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Research Article

Differentiation and Histological Study of the Ovary in Shrimp *Macrobrachium Vollenhovenii* Herklots, 1857 (Decapoda Palaemonidae) Of the Bandama River in Côte D'Ivoire

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Abstract: In Côte d'Ivoire, *Macrobrachium vollenhovenii* is a common shrimp species of lagoon area. Microscopical and Histological observations of this shrimp made with the naked eye, the photographic binocular magnifying glass and the light microscope highlighted some criteria for identifying the stages of the ovogenesis. The morphology of these shrimps corresponds to the general description of the Palaemonidae shrimp. The sexual maturity of females includes 5 stages, identifiable through internal criteria. During sexual maturity occurs the differentiation of the ovary during ovogenesis. The ovary presented as orange viscous mass appears in the females of the stage I as two adjoining lobes. At the stage III ovary increases and one notes at the stage IV hypertrophy of the ovary that spreads in the whole cephalothorax of the animal. The volum of the ovary decreases at the stage V. Investigations with light microscope after histological treatment allowed following the different stages of ovogenesis. The process of the ovogenesis comprises five stages in which ovogonia change successively in oocytes and in follicles and has a

single layer of follicular cells, which disappears before the eggs laying. Follicles are transformed in a telolecithal egg. It then starts directly its embryonic development.

Keys words: Females shrimps; *Macrobrachium vollenhovenii*; sexual maturity; Oogenesis.

1. INTRODUCTION

Shrimp is an essential component of the global diet. They account for 4.2% of the world's total fish production¹. These Shrimps are marine decapods or belonging to the lagoon sectors of the coast and to continental waterways. The distribution of the different species is related to the different waterways considered. In West Africa, very little work relates to fresh water shrimp. In Côte d'Ivoire, all statistics on shrimp production prior to the 1990s carried out by the Ivorian Refrigerating Armament Company² concern only maritime production. It is to remedy this situation that Gooré Bi undertook in 1998, the census of the specimens encountered in the lagoons of Côte d'Ivoire. Among the specimens of the lagoon sectors in Africa there are ten species of shrimp belonging to the genus *Macrobrachium*^{3,4}. In the Bia River in Côte d'Ivoire, are found the species *Macrobrachium vollenhovenii* (Herklots, 1857), *Macrobrachium macrobrachion* (Herklots, 1857), *Macrobrachium felicinum* (Holthuis, 1949), *Macrobrachium raridens* (Hilgendorf, 1893), *Macrobrachium dux* (Lenz, 1910). Only the first two species show important populations and the most common is *Macrobrachium vollenhovenii* (Gooré Bi, 1998). The work of various authors^{1,5-9} on Caridea shrimps such as *Macrobrachium* of the Côte d'Ivoire lagoons relates to the diversity of shrimps, their biology and the ecology of species. The studies carried¹⁰⁻¹⁴ out in *Macrobrachium vollenhovenii* focus on the seasonal breeding cycle, adult biology, and post-larval development. The study of the embryonic development in *Macrobrachium macrobrachion* was carried out by¹⁵.

The present work on oogenesis in *Macrobrachium vollenhovenii* complements those of previous authors. They relate respectively to some indications concerning the differentiation and histology of oogenesis during sexual maturity.

2. MATERIALS AND METHODS

2.1 Biological Materials: The biological material used in this study consists of shrimp exploited in the Bandama River. However, the emphasis was placed on a species of economic interest, namely *Macrobrachium vollenhovenii*. All specimens come from commercial fishing in the localities of Taabo, Singrobo and Tiassalé in Côte d'Ivoire.

2.2 Shrimp processing: In the field, the captured animals are immediately packaged with ice in a cooler to avoid alteration of the ovaries. In the laboratory the specimens have been frozen and the various manipulations are carried out after an (1) hour freezing in a freezer (LIEBHERR). All manipulations are made immediately in a very short period of time in order to avoid the alteration of the reproductive organs which tend to liquefy rapidly. The shrimp caught are weighed to the nearest gram and then dissected. They are open on the dorsal side at the level of the cephalothorax in order to expose the lobes of the ovary which are photographed using a camera (LUMIX). The gonads of each

specimen are subsequently removed. The color of the gonads is noted and then they are fixed by immersion in aqueous Bouin for histological studies.

2.3 Determining the stages of sexual maturity: One aspect of the study of the sexual cycle is based on the description of the different stages of evolution of the gonads. This was made on the basis of the macroscopic and microscopic criteria of the gonads established by Berté¹⁶. The determination of the maturity stages was made according to the maturity scale defined by many authors¹⁷⁻²⁰.

In females, the criteria for the macroscopic identification of ovarian maturation and the stages of oogenesis are color, the volume occupied in the cephalothorax and the appearance of the ovaries.

2.4 Histological technique: To conduct histological studies, works of reference are those of authors²¹⁻²⁵. The pregnant shrimp are dissected, the removed gonads are fixed by immersion in aqueous Bouin, and dehydrated in ascending series of ethanol (70°, 95° and 100°). Afterwards samples were pre-impregnated in butanol. The impregnation and the embedding were carried out in paraplast (Paraplast Monoject scientific Division of Sherwood Medical. Athy, CO. Kildare, Ireland). Sections of 7µm thickness were realized on a microtome REICHERT-JUNG or MICROM, and stained with hemalun and eosin. In order to obtain suitable section without tearing, the histological blocks started were constantly soaked from 12 to 24 hours in running water. This soaking allows the easy realization of the section especially in the blocks of ovary very rich in yolks. This operation was repeated whenever the blocks crumbled during the realization of the section. The sections were also collodionated (Collodion 4% Merk) to avoid their detachment. Observations and photographs were carried out on a light ZEISS microscope.

