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Ovarian development and spawning of *Macrobrachium amazonicum* (Crustacea, Decapoda)

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ABSTRACT. *Macrobrachium amazonicum* (Heller, 1862) is the most important species for aquaculture native to South America. This study evaluates the phenotypic plasticity of females of *Macrobrachium amazonicum* with respect to the gonadal development, and determines the spawning type. Our study consisted of macro and microscopic analysis of the reproductive tract. Ovaries exhibited the following reproductive cells in developmental sequence: oogonia (OO) - mean diameter and standard deviation of 25.4 ± 6.5 µm; previtellogenic oocyte (PVO) - 61.7 ± 10.7 µm, vitellogenic oocyte (VO) - 113.9 ± 24.5 µm; and mature oocyte (MO) - 308.7 ± 56.3 µm. Ovaries increased in volume due to vitellogenesis and changing of basophilic to acidophilic composition. Follicular cells, atresic oocytes and postovulatory follicles were also analyzed. By combining macro and microscopic analysis, the ovaries of *M. amazonicum* were classified into six stages: Immature, In maturation, Mature, Spawned in maturation, Spawned and Resting. The ovarian development of *M. amazonicum* followed a standard pattern even among different populations. Considering our results and the evidence in literature, we conclude that spawning for this species is parceled or multiple, that is, synchronous in more than two groups (clutches of oocytes). Such observations provide basis for further studies addressing sustainable management strategies for species conservation and contribute to elucidate the biology of their specimens.

KEYWORDS. Reproductive biology, maturation, clutches of oocytes, histology, Amazon River prawn.

RESUMO. Desenvolvimento ovariano e desova de *Macrobrachium amazonicum* (Crustacea, Decapoda). Das espécies nativas de camarões da América do Sul, *Macrobrachium amazonicum* (Heller, 1862) é a mais importante para fins de aquicultura, possuindo elevado valor comercial. Devido à sua importância, este estudo objetivou avaliar a plasticidade fenotípica de fêmeas da espécie em relação ao desenvolvimento gonadal, e determinar o tipo de desova. Para tal, foi realizada análise macro e microscópica do aparelho reprodutor. Observaram-se ovários apresentando as seguintes células reprodutivas em sequência de desenvolvimento: oogônias (OO) com diâmetro médio e desvio padrão de 25,4 ± 6,5 µm, oócito pré-vitelogênico (OPV) com 61,7 ± 10,7 µm, oócito vitelogênico (OV) com 113,9 ± 24,5 µm, e oócito maturo (OM) com 308,7 ± 56,3 µm, havendo um aumento do volume devido ao processo de vitelogênese e uma alteração de composição basófila para acidófila. Além destas células, observaram-se células foliculares, oócitos atrésicos e folículos pós-ovulatórios. Unindo análise macro e microscópica, os ovários de *M. amazonicum* puderam ser classificados em seis estágios: Imaturo, Em maturação, Maturo, Desovado em maturação, Desovado e Repouso. Verificou-se que o desenvolvimento ovariano de *M. amazonicum* segue um padrão para as diferentes populações. Diante dos resultados encontrados e evidências na literatura, constata-se que a desova para esta espécie é do tipo parcelada ou múltipla, ou seja, sincrônica em mais de dois grupos (lotes de oócitos). Estas observações oferecem uma base para estudos que contribuirão para a criação de medidas de manejo sustentáveis para a manutenção da espécie na natureza, bem como contribuem para elucidar a biologia de seus indivíduos.

PALAVRAS-CHAVE. Biologia reprodutiva, maturação, lotes de oócitos, histologia, camarão da Amazônia.

