Sexual maturity scale of the swimmingcrab *Callinectespallidus* (Rochebrune, 1883) from Lake Nokoué in South Benin, West Africa

Comment [SP1]: *Callinectes pallidus*

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 withmacroscopic 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 ~~2018~~ 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 *Callinectes 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 *Callinectespallidus* 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 oocytesdiameters of the different oocyte development stages ($p<0.05$).
Conclusion: Theoutcomes of this constitute an important database for the assessment of the reproductive parameters of the species in the water bodies of its exploitation

Keywords: Macroscopic; histology; gonads; stages; oocytes.

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1. INTRODUCTION

Crabs of the genus *Callinectes* are distributed both north and south from the central Atlantic coastal region as well as in the eastern tropical Pacific across the connections 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 *Callinectesamnicola*; *Callinectespallidus*; *Callinectesmarginatus* [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 *Callinectesamnicola* and *Callinectespallidus* occupy 86% and 12% of the total catches of swimming crabs, respectively, out of the seven species identified [8].

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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 *Callinectespallidus* whose ecology differs from that of *Callinectesamnicola*, no reproductive parameters are

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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 *Portunuspelagicus* (Portunidae), a species of the same family and similar ecology to *Callinectespallidus* 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 *Callinectespallidus*.

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2. MATERIAL AND METHODS

2.1. Study area

Located in southeastern Benin between parallels 2°24' and 2°37' North and meridians 6°23' and 6°28' East, Lake Nokoué, with its 150 km² of surface area, is the largest brackish water area in the country [19]. It is 20 km long (East-West), 11 km wide (North-South) with a depth of between 0.4 m and 3.4 m, and communicates to the North with the Sô and Ouémé deltas via large flooded meadows, to the east through the Totché canal with the Porto Novo lagoon (5 km long and 150 m wide and permanently open) and temporarily with the sea to the south, through the Cotonou channel (4.5 km long and 250 m wide). The hydrodynamics of Lake Nokoué is mainly controlled by the seasonal regime of continental inflows from the Sô and Ouémé rivers [20].

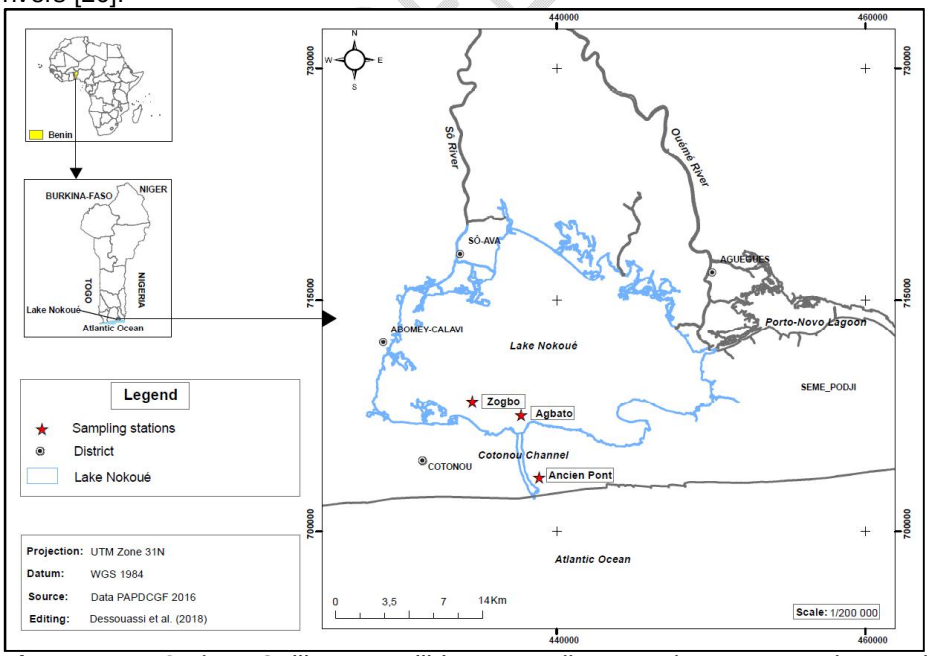

Figure 1: Crabs *Callinectespallidus* sampling stations on Lake Nokoué, southernBenin, West Africa.