3. RESULTS

3.1 Internal anatomy and gonad differentiation in females of *Macrobrachium vollenhovenii*:

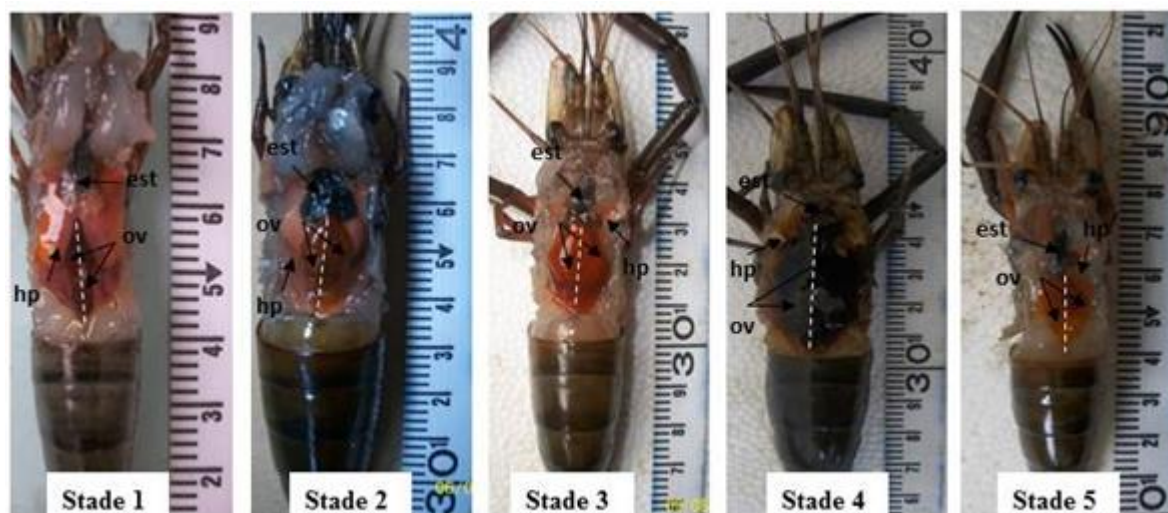
Female reproductive organs consist of paired spermathecas and a pair of compact and elongated ovaries which spread on the dorsal side of the stomach (Figure 2). The ovary is constituted by two contiguous lobes. At the posterior extremities, the gonads arrange a small space in the central axis for the passage of the cardiovascular tube. The oviduct, a thin-walled tube, begins at the median edge of each ovary and extends vertically downwards to the genital orifice located at the internal surface of the third pair of coxopodites of the locomotive appendages.

3.2 Sexual maturity scale and ovarian differentiation in the female of *Macrobrachium vollenhovenii* (Figure 1) and (Table I):

In females of stage 1 the gonads are two parallel adjoined lobes with pink color. The diameter of the viscous mass of the ovary is 0.43 ± 0.06 cm over a length of 1.27 ± 0.12 cm. (Figure 1).

- In stage 2 the parallel paired gonads have pale yellow or orange color. The diameter of the viscous mass is 0.73 ± 0.31 cm and measures 1.73 ± 0.50 cm in length. (Figure 1).
- In stage 3 the gonads constituted by two parallel lobes have a dark orange color and have a diameter of 1.67 ± 0.31 cm. The length of the gonads was 3.07 ± 0.61 cm. (Figure 1).






- The gonads formed by two parallel adjoined lobes in females of stage 4 are reddish brown or yellowish brown. The diameter of the viscous mass is 2.67 ± 0.64 cm and measures 4.30 ± 0.26 cm in length. (Figure 1).
- Finally, in stage 5, the pair of ovaries presented as two parallel lobes has a light reddish brown or orange-brown color. They are quite developed. The diameter of the viscous mass is 1.02 ± 0.38 cm. The gonads length is 2.07 ± 0.31 cm. (Figure 1).



Est: stomach; **ov:** ovaries; **hp:** hepatopancreas

Figure 1: The Stages of sexual maturity and the differentiation of the ovary in the female of *Macrobrachium vollenhovenii*

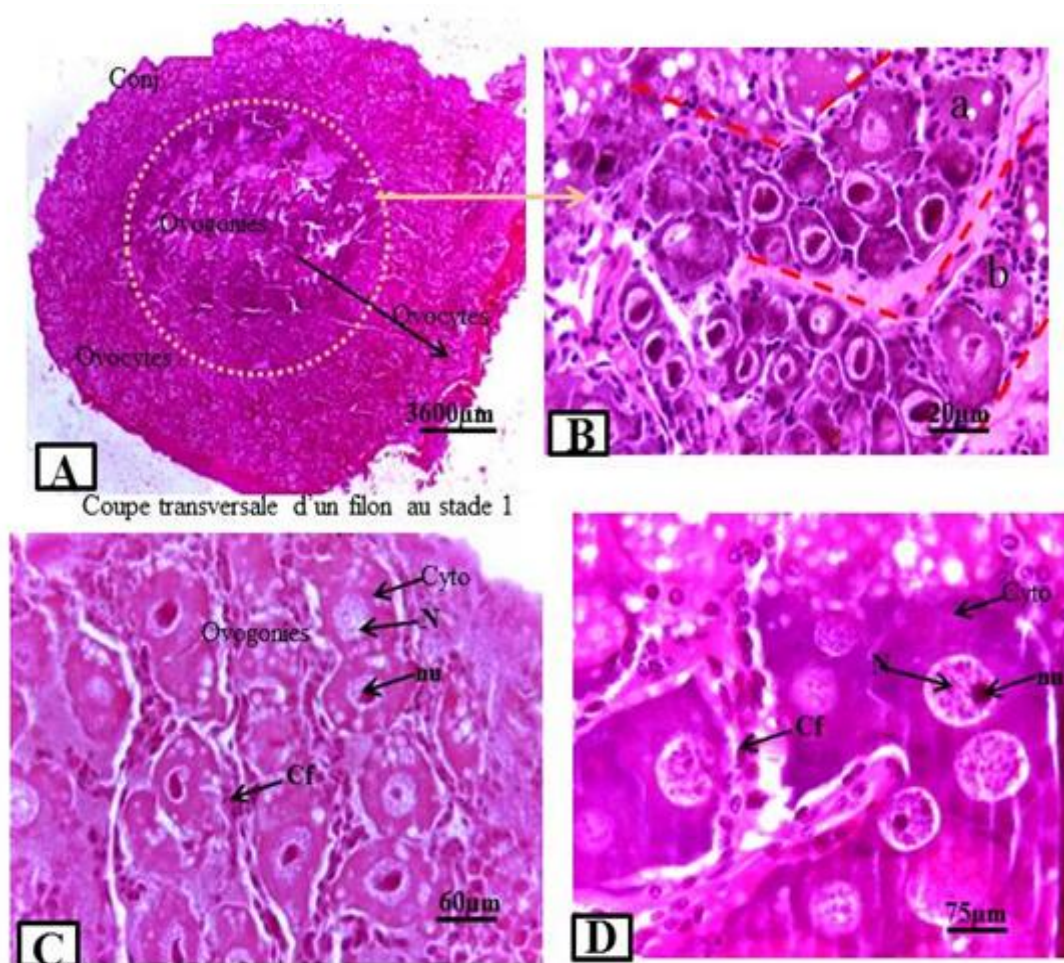
Table I: Macroscopic scale of identification of the stages of sexual maturity of the females based on the characteristics of the isolated ovaries

Stages	Characteristics	Macroscopic appearance
1	Immature: Flattened ovary with small size, occupies the third of the cephalothorax. It is pink and more or less turgid. Ovary presents filiformes or rarely spots with the well-defined outline. The setae on the pleiopods are whitish.	
2	Beginning of maturation: Ovary is less developed. It is turgid, larger in volume than stage 1. It occupies more than the third of the cephalothorax. Its color is pale yellow or pale orange. Presence of a pink spots with a well-defined outline. Presence of whitish setae on the pleiopods.	
3	Mature: Developed, turgid ovary, with orange-yellow, light orange or dark orange color. It occupies two thirds or rarely three thirds of the cephalothorax. Presence of brownish setae on the pleiopods.	
4	Laying: The ovary is very voluminous, reddish-brown or yellowish-brown, occupying the entire cephalothorax. Some oocytes or mature follicles with droplets appearance are visible through the ovarian membrane. The setae on the pleiopods are also brownish.	
5	Post-laying: flaccid and brown ovary, occupies a small portion of the cephalothorax ; or slender, slightly bulky ovary, occupies all the cephalothorax. It is reddish-brown or orange-brown. The females are ovigerous specimens or not with presence of blackish setae on the pleiopods.	

