Sexual Maturity Scale of the Swimming Crab *Callinectes pallidus* (Rochebrune, 1883) from Lake Nokoué in South Benin, West Africa

ABSTRACT

Aims: Reproductive parameters are important tools for the sustainable management of halieutic species in the water bodies of its exploitation. This study intends to characterize the sexual maturity scale of swimming crab *Callinectes pallidus*, perform testicular and ovarian structures in order to develop a specific maturity scale.

Study Design: This study was carried out with macroscopic and microscopic observations of 394 specimens of crabs including 206 females and 188 males.

Place and Duration of Study: The specimens used in this study were collected monthly from March to July 2018 from small scale fisheries of Lake Nokoué (Benin).

Methodology: At the laboratory, each crab specimen is identified and its sex determined. Grids for describing each sexual maturity stage of the crab *C. pallidus* were developed based on the Zairon et al. (2015) scale. Histological sections of female and male gonads were analyzed in order to certify the different stages of sexual development noted from the macroscopic study.

Results: Macroscopic analysis of external and internal anatomy and histological study of male and female gonads of *C. pallidus* allowed to establish a sexual maturity scale with 5 stages in females and 4 stages in males respectively. Macroscopic observations made with a binocular magnifying glass allowed to describe the characteristic features of the sexual development cycle of the species. The histological study confirmed the results of the macroscopic analysis. Significant difference was recorded between the mean oocytes diameters of the different oocyte development stages ($p<0.05$).

Conclusion: The outcomes of this constitute an important database for the assessment of the reproductive parameters of the species in the water bodies of its exploitation. The stages IV and V are the mature steps in the female development whereas the stages III and IV are that of the male. It is recommended to allow *C. pallidus* reach the sexual maturity (5,95 cm for female and 6,38 cm for male) before their exploitation; to ensure the sustainable management of the species.

Keywords: Callinectes pallidus; macroscopic; histology; gonads; stages; oocytes.

1. INTRODUCTION

Crabs of the genus *Callinectes* are widely distributed in central Atlantic coastal region as well as in tropical Pacific and along tropical West Africa [1]. They inhabit shallow coastal waters, marine waters, brackish and fresh waters [1]. They are represented in West African lagoon systems by three species namely *Callinectes amnicola*; *Callinectes pallidus*; *Callinectes marginatus* [2-4]. In Benin, three species of this genus are encountered in water bodies [5-8]. Monitoring of the exploitation of swimming crabs in the Lake Nokoué-Lagoon of Porto-Novo complex showed that the species *Callinectes amnicola* and *C. pallidus* occupy 86% and 12% of the total catches of swimming crabs, respectively, out of the seven species identified [8].

Sexual maturity scales based on the differentiation of reproductive organs of *C. amnicola* [9,10] were used for the determination of reproductive parameters of the species in the lagoon complexes of South Benin. As for *C. pallidus* whose ecology differs from that of *Callinectes amnicola*, no reproductive parameters are available throughout its range. Several scales of sexual maturity are used in portunid crabs. These scales range from the simplest, poorly illustrated [11-14] to the most detailed, specific with full illustration of genital development [9,10,15,16,17].

The sexual maturity scale of [14], related to *Portunus pelagicus* (Portunidae), a species of the same family and similar ecology to *C. pallidus* was used in the determination of the reproductive parameters of the latter in the lagoon complexes of South Benin [18]. This sexual maturity scale has 4 stages in females and 3 stages in males. It is a scale whose stages have not been characterized anatomically and histologically. Its use requires enough experience on the reproductive organs of portunid crabs.

This study intends to characterize the sexual maturity scale of [14], perform testicular and ovarian structures in order to develop a specific maturity scale for *C. pallidus*.

2. MATERIALS AND METHODS

2.1 Study Area

Located in southeastern Benin, Lake Nokoué, with its 150 km² of surface area, is the largest brackish water area in the country [19] (Fig. 1). It is 20 km long (East-West), 11 km wide (North-South) with a depth of between 0.4 m and 3.4 m. The lake communicates to the North with the Sô and Ouémé deltas via large flooded meadows. To the east, it flows through the Totché canal with the Porto Novo lagoon and temporarily with the sea to the south, through the Cotonou channel. The hydrodynamics of Lake Nokoué is mainly controlled by the seasonal regime of continental inflows from the Sô and Ouémé rivers [20].

