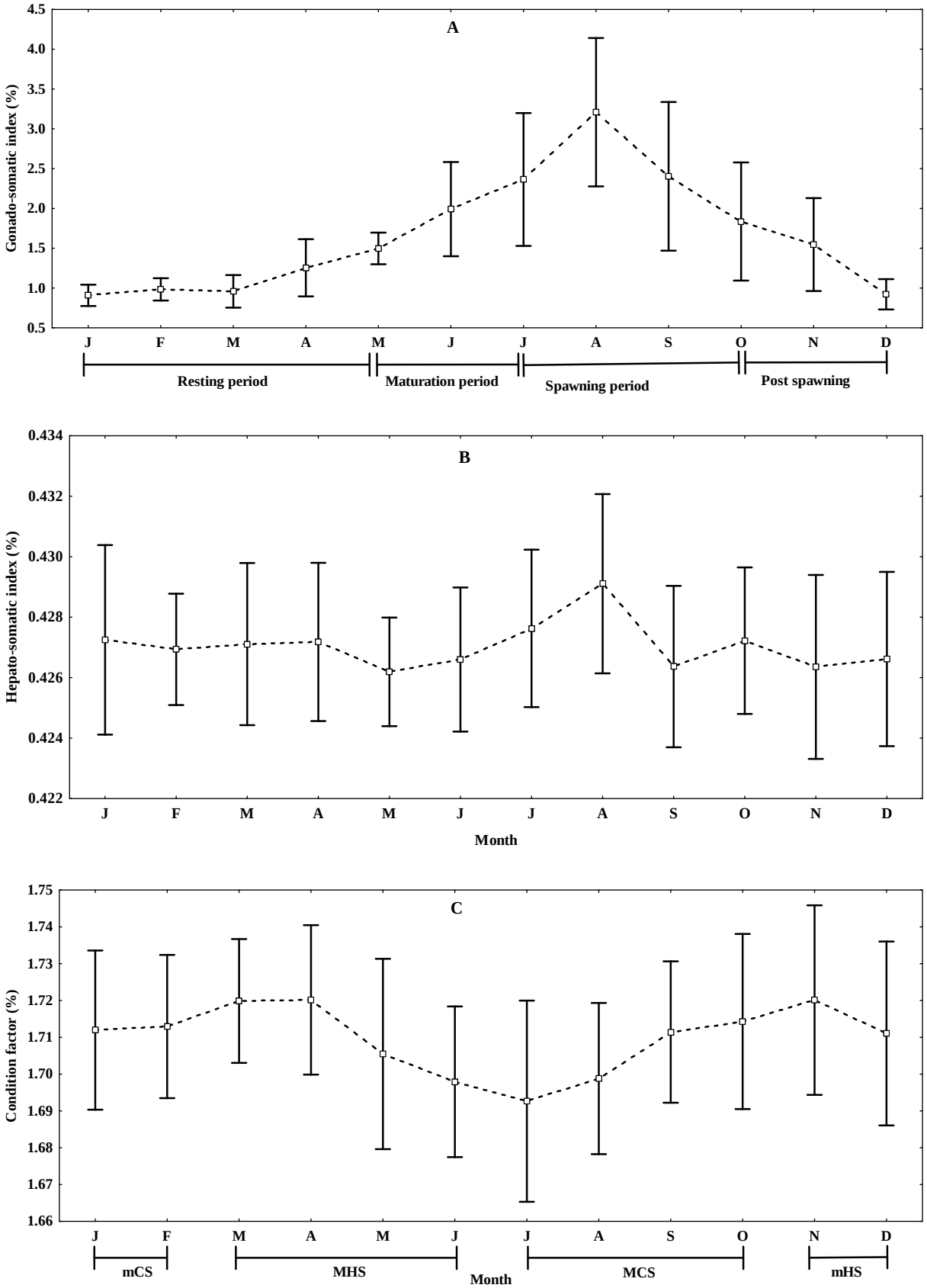

FIGURE 3. Monthly variation of GSI, HSI, and K_c in female frigate tuna caught with gillnets in Ivorian shelf waters from January to December 2004. mCS = minor cool season; MHS = main warm water season; MUS = main upwelling season; mHS = minor warm water season

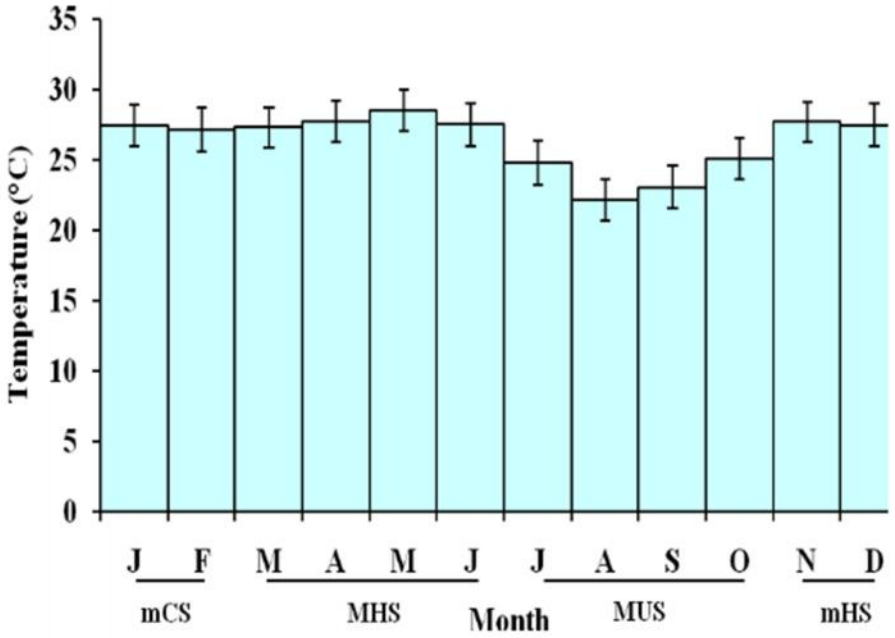

FIGURE 4. SST variation on continental shelf of Côte d'Ivoire throughout the study period from January to December 2004. mCS = minor cool season; MHS = main warm water season; MUS = main upwelling season; mHS = minor warm water season.