Histological study of the ovaries in females of *Macrobrachium vollenhovenii*: Microscopically, five stages were identified in the female of *Macrobrachium vollenhovenii*.

Stage I (Immature) (Figures 2A, 2B, 2C): the stage I of the sexual maturity scale is considered as the immature stage. The setting up of the ovary begins with the formation of two lobes surrounded by a conjunctive envelope (Figure 2A). The two lobes of the ovary contain clusters of ovogonia, which are small cells with 2 μm to 5 μm diameter. They are initially regrouped in the center of the lobes. This area is considered as the germinative zone (**Figures 2A, 2B, 2C**). Ovogonia are constituted by of a plasmic membrane surrounding a homogeneous cytoplasm. In the cytoplasm one distinguishes a round nucleus containing a single nucleolus. Each cell is surrounded by a layer of follicular cells (**Figures 2A, 2B and 2C**).

Stage II (beginning of maturation) (Figures 4A, 4B, 4C, 4D): During the stage II, occur the formation, proliferation and size increase of oocytes (Figure 2D) and (Figures 3A, 3B, 3C, 3D). In the lobe, ovogonia divides mitotically, its size increased and giving oocytes (**Figures 3A, 3B, 3C, 3D**). Oocyte formation occurs centrifugally (**Figure 3A**). Their diameter is about 5 μm . Their size gradually increases from the central zone of the lobe towards the periphery. Structurally, they have the same features as ovogonia. They have a peripheral plasmic membrane, a homogeneous cytoplasm and a nucleus containing a single nucleolus. (**Figure 3C, 3D**). In the oocyte cytoplasm, microvesicles of yolk with less than one micron in diameter are distinguished, and these oocytes are considered to be at the beginning of vitellogenesis. In the oocytes occurs vacuolization phenomenon (**Figure 3C and 3D**). Follicular cells persist around the oocytes (Figs. 3C and 3D). One can deduce that in stages I and II the ovaries include ovogonia and oocytes and correspond to the previtellogenesis phase.



A: Cross sectional trough a lobe of the ovary of the female of the stage 1. The ovary is constituted by of ovogonia and oocyte. It is surrounded by a thin conjunctive envelope. The area of ovogonia located in the center at the level of the germinative zone is surrounded by mature oocytes.

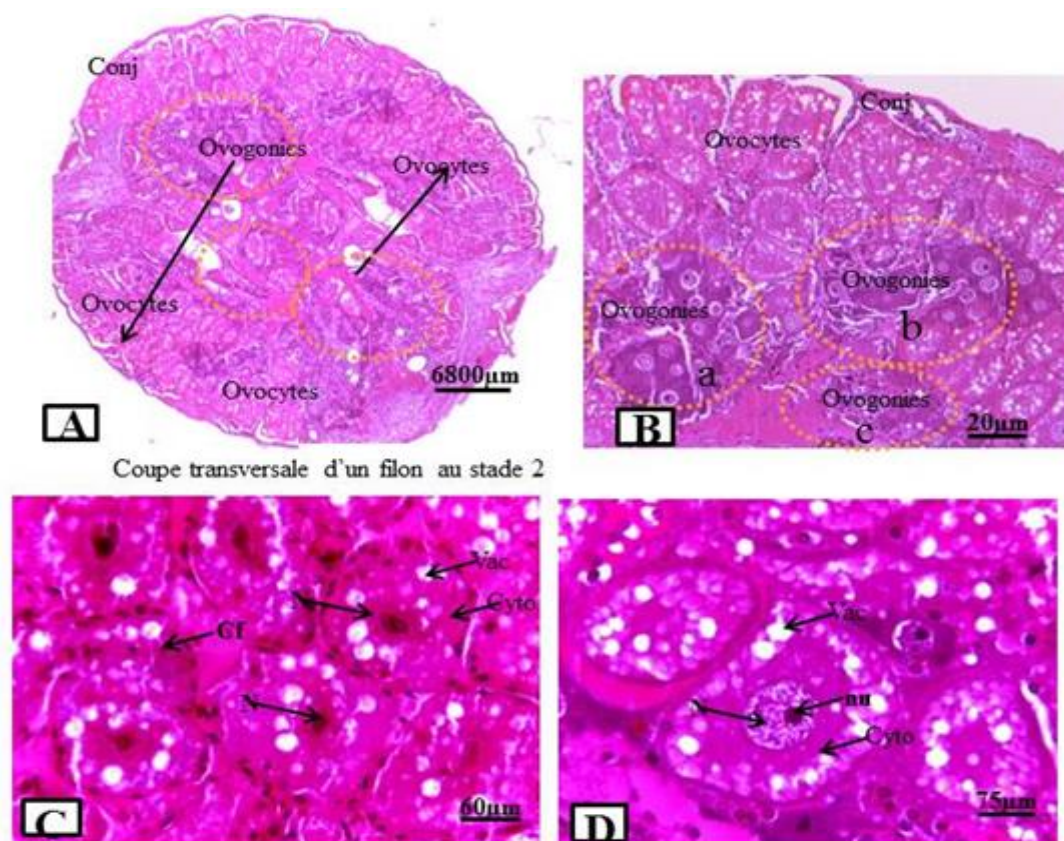
B: Portion of the central area of the ovary (Figure A) showing two adjoined lobes (A) and (b) of ovogonia.

C: Details of the central germinative area of the ovary (Figure A). The ovogonia are composed of a nucleus, an eosinophilic cytoplasm surrounded by the plasma membrane and a layer of follicular cells.

D: details of a portion of the peripheral region of the ovary composed of oocytes.

Conj : connective; **N**: nucleus; **NU**: nucleolus; **Cf**: follicular cells.

Figure 2: Histological study of oogenesis in *Macrobrachium vollenhovenii*: Section at the level of the ovary at stage 1.