2.2 Data Collection

The crab *C. pallidus* is found exclusively on water bodies directly in contact with marine waters namely Lake Nokoué in the East lagoon complex and the coastal lagoon in the West lagoon complex [21]. The specimens (*C. pallidus*) used in this study are from monthly collections from Lake Nokoué from March 2018 to July 2018 from small scale fisheries. Their weights were comprised respectively between 5.62 to 83.41 gramme for male and 6.93 to 47.67 gramme for female. They are collected from the Ancien Pont, Agbato and Zogbo sampling stations where the species is most abundant (Fig. 1). The collected samples are gently transported in a cooler at 0°C from the station to the laboratory in order to preserve the specimens.

2.3 Genitalia Morphology of *C. pallidus* and Specimen Sex Determination

At the laboratory, each specimen is identified according to the taxonomic key [4] and its sex determined as shown in Fig. 2 pictures for male and Fig. 3 for female.

To maintain the internal (sexual) organs for observation, the sample were subjected to a one-hour heat shock at 18°C using a Samsung refrigerator [9].

Grids for describing each stage of sexual maturity of the crab *C. pallidus* were developed based on the [14] scale for *Portunus pelagicus* (Portunidae) (Table 1).

They were informed by binocular loupe observations of 394 specimens including 206 females and 188 males.

2.4 Gonadal Histology

The microscopic study was carried out through histological sections of female and male gonads in order to certify the different stages of sexual development noted from the macroscopic study.

Fresh crabs sample previously submitted to heat shock are dissected.

The histological study of the ovaries and testes of *C. pallidus* included 64 specimens of which 37 were females (stage II: 9; stage III: 11; stage IV: 12; stage V: 5) and 27 males (stage III: 13; stage IV: 14).

The collected gonads are preserved in pillboxes with 10% buffered formalin and labelled according to the stages. The samples are then processed in the Histology Laboratory according to the histological technique which includes: preparation of cassettes and fixation of tissues, circulation, embedding, microtome sectioning, spreading, staining and mounting according to [14] and [22].