2.2. Data collection

The crab *Callinectespallidus* 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 (*Callinectespallidus*) used in this study are from monthly collections from Lake Nokoué from March 2018 to July 2018 from small scale fisheries. They are collected from the Ancien Pont, Agbato and Zogbo sampling stations where the species is most abundant (**Figure 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.

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2.3. Description of the external morphology and macroscopic view of the genitalia of *Callinectespallidus*

At the laboratory, each specimen is identified according to the taxonomic key [4] and its sex determined. To maintain the internal organs for observation, the sample were subjected to a one-hour heat shock at 18°C using a Samsung refrigerator [9].

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Grids for describing each stage of sexual maturity of the crab *Callinectespallidus* were developed based on the [14] scale for *Portunuspelagicus*(Portunidae) (**Table 1**). They were informed by binocular loupe observations of 394 specimens including 206 females and 188 males.

Table 1: Sexualmaturityscale for *Portunuspelagicus*

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

Source: [14]

2.4. Microscopic study of gonads

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.

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Fresh crabs sample previously submitted to heat shock are dissected.

The histological study of the ovaries and testes of *Callinectespallidus*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. The preparation of the cassettes consists of cutting the gonads into small pieces and placing them on cassettes; the choice of fixative was made on 10% buffered formalin because it is less expensive, easy to prepare, and stable. Moreover, this fixative penetrates well the tissues. The duration of fixation was 3 days on average. The circulation consisted in making the pieces of tissue stay in a series of liquids, in order to confer them a rigidity favorable to the thin section; it was carried out in three phases: dehydration, clearing and impregnation. The fabrics after impregnation are coated with kerosene for the manufacture of blocks. The melted kerosene is poured into the molds that serve as supports for the blocks that will be formed. The block-mold assembly is cooled in a refrigerator. The kerosene blocks containing the embedded parts are cut into 5µm thick slices on a

rotary microtome of the brand AO Scientific Instruments 820. The slices are spread on slides on a hot plate set at 45°C. To do this, clean and engraved slides are covered with water and glycerine albumin. These slides are then placed in an oven at 40°C for one hour after drying; finally they are left to cool for at least 15 minutes. The staining is done with hemathein-eosin; it allows to highlight the nucleus, the cytoplasm and the collagen. Hematein is a basic dye that binds to nuclear structures while eosin is an acidic dye with an affinity for the cytoplasm and collagen fibers.
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

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.

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Table 2: Stages of sexual development and macroscopic description in female *Callinectespallidus*

Stages	Description	Size of specimens (carapace width in cm)	Externalanatomy (macroscopicview)	Internalanatomy (macroscopicview)
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

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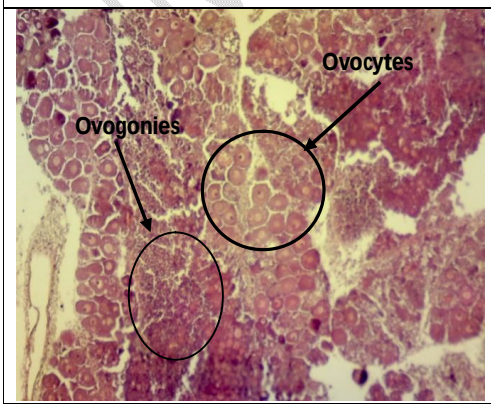
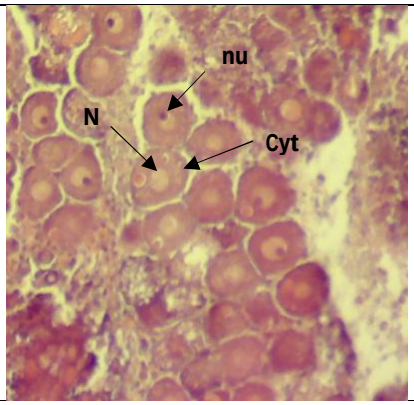
Table 3: Sexual development stages and macroscopic description in male *Callinectespallidus.*