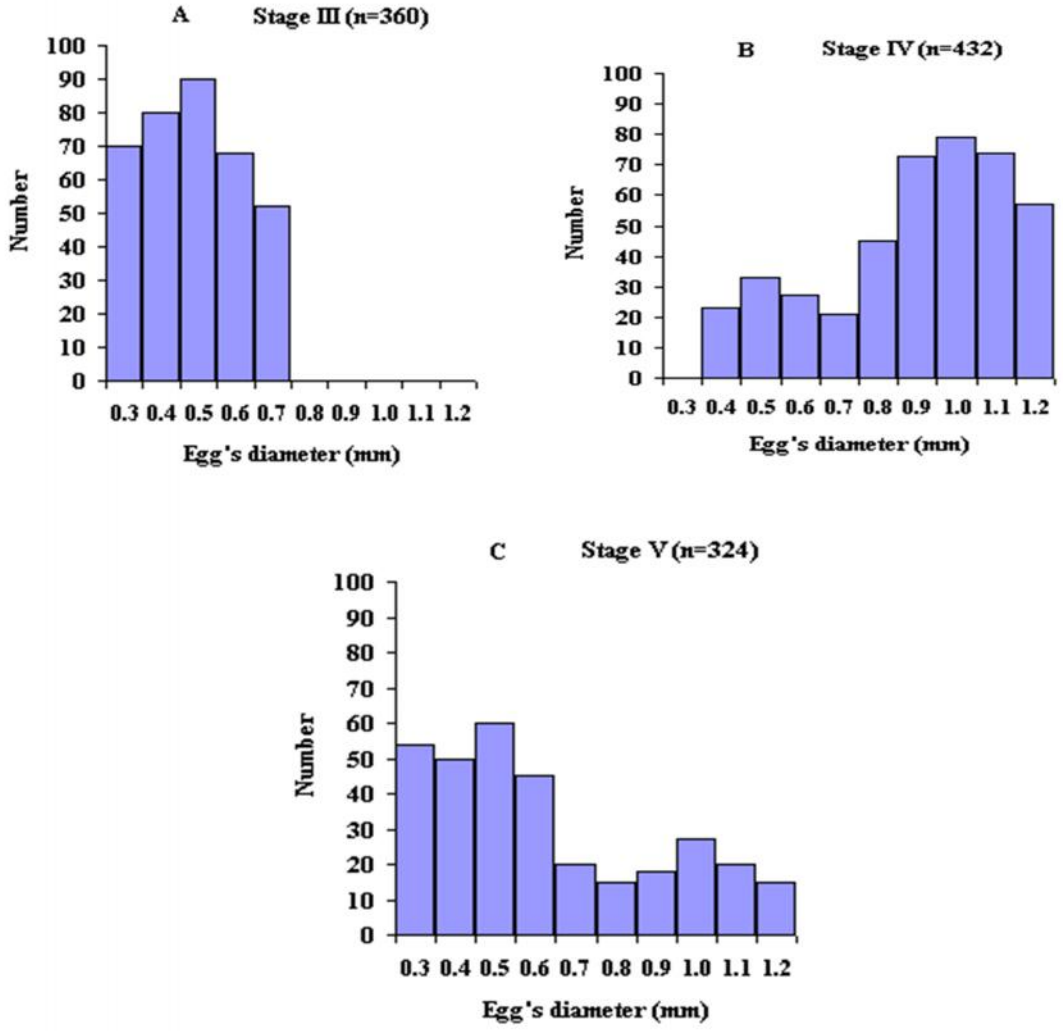

FIGURE 5. Frequency distribution of eggs diameter in the ovaries of frigate tuna *A. thazard* at stages III, IV, and V

Eggs length frequency distribution is shown in Figure 5. There appeared to be much more eggs measuring in size from 0.3 to 0.7 mm in ovaries at stage III than there were in ovaries at stages IV and V (Figs. 5A, 5B, and 5C). In addition, ovaries at stage IV predominantly contained eggs that measured between 0.8 and 1.2 mm. Although ovaries at stage V contained eggs measuring in size from 0.3 to 1.2 mm, eggs of 0.5 mm seemed to be more abundant within them (Fig. 5C). Overall, each stage was characterized either by one or by two eggs-size-classes in which eggs measuring 0.5 or 1.0 mm proved to be the most numerous. Absolute fecundity was estimated for frigate tuna (Fig. 6). This species can spawn up to 305,000 and 891,000 eggs (mean value = 544,920 ± 161 eggs) in females measuring in size between 33 and 45 cm FL. Relative fecundity was estimated to be 470-666 eggs/g (mean value = 539 ± 42 eggs/g). Absolute fecundity was positively correlated with body weight of specimens (Figure 6A), which suggests that fecundity is likely to be greater in larger females than in small ones. Absolute fecundity was also found to correlate with ovary weight as shown in Figure 6B through the equation AF = 136.11 + 13.416×OW.

