ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 09, Issue, 11, pp. 31851-31859, November, 2019

RESEARCH ARTICLE OPEN ACCESS

HISTOLOGICAL AND ULTRASTRUCTURAL CHARACTERISTICS IN OVARIES OF THE FEMALE OF THE SOMPAT GRUNT *POMADASYS JUBELINI* (CUVIER, 1830) PERCIFORMES, HAEMULIDAE FROM COTE D'IVOIRE LAGOONS

Bodji Iridjé Marcelle[1*], N'ZI Konan Gervais[1], OUATTARA Nahoua Issa[1], D'Almeida Anne Marie[2] and ATSE Boua Celestin[3]

[1]Hydrobiology Laboratory, University Felix Houphouet Boigny, Cote d'Ivoire
[2]Biology Cellular Laboratory, University Felix Houphouet Boigny, Cote d'Ivoire
[3]Laboratory of Aquaculture, Oceanological Research Centre BPV, Abidjan, Cote d'Ivoire

ARTICLE INFO

Article History:
Received 02nd August, 2019
Received in revised form
28th September, 2019
Accepted 01st October, 2019
Published online 30th November, 2019

Key Words:

Histology, Ultrastructure, Gonadal development, Sompat grunt, Cote d'Ivoire lagoons.

Corresponding author:
Bodji Iridjé Marcelle

ABSTRACT

This present investigation was designed to describe structure and ultra structure of ovaries of Sompat grunt *Pomadasys jubelini*. Sampling were done monthly in Grand-Lahou, Ebrie and Aby lagoons (Côte d'Ivoire) from January 2007 to December 2008. Histological Techniques were made using the paraffin method. Ovaries of sompat gunt are consisting of two contiguous lobes. Six stages of maturity were observed. The ovaries display a variety of colours from transparent or pink to pale yellow, orange and reddish. Ultrastructure observation showed two types of ovaries. An ovarian differentiation divided into four stages observed in fish who fork length is from 10.70 to 30.90 cm, and an undifferentiated gonads divided into two stages observed in fish who fork length is from 8.50 to 25.60 cm. Undifferentiated gonads were characterized by many primary oogonia and young oocytes. Cells are previtellogenic oocytes. Differentiated ovaries are characterized by heterogeneous cytoplasm with some vacuoles and vitellus.

Citation: Bodji Iridjé Marcelle, N'ZI Konan Gervais, OUATTARA Nahoua Issa, D'Almeida Anne Marie and ATSE Boua Celestin. 2019. "Histological and ultrastructural characteristics in ovaries of the female of the sompat grunt *pomadasys jubelini* (Cuvier, 1830) Perciformes, Haemulidae from Cote D'ivoire lagoons", *International Journal of Development Research*, 09, (11), 31851-31859.

INTRODUCTION

Haemulidae is a family of fishes in the order of Perciformes known commonly as grunts. There are about 134 species in 19 genera. These fish are widely distributed in tropical fresh, brackish, and salt waters around the world (Nelson, 1994). They are bottom-feeding predators, and named for their ability to produce sound by grinding their teeth (Tavera *et al*., 2012).
In Côte d'Ivoire, this family has three genera in which genus *Pomadasys*, are represented by four species, *Pomadasys rogeri* (Cuvier, 1830), *P. incisus* (Bowdich, 1825), *P. peroteti* (Cuvier, 1830) and *P. jubelini* (Cuvier, 1830). Among these species, *Pomadasys jubelini*, focus of this study, is one of the species most often fished and sold in the ivorian markets. This species is very appreciated by local population because of its high quality flesh. Despite its worldwide importance, histological and ultra structural characteristics in ovaries of *Pomadasys jubelini* has not been enough studied.

Pomadasys jubelini is a perciform teleost. It is classified as a differentiated gonochorist species, in which an indifferent gonad develops directly into a testis or ovary. In addition, for Nakamura *et al*. (1998), careful histological observations of the process of morphogenesis of the gonads are primary importance for a precise understanding of the mechanisms of gonadal sex differentiation. Histology refers to the study of the individual parts and structures which make up a cell, and the relationship between structure and function. It is could help to develop efficient methods of directing sexual development in aquaculture species, since they can provide a guide for determining the hormone-sensitive period in cases of sex manipulation by exogenous steroid treatment (Hunter and Donaldson, 1983; Foyle, 1993). Gonadal maturation, oocyte growth and ovarian developent, have been described in many gonochoric fish (Arocha, 2002; Mylonas *et al*., 1998; Linhart *et al*.,1995; Selman *et al*.,1993; Matsuyama *et al*.,1991; Begovac and Wallace, 1988). However, a detailed histological description of ovarian development in *Pomadasys*