Stages	Description	Size of specimens (Length inter spine)	Externalanatomy;macroscopicview	Internalanatomy;macroscopicview
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	Abdomen detachable 2nd segment of telson soft	Testicles visible and thin; genital canals visible and developed whitish color
IV		6.38 – 10.73 cm	Abdomen detachable 2nd segment of telson soft	visible and developed testes; developed genital canals anterior whitish; posterior translucent

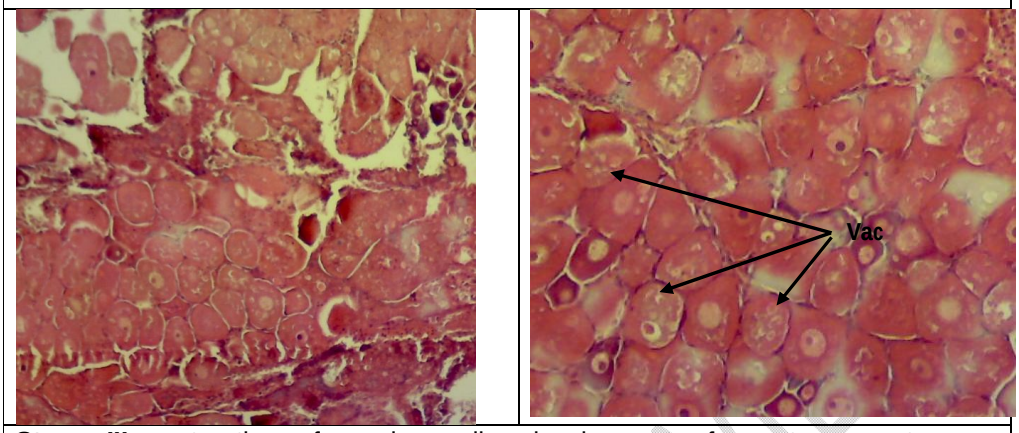
Microscopic study

The histological study of the ovaries and testes of *Callinectespallidus*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, and6. The curves of the variation of oocyte size by stage of maturity are presented in Figure 2. The ANOVA test showed a significant difference between the different stages of oocyte development ($p<0.05$) (Table 3).

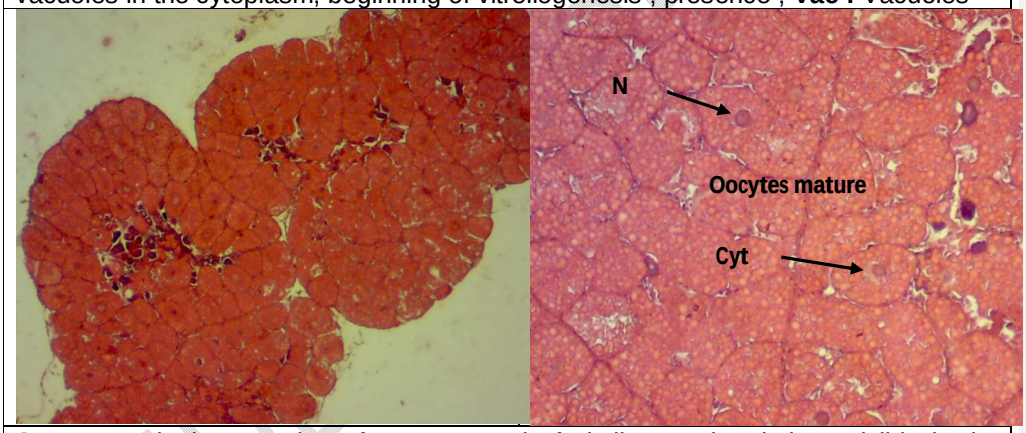
Table 4: View of histological sections of female gonads of the crab *Callinectespallidus*