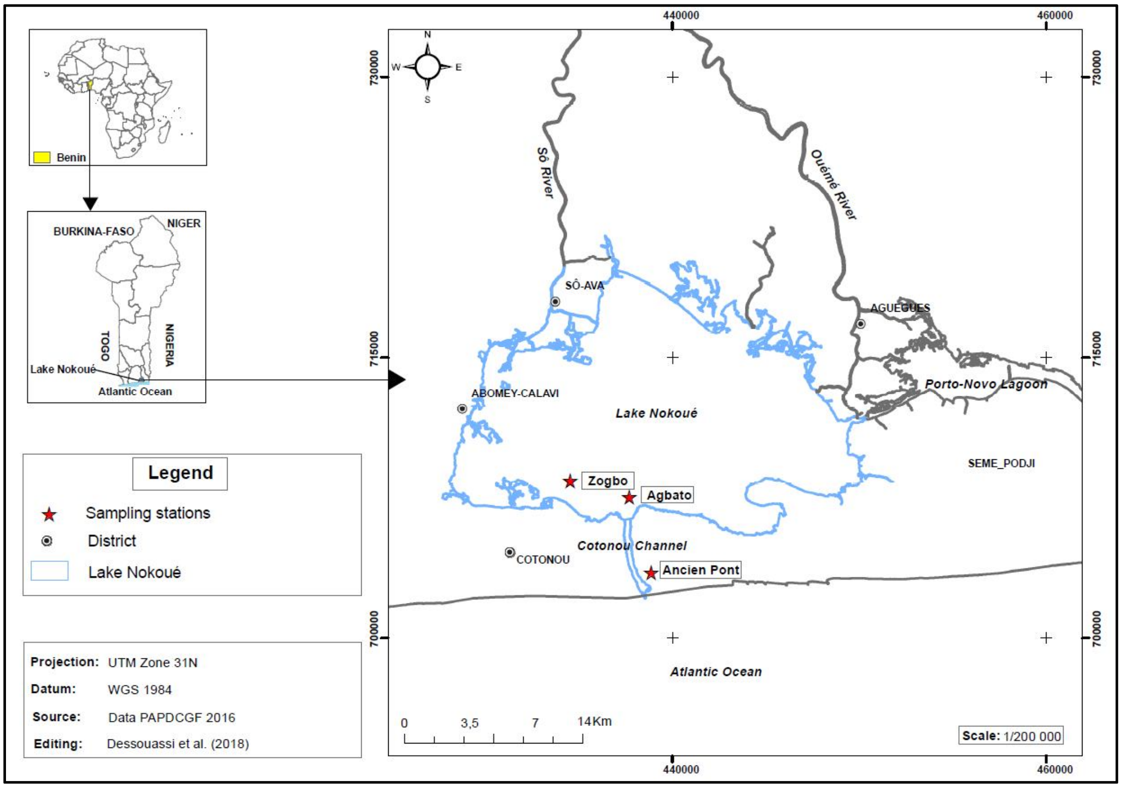
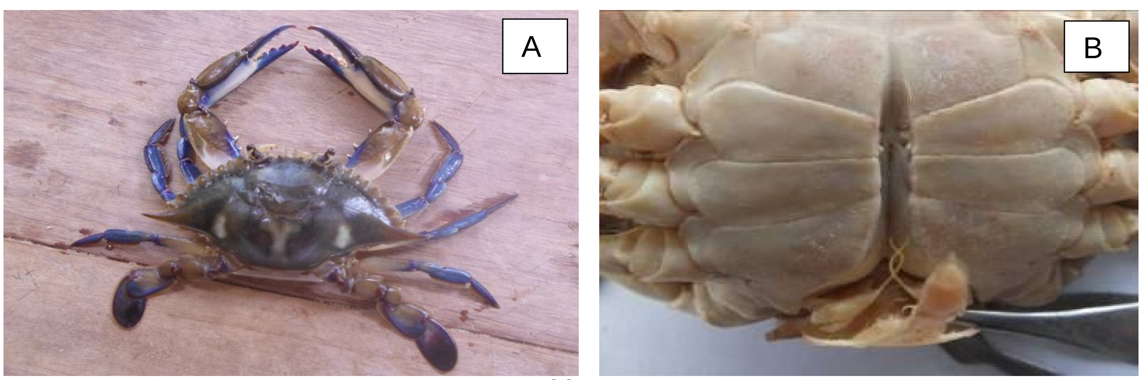


Fig. 1. Crabs *Callinectes pallidus* sampling stations on Lake Nokoué, southern Benin, West



Africa

Fig. 2. A: *Callinectes pallidus* male dorsal face B: *Callinectes pallidus* male abdomen

Fig. 3. A: *Callinectes pallidus* female immature abdomen B: *Callinectes pallidus* male abdomen

Table 1. Sexual maturity scale for *Portunus pelagicus*

Stades/Sexe	Female	Male
I	Immature	Immature
II	Early maturation	In maturation
III	Advanced maturation	Mature
IV	Mature	-----------

Source: [14]

Histological sections were taken on an Optum/Jeulin light microscope at X40 and X100 magnifications. Images were processed using Scope imag 9-0 H1C processing software.

The diameter of the oocytes of the different developmental stages determined were measured using Vistametrix software (SkillCrest Version 1.36.0, version 2009).

2.5 Statistical Analysis

Analysis of Variance with one classification criterion (Anova1) was performed to assess the variation in the mean sizes of the oocytes of the different stages determined.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Macroscopic study

As a result of the external and internal macroscopic observations, five (5) stages are distinguished for females and four (4) for males in their development. Tables 2 and 3 summarize the macroscopic analysis.

3.1.2 Microscopic study

The histological study of the ovaries and testes of *C. pallidus* included 64 specimens of which 37 were females (stage II: 9; stage III: 11; stage IV: 12; stage V: 5) and 27 males (stage III: 13; stage IV: 14). The different stages of oocyte and testicular development observed on gonadal tissues are recorded in Tables 4, and 6. The curves of the variation of oocyte size by stage of maturity are presented in Fig. 4. The ANOVA test showed a significant difference between the different stages of oocyte development ($p<0.05$) (Table 5, Fig.4).