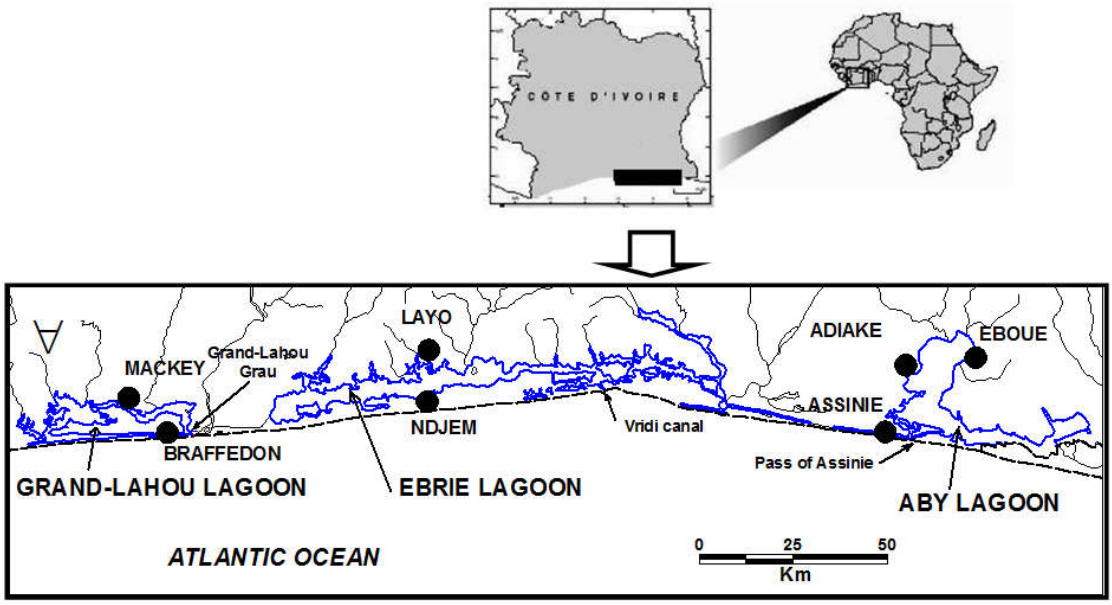

Figure 1. Sampling areas and samples sites (●) in Lagoons complex Grand-Lahou, Ebrie and Aby (Cote d'Ivoire) from January 2007 to December 2008

jubelini species are still lacking. In order to control the problem of declining stocks, to attain food self-sufficiency, one resolution is in the control of species biology and their breeding cycle. These are useful to envisage a sustainable fish farming. Although it has significant nutritional, economic interests in Cote d'Ivoire, *Pomadasys jubelini* has not yet been enough studied. The objectives of the present study is therefore to present a full description of morphological and histological changes during *Pomadasys jubelini* ovaries maturation.

MATERIAL AND METHODS

Study area: Study is conducted in Grand-Lahou, Ebrie and Aby lagoons (Côte d'Ivoire). These lagoons are located between 2°50 and 5°25 west longitude and 5°25 north latitude (Durand and Skubich, 1982) and have an equatorial climate, including two rainy seasons (April - July and October - November), and two dry seasons (December - March and August - September).

Sampling: Fish samples were collected monthly from January 2007 to December 2008 in Grand-Lahou lagoon (Braffedon and Mackey), Ebrie lagoon (Layo and N'djem) and Aby lagoon (Adiake, Eboue and Assinie) (Figure 1) using gill nets with mesh sizes of 10, 12, 15, 20, 25, 30, 35, 40 and 50 mm. This combination of various gill nets mesh sizes was employed to have a wide range of fish size. Sampling was carried out at various day periods in various areas of the lagoon. The night sampling was done between 5.00 p.m to 6.00 a.m while the day sampling was between 7:00 a.m. to 3:00 p.m. Fish captured were identified according Bauchot (1992) identification keys.

Gonad maturity stages: The macroscopic examination of gonad maturity stages was recorded using Fantodji's (1987) criteria which were modified according to the results of the research and field observations. Gonadal maturity stages were recognized, in females, by six stages. Stage I = immature; II = resting; III = developing; IV = ripe; V = ripe running and VI = spent.

Histological techniques: Histological Techniques were made using the paraffin method. The gonadswerefixed by immersion in Alcoholic Bouin solutionfixative. Gonads were dehydrated in a graded ethanol series. The samples were post fixed in butanol solution. Subsequently, for the impregnation, tissues underwent three successive baths of liquid paraffin in an oven at 60 ºC. Paraffin-embedded sections have been cut at 5 µm using a Leitz-Wetzlar microtome type and stained using haematoxylin and eosin. Ultrathin sections were examined under a Motic BA300 Polorizing Microscope type up to 1000x magnification.

RESULTS AND DISCUSSION

Macroscopic differentiation of ovaries of Pomadasys jubelini: In females of *Pomadasys jubelini*, ovaries are peer organs consisting of two contiguous lobes. Six stages of maturity were observed in which the ovaries changed in appearance, size, shape and color (Table I).

- At stage 1, females specimens have a fork length between 8.50 and 22.90 cm. Their ovaries are two filiform lobes that measure 2.94 cm length with 0.10 cm diameter. They are transparent or pink color (Figure 2A).
- At stage 2, a size of females are between 10.40 and 25.60 cm and have fully developed ovaries. These gonads are cylindrical and have 2.99 cm length with 0.20 cm diameter. Their color varies from pink to pale yellow (Figure 2B).
- At stage 3, a fork length of females is between 10.70 and 28.80 cm. Their ovaries are relatively developed, cylindrical and have 3.98 cm size with 0.40 cm diameter. They are yellow pink color and oocytes are well visible (Figure 2C).
- At stage 4, females have a fork length between 12.40 and 32.70 cm. The developed gonads size are 5.93 cm with maximum diameter of 1.20 cm. The gonads color vary from light orange to dark orange. Oocytes are well visible but non-expellable to the pressure of abdomen (Figure 2D).