Macroscopic and histological characteristics of the ovaries of frigate tuna

Macroscopically observation of ovaries on the basis of external features along with histological examination of ovarian cells moved us to distinguish six stages in relation to six types of ovaries as follows (Figs. 7-12):

(i) The immature stage (I): Ovaries were cylindrical, almost threadlike, with two tapering ends (Fig. 7A). Immature stage was also characterized by a large amount of oogonia and pre vitellogenic oocytes which were very small and spherical cells, each with thin and indistinct peripheral cytoplasm (Fig. 7B).

(ii) At the onset of maturation (stage II), ovaries increased in size and they were pinkish in colour (Fig. 8A). This stage showed primary oocytes that were mostly oval or round. Two rows of these oocytes separated by interstitial conjunctive tissue (ICT) are shown in Figure 8B.

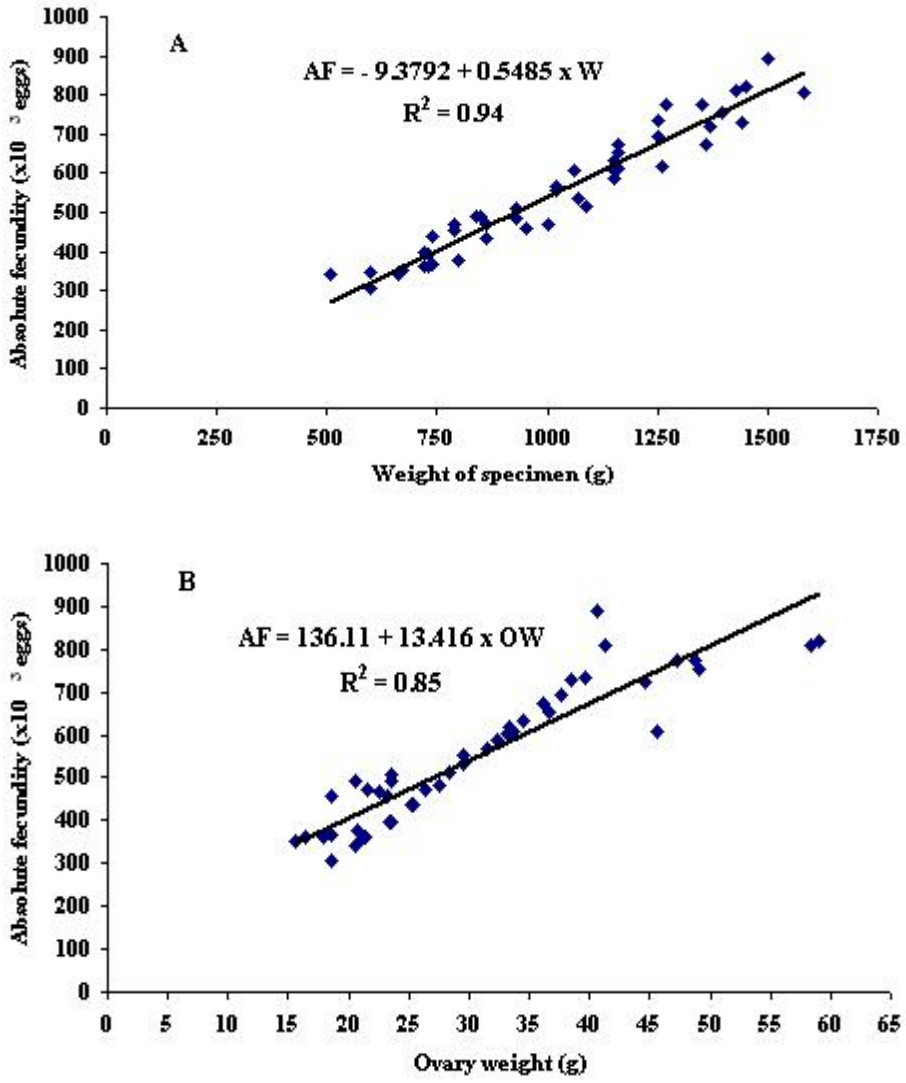

FIGURE 6. Relationships between AF and TW (A), AF and OW (B) in female frigate tuna sampled from January to December 2004 at Abidjan fishing port