Macrobrachium species are especially important due to its scientific and economic relevance (Fransozo *et al.*, 2004). *Macrobrachium amazonicum* (Heller, 1862) is distributed throughout the eastern coast of South America, from Venezuela to Argentina, and from Ecuador to Peru on the west coast (Melo, 2003). In Brazil, this species occurs throughout the country across different states (see Coelho & Ramos-Porto, 1985; Bialetzki *et al.*, 1997; Melo, 2003; Magalhães *et al.*, 2005; Cruz *et al.*, 2010; Pileggi *et al.*, 2013). *Macrobrachium amazonicum* is present in the basins of the Amazonas, São Francisco, Northern and Northeast coast, Paraná and Paraguay (Coelho & Ramos-Porto, 1985), and also in the Orinoco basin (Holthuis, 1952). The species inhabits both inland and coastal areas (Coelho & Ramos-Porto, 1985).

Studies on females of *M. amazonicum* collected from natural environments in the Northern region of Brazil have been focused on the oocyte development (Chaves & Magalhães, 1993), gonadal development, morphometry and the ultrastructure of germ cells (Ferreira *et al.*, 2012). In the Southeast region, K. Ribeiro (unpubl. data) studied the gonadal development and the ultrastructure of germ cells of females from prawn nurseries. On the other hand, studies on these features aiming at better understanding the species, considering its high probability of significant phenotypic adaptation and plasticity across its wide distribution range are scarce in the Northeast and other regions of Brazil.

Macrobrachium amazonicum has the greatest potential for cultivation among the native prawn species of South

America, which can be maximized by the proper management of sites where the species naturally occurs and is harvested by commercial fishing (Moraes-Riodades & Valenti, 2004; Silva *et al.*, 2007; Marques & Moraes-Valenti, 2012). Therefore, data on the reproductive biology of *M. amazonicum* is a helpful tool to aquaculture and to establish strategies for biodiversity conservation (Mossolin & Bueno, 2002). The pilot project of *M. amazonicum* cultivation started in 1996. In spite of the giant river prawn *Macrobrachium rosenbergii* (De Man, 1879) being intensively cultivated, Brazilian consumers have shown preference for the first species due to its marked taste and firmer muscle texture (Moraes-Riodades & Valenti, 2001). The way females release mature oocytes within a reproductive period defines the type of spawn (Vazzoler, 1996). Knowledge of the type of spawning can help with conservation strategies. Moreover, defining the gonadal developmental stages of individuals through histological analysis provides foundation for reproductive studies besides contributing to the general understanding of the species biology. Thus, this study evaluates the phenotypic plasticity of *M. amazonicum* females regarding their gonadal development through macro and microscopic analysis and determines the spawning type based on a population inhabiting the semiarid Northeastern Brazil.

MATERIAL AND METHODS

Study area and sampling. The specimens were collected from a reservoir known as Cachoeira II, located in the city of Serra Talhada, in the state of Pernambuco, Brazil (07°57'00"S, 38°20'00"W). Collections were carried out upstream the reservoir from November 2014 to January 2015 and from September 2016 to August 2017 by using repurposed PET bottle-handmade traps with opening of 3 cm diameter and specimens were baited with cassava flour. The traps were set at dusk (4:00 p.m.) among aquatic macrophytes at the research site and then retrieved the following morning (5:00 a.m.). We transported the live captured specimens inside the traps with water from the collection site to the laboratory.

Laboratory procedures and data analysis. The specimens were cryoanesthetized at -10 °C for approximately 20 min, quantified and identified for species by using the identification method proposed by Melo (2003). The presence or absence of the male appendix located in the endopodite of the second pair of pleopods was the feature to determine the sex of species (Lima *et al.*, 2014). We measured the total length (TL, distance between the distal margin of the face and the distal margin of the telson) using a digital caliper with 0.01mm resolution for obtaining somatic information.

For the analysis of gonadal development, we removed the exoskeleton from the cephalothorax of the specimens with the help of tweezers, exposing the organs of this compartment. We examined the ovaries under a stereomicroscope and we classified the developmental stages macroscopically by observing the dimension, color and organ texture, following a method adapted from K. Ribeiro (unpubl. data). We noted the color of the ovaries according to the RGB scale (Robinson *et al.*, 1995).