- At stage 5, specimens have a size between 14.40 and 31.50 cm. Their ovaries occupy the entire visceral cavity and measure 8.03 cm length with a diameter of 1.50 cm. they ovaries are voluminous, turgid and have an orange color. These gonads contain hyaline-type oocytes that are perfectly visible and expellable at the slightest abdominal pressure (Figure 2E).
- At stage 6, a fork length of females vary between 11.36 and 30.90 cm. They present two flaccid, reddish ovaries containing some residual oocytes. The size of ovaries are 7.83 cm with a diameter of 1.20 cm (Figure 2F).

In general, oogenesis of *Pomadasys jubelini* is divided into six main stages that result in the formation of mature oocytes ready to be laid and fertilized and then, to a period of sexual rest during which gonad regenerates. The oocyte development pattern observed in *P. jubelini* is comparable to that described by Fantodji (1987) and follows the basic progression as described for other Haemulidae (García-Cagide 1986; Granado 1989).

lobules contain sex cells i.e primary oogonia and young oocytes. Ovogonies have 40 µm diameter and oocytes size is about 50 µm. The cells are oval-shaped. From outside to inside, cells are composed to cytoplasmic membrane and a very eosinophilic cytoplasm. In its center, there is a nucleus with diameter between 13 to 20 µm and containing one to three nucleoli distributed in the nucleoplasm. The nucleo-cytoplasmic ratio varies from 48-50 % with an average of 49 ± 1.41 % (Figure 3).

- Stage 2, the two lobes of ovaries in cross sectional showed an envelope consisting of an entanglement of connective tissues. It has also been noted the subdivision of lobes into lobules, separated by connective tissue. In the lobules, majority of cells are oocytes with a diameter between 52 and 73 µm. There are some rare ovogonies in the central region of the ovary. Oocytes also consist of a plasma membrane and an eosinophilic cytoplasm. The nucleus of the cells is between 20 - 29 µm diameter and contains chromatin clusters. Cells are previtellogenic oocytes.

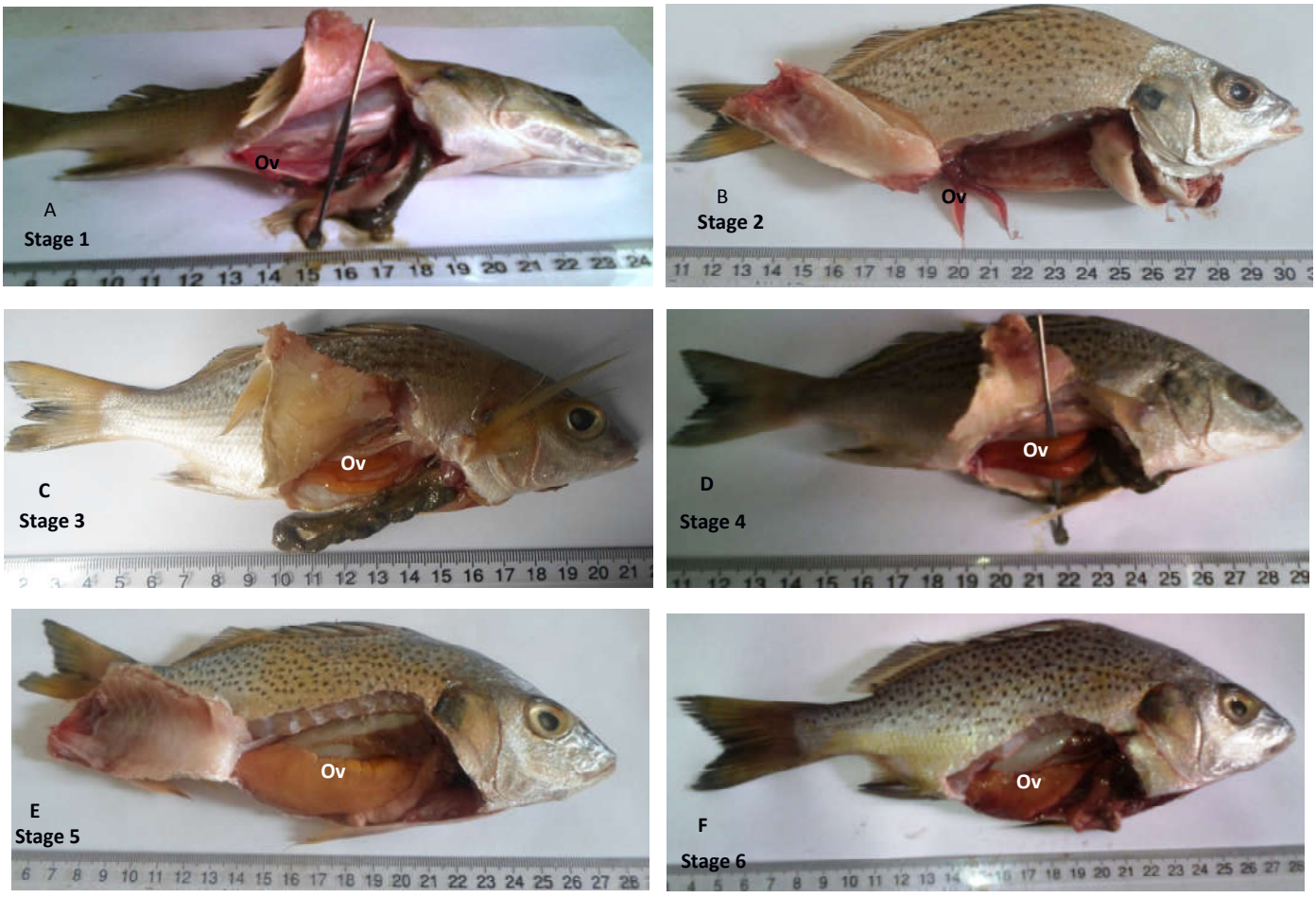

Figure 2. In toto observation of ovaries during sexual maturity of female of *Pomadasys jubelini*.Ov = ovary

Microscopic description of female gonads

Microscopic examination of the gonads of *Pomadasys jubelini* involves the following six stages of maturity. Un differentiated gonads are observed in juvenile fish of 8.50 to 25.60 cm fork length and two stages are noted.