Ovogenesis in the crab *Callinectespallidus*	
Stage I invisible ovary	
	

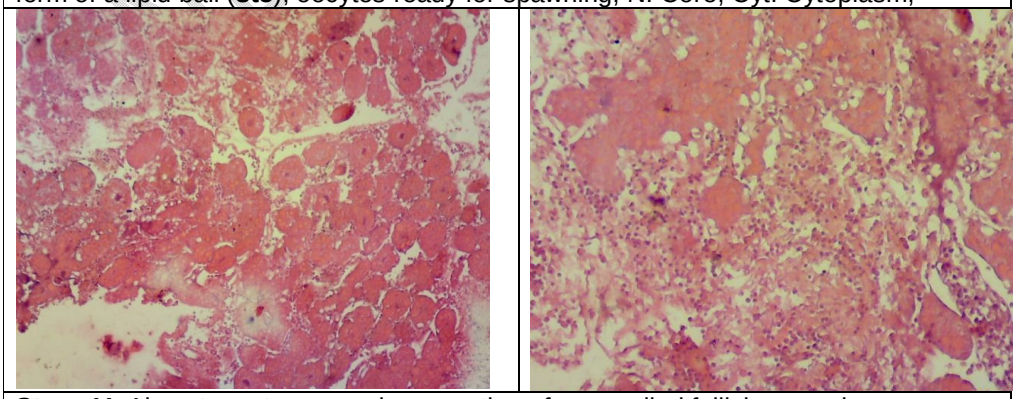
Stage II: Development of ovarian cells (ovogonia) oocytes at different stages of development (**st1**, **st2**); **N:** nucleus; **nu:** nucleolus; **Cyt:** Cytoplasm



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

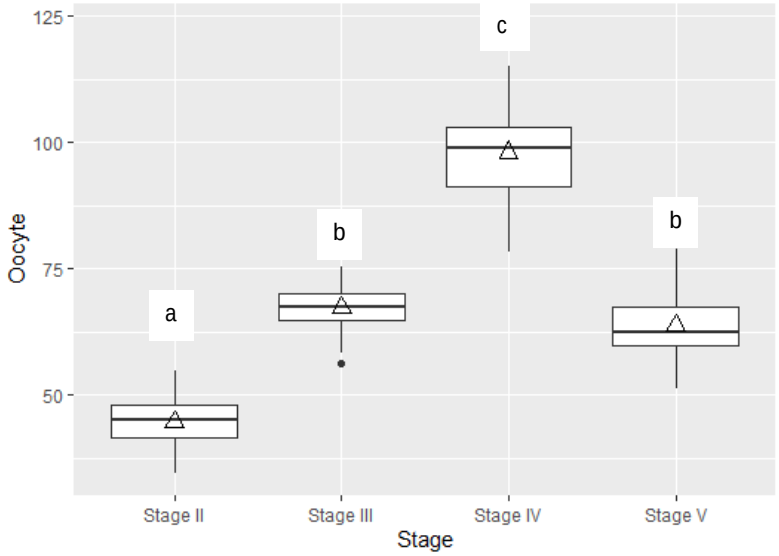
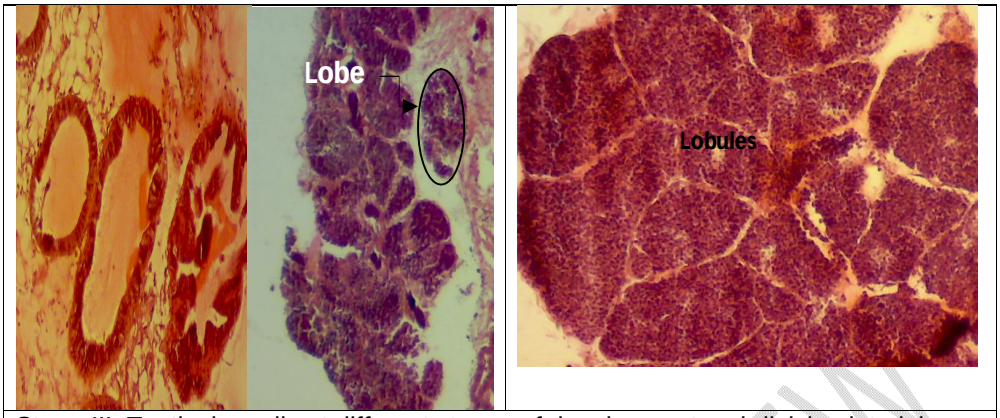