A: Cross section of a branch of the ovary in the female at the stage 2. The Ovary composed in majority of oocytes is surrounded by a thin conjunctive envelope. The areas of ovogonia located in the center at the level of the germinative zone persist. The formation of oocytes occurs by centrifugal way (arrow).

B: Portion of the ovary showing contiguous areas (a, b and c) of ovogonia surrounded by enlarged oocytes.

C: Details of the peripheral area of the ovary (Figure A). The oocytes are surrounded by a layer of follicular cells. One notes the appearance of vacuoles in the cytoplasm.

D: Details of the peripheral region of the ovary constituted by oocytes. The oocytes are composed by a nucleus, an eosinophilic cytoplasm and surrounded by the plasma membrane. The vacuolization is limited to the periphery of the cytoplasm near the plasma membrane.

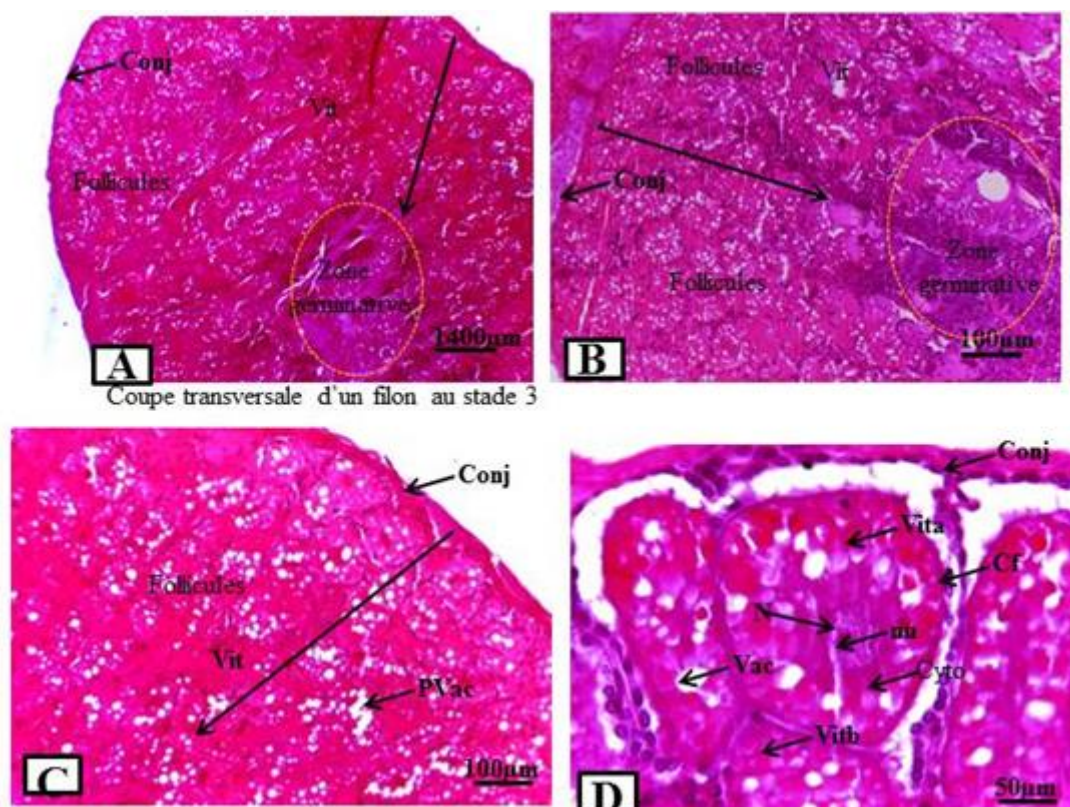
Conj : connective tissue; **N**: nucleus ; **nu**: nucleolus; **Cf**: follicular cells; **Vac**: vacuoles.

Figure 3: Histological study of oogenesis in *Macrobrachium vollenhovenii*: section at the level of the ovary at stage 2.

Stage III (mature) (Figures 4A, 4B, 4C, 4D) : The ovary at stage III is constituted by cluster of cells which are in vitellogenesis (**Figures 4A, 4B, 4C, 4D**). The ovaries are formed by two lobes containing only follicles in vitellogenesis stage and they are lined with a connective tissue (**Figures 4A, 4B, 4C**). These follicles are derived from oocytes. During the vitellogenesis, in the mature oocytes, the synthesis of yolk is asynchrone. Vesicles of yolk invade gradually the entire cell. This cell becomes a follicle. The young follicles have a diameter of 10, 30 or 40 µm (**Figures. 4A, 4B, 4C,**

4D). On some sections, the ovary has three concentric regions: the central zone of the ovogonia, the zone of the oocytes and the peripheral zone composed by follicles (**Figures 3B, 4A, 4B**).

These follicles are formed from the outside towards the inside of an envelope and a cytoplasm filled with enlarged vesicles of secretion (**Figure 4D**). The diameter of the vesicles is about a few microns. The follicles includes a nucleus containing a single large nucleolus (**Figure 4D**). In these follicles the phenomenon of vacuolization continues. (**Figures 4C and 4D**).



A: Cross sectional trough a lobe of the ovary in the female of stage 3. The ovary at the beginning of vitellogenesis is composed of a thin conjunctive envelope surrounding the follicles containing vesicles of yolk.

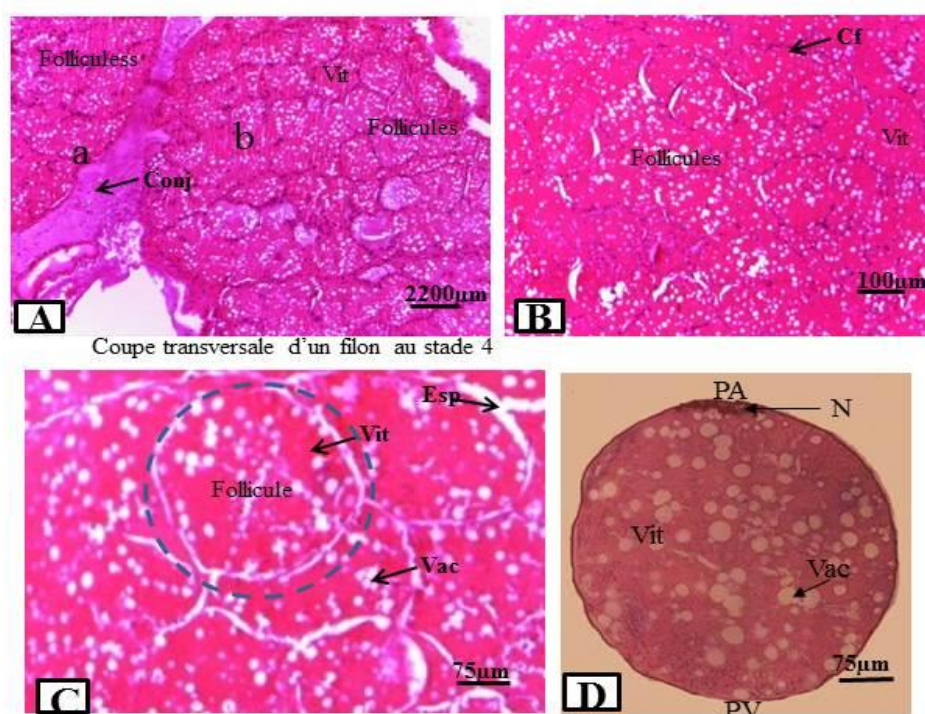
B and C: In the young follicles, the synthesis of yolk occurs centripetally (arrows). Following the plan of section, the nucleus and the nucleolus are not visible on the micrographs. The cytoplasm of the follicle contains multitudes of vesicles of yolk. The plasma membrane is lined by a layer of follicular cells. The phenomenon of vacuolization is more intense.