Ovaries at different stages of development were removed and deposited in Davidson's solution for a period of 24 to 48h. These ovaries were submitted to the standard histological routine: embedded in paraffin, cut at 5 µm and stained with Harris Hematoxylin-Aqueous Eosin (adapted from Junqueira & Junqueira, 1983) and the Gomori Trichrome (adapted from Tolosa *et al.*, 2003) for tissue differentiation.

We measured reproductive cells using the UTHSCSA ImageTool 3.0 program (Wilcox *et al.*, 1996) to ascertain both total and nucleus diameters. For each cell stage, a total of thirty random cells were selected within the different gonadal stages, which was considered satisfactory. Images for this analysis were obtained via digital photo camera attached to the optical microscope, and we manually adjusted the zoom according to the settings that best captured images of the components. We performed initial tests to evaluate whether these variables had statistical significant differences ($p < 0.05$), distribution errors (Kolmogorov-Smirnov test) and homoscedasticity of the variances (Goldfeld-Quandt test). Due to the non-normality of errors in the total diameters of cells, data were log-transformed to parametric statistics. The cell diameters (comparison between the total diameters, and also between the diameters of the nucleus) were compared by ANOVA, followed by the Tukey test for means comparison. Analyses were performed using the softwares Microsoft Excel 2010 and BioEstat 5.3 (Ayres *et al.*, 2007). For the spawning type analysis, we collected three gonads for each maturational stage and measured all cells for total diameter, subsequently, the relative frequencies by diameter class were analyzed by using the classification method proposed by Marza (1938) and cited by Vazzoler (1996), in which the type of spawning can be synchronous in one group, synchronous in two groups, synchronous in more than two groups or asynchronous.

RESULTS

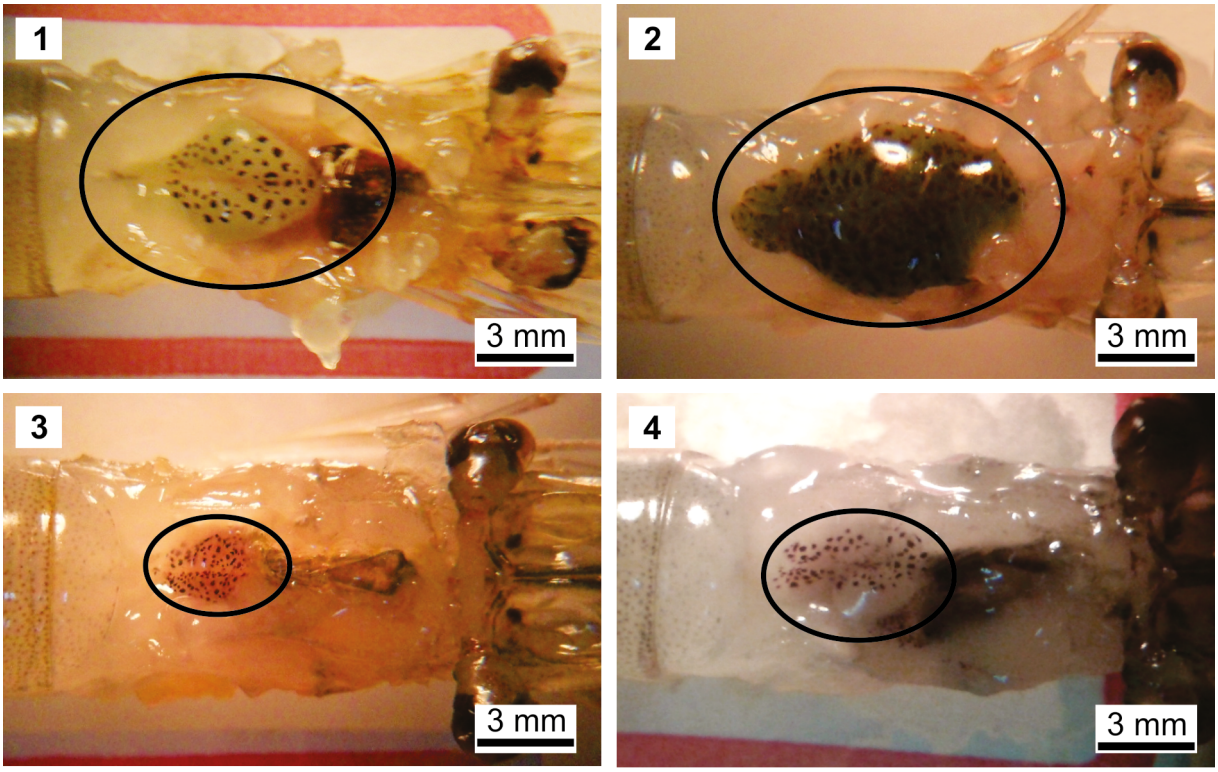
Reproductive system. The female reproductive system of *M. amazonicum* was located in the dorsal part of the cephalothorax. Bilaterally symmetrical, the system exhibited a pair of ovaries with a median commissure and a pair of oviducts; channels of thin and translucent tubes extending to the gonopores located at the base of the coxa of the third pair of pereiopods. The ovaries were located above the hepatopancreas between the heart and the stomach. Two channels emerge from the ovaries with same structure of the oviducts. One channel connected the hepatopancreas to the middle region of the two organs, and the second channel connected the posterior portion of the gonad to the abdominal compartment. The heart was always above the posterior portion of the gonad. Ovaries were initially small and above the posterior portion of the hepatopancreas; as the ovaries was maturing, their volume was increased, reaching up to the first abdominal segment