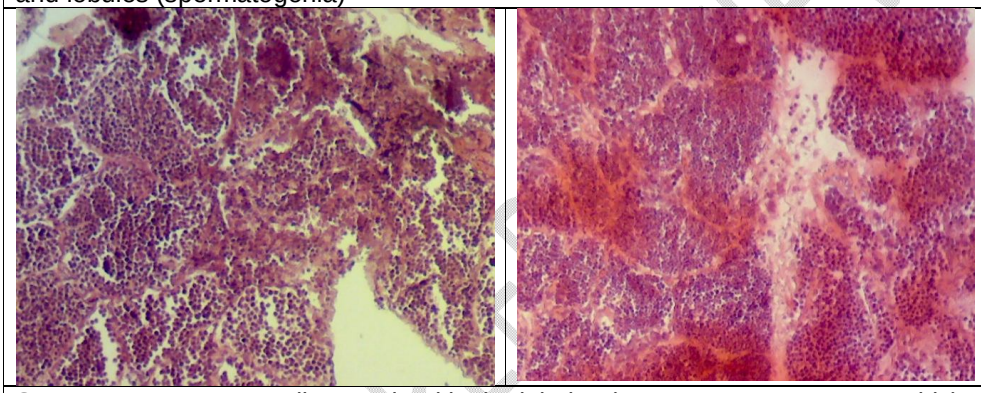
Figure 2: *C. pallidus* female Oocyte size variation by stage

Table 6: View of histological sections of male gonads in the crab ***~~Callinectespallidus~~Callinectes pallidus***

Spermatogenesis in the crab *~~Callinectespallidus~~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

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.

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 [22].

Females

In females, a total of five (05) stages of gonad development were observed. The same results were reported by [23, 24] on *Portunuspelagicus*. on the other hand, [9] described eight (8) stages of development in *Callinectesamnicola*. 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, 24]. 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 *Callinectesamnocola* and *Portunuspelagicus*

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respectively by [10, 24]. 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 *Callinectesamnicola* and *Macrobrachiumvollenhovenii*[10, 25], in *Macrobrachiumolfersi*[26].

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 [25]. 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 [27]. 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 [27].

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 [24, 25] 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 [23, 24]. 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 *Callinectesamnicola* and *Cardisomaarmatum*[9, 25], *Portunuspelagicus*[23, 24].

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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[23]. 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 *Callinectesamnicola* which is a key element of differentiation. At the microscopic level, there is no great difference in spermatogenesis.

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CONCLUSION

Growth in size in *Callinectespallidus* 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 spermathecaealready 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.

REFERENCES

1. Williams AB. The Swimming crabs of the genus *Callinectes* (Décapoda :Portunidae),Fisheries bulletin. 1974 ; 72 (3).

2. Lhomme F. Exploitable crustaceans. In Environment and Aquatic Resources of Ivory Coast Volume II-The Lagoon Environments. ORSTOM (Editions): Paris. 1994; 229-238.

3. Monod T. Hippidea and West African Brachyura. Memoir of the French Institute of Black Africa, N°45, IFAN: Dakar.1956.

4. Manning RB, Holthuis LB. West African Brachyuran Crabs (Crustacea: Decapoda). Smithsonian Contributions to Zoology.1981; 306: 379 p.

5. Murai T, Degbey J-B, Hounkpè C. Atlas of fish and shellfish in Benin. Fresh and brackish waters. MAEP/DP:Cotonou.2003.

6. AdandedjanD. Diversity and determinism of benthic macroinvertebrate populations in two lagoons in southern Benin: the Porto-Novo Lagoon and the Coastal Lagoon. Doctoral thesis, University of Abomey-Calavi, Abomey-Calavi. 2012; 239.

7. Aka NAD, Allabi ACE, Dreyfuss G, Kinde-Gazard D, Tawo L, Rondelaud D, Bouteille B, Avode G, Anagonou SY, Gninafon M, Massougbodji A, Dumas M. Epidemiological observations on the first case of human paragonimiasis and potential intermediate hosts of Paragonimus sp. in Benin.Bull Soc PatholExot. 1999; 92:191-194.

8. Dessouassi CE, Lédéroun D, Chikou A, Lalèyè PA. Diversity and characteristics of lagoon crabs in southern Benin, West Africa. Africa Science.2018; 14(6): 366-387.

9. d'Almeida MA, Sankare Y, Koua HK. Microscopic study of oogenesis in female Callinectesamnicola, de Rochebrune, 1883 (DecapodaPortunidae). J, Rech, Sci, Univ, Lomé (Togo), Series A). 2006; 8 (2): 9 - 18.