Table 2. Stages of sexual development and macroscopic description in female *Callinectes pallidus*

Stages	Description	Size of specimens (carapace width in cm)	External anatomy (macroscopic view)	Internal anatomy (macroscopic view)
I	Immature	4.77 – 6.03	Abdomen triangular; not detachable	Spermathecae invisible or visible (fine) and ovary invisible
II	Early maturation	5.22 – 8.34	Abdomen semi-circular;detachable	Spermathecae visible and transparent; ovary invisible
III	Advanced maturation	6.24 – 7.21	Abdomen semi-circular; detachable	Developed spermathecae transparent; ovary visible as H
IV	Mature	5.95 – 7.77	Abdomen semi-circular; detachable	Spermathecae resorbed; developed ovary spread throughout carapace; very orange, light orange coloration
V		5.95 – 8.35	Abdomen semicircular pleopods bearing sprouts of color: yellow orange, brown or black ovigerous	Abdomen semicircular pleopods bearing sprouts of color: yellow orange, brown or black ovigerous

Table 3. Sexual development stages and macroscopic description in male *Callinectes pallidus*

Stages	Description	Size of specimens	External anatomy; macroscopic view	Internal anatomy; macroscopic view

		(Length inter spine)		
I	Immature	4.5 – 7.06 cm	Abdomen not detachable	Testes and genitalia invisible
II	In maturation	5.82 – 10.12 cm	Abdomen not detachable	Testes not visible; genital ducts visible two whitish masses
III	Mature	6.8 – 10.22 cm	Abdomendetachable 2nd segment of telson soft	Testicles visible and thin; genital canals visible and developed whitish color
IV	Mature	6.38 – 10.73 cm	Abdomen detachable 2nd segment of telson soft	visible and developed testes; developed genital canals anterior whitish; posterior translucent

3.2 Discussion

During sexual maturation, there are macroscopic (external: shape of the abdomen, mobility; internal: gonads, size, coloration, etc.) and microscopic (ovarian cell development) differentiations in most decapods [10].

Ovarian development could be separated into two distinct stages: (1) initial oocyte proliferation and growth (corresponding to ovarian stages St1 and St2), followed by (2) vitellogenesis. Vitellogenesis takes place in two stages, (a) St3 ovarian stage and (b) St4 and St5 ovarian stages which lead to egg laying [23].