D: The young follicle is composed of a nucleus with a large nucleolus, a cytoplasm containing the yolk, and the plasma membrane lined with a layer of follicular cells. The phenomenon of vacuolization continues.

Conj : connective tissue ; **N:** nucleus; **nu:** nucleolus; **Cf :** follicular cells; **Vit :** yolk; **Vita:** yolk of type (a) red; **Vitb:** yolk of type (B) violet; **Vac:** vacuoles.

Figure 4: Histological study of oogenesis in *Macrobrachium vollenhovenii*: section at the level of the ovary at stage 3.

Stage IV (laying) (Figures 5A, 5B, 5C): In stage 4 (Figures 5A, 5B, 5C) the cells are large follicles with structure similar to that of stage three. They have a larger diameter between 120 and 150 μm . They contain enlarged yolks platelets resulting from the fusion of some vesicles of yolk (Figure 5C). At this stage, the hypertrophied ovaries occupy almost the whole volume of the cephalothorax. This hypertrophy results from the increase in the volume of the follicles and corresponding to the stage of great growth. The follicular cells persist around the follicles, against the oocyte membrane (Figure 5C). Initial vacuolization in oocytes persists (Figures 5B, 5C). On notes the coalescence of some vacuoles that is materialized by the presence of wide white areas within the cytoplasm. A phenomenon of spacing allows the individualization of the follicles which leads to the formation of telolecithal eggs.



A: Cross section through the two contiguous lobes of the ovary in females of the stage 4 and 5. The two lobes (A) and (b) of the ovary in vitellogenesis are lined with a conjunctive envelope surrounding the follicles containing vesicles of yolk.

B: Each follicle is lined by a layer of follicular cells forming a purplish layer around this last. The phenomenon of vacuolization is always present.

C: According to the plan of the section of the follicle, the nucleus and the nucleolus are not visible. The cytoplasm contains of platelets of yolk resulting from the fusion of the vesicles. The occurrence of a phenomenon of spacing induces the individualization of follicles that will give the eggs. The phenomenon of vacuolization continues.

D: telolecithal egg resulting from the follicle of stage 5.

Conj: connective tissue ; **Vit :** yolk ; **N:** nucleus; **nu:** nucleolus; **PA:** animal pole ; **PV:** vegetal pole; **Cf:** follicular cells ; **Vac:** vacuole ; **Esp:** spacing.

Figure 5: Histological study of oogenesis in *Macrobrachium vollenhovenii*: section at the level of the ovary at stages 4 and 5.

The ovary of stage IV crabs, therefore, includes follicles in which vitellogenesis has already taken place. It consists of clusters of follicles constituted by:

- A nucleus poor in chromatin in which a large nucleolus is found.
- A cytoplasm comprising vesicles of yolk of variable size (Figs. 5C, 5D).
- An oocyte membrane.
- Follicular cells with an oval nucleus and an elongated cytoplasm line these follicles.

The ovary in stages III, IV and V comprise only follicles. They correspond to the Vitellogenesis phase. The structure of the follicles in stage IV is similar to that of stage V.

Stage V (post-laying) (Figure 5D): The follicles at this stage are considered as fertilized eggs, the mating having already taken place (Figure 5D) and fertilization being internal in the shrimp. The diameter of these follicles is approximately 250 μm (Fig. 5D). At the level of these follicles, one distinguishes the oocyte membrane which is considered as the membrane of fertilization. The follicles are surrounded by a layer of follicular cells constituted by stretched shaped nucleus. The cytoplasm contains yolk which has lost its platelet appearance (Figure 5C). This yolk is homogeneous. In these follicles, well-individualized nuclei are eccentric. Each follicle constitutes a cellular entity (Fig. 5C and 5D). The fact that the nucleus is eccentric supposes that it is a telolecithal egg with the nucleus at a pole (Figure 5D). These follicles will be emitted through the gonopores and begin their embryonic development.

At the end of all these stages, the follicles are emitted and the ovary become an external ovarian cluster comprising a multitude of fertilized eggs each surrounded by a fertilization membrane ((Figure 5C). The yolk in the eggs has an amorphous appearance following a coalescence of the vesicles. Each egg is telolecithal type (Fig. 5D) constituted by a nucleus and a cytoplasm forming the cicatrice at the animal pole, an abundant yolk at the vegetal pole. All the elements are bordered by a fertilization membrane.

5. DISCUSSION

Sexual maturity in females of *Macrobrachium vollenhovenii* occurs in 5 stages. During the differentiation process, the diameter and length of the gonads increase from stage 1 to stage 3 or 4 in females. Following the release of gametes, gonad size decreases in stage 5 in females. ¹⁷ described the same morphological evolution in females of *Macrobrachium birmanicum*. In *Macrobrachium olfersi*, ²⁰ 5 stages of the differentiation of the ovary in females was also reported.

In the genus *Macrobrachium* and as mentioned ²⁶ in *Ranina ranina* crab, ^{27,28} in the freshwater crab *Callinectes amnicola*; in the land crab *Cardisoma armatum*²⁹, on notes the variation of ovary color from yellow to dark yellow or brown during sexual maturity.

According to ^{30, 31,32}, in the crustaceans the variation of the color of the ovary during its differentiation is due to the presence of minerals and pigments of carotenoids which is accumulated there.

Histologically, the distinctions between the different stages of maturity are based on the differentiation of the different sexual cells, which are ovogonia, oocytes and follicles. They relate to cell size, the appearance of nuclei and cytoplasmic inclusions. In *Macrobrachium vollenhovenii* the rough of the lobes of ovary is constituted by the germinative area which intervenes in oogenesis. The process of oogenesis comprises five stages grouped into three major stages in which the ovogonia is

transformed successively into oocytes and follicles. The same stages are found during oogenesis in *Callinectes amnicola*^{27,28} and in *Cardisoma armatum*²⁹. The process of oogenesis involving a germinative zone and the stages of transformation of the oocytes was indicated by³³ in his work on the upper crustaceans.