- Stage 1, each lobe of both ovaries is subdivided into lobules and enveloped in a conjunctive membrane. The lobules are separated by connective tissues. Most

The nucleo-cytoplasmic ratio at this stage varies between 38-40 % with an average of 39 ± 1.06 % (Figure 4).

Stages 1 and 2 are identified by the presence of ovogonies that multiply by mitotic divisions and become primary oocytes. These primary oocytes is characterized by a central, rounded or spherical and voluminous nuclei. These stages correspond to previtellogenesis. These observations are similar to those reported by Migaud *et al*. (2003) who describe these stages as primary growth of the oocyte.

Table 1. Gonad photography illustrating macroscopic scale of sexual maturity of females of *Pomadasysjubelini*

Main characteristics of gonad	Gonadmacroscopic aspect
Stage 1. Small ovaries formed of two filiform divisions, translucent and pinkish	Posterior, Lobes, Front, 1 cm
Stage 2. Ovaries are cylindrical and in development. Pink or pale yellow colour	1 cm, Oviduct
Stage 3. Ovaries relatively developed, yellow pink color with oocytes clearly visible through the ovarian membrane	1 cm
Stage 4. Ovaries developed, pale orange or dark orange oocytes, granular ovarian	1 cm
Stage 5. Ovaries voluminous, turgid and orange color ; ovarian membrane very fine; hyaline oocytes perfectly visible and expulsable at the slightest abdominal pressure	1 cm
Stage 6. Soft ovaries with reddish tint color and containing some residual oocytes	1 cm

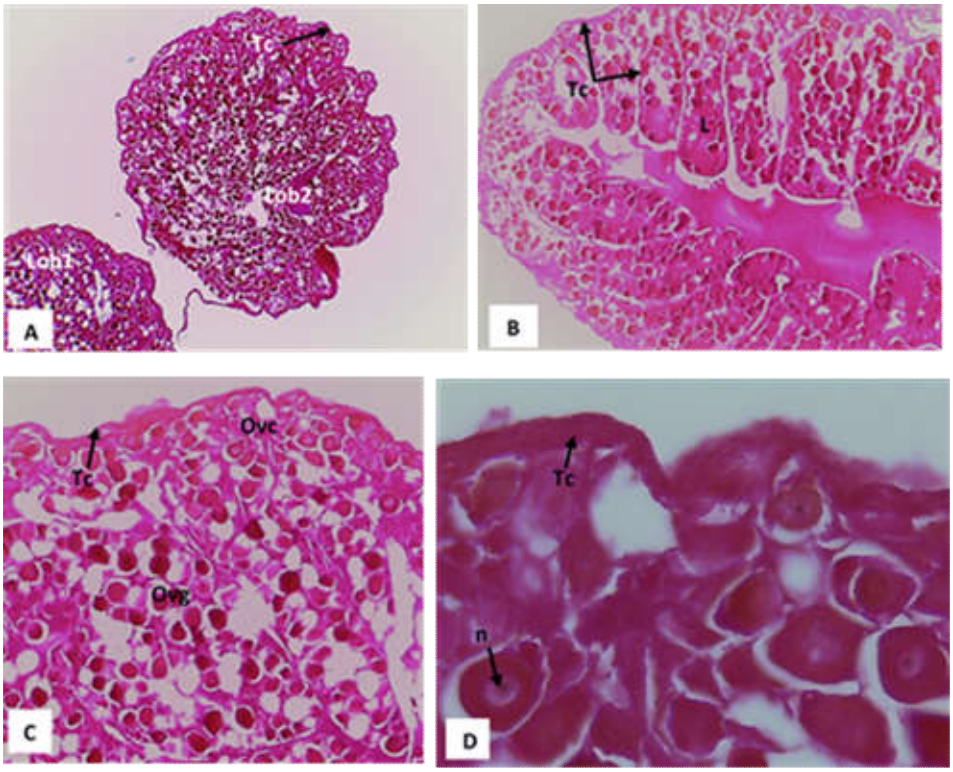

Figure 3. Cross sections in ovary of *Pomadasys jubelini* at stage 1 of sexual maturity. Haematoxylin and eosin stain. A: Global view of the two lobes of ovaries in cross section (G x 40). B: Overview of a portion of one of the ovaries showing subdivision of lobes into lobules. Lobules located on the periphery of the ovary (G x 100). C: Each lobe are comprised of germinal cells arranged in gradient, the youngest cells at the base and the oldest one at the periphery (G x 100). D: Overview of a peripheral zone showing ovocytes constituted by a single nucleolus (G x 400). E: Global view of the peripheral zone of ovary showing oocytes containing at least three nucleoli (G x 400). Detailed view of an ovarian portion with ovogonies (Ovg) and young oocytes (Ovc) (G x 400). Tc : connective tissue ; Lob1 : lobe 1 ; Lob2 : lobe 2 ; L : Lobule; N : nucleus ; n : nucleoli ; Cyto : cytoplasm ; CM : cytoplasmic membrane