3.2.1 Females

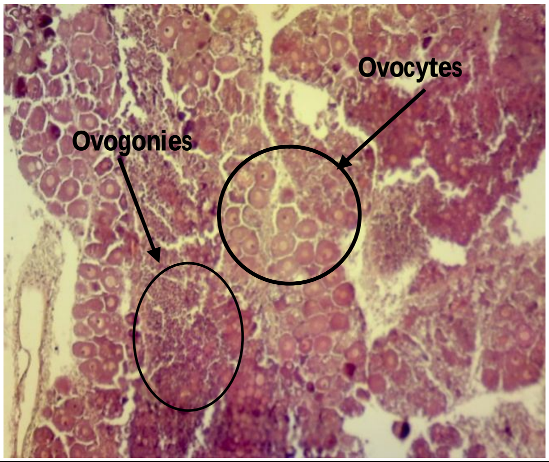
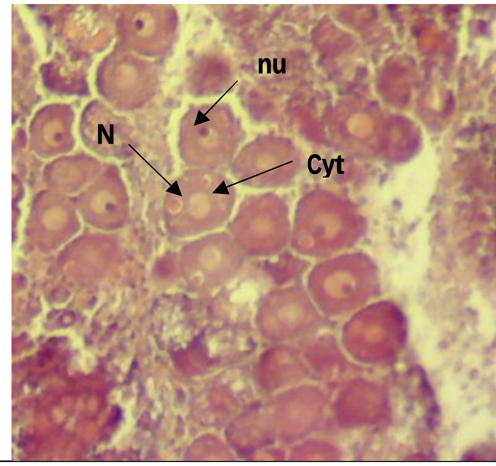
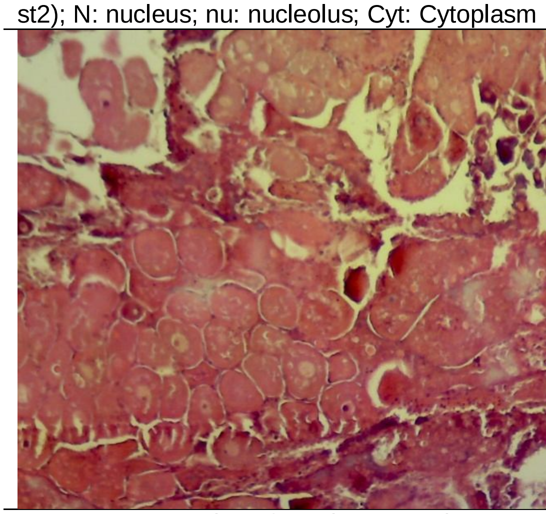
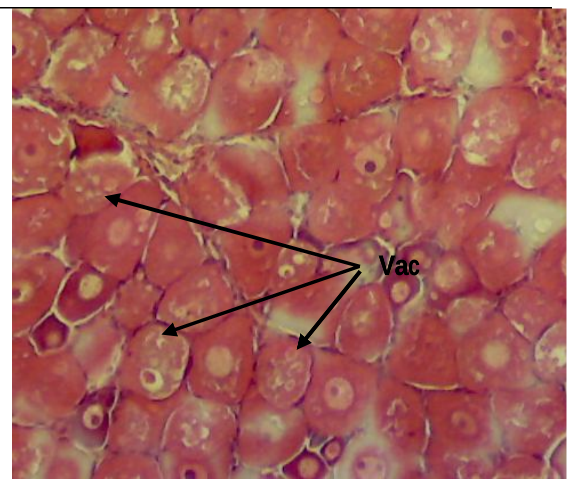
In females, a total of five (05) stages of gonad development were observed. The same results were reported by [24,25] on *Portunus pelagicus*. on the other hand, [9] described eight (8) stages of development in *Callinectes amnicola*. For stage I individuals, the abdomen is non-detachable triangular in shape with the presence or absence of thin, transparent spermathecae; the gonads are very thin and almost invisible [10,25]. From stages II to IV, the abdomen is semicircular in color yellowish at first then brown in the most mature and detachable ones [10]; the spermathecae are clearly visible as well as the ovaries at different stages of development. Its similar characteristics are described in *Callinectes amnocola* and *Portunus pelagicus* respectively by [10,25]. The ovaries mainly studied range from a small filament of yellowish color barely identifiable in stage II to a true organ of yellow color (stage III) then orange filling the entire carapace (stage IV). Following the emission of gametes (ovigerous eggs), the size of the gonads decreases considerably at stage V with post-ovigerous residues. The same observation is made in *Callinectes amnicola* and *Macrobrachium vollenhovenii* [10,26], in *Macrobrachium olfersi* [27].

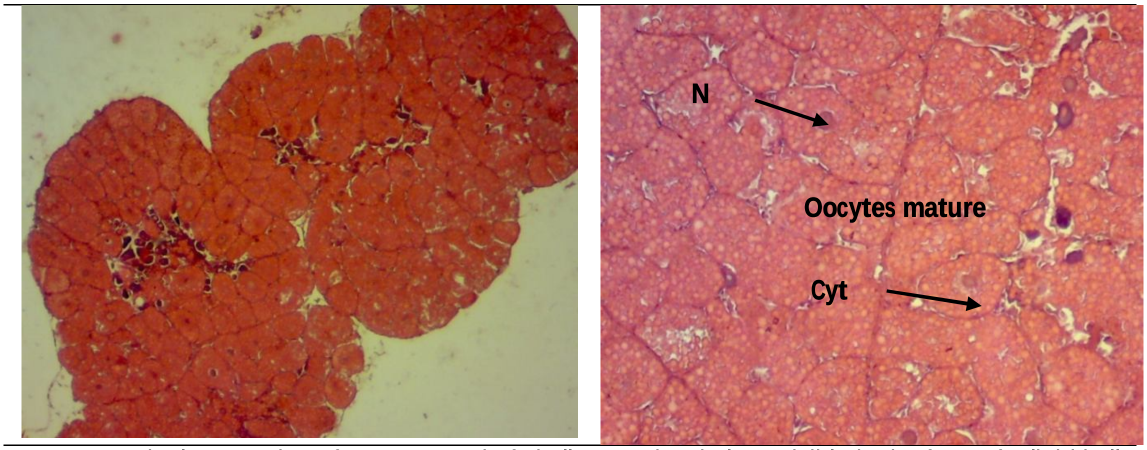
Different structures and microscopic phases could be observed during the ovarian development cycle in order to confirm the macroscopic observations in *C. pallidus* from Lake Nokoué. The distinctions between the different stages of maturity are based on the differentiation of the different levels of development of ovarian cells, namely oogonia, oocytes and follicles. These are the size of the cells, the appearance of the nuclei and cytoplasmic inclusions [26]. Each phase is characterized by the relative frequency of the different oocyte stages and post-ovulatory follicles.