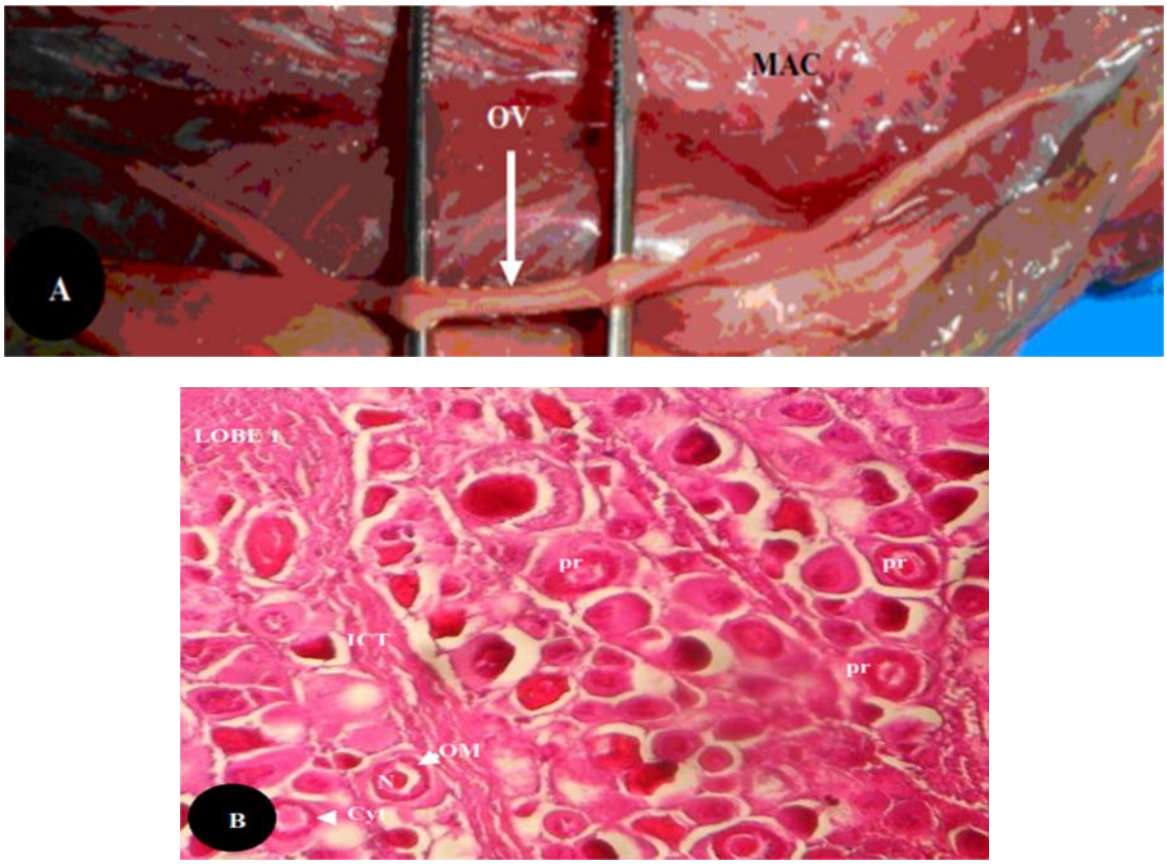

FIGURE 7. Ovaries of an immature female *Auxis thazard* (stage I). (A) Overall appearance and localization of the two-thread-like immature ovaries. (B) Cross-section of an immature ovary showing two lobes of germ cells, with an interstitial conjunctive tissue (ICT) keeping them apart. We can see pre vitellogenic oocytes (pr) with their nuclei (N), cytoplasm (Cyt), and oocyte membrane (OM). (M × 200) (Hematoxylin and Eosin). MAC = muscles of abdominal cavity ; OV = ovaries.

(iii) At the developing or maturing stage (III), nearly ripe ovaries were orange yellow in colour, with bloody vessels on their surface that are conspicuous (Fig. 9A). Eggs were distinguishable with the naked eye when looking through the ovarian membrane. The maturing stage was also featured by vacuolization (i.e. appearance of vacuoles) and by the appearance of the zona radiata as constituent of the oocyte wall. The vacuoles were at first few in number and small in size, yet scattered within the oocytes' cytoplasm (Fig. 9B). At this stage, oocytes measured 0.59 ± 0.13 mm in diameter (mean size).

(iv). At the spawning stage (IV), ovaries reached maximum development in thickness and width, which undoubtedly made blood vessels on their surface more conspicuous (Fig. 10A). Ovaries occupied the entire length of the body cavity, almost leaving little portion for the viscera to display. With the ovarian membrane getting thinner, eggs became more distinguishable with the naked eye. The belly of some individuals got so swollen that a slight pressing on the belly generally made eggs release out. Follicles, or eggs, increased in diameter and reached maximum size (mean value = 1.04 ± 0.15 mm across the long axis). The vacuoles increased in size and intermixed with the yolk granules. Both vacuoles and yolk granules scattered within the cytoplasm (Fig. 10B).

(v) At the spent stage (V), there appeared to be a discharge of a considerable amount of ripe ovaries in the course of spawning (Fig. 11A). Ovaries were severely flaccid and collapsed, which caused them to decrease in weight, compared to the ones at the preceding stage. Vascularization was prominent, which moved the ovaries to be reddish in colour, as is generally the case in fish at such a stage. The spent period was also characterized chiefly by the presence of empty follicles. The ovary also displayed various peculiarities at this stage of the spawning season. It may closely resemble the ripe ovary in having mature follicles that are not yet released. Besides atretic oocytes, empty follicles can be observed. The discharge of ripe ovaries during the spawning period resulted in numerous empty follicles being present within the ovaries (Fig. 11B).

(vi) The recovery and resting adult stage (VI) is the stage at which some ovaries were reduced in size. They could easily be likened to ovaries at stage II, had they not slightly been more vascularized after spawning (Fig. 12A). New generation of small oocytes made their appearance. The number of atretic oocytes and empty follicles increased, which made this stage different from the preceding stage V (Fig. 12B).