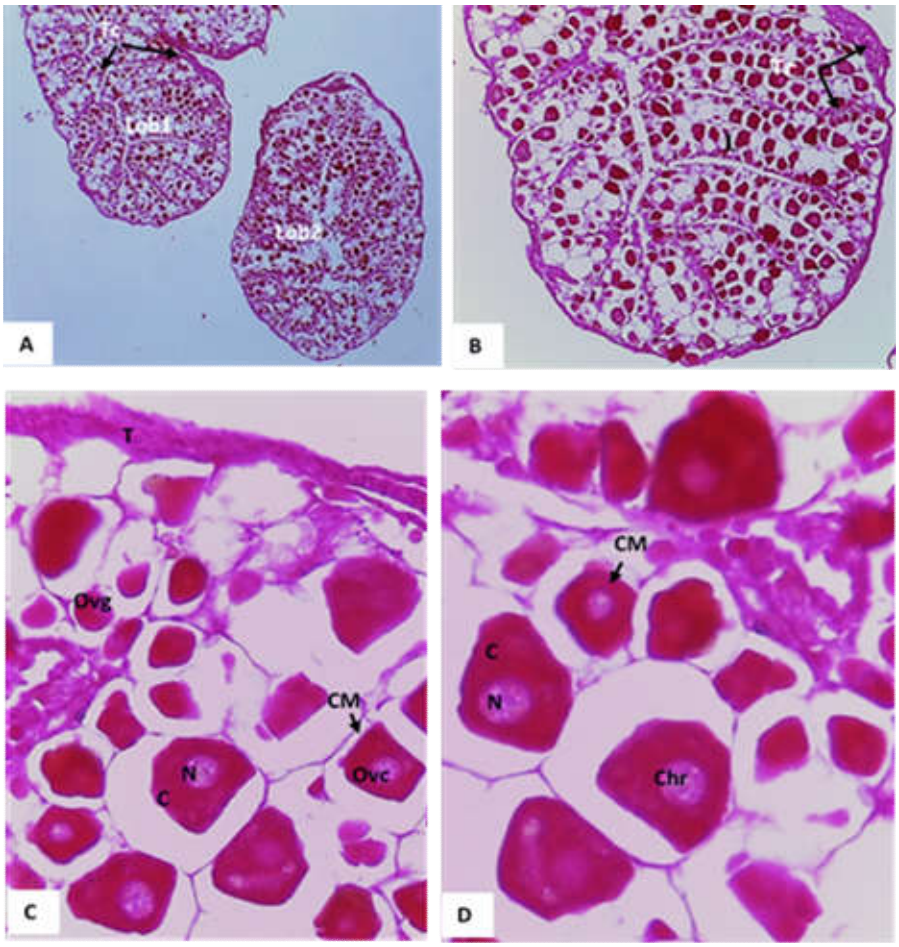

Figure 4. Cross sections in ovary of *Pomadasys jubelini* at stage 2 of sexual maturity. Haematoxylin and eosin stain. A: Global view of the two lobes of ovaries in cross section (G x 40). B: Overview of a portion of one of the ovaries showing subdivision of lobes into lobules. Lobules located on the periphery of the ovary (G x 100). C: Detailed view of an ovarian portion with oocytes (Ovc) and ovogonies (Ovg) at central region of cell (G x 400). D: Detailed view of an ovarian portion highlighting oocytes at stage 2 (G x 400). Detailed view of an ovarian portion with ovogonies (Ovg) and young oocytes (Ovc) (G x 400). Tc: connective tissue; Lob1: lobe 1; Lob2: lobe 2; L: Lobule; N: nucleus; Cyto: cytoplasm; CM: cytoplasmic membrane. Chr: Heap of chromatin

Ovarian differentiation may be divided into four stages. Females fish are between 10.70 and 30.90 cm fork length.

- Stage 3, a qualitative and quantitative change in the ovary is observed. There is no more subdivision of the lobes into lobules because the connective tissue has faded. The oocytes characteristic of this stage are follicles with a diameter between 124 and 150 µm. Nuclei of follicles are between 38-50 µm and have a heterogeneous cytoplasm. In the cytoplasm of the cells, there are some vacuoles and vitellus. Between follicular cell layer and cytoplasm is formed a zone of striated appearance which corresponds to the zona radiata. The cellular base around the follicle forms the theca. The nucleo-cytoplasmic ratio at this stage varies between 25 - 40 % with an average of 32.50 ± 10.60 %. This stage corresponds to the primary vitellogenesis (Figure 5).
- Stage 4, in the ovary, the connective tissue has faded as in stage 3. Size of follicles increases to 400 µm. Within follicles, a nucleus (40 to 90 µm size), and a cytoplasm containing numerous vitelline vacuoles and a finely granular vitellus, can be observed. In each follicle, nucleus tends to be flattened and the vitelline vacuoles are perinuclear. Two types of granular vitellus were observed: purplish and pinkish. Nucleo-cytoplasmic ratio at this stage varies between 16 - 26 % with an average of 21.50 ± 7.11 %. This stage corresponds to the secondary vitellogenesis (Figure 6).