The process of oogenesis begins with a germinative zone and the stages of oocyte transformation follow in higher crustaceans [28]. The oogonia are present in all females regardless of their developmental stages and they are grouped at the periphery of the ovary in the ovarian lobules [28].

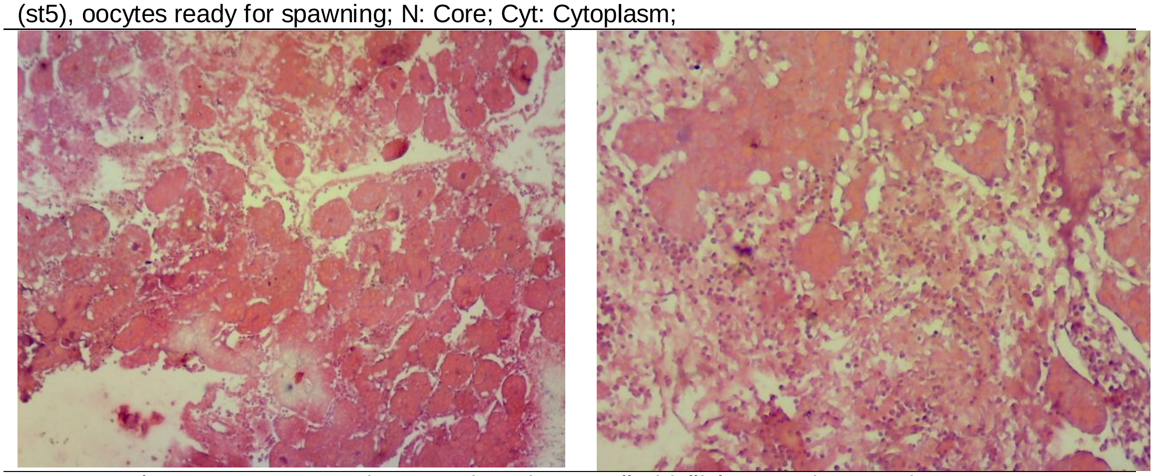
The multiplication and development of ovarian cells marking the beginning of the previtellogenic phase St1 and St2 (stage II) causes the yellowish coloration of the gonads to appear [25,26] then follows the actual vitellogenic phase St3, St4 and St5 (Stages III, IV and V) which is characterized by the incorporation of the yolk by the oocytes with the presence of granules at the periphery of the oocytes (stage III) and finally a complete occupation (Stage IV) corresponding to the end of vitellogenesis. This phase allows a rapid increase in the volume of the gonads and gives it a bright yellow and orange color [24,25]. Stage V spawning already takes place where we observe the presence of St3 type oocytes in majority and follicles in resorption. The same stages are found during oogenesis in *Callinectes amnicola* and *Cardisoma armatum* [9,26], *Portunus pelagicus* [24,25].