and the rostral beginning, partially covering the stomach. Visually, they were initially smooth and gradually having a granular appearance during maturation due to the presence of oocytes, which enabled us to visually categorize them with the naked eye. Throughout development, there was increasing amount of dark chromatophores along the dorsal surface of the organ.

Macroscopic gonadal development. A total of 533 individuals were macroscopically analyzed. The TL of these individuals ranged from 14.5 to 80.6 mm, with mean and standard deviation of 52.4 ± 9.6 mm. The gonadal development divides into six distinct macro stages, as follows: 1st) Not developed: ovaries were translucent and small organs. The mean TL of the individuals at this stage was 38.2 ± 6.1 mm. 2nd) Initial development: ovaries exhibited a whitish color (RGB: 249-255-249) and higher volume compared to the previous stage. Mean TL of individuals: 51.0 ± 8.8 mm. 3rd) In advanced development: ovaries exhibited a greenish coloration (RGB: 134-242-115) and even larger volume. Mean TL of individuals: 57.6 ± 8.0 mm. 4th) Developed: ovaries exhibited intense green coloration (RGB: 40-100-32) and the largest volume the organ can reach. Mean TL of individuals: 56.0 ± 7.3 mm. 5th) Spawned in maturation in ovigerous females: ovaries exhibited the same characteristics of the 3rd or 4th stage; however, individuals showed eggs attached to the pleopods in the incubation chamber. Mean TL of individuals: 56.5 ± 8.3 mm. 6th) Spawned: ovaries exhibited characteristics similar to the 1st or 2nd stage, but had flaccid appearance and large accumulation of chromatophores along the dorsal surface of the organ. Ovaries similar to the 2nd stage may be entering a new cycle. Mean TL of individuals: 52.1 ± 5.7 mm. In the 5th stage, there were also ovigerous females exhibiting ovaries with characteristics of the 6th stage. Figs 1-4 show the stages of ovarian development of *M. amazonicum*.

Ovaries histology. A total of 190 ovaries were microscopically analyzed. Gonad selection was randomly within the different stages of development and this quantity was considered satisfactory. The development of reproductive cells changes from the initial stages occurring in the center of the organ (germinative zone) and as cells mature, they advance to the periphery of the organ (maturation zone). Follicular cells were present inside the ovaries and assisted in vitellogenesis. Initially, reproductive cells had a basophilic character and later become acidophilic in the maturation zone. Nuclei, nucleoli and follicular cells retained their basophilic structures and varied the degree of color intensity.