10. d'Almeida MA, Sankaré Y, Koua HK. Scale of sexual maturity and differentiation of the reproductive organs of the female crab callinectesamnicola (from rochebrune, 1883; decapodaportunidae), lagoon waters of Ivory Coast. J, Rech, Sci, Univ, Lomé (Togo) Series A.2010; 12(1): 27-44.

11. Costa TM, Neigreiros-Fransozo MLN. The reproductive cycle of Callinectes danae Smith, 1869 (Decapoda, Portunidae) in the Ubatuba region, Brazil. Crustaceana. 1998; 71 (6): 615–627

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12. Arimoro FO, Idoro B. Ecological studies and biology of Callinectes amnicola (Family: Portunidae) in the Lower Reaches of Warri River, Delta State, Nigeria. World Journal of Zoology.2007; 2 (2): 57-66.

13. doNascimento FA, Zara FJ. Development of the male reproductive system in Callinectes ornatus, Ordway, 1863 (Brachyra: Portunidea). Nauplius. 2013; 21:2, 161-177

14. Zairion YW, Mennofatria B, Achmad F. Reproductive Biology of the Blue Swimming Crab Portunus pelagicus (Brachyura: Portunidae) in East Lampung Waters, Indonesia: Fecundity and Reproductive Potential. Tropical Life Sciences Research. 2015;26(1): 67–85.

15. Soundarapandia P, Varadharajan DA. Reproductive biology of the commercially important Portunid crab, Portunus sanguinolentus (herbest). Journal of Marine science. Res. Dev. 2013; 3:2.

16. de Andrade LS, da Costa RC, Antinio LC, Israel FF, Gustavo SS, Adilson F. Reproductive and population traits of the swinning crab Archelousspirimanus (Crustacea: Decapoda) in an upwelling region in southeastern Brazil. Nauplis. 2017; 25-th 004

17. Sukumaran KK, Neelakantan B. Relatve growth and sexual maturity in the marine crabs, Portunus (Portunus) sanguinolentus (Herbst) and Portunus (Portunus) pelagicus. 1996.

18. Dessouassi CE., Gangbe L., Agadjihouede H, Montchowui E., LaleyeAP.. Reproduction of the main species of swimming crabs exploited in the lagoons of southern Benin. International Journal of Innovation and Scientific Research, 2019 40(2): 287-305.

19. LalèyèPA.. Comparative ecology of two species of Chrisichthys, siluriform fish (Claroteidae) from the lagoon complex of Lake Nokoué-Porto-Novo lagoon in Benin. Doctoral thesis in Sciences, University of Liège (Belgium), 1995 152 p. + Appendices.

20. Gnohossou P, Lalèyè P, Atachi P, Moreau J, DautaC.. Influence of salinity on the spatial distribution of invertebrates in Lake Nokoué. 3rd International Conference of the Pan African Fisheries Society, Cotonou (Benin), 10-14 Nov. 2003. PICARTS (Ed.)- Benin. (Proceedings in press in Studies in Afrotropical zoology, 2003 2005-Belgium)

21. DessouassiCE.. Diversity and exploitation of crabs in the lagoon complexes of southern Benin. Dissertation submitted for obtaining the degree of Doctor of Agricultural Sciences. Option: Development and Management of Natural Resources. University of Abomey-Calavi, 2020. 293p

22. Stewart J, Kennelly SJ, Hoegh-Guldberg O. Size at sexual maturity and the reproductive biology of two species of scyllarid lobster from New South Wales and Victoria, Australia. Crustaceana. 1997; 70: 344-367.

23. Efrizal E, Arshad AB, Kamarudin MS, Saad CR, Amin SMN. Some Aspects of Reproductive Biology of Blue Swimming Crab (Portunus pelagicus (Linnaeus, 1758)) Under Laboratory Conditions. J. Fish. Aquat. Science. 2015; 10:77–91. https://doi.org/10.3923/jfas.2015.77.91

24. Liu Z, Wu X, Wang W, Yan B, Cheng Y. Size distribution and monthly variation of ovarian development for the female blue swimmer crab, Portunus pelagicus