Table 4. View of histological sections of female gonads of the crab *Callinectes pallidus*

Ovogenesis in the crab *Callinectes pallidus*
Stage I: invisible ovary
 
Stage II: Development of ovarian cells (ovogonia) oocytes at different stages of development (st1, st2); N: nucleus; nu: nucleolus; Cyt: Cytoplasm
 
Stage III: maturation of ovarian cells; development of oocytes; oocytes are surrounded by a layer of follicular cells (st3, st4). We note the appearance of vacuoles in the cytoplasm; beginning of vitrellogenesis ; presence ; Vac : Vacuoles



Stage IV: Final maturation of oocytes; end of vitellogenesis: viteluse visible in the form of a lipid ball (st5), oocytes ready for spawning; N: Core; Cyt: Cytoplasm;



Stage V: Almost empty ovary; degeneration of unexpelled follicles, ovarian membranes; presence of oocytes at the beginning of vitrellogenesis (st3, st4).

Table 5. Oocyte size by stage of *C. pallidus* females (ANOVA p<0.05)

Oocyte stages	Mean (µm)	Standard deviation (µm)	Variation (µm)	Total number of oocytes
Stage II	448.3	4.61	34.6 – 54.7	61
Stage III	674.1	4.03	56.2 – 75.4	61
Stage IV	980.4	9.53	78.2 – 123	61
Stage V	639.5	6.48	51.3 – 78.8	61

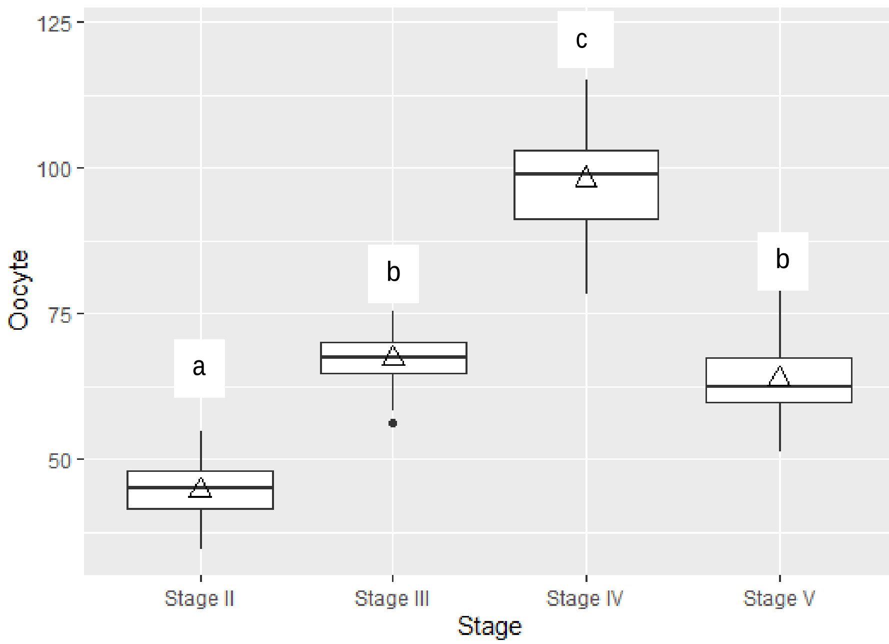
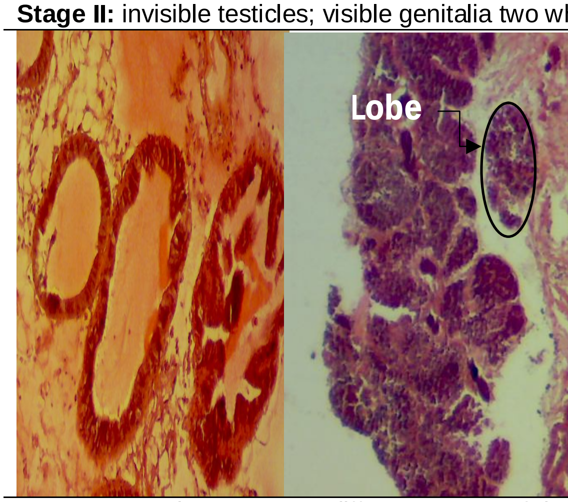
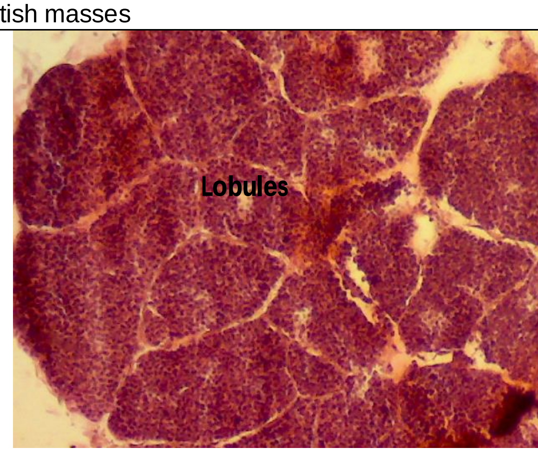
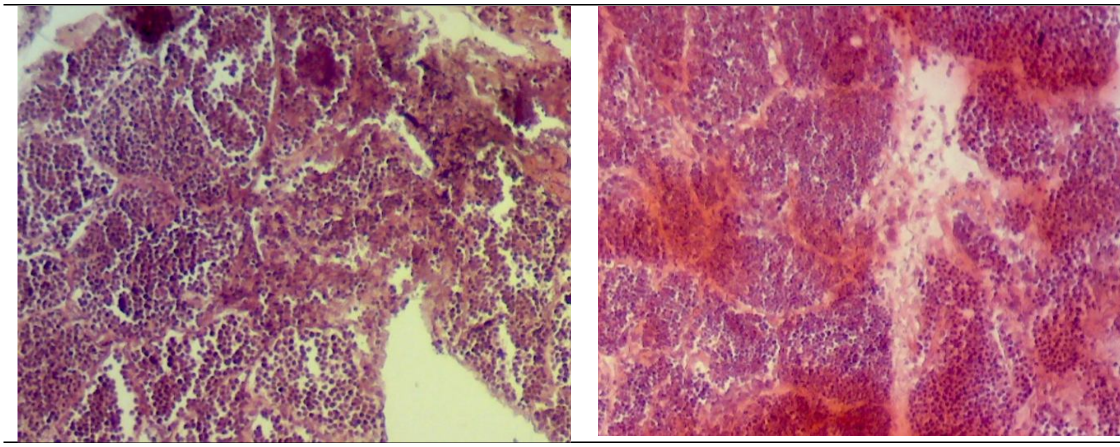