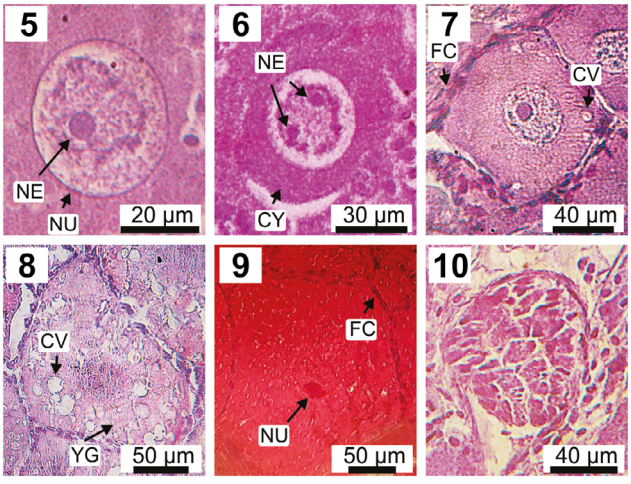
Oocyte development showed four cellular stages: 1st) Oogonia - OO: spherical cells with reduced cytoplasm. The nucleus exhibited the dispersed chromatin. Some cells had tiny nucleoli, with only a slightly larger nucleolus when these cells were more developed. The oogonia were the smallest cells in the developmental process of the oocyte. The mean total cell diameter was 25.4 ± 6.5 µm and the diameter of the nucleus was 15.0 ± 4.0 µm, representing 58.8% of the cell size. 2nd) Previtellogenic oocytes - PVO: larger than OO and characterized by a polyhedral to oval shape with clear and agranular cytoplasm. One nucleolus generally oriented at the



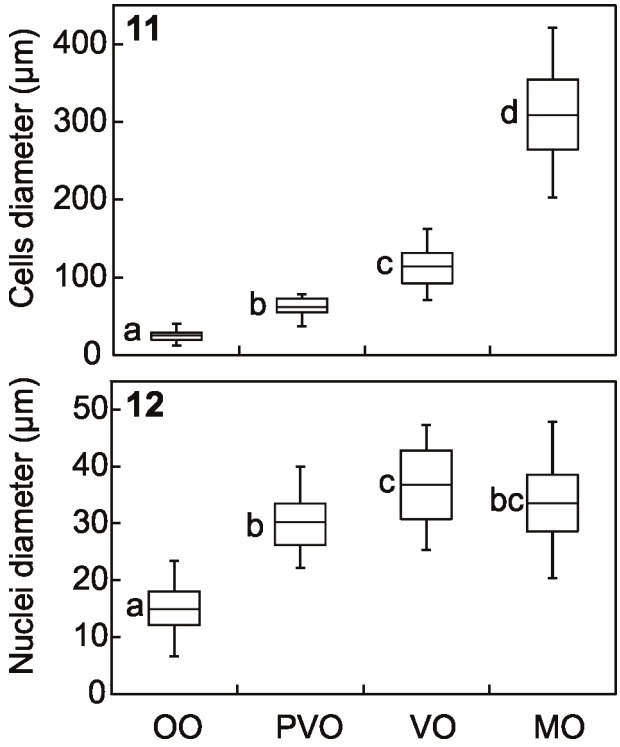
Figs 1-4. Stages of ovarian development of *Macrobrachium amazonicum* (Heller, 1862) captured in the Cachoeira II reservoir between November 2014 and August 2017. The organ is demarcated by an ellipse: 1, In advanced development; 2, Developed; 3, Spawned similar to an undeveloped organ; 4, Spawned similar to an early developing organ.