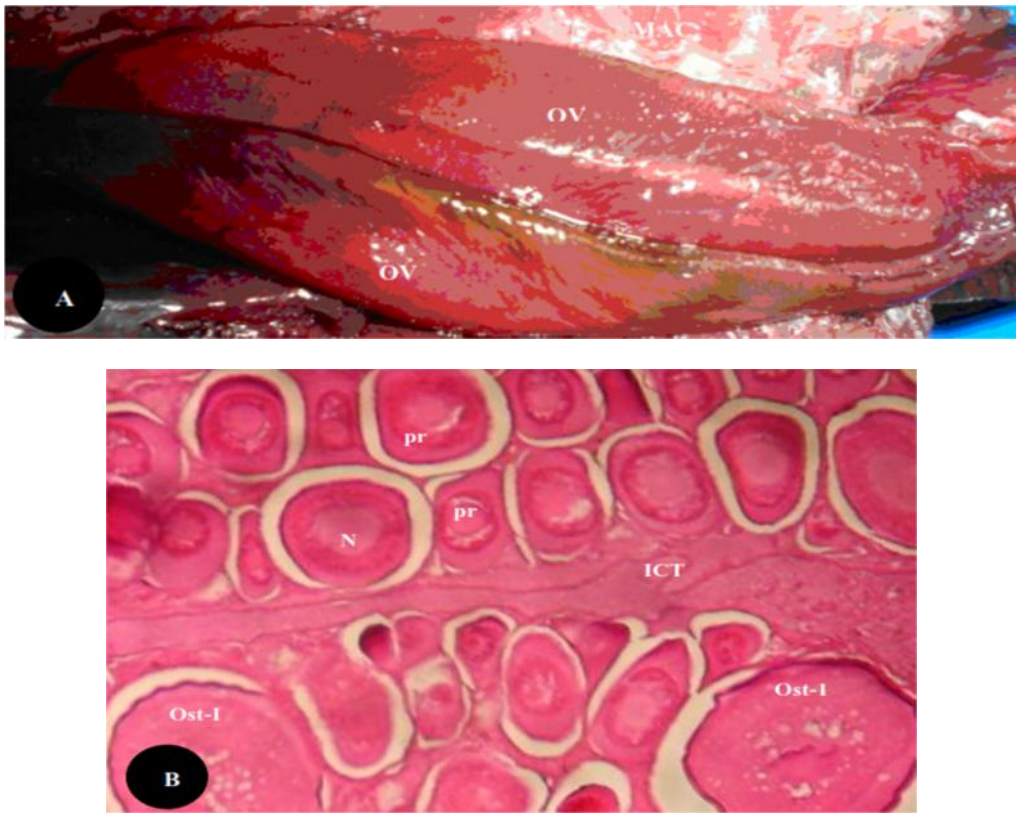

FIGURE 8. Ovaries of female *Auxis thazard* at stage II (onset of maturation). (A) Overall appearance of the ovaries of a female frigate tuna *A. thazard* at Stage II. (B) Cross-section showing details of the observation of ovaries of frigate tuna at Stage II. Appearance of primary vitellogenic oocytes (Ost-1) in addition to pre vitellogenic oocytes (pr). (M × 200) (Hematoxylin and Eosin). MAC = muscles of abdominal cavity ; OV = ovaries ; N = nucleus ; Ost-1 = primary vitellogenic oocytes ; pr = pre vitellogenic oocytes ; ICT = interstitial conjunctive tissue.