The ovogonia and oocytes observed in *M. vollenhovenii* show variable shape. This variability of shape was mentioned in the crab *Callinectes amnicola*²⁸. Moreover, the sexual cells from ovogonia stage to the follicle stage in *M. vollenhovenii*, in *Callinectes amnicola*²⁸ as well as in *Cardisoma armatum*²⁹ possess a single large nucleolus in the nucleus unlike fish.^{34,35} described young oocytes of polyhedral shape with a spherical nucleus with several nucleoli in the fish *Tylochromis jentinki* and *Pellonula leonensis*.

In *M. vollenhovenii*, the spatial, horizontal and centrifugal disposition of the oocyte follows a maturation according a gradient. Very young oocytes cluster are in the center, the oldest oocytes are at the periphery. This disposition was also observed in the swimming crab *Callinectes amnicola*²⁸ as well as in the land crab *Cardisoma armatum*²⁹. In *Mya arenaria* (shellfish), on the other hand, the maturation of the oocytes is centripetal.

During the maturation of the oocyte, the oocyte is surrounded by only one layer of follicular cells. This feature was also observed in *Callinectes amnicola*²⁸ and in *Macrobrachium olfersi*²⁰ as well as in *Cardisoma armatum*²⁹. The follicular cells would have a nutritive function^{37, 38}.

All the follicles are surrounded by an oocyte membrane. Dollander and Fenart³⁹ pointed out that an *ovulofollicular* membrane of *mucopolysaccharides* nature is formed between the surface of the oocyte and the layers of follicular cells. This latter allows the transfer of substance inside the oocyte.

The transformation of the oocyte or oogenesis in *M. vollenhovenii* involves two phases. A phase of *previtellogenesis* corresponding to stages 1 and 2 and a phase of *vitellogenesis* corresponding to stage 3 to stage 5. Both phases have been described by¹⁴ in *M. vollenhovenii*. They were observed during *Macrobrachium macrobrachion* oogenesis by¹⁴.²⁸ reported this phenomenon in the crab *Callinectes amnicola*.²⁹ reported similar finding in *Cardisoma armatum*.³³ mentioned the existence of the two phases in the upper crustaceans and²⁶ in the crab *Ranina ranina*.

Follicle transformation processes are both nuclear and cytoplasmic. The cytoplasmic transformations described by^{38,40} are the accumulations of reserves which are made in two phases : (a) Accumulation of protein reserves during *previtellogenesis* by the presence of granular endoplasmic reticulum and ribosomes^{28,33,38,41} ; (b) accumulation of yolk reserves during *vitellogenesis* characterized by the presence of vesicles and yolk platelets. These structures have been observed in *M. vollenhovenii*.

The accumulation of reserves can be both endogenous and exogenous. During the accumulation of yolk reserves, substances in particular lipids pass into the follicle through the membrane. The follicle increased in size and volume. Similar observation was made in the same species by⁴². In *M. vollenhovenii*, the diameter of the cells increases from 2 µm to 40 µm on average during *previtellogenesis* and reaches 200 to 250 µm during *vitellogenesis*. This phenomenon is designated³³ phase of great growth and produces a considerable increase in size and volume of the follicle. Similar results were obtained in *Callinectes amnicola*, the cell diameter increases from 10 µm to 60 µm during *previtellogenesis* to 300 µm at *vitellogenesis* phase^{27,28}. In *Cardisoma armatum*²⁹, the diameter of the cells increases from 10 µm to 60 µm on average during *previtellogenesis* at nearly 200 to 300 µm at *vitellogenesis* phase.

According to⁴³⁻⁴⁵ in the amphipods the ovary has a germinative zone containing primary ovogonia that evolve into oocytes. The oocytes in *previtellogenesis* are follicles with 80 µm diameter, which

grow up to reach the diameter of 230 μm during the vitellogenesis. The diameter then passes from 230 to 750 μm at the end of the vitellogenesis^{46, 47}.

In *M. vollehovenii* fertilization is internal. ¹⁴ has showed it in the same species. Fertilization of the crab *Callinectes amnicola*⁴⁸; of the shrimp *M. macrobrachion* ¹⁵ and *Cardisoma armatum*⁴⁹, is also internal. It is therefore fertilized eggs which are emitted by gravid females. This finding was made in *M. vollehovenii* and *M. macrobrachion* respectively by ^{14,15}. These results are in agreement with those of ⁵⁰ who pointed out that crustaceans only emit fertilized eggs. According ⁵¹, Crustacean eggs are centrolecithal or telolecithal. Those of *M. vollehovenii* similar to those of *Callinectes amnicola*, *Macrobrachium macrobrachion* and *Cardisoma armatum* are telolecithal ^{15,28,48,49}.

6.CONCLUSION

The growth in size in *M. vollehovenii* takes place during sexual maturity. It is related to anatomical changes that relate to the differentiation of the reproductive organs. The ovary, viscous mass evolves into two major phases. The first that constitutes the maturation of the ovary and which is internal, takes place in pregnant females. The second external, occurs in ovigerous females. Considering the evolution of the ovary during the first five stages, it is carried out in three phases: the stage of formation which corresponds to stages I and II; the phase of growth corresponding to stage III to V. Ovigerous females constitute the third phase. The data obtained were used as the basis for the histological study of the ovary. In the female of *M. vollehovenii* the oogenesis takes place at the level of an ovary and follows the known classical pattern. The oocytes resulting from the ovogonia, following the phenomena of previtellogenesis and vitellogenesis are transformed into follicles and then into a telolecithal egg. In the female of *M. vollehovenii* the gravid stages III to V are the capital stages during which the accumulation of reserve occurs for the development of an embryo. The ovogenesis of *M. vollehovenii* allows emission of millions fertilized eggs by a female.

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REFERENCES

1. G. Gooré Bi., J. N. Kouassi et E.F. Thys Van Den Audenaerde, Critères pratiques d'identification et peuplement des crevettes (*CARIDEA*) de la rivière Bia (Côte d'Ivoire). *Bulletin de l'Ifan Ch. A. Diop, Dakar T. LII, sér. A*, n° 1-2, 2002-2007. 163-186. species from Ivory Coast (Crustacean, Decapoda, Palaemonidae) *Rev. Zool. Afr.*, 1980, 94, (2). 317-326.
2. Anonyme 2, Annuaire des statistiques de production animale n°1. Institut National de la Statistique, E, 1993, 72 p.
3. T. Monod, Crevettes et crabes des côtes occidentales d'Afrique. Mémoire des Institut Fondamental d'Afrique noire, 1966, 77, 103-234.
4. C.B. Powell, The genus *Macrobrachium* in West Africa : *M. thysi* a new large egged species from Ivory Coast (Crustacean, Decapoda, Palaemonidae) *Rev. Zool. Afr.*, 1980, 94, (2). 317-326.