periphery of the nucleus. We also observed a second nucleolus in some cells. The granulations within the nucleus remained evident. The mean total cell diameter was 61.7 ± 10.7 µm and the diameter of the nucleus was 30.2 ± 4.8 µm (48.9% of the cell). These two cell types comprised the germinative zone, being basophilic cells with spherical-shaped follicular cells dispersed in their adjacency. In the maturation stage, we found: 3rd) Vitellogenic oocytes - VO: oval or irregular shaped and marked by higher acidophilic levels, more abundant cytoplasm than in the previous stage and the presence of yolk granules and cytoplasmic vesicles. At this stage we found oval-shaped follicular cells beginning to surround the oocytes. The nucleus showed a nucleolus. During maturation, these cells could be found in both early and advanced stages. The mean total cell diameter was 113.9 ± 24.5 µm and the diameter of the nucleus was 36.8 ± 6.7 µm (32.3% of the cell). 4th) Mature oocytes - MO: exhibited strongly acidophilic cytoplasm, full of yolk granules and cytoplasmic vesicles. The follicular cells displayed themselves as a cord-like coating encircling these oocytes, they displayed themselves as pavement cells. MOs were the largest cells, with the nucleus having a small size reduction and being rarely sectioned. The mean total cell diameter was 308.7 ± 56.3 µm and the diameter of the nucleus was 33.5 ± 6.8 µm (10.9% of the cell). The increase in cell size at each stage is due to the process of vitellogenesis, increasing the amount of yolk granules and cytoplasmic vesicles. Atresic oocytes - AO were also observed, which were irregular and disorganized structures, resulting from the MO retention, which were then reabsorbed by the organism. Figs 5-10 show the stages of development of the reproductive cells. After the release of MO to be fertilized, the ovaries exhibited postovulatory follicles characterized by cell coatings that previously surrounded the oocytes. Total cell diameters showed significant difference [$F(3, 116) = 694.11$, $p<0.01$], as well as the diameters of the nuclei [$F(3, 116) = 86.15$, $p<0.01$]. The nuclei diameters increased from OO to VOs and reduced when mature. The Tukey test showed no differences between PVO and MO and between VO and MO of the nuclei diameters (Figs 11, 12).

Stages of maturation. The presence of reproductive cells in the ovaries of *M. amazonicum* combined with macroscopic analysis allowed us to classify this organ into six stages of maturation, as follows: 1st) Immature: gonad was entirely composed of the germinative zone. Analogous to 1st macro stage. 2nd) In maturation: gonad composed of early and advanced phase PVO and VO. Initially analogous to 2nd macro stage and later with the 3rd macro stage as maturation advances. 3rd) Mature: gonad predominantly composed of MO. Analogous to the 4th macro stage. 4th) Spawned in maturation: gonad composed of PVO and VO and/or MO, exhibiting postovulatory follicles. Analogous to the 5th macro stage. 5th) Spawned: gonad composed of postovulatory follicles, AO and OO. Analogous to the 6th macro stage. 6th) Resting: gonad full of dispersed follicular cells and OO. Also analogous to the 6th macro stage. Regardless of the stage, ovaries exhibited OO and PVO, indicating the presence of a reserve cellular stock. Figs 13-18 show the maturation stages of the ovaries of *M. amazonicum*.