Stages 3 and 4 are characterized by an increase in the size of the oocytes. Indeed, at these stages, there is an accumulation of vitellus in the form of vesicles of a mucopolysaccharide or glycoprotein in the oocyte cytoplasm, thus increasing its size and compressing it around the nucleus. This phase is the beginning of vitellogenesis as revealed in most teleosts fish by Rinchard *et al.* (1998), Palazón-Fernández (2007) and Albieri *et al.* (2010).

- Stage 5. At this stage, the germ cells are more numerous and heterogeneous. Most follicles reach their maximum size and measure between 530 and 640 µm. The follicular cytoplasm contains numerous vitelline vacuoles of about 10 µm size and has a reduced nucleus. Envelopes of follicles reinforced by the development of zona radiata are composed of several layers of cells. There is also the formation of a central circular ring in some follicles. They mark the beginning of follicular atresia. This stage corresponds to the end of vitellogenesis (Figure 7).

Stage 5 corresponds to the stage of hyaline oocytes or final oocyte maturation. Many vitelline inclusions are observed. Indeed, the process of oocyte maturation is dependent on steroid hormones. It is accompanied by important changes in the cytoplasm and in the vitellus. Also, the oocyte undergoes significant hydration result of a rapid increase in its volume during this stage. This

stage corresponds to vitellogenesis proper. In this present study, during the oocyte maturation phase, we noted very high refractivity of the vitelline globules which, by coalescence, gradually gave to the oocytes, a hyaline structural aspect. In stage 6, ovary changes in appearance, shape, colour and thickness. Within it, there are follicles in atresia and in resorption, and oocytes at stages 1 and 2. The cytoplasm, cytoplasmic membrane, nucleus, vitelline inclusions and zona radiata are falling into decay.

At the end of the atresia, we are witnessing the degeneration of the ovary (Figure 8).Stage 6 or ovary post-spawning gives an indication on patterns of reproductive strategy of *Pomadasys jubelini*. This stage revealed the presence of follicular atresia that correspond to follicular degeneration. Zona radiata loses its striated appearance and has a disorderly configuration. The presence of oocytes similar to those of stages 1 and 2 were also noted. These observations are consistent with those of Fantodji (1987) and Migaud (2002).