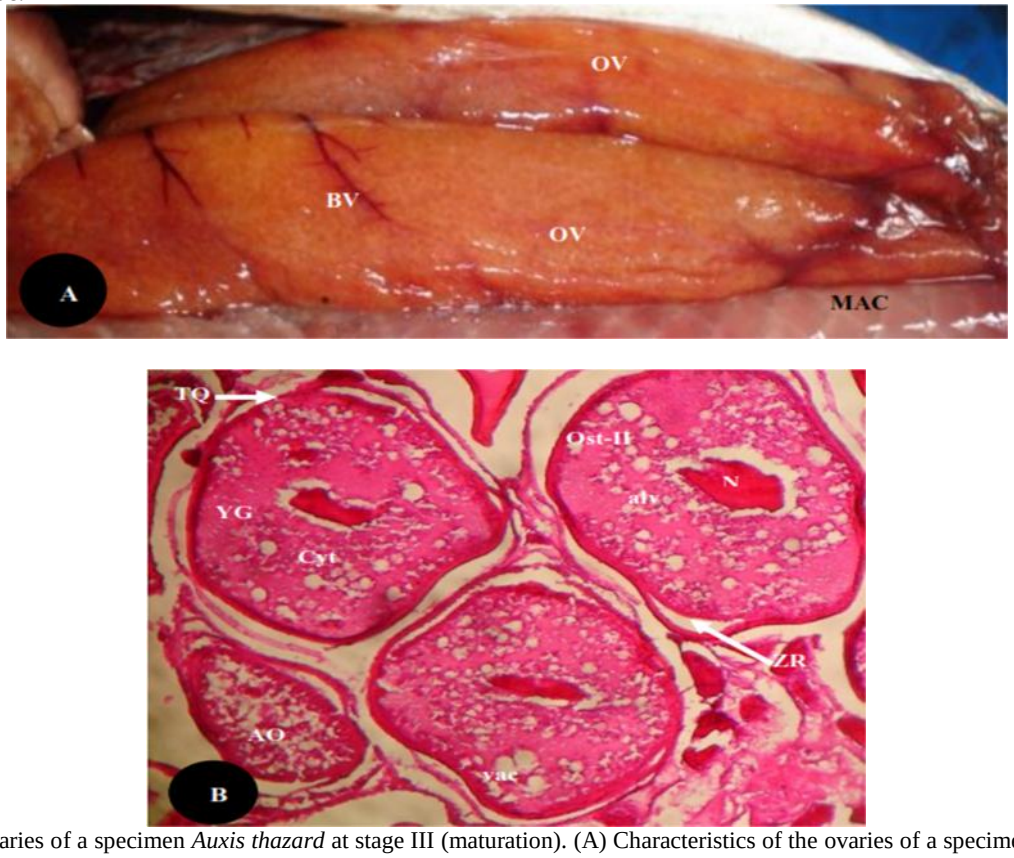

FIGURE 9. Ovaries of a specimen *Auxis thazard* at stage III (maturation). (A) Characteristics of the ovaries of a specimen *A. thazard* at Stage III. The ovaries (OV) are orange yellow, with blood vessels (BV) on their surface. (B) Cross-section of an ovary of frigate tuna at Stage III showing eggs nearing onset of secondary vitellogenic division (Ost-II). (M × 250) (Hematoxylin and Eosin). MAC = muscles of abdominal cavity ; OV = ovaries ; BV = blood vessels ; alv = alveoli ; Cyt = cytoplasm ; N = nucleus ; Ost-II = secondary vitellogenic oocyte ; AF = atretic follicle ; TQ = theca ; Vac = vacuoles ; YG = yolk granules ; ZR = zona radiata.

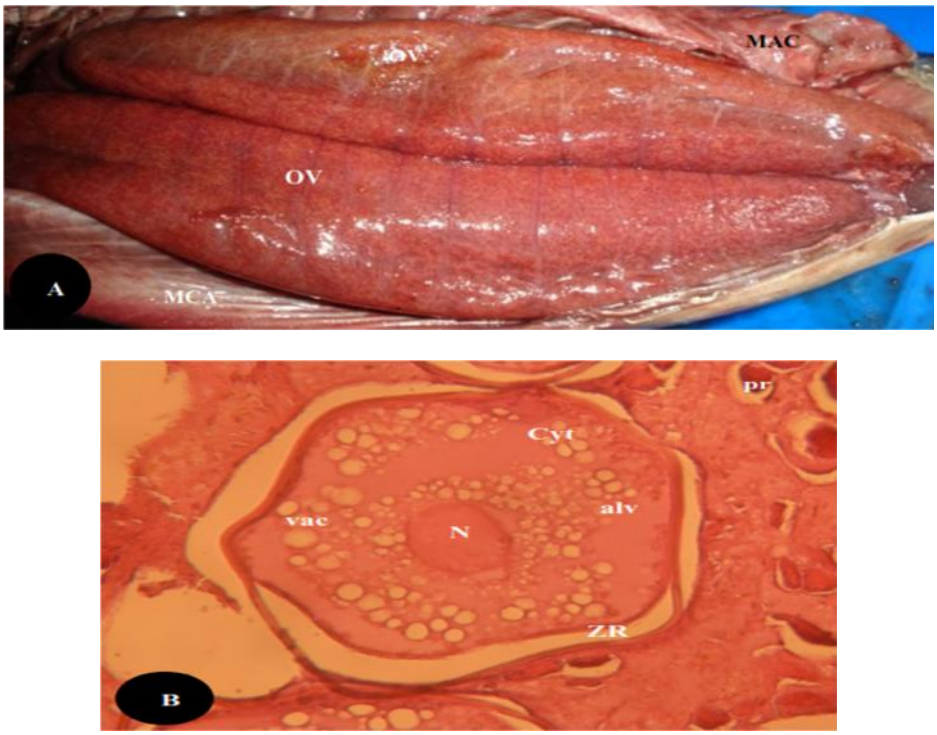

FIGURE 10. Ovaries of a specimen *Auxis thazard* at stage IV (spawning, gravid female). (A) Overall appearance of the ovaries in a mature specimen *A.. thazard* at spawning stage IV. (B) Cross-section of the ripe ovary of a specimen frigate tuna at spawning stage. Vacuoles (vac), alveoli (alv), and yolk granules (YG) are mixed up and they scattered within the cytoplasm (Cyt). The nucleus (N) occupies a central position. (M × 250) (Hematoxylin and Eosin). MAC = muscles of abdominal cavity ; OV = ovaries; N nucleus ; pr = pre vitellogenic oocyte; Vac = vacuoles.