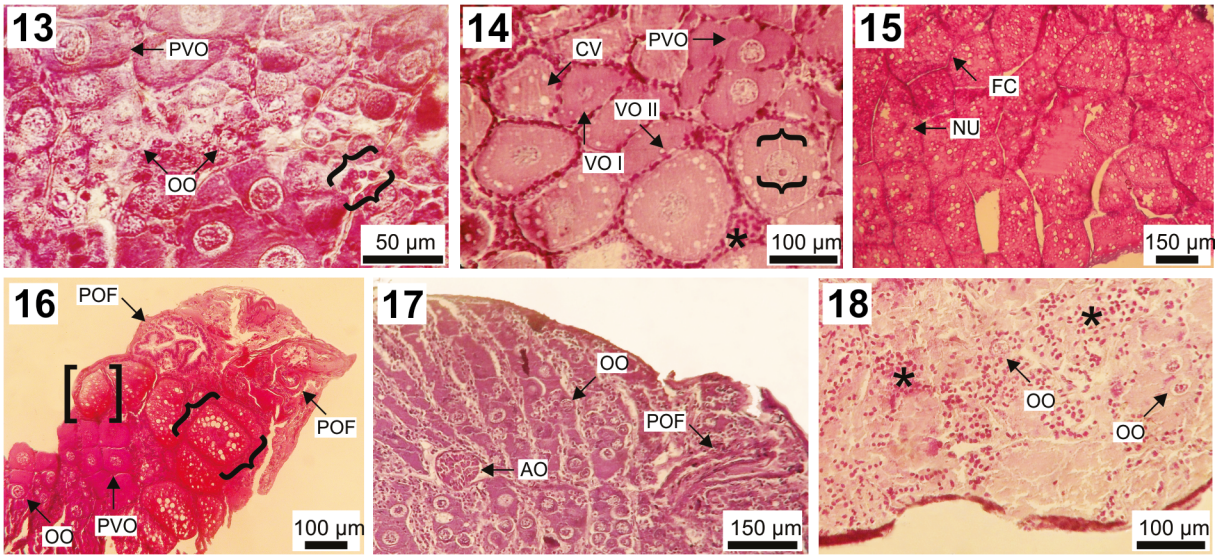


Figs 5-10. Photomicrographs of germ cells of the ovary of *Macrobrachium amazonicum* (Heller, 1862) at different stages of development, captured in the Cachoeira II reservoir between November 2014 and August 2017: 5, Oogonia: nucleus (NU) and nucleolus (NE). The granulations within the nucleus characterize the chromatin; 6, Previtellogenic oocyte: cytoplasm (CY) and two nucleoli (NE) within the nucleus; 7, Initial vitellogenic oocyte: formation of cytoplasmic vesicle (CV) and spherical follicular cells (FC) around the cell; 8, Advanced vitellogenic oocyte: cytoplasmic vesicles (CV) and yolk granules (YG); 9, Mature oocyte: follicular cells (FC) forming a cord-like coating around the oocyte, with center nucleus (NU); 10, Atretic oocyte: showing cellular disorganization. Color: Hematoxylin-Eosin (5, 6, 9, 10); Gomori trichrome (7, 8).