5. G. Gooré Bi, V. N'Douba, G. Gourene et J. N. Kouassi, Etude statistique de la pêche et de la commercialisation de deux espèces de crevettes d'eau douce d'intérêt économique, *Macrobrachium Vollenhovenii* et *Macrobrachium macrobrachion* de la rivière Bia dans le village de Biaka (Côte d'Ivoire). *Bioterre, Rev. Inter. Sci. De la Vie et de la terre*, 2001a, Vol. 2, n° 1. 35-42.
6. 6-G. Gooré Bi, V. N'Douba., G. Gourene et J. N. Kouassi, Rythme d'activité et rythme alimentaire de deux espèces de crevettes d'eau douce ouest africaines : cas de la rivière Bia (Côte d'Ivoire). *Science et technique, Sciences naturelles et agronomie*, 2001b, Vol 25, N°2. 17-24.
7. G. Gooré Bi, G. Goorene, V. N'Douba, et J.N. Kouassi, Stratégie de reproduction de deux espèces de crevettes d'eau douce *Macrobrachium Vollenhovenii* (HERKLOTS 1857) et *Macrobrachium macrobrachion* (HERKLOTS 1851) de la rivière Bia (Côte d'Ivoire). *Bioterre, Rev. Inter. Sci. De la Vie et de la terre*, 2004, Vol. 4, N° 1. 115-127.
8. G. Gooré Bi., J. N. Kouassi, et E.F. Thys Van Den Audenaerde, Critères pratiques d'identification et peuplement des crevettes (*CARIDEA*) de la rivière Bia (Côte d'Ivoire). *Bulletin de l'Ifan Ch. A. Diop, Dakar T. LII, sér. A*, n° 1-2, 2002-2007. 163-186.
9. K. G. N'Zi, G. Goore Bi., E. P. Kouamelan, V. N'Douba, T. Kone et F. Ollevier, Diversité Biologique des crevettes d'un petit bassin côtier ouest africain de Côte d'Ivoire en relation avec les variables environnementales. *Science et technique, Sciences naturelles et agronomie*. 2003, Vol 27, n° 1 et 2. 17-27.
10. J.P. Ville, Recherche sur la production de *Macrobrachium* des lagunes ivoiriennes. Migrations liées à la reproduction chez les *Macrobrachium* de Côte d'Ivoire. *Ann. Univ. Abidjan, sér. E*, 1970a, 3. 253-261.
11. J.P. Ville, Recherche sur la production de *Macrobrachium* des lagunes ivoiriennes. Migrations liées à la reproduction chez les *Macrobrachium* de Côte d'Ivoire. *Ann. Univ. Abidjan, sér. E*, 1970 b, 3. 253-268.
12. J.P. Ville, Ecologie des *Macrobrachium* de Côte d'Ivoire. Rôle de la température et de la salinité *Ann. Univ. Abidjan, sér. E*, 1971 a, 4. 317-324.
13. J.P. Ville, Ecologie des *Macrobrachium* de Côte d'Ivoire. Description des premiers stades larvaires de *Macrobrachium vollenhovenii*. (Herklots 1851). *Ann. Univ. Abidjan, sér. E*, 1971 b, 4. 325-341.
14. J.P. Ville, Cycle ovarien saisonnier chez *Macrobrachium vollenhovenii*. (Herklots 1851), Décapode, Palaemonidae, en Côte d'Ivoire *Ann. Univ. Abidjan, sér. E*, 1972, 5 (1). 561-576.
15. M.A. d'Almeida, G. Kouassi-Atta, H. K. Koua, G. Gooré Bi, V. N'Douba, A. Fantodji, A. Ehouman, A. S. N'Diaye, Caractères généraux et étude du développement embryonnaire de la crevette *Macrobrachium macrobrachion*, Herklots, 1857 (Decapoda, Palaemonidae) des lagunes de Côte d'Ivoire", *Revue Française d'Histotechnologie*, 2013, vol. 26, n°1: 123-140.
16. S. Berté, Biologie et écologie de *Distichodus rostratus* (Günther, 1864) dans un hydrosystème particulier en Côte d'Ivoire. Thèse de Doctorat, Université d'Abidjan-Cocody (Cote d'Ivoire). 2009, 180 p.

17. S.R. Singh et D. Roy, Reproductive cycle of the freshwater prawn *Macrobrachium birmanicum* choprai (Tiwari). *Asian fisheries science*, 1994, 7: 77-89.
18. C.M.S Sampaio, R.R. Silva, J.A. Santos, et S.P. Sales, 2007, Reproductive cycle of *Macrobrachium amazonicum* females (Crustacea, Palaemonidae). *Brazilian Journal of Biology*, 1965, 67 (3): 551-559.
19. J.C.Kim, W.C. Ma, C.W. Oh, et S.G. Paik, Reproduction and growth of the freshwater prawn, *Palaemon paucidens* (Decapoda: Palaemonidae) in a Lake of Korea. *Journal of Environmental Biology*, 2008, 29 (2): 163 – 168.
20. N. Giovannetti, Caracterização do ciclo da vitelogenese do camarão de agua doce *Macrobrachium olfersi* (Wiegmann, 1836) (Crustacea decapoda Palaemonidae). Programma depos-graduação em ciências biologicas (Biologia Celular e molecular), 2010, 82p
21. G.L. Humason, Animal tissue techniques. W. H. Freeman and Company. San Francisco. 2^{ème} édition, 1967. 4-226.
22. R. Martoja, M. Martoja-Pierson, Initiation aux techniques de l'histologie Animale. Masson et Cie, (1967) 8-125.
23. M. Gabe, Techniques histologiques. Masson et Cie, Editeurs, 1968, 28-262.
24. C. Nezelof, P. Galle et N. Hinglais, Les examens de laboratoires. Techniques microscopiques. Flammarion Médecine-Sciences, 1972, 55-183.
25. M. Locquin et M. Langeron, Manuel de microscopie. Masson, 1978, 2-224.
26. M. Minagawa, J. R. Chiu, M. kudo, F. Ito, and F. Takashima, Female reproductive biology and oocyte development of the red frog crab, *Ranina ranina*, off Hachijojima, Izu Islands, Japan, *Marine Biology* 115, (1993) 613-623.
27. M.A. d'Almeida, Cycle de reproduction du crabe d'eau douce *Callinectes amnicola* (de Rochebrune, 1883; Decapoda, Portunidae). Des eaux lagunaires de Côte d'Ivoire. Doctorat d'Etat. Université de Cocody-Abidjan. (Côte d'Ivoire), 1999, 169 p.
28. M.A. d'Almeida, Y. Sankare, H. K. Koua, Etude microscopique de l'ovogenèse chez la femelle de *Callinectes amnicola*, de Rochebrune, 1883 (Decapoda Portunidae). *J. Rech. Sci. Univ. Lomé* (Togo). Série A,), 2006, 8 (19-18).
29. M.A. d'Almeida, 2016, Différenciation et étude histologique de l'ovaire chez le crabe terrestre, *Cardisoma armatum*, herklots, 1851(decapoda gecarcinidae) de côte d'ivoire. *Afrique SCIENCE*, 2016, 12 (5) 12- 24. ISSN 18-13-548x [http ::www.afriquescience.info](http://www.afriquescience.info).
30. M.J. Ceccaldi, Recherche sur la biologie des associations entre protéines et caroténoïdes chez les Crustacés Décapodes, aspects métaboliques et moléculaire. Thèse Marseille, 1968, 1-479.
31. J.L.M. Martin, Evolution des protéines de l'ovaire chez *Carcinus maenas* L. femelles durant l'ovogenèse. *Téthys*, Fr., 1972, 3, 251-254.
32. J.L.M. Martin, Etude comparée du métabolisme et des facteurs de concentration de Fe, Cu, Zn, Mn, CO et Mg dans l'ovaire de *Carcinus maenas* L et *Cancer irroratus* durant l'ovogenèse. *Cah. Biol., Fr.*, 1975, 16, 149-157.