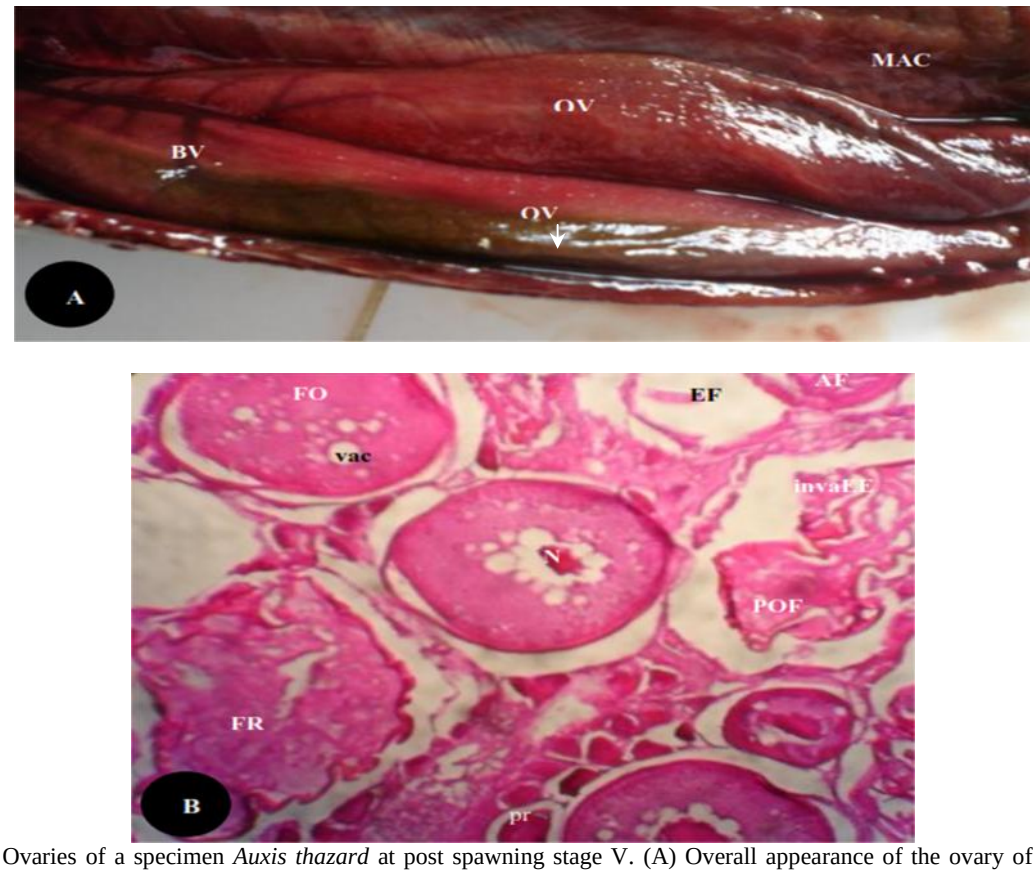

FIGURE 11. Ovaries of a specimen *Auxis thazard* at post spawning stage V. (A) Overall appearance of the ovary of a specimen *A. thazard* at post spawning stage V. (B) Cross-section of the ovary of a post spawner showing in central position a secondary yolk stage follicle (FO) with its nucleus (N). The follicular epithelium (FE) underwent invagination after spawning. Atretic follicles (AF), follicles in resorption state (FR), empty follicles (EF), and post-ovulatory follicles (POF) can be seen. (M × 200) (Hematoxylin and Eosin). MAC = muscles of abdominal cavity ; OV = ovaries ; BV = blood vessels; AF = atretic follicle ; FR = follicle in resorption state ; POF = post ovulatory follicle; FO = mature follicle ; EF = empty follicle ; N = nucleus ; invaFE = invagination of the follicular epithelium ; pr = pre vitellogenic oocyte.

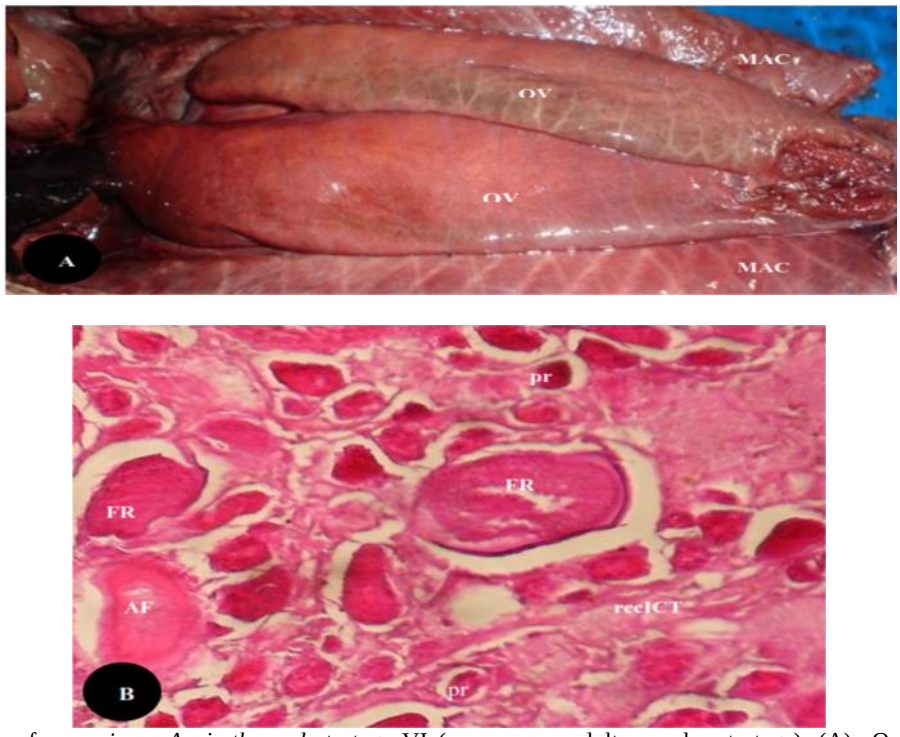

FIGURE 12. Ovaries of a specimen *Auxis thazard* at stage VI (recovery or adult sexual rest stage). (A) Overall appearance and characteristics of the ovaries of a resting adult *A. thazard*. (B) Cross-section of the ovary of a specimen frigate tuna at resting stage. (M × 200) (Hematoxylin and Eosin). MAC = muscles of abdominal cavity ; OV = ovaries. AF = atretic follicle; FR = follicle in resorption state ; pr = pre vitellogenic oocyte ; recICT = reconstitution of the interstitial conjunctive tissue.