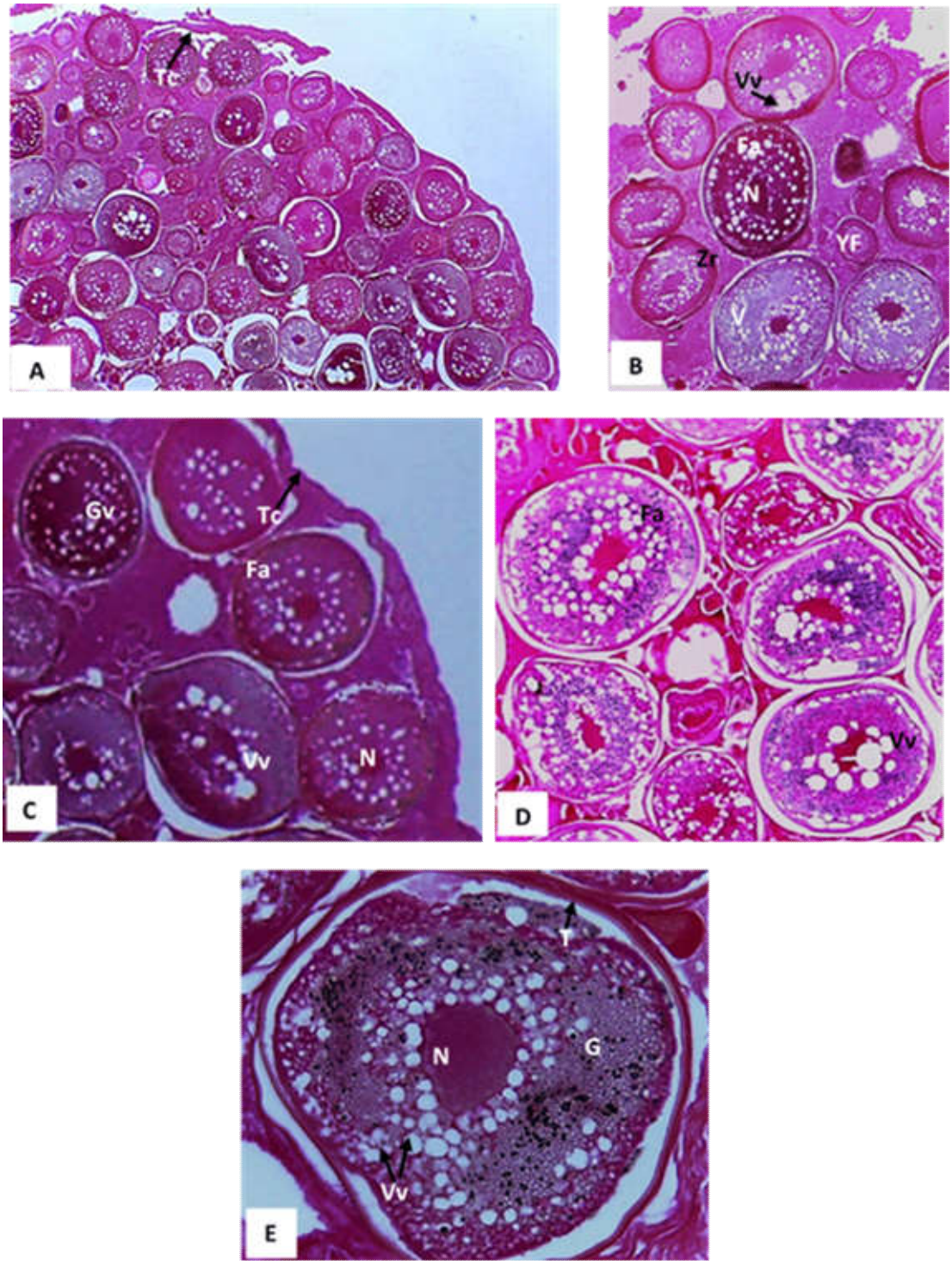

Figure 5. Cross sections in ovary of *Pomadasys jubelini* at stage 3 of sexual maturity. Haematoxylin and eosin stain. A: Global view of an ovarian portion withfollicles (G x 40). B: Global view of an ovarian portion showing young (YF) and oldest follicles (G x 100). C: Global view of an ovarian portion showing oldest follicles with many vacuoles and granular vitellus (G x 100). D: Overview of an ovarian portion with oldest follicles and vacuoles that merged (G x 100). E : Detailed view of follicles at stage 3 (G x 400). Tc: connective tissue; N: nucleus ; Cyto: cytoplasm; Vv : Vitelline vacuole ; Gv : Vitellus granular; Z: zona radiata; Th: Theca ; Cf : Follicular cell

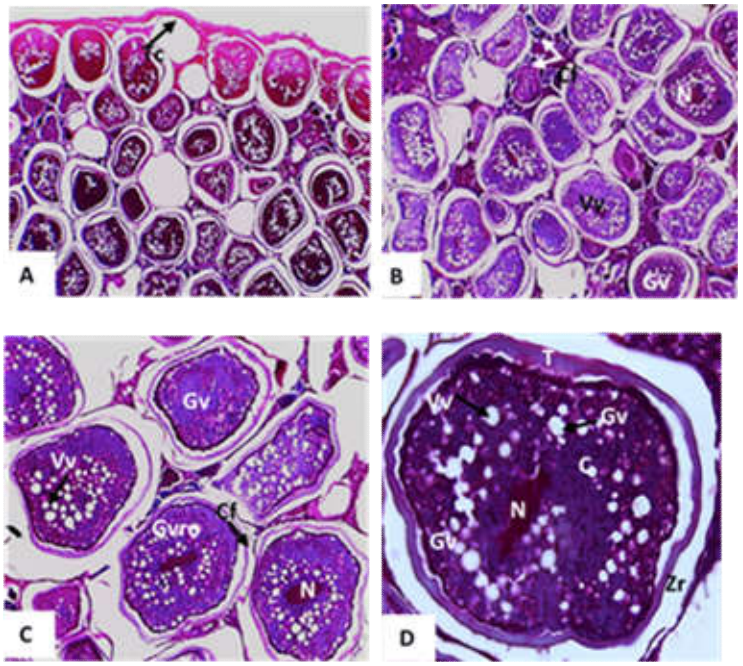

Figure 6. Cross sections in ovary of *Pomadasys jubelini* at stage 4 of sexual maturity. Haematoxylin and eosin stain. A : Global view of an ovarian portion with follicles lined up in each lobule (G x 40). B :Global view of an ovarian portion pointing out significant follicles cells around the oocyte envelope. C : Detailed view of an ovarian portion pointing out two types of vitellus (G x 400). D : Detailed view of an ovarian portion including details of follicles at stage 4 (G x 400). Tc : connective tissue ; N : nucleus ; Cyto : cytoplasm ; Vv : Vitelline vacuole ; Z : zona radiata ; Gv : Vitellus granular ; Gvvi : purplish vitellus ; Gvro : pinkish vitellus ; Th : Theca ; Cf : Follicular cell