Fig. 4. *C. pallidus* female Oocyte size variation by stage

Table 6. View of histological sections of male gonads in the crab *C. pallidus*

Spermatogenesis in the crab *Callinectes pallidus*
Stage I: invisible testicles **Stage II:** invisible testicles; visible genitalia two whitish masses
 
Stage III: Testicular cells at different stages of development; subdivision into lobes and lobules (spermatogonia)



Stage IV: Mature sex cells contained in the lobules (spermatocytes or spermatids); formation of spermatophores

3.2.2 Males

In males, differentiations are noted by the presence of genital canals and testes. In fact, in immature individuals (stage I), neither genital canals nor testes are observed, but already in juveniles, the presence of genital canals in the form of two whitish masses is noted; the testes being still very thin are merged with the membrane of the exoskeleton. For stages I and II, the telson is adherent. For stages III and IV, the genital canals are well distinguished as well as the testicles [24]. The difference is noted by the size and the coloration of the genital canals. Not only is the telson mobile but its second segment is soft which is not the case in mature males of *Callinectes amnicola* which is a key element of differentiation. At the microscopic level, there is no great difference in spermatogenesis.

4. CONCLUSION

Growth in size in *C. pallidus* takes place during sexual maturity. It is characterized by anatomical modifications which relate to the differentiation of the reproductive organs. In the females, we note the establishment of spermathecae already in stage I. The spermathecae are more developed from stage II to stage IV combined with the development of the ovary in the form of a yellowish filament in stage II then in the form of a yellow H in stage III and orange in stage IV. The resorption of spermathecae in grained females with the residual ovary of variable color (yellowish, yellow, orange). In males, the presence of genital canals is observed in stages II to IV. The testes are visible only in stages III and IV. The data obtained served as a basis for the histological study which confirmed the macroscopic observations. Its results constitute an important database for the study of the reproductive parameters of the species in the water bodies of its exploitation. It is recommended to allow *C. pallidus* attain the sexual maturity (5.95 cm for female and 6,38 cm for male) before catching to ensure the sustainable management of the species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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