DISCUSSION

Length-weight relationship is one of the commonest ways for determining the type of growth in fishes. That relationship generally is exponential-like, fitting in with the equation of Le Cren (1951). According to Fréon (1979), the coefficient "b" of that equation which varies between 2 and 4, yet close to 3 in many cases, is an indication of the overall body shape of fishes. In frigate tuna where the coefficient "b" is superior to 3, it does indicate a faster increase of the fish in weight rather than in length (See Micha, 1973; Ricker, 1980). Konstantinova and Chur (1976) reported sexual maturity to be 30 cm FL for females and males frigate tuna. Therefore, size at first sexual maturity for female *Auxis thazard* obtained here would appear to be of the right order by comparison with these results. Furthermore, patterns in the variations of GSI and HSI reinforce this assertion. In fact, in continental shelf waters of Côte d'Ivoire, most of individuals *Auxis thazard* are immature or are in adult sexual rest phase from December to May. Gonad maturation phase, which occurs as gonads' weights reach 2% of body weight (Postel, 1950; Frade and Postel, 1955), started in May but lingered on untill July. Starting June, gravid frigate tuna appeared in the fishery, although spawning got intensified in August and lessened (or weakened) in November. Therefore, GSI which expresses gonads' tendency to increase or decrease, depending on whether fish in spawning condition are numerous or not, showed varied amplitudes according to months, of which the ones from June to November coincided with higher GSI values.

Patterns in the proportion of fish by maturity developmental stage suggested that spawning took place from June to November with fish in spawning condition only being observed during this period. During the gonad maturation phase (from May to July) females frigate tuna undoubtedly fed actively in order for them to store up energetic reserves within the liver. Hence, during that period, both the GSI and HSI increased. According to Htun-Han (1978), the increase in the GSI is generally associated with accumulation of higher levels of proteins and lipids within the gonads. In fact, before gonads in general or especially ovaries get mature, they undergo various morphological and physiological modifications whose final result is to get the fish ready for spawning. According to Koné (2000), in order for them to successfully get their gonads mature, fatty-fish actively feed and, thereby, store up energetic reserves within their muscles, their perivisceral mesenteries and beneath their skins. Those energetic reserves thereafter first reach the liver and then the gonads so as to provide for the further energetic needs of the fish in the course of the reproduction. For those kind of fish, the peak of GSI coincides with that of the HSI (Lahaye, 1980), as show the results we obtained.

The condition factor has been mentioned as a measure of the degree of fatness, that of gonad development and of a suitable environmental condition with regard to the feeding conditions (MacGregor, 1959). Decrease of the condition factor during the gonad maturation phase shows that frigate tuna at that period got thinner, possibly because they scarcely fed intensely. Energetic reserves stored up within the liver were being used because energy supply from food consumption insufficiently compensated for energy requirements from muscular origin, which contributed to the lessening of the condition factor. However, the increase of the condition factor from July to November was due to the increase in weight of frigate

tuna as a result of heavy consumption of food favoured by the plankton bloom at that period (Binet, 1993).

The presence of gravid frigate tuna from June to November showed that the spawning occurred within a temperature range of 27.56°C and 28.53°C (during the warm water season). It did occur during the cool water season at temperatures varying from 22.14°C to 25.15°C. For example, in Senegal (a western African country), the spawning period of frigate tuna occurs from June to November, with temperature of superficial layers of the seawater over 24°C (Frade and Postel, 1955; Conand, 1970). Temperatures we mention in our study are among the ones frigate tuna larvae can bear. According to Valeiras and Abad (2010), frigate tuna larvae are tolerant of a wide range of temperature as they can live in waters with temperature between 21.6°C and 30.5°C. Tunas in general are known to be batch spawners (Cayré, 1984; FAO, 2012). This implies that various eggs at different developmental stages be observed within each ovary, which gives way for eggs with different diameter to display. Hence, in the course of reproduction, not all eggs are released right away. Only eggs with maximum size of about 1.04 mm are such-like ones. Albaret (1976) explains the multiple spawning process. Within the spawning period a cyclic process that will occur repeatedly over a certain time sets in. After normal maturation, which occurs from stage I to stage V, the ovary functions as if returning back to the end of stage III or the onset of stage IV. This occurs a certain time throughout the spawning season (Albaret, 1976). Additionally, the presence of modes in eggs frequency distribution was observed in tuna species such as yellowfin (*Thunnus albacares*), skipjack (*Katsuwonus pelamis*) and bigeye tuna (*Thunnus obesus*) (See Cayré et al., 1988).

The bimodal eggs-frequency distribution revealed the existence within the ovaries of more than a single cohort of eggs awaiting discharge. In addition, the observation of histological slides showed gradual phases in eggs growth where the most evolved eggs (i.e. mature follicles) are the ones that contain the most vacuoles. Vacuoles are tiny holes where diverse inclusions store up. Vacuolization (i.e. vacuoles occurrence) coincides with the developmental phase at which peripheral vacuoles and zona radiata coexist within the eggs. The zona radiata sustains the oocyte's (i.e. the egg's) wall (Mohamed and Al-Absawy, 2010). Ovaries at post spawning and adult rest stages give us much more insight regarding the type and characteristics of the spawning of *Auxis thazard*. In fact, ovaries at these stages enclose, besides atretic follicles and follicles in resorption state, numerous tiny cells that are a part of a new generation of eggs that are to be released later. The existence of such a cellular arrangement lies in the batch spawning that is peculiar to multiple spawners, of which frigate tuna (**C**hur, 1972; Rudomiotkina, 1983; Collette and Nauen, 1983; Valeiras and Abad, 2010).

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