33. H. Charniaux-Cotton, L'ovogenèse et sa régulation chez les Crustacés supérieurs. *Ann. Biol. Anim. Bioch. Biophys*, 1975, 15 (4), 715-724.
34. K.J. Konan, Étude des paramètres de populations, de la biologie de reproduction et des stratégies alimentaires du Cichlidae *Tylochromis jentinki* (Steindachner, 1895) dans la lagune Ebrié (Côte d'Ivoire). Thèse de Doctorat. Université de Cocody-Abidjan, 2010, 176 p.
35. N. Koné, Étude de la pêche, des paramètres des populations et de la biologie de la reproduction de du Clupéidae *Pellonula leonensis* Boulenger, 1916 dans les lacs de barrages de Kossou et de Taabo (fleuve Bandama, Côte d'Ivoire). Thèse de Doctorat. Université Félix Houphouët Boigny, Abidjan (Côte d'Ivoire), 2012, 174 p.
36. J.W. Ropes et A.P. Stickney, Reproductive cycle of *Mya arenaria* in new England. Reprinted from biological Bulletin, 1965, vol. 128, n° 2, 315-327.
37. J. Raccaud-Schoeller, Les Insectes, Physiologie Développement. Masson. Paris, 1980, 195-265.
38. A. Fantodji et A. S. N'Diaye, Etude de la reproduction chez *Pomadasys jubelini*. Evolution et organisation des gonades au cours de l'année. *Annales Université Abidjan, serie C, (Sciences)* tome XVII, 1981, 141-159.
39. A.DOLLANDER et R. FENART, Eléments de Embryologie. Embryologie générale 1. Flammarion. 4^{ème} édition, 1979, 68-130.
40. A. Lucas, Les gamètes des Mollusques. *Haliotis*, vol. 1, n° 2, 1971, 185-214.
41. J.J. Albaret, La reproduction de l'albacore (*Thunus albacares*, Bonnaterre 1788) dans le golfe de Guinée. Cahier Orstom Serie Océanographie, 1977, vol 15, n°4, 389-419.
42. N.F. Oulé, P. Tawari-Fufeyin et J.C Okonkwo, Reproductive biology of freshwater prawn *Macrobrachium vollenhovenii* (Herklot, 1857) caught in warri River, Nigeria. *Banat's Journal of Biotechnology*. DOI: 10.7904/2068 – 4738 – 3(2) – 2012, 86, 11p.
43. H. Charniaux-Cotton, Données nouvelles concernant la vitellogenèse des Crustacés Malacostracés obtenues chez l'Amphipode *Orchestia gammarellus* (Pallas) : folliculogenèse à partir d'un tissu permanent : action du busulfan ; action inhibitrice de l'hormone juvénile. *C.R. Acad. Sci.*, 1974, 279: 563-566.
44. H. Charniaux-Cotton, Experimental studies of reproduction in Malacostraca Crustaceans. Description of vitellogenesis and of its endocrine control. *Advances in Invertebrates reproduction*. N.H. Clark, Jr. And T.S. Adam Ed., Elsevier North Holland, Inc., 1980, 177-186.
45. H. Charniaux-Cotton et C. Zerbib, Ultrastructure des phases de l'ovogenèse et origine du vitellus protéique chez l'Amphipode *Orchestia gammarellus* (Pallas). *Bull. Soc. Sci. Nat.*, Tunisie, 1979, 14, 21-28
46. C. Zerbib, Premières observations des granules corticaux dans l'ovocyte d'un Crustacé, l'Amphipode *Orchestia gammarrellus* (Pallas). *C.R. Acad. Sci.*, 281, 1975, 1345-1347.
47. C. Zerbib., Etude structurale de l'ovocyte en vitellogenèse Chez les Écrevisses *Astacus astacus* et *Astacus leptodactylus*. *Int. J. Invertebr. Reprod.*, NLD, 1, 1979, 289-295.

48. M. A. d'Almeida, H. K. Koua ; A. Fantodji, Y. Sankare, Sankare, A Sankare et A. S. N'Diaye, Etude microscopique du développement embryonnaire du crabe *Callinectes amnicola* (de Rochebrune, 1883 ; Decapoda Portunidae) des eaux lagunaires de Côte d'Ivoire. *Rev. Fr. Histotechnol.*, 2008, 21 (1), 59-74.
49. M.A. d'Almeida., H. K. Koua, A. Fantodji, A. Ehouman, A. S. N'Diaye, Caractères généraux et étude histologique du développement embryonnaire du crabe terrestre, *Cardisoma armatum*, Herklots, 1851 (Decapoda Gecarcinidae) de côte d'Ivoire. *Afrique Science*, 2014, 10 (2) 338- 355. ISSN 18-13-548x [http : www.afriquescience.info](http://www.afriquescience.info).
50. A. Beaumont et P Cassier,. Biologie animale des Protozoaires aux Métazoaires épithélioneuriens tome 2. Dunod., 1983, 579-925.
51. T. H. Waterman and F. A. JR. Chace, In the physiology of crustacean. Volum I : Metabolism and growth. TALBOT WATERMAN. Academic Press New-York and London, 1960, 1-33.

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