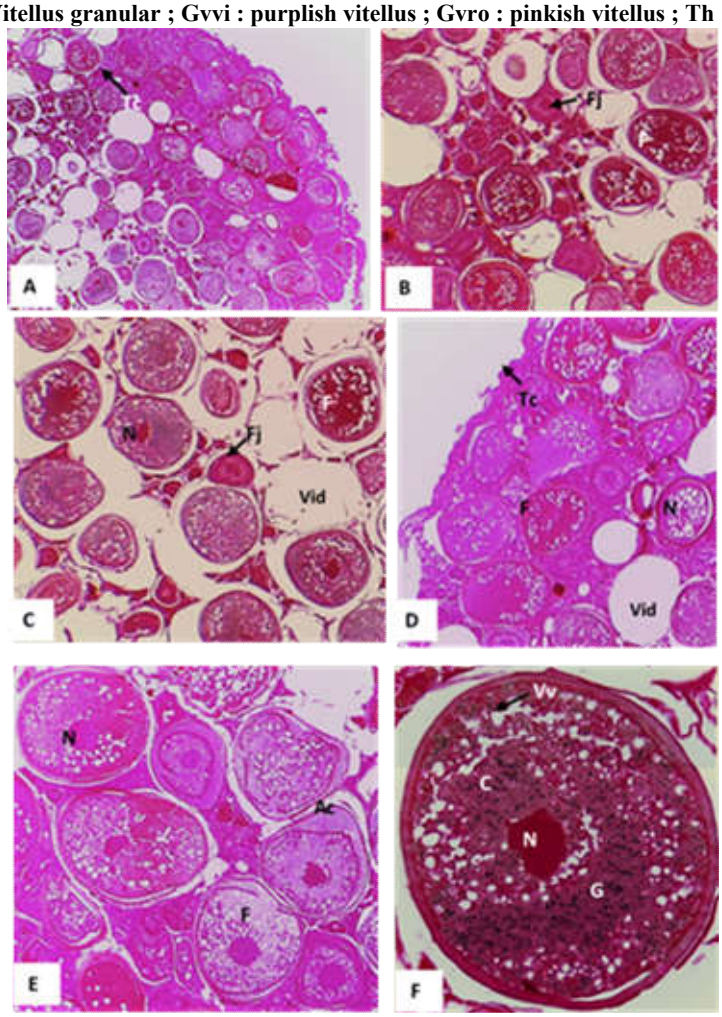

Figure 7. Cross sections in ovary of *Pomadasys jubelini* at stage 5 of sexual maturity. Haematoxylin and eosin stain. A : Global view of an ovarian portion with a heterogeneity of sexual cells (G x 40). B : Detailed view of an ovary portion showing a recovery area (Zrc) of young follicles (Fj) and mature follicles (Fm)(G x 100). C : Detailed view of an ovarian portion with some young and mature follicles (G x 100). D :Detailed view of an ovarian portion pointing out mature follicles in the peripheral zone of cell (G x 100). E : Detailed view of an ovarian portion showing follicles in atresia (G x 100). Detailed view of an ovarian portion including details of follicles at stage 5 (G x 400). Tc :connective tissue ; F : follicles ; Ac : Circular ring ; N : nucleus ; Cyto : cytoplasm ; Vv : Vitelline vacuole ; Zr : zona radiata ; Th : Theca.

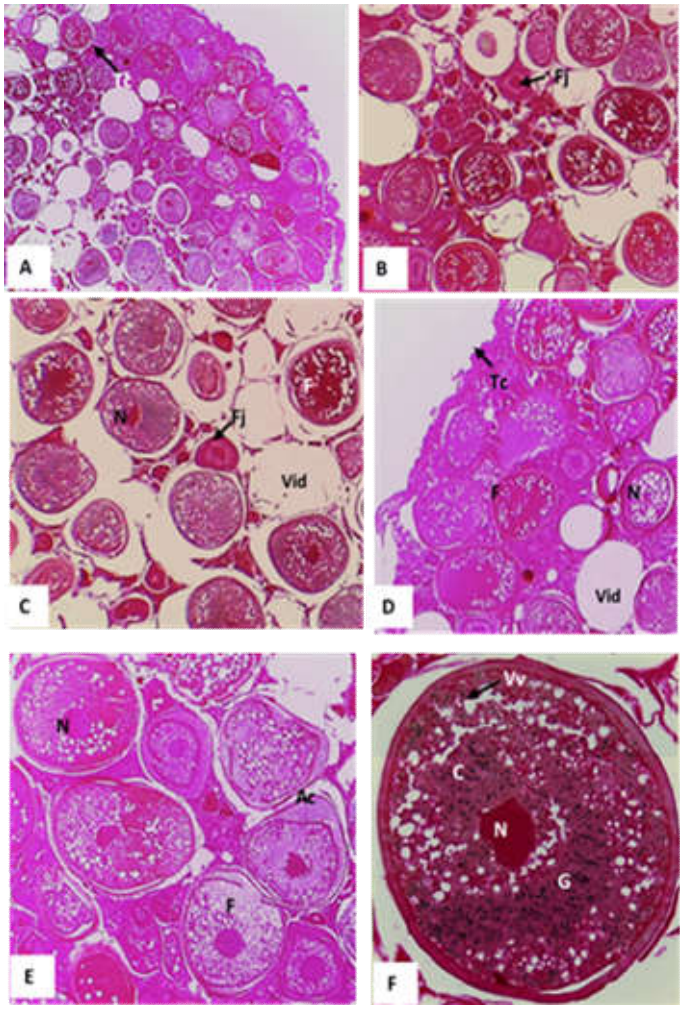

Figure 8. Cross sections in ovary of *Pomadasys jubelini* at stage 6 of sexual maturity. Haematoxylin and eosin stain. A : Overview of an ovarian portion with atretic follicles and some oocytes (Ovc) ;B : Detailed view of an ovary portion with atresia follicles with crenelated membranes (G x 100). C : Description of circular ring in atresia follicles (G x 100). D1 à D3 : Different stages of follicular atresia ; D1 : Formation of the circular ring (G x 400) ; D2 : Deformation of the follicular membrane ; D3 : Deformation and invagination of the follicular membrane and vitellus degradation with the nucleus, which presents contrasts in the form of streaks (G x 400). Tc : connective tissue ; Mcr : crenelated membranes ; N : nucleus ; Ac : Circular ring ; F : follicles ; Iv : vitelline inclusion

Conclusion

This study will contribute valuable knowledge needed for fisheries management and aquaculture of *Pomadasys jubelini* by increasing the knowledge of reproductive biology of this species. Histologically, six stages of sexual maturity characterize oogenesis in females of *P. jubelini*.

Acknowledgments

The authors thank Professor D'Almeida Marie Anne (Laboratory of Molecular and Cellular Biology, University Felix Houphouet-Boigny) for reading the manuscript, for useful suggestions and for technical assistance in microscopy; DrAtsé Boua (Aquaculture laboratory, Ocean Research Center of Cote d'Ivoire) for supervising research program on the domestication of local species of aquaculture interest through full control of the biological cycle.

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