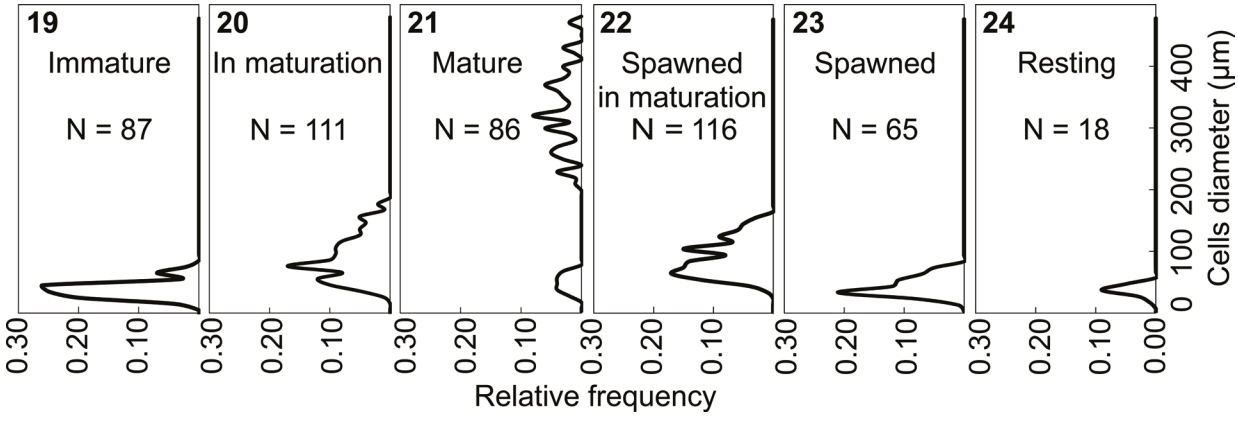


Figs 11, 12. Graphical representation of reproductive cells of *Macrobrachium amazonicum* (Heller, 1862) females captured in the Cachoeira II reservoir between November 2014 and August 2017: 11, cells diameter; 12, nuclei diameter, showing maximum observation (upper bar), minimum observation (lower bar), third quartile (top of the box), first quartile (bottom of box) and average (inner bar) (OO, oogonia; PVO, previtellogenic oocyte; VO, vitellogenic oocyte; MO, mature oocyte). Horizontally, different letters indicate statistical difference ($p<0.01$), verified by the Tukey test.

Spawning. According to the frequency of distribution of reproductive cell diameter (Figs 19-24), the cells of the reserve stock belong to classes between 0 and 100 µm. Ovaries "in maturation" (Fig. 20) showed high levels of oocytes at different stages of development (modal groups) and ovaries in the "spawned in maturation" (Fig. 22) indicate clutches of oocytes remained after elimination of a clutch of mature oocytes. These findings suggest spawning is parceled or multiple and therefore, synchronous in more than two groups. This means individuals spawn two or more times during each reproductive period.



Figs 13-18. Ovary sections of *Macrobrachium amazonicum* (Heller, 1862) at different maturation stages, captured in the Cachoeira II reservoir between November 2014 and August 2017: 13, Immature: oogonia (OO), previtellogenic oocyte (PVO) and follicular cells (between { }); 14, In maturation: previtellogenic oocyte (PVO), initial vitellogenic oocyte (VO I), advanced vitellogenic oocyte (VO II), and cytoplasmic vesicle formation (CV). Clear nucleus and nucleolus (between { }), and spherical follicular cell nodes (star), also around the oocytes; 15, Mature: follicular cells forming a lining around the oocytes, where they are barely perceptible due to their level of proximity and flattening (FC), and small nucleus (NU). The crystallized appearance is due to the accumulation of yolk granules, and the several small uncolored spots characterize the cytoplasmic vesicles; 16, Spawned in maturation: oogonia displaying their nuclei and nucleoli (OO), previtellogenic oocyte (PVO), initial vitellogenic oocyte (between [ ]), advanced vitellogenic oocyte (between { }) and postovulatory follicles (POF); 17, Spawned: oogonia (OO), atretic oocyte (AO) and postovulatory follicle (POF); 18, Resting: oogonia (OO) and follicular cell nodes (star). Color: Gomori trichrome (13, 14, 16, 18); Hematoxylin-Eosin (15, 17).



Figs 19-24. Distribution of reproductive cell diameters of *Macrobachium amazonicum* (Heller, 1862) females captured in the Cachoeira II reservoir, between November 2014 and August 2017, in different stages of gonadal maturation: 19, Immature; 20, In maturation; 21, Mature; 22, Spawned in maturation; 23, Spawned; 24, Resting. The peaks of elevations in the curves indicate modal groups (N, number of oocytes measured).

DISCUSSION

The arrangement of the ovaries of *M. amazonicum* in this study corroborates the findings of FERREIRA *et al.* (2012) in females collected from a natural habitat in the Northeastern region of the state of Pará (Brazil), who described ovaries with bilateral symmetry exhibiting a commissure and those observed by K. Ribeiro (unpubl. data) in females from prawn nurseries in the state of São Paulo (Brazil), who reported the paired ovaries located above the hepatopancreas. In addition, the gonadal development in the cephalothoracic cavity towards the stomach and the start of the abdomen, as well as changes in color - firstly from transparent to whitish and increasing tones of green in later stages are also findings

already reported by K. Ribeiro (unpubl. data). The stages of development of an organ can be described as perceptible changes occur. However, such phase separation is subjective according to the observer, which may lead to discrepancies. Thus, in spite of the disagreement about this classification (Tab. I) regarding the main components of the studies of these authors and the method of classification used, a consistent pattern is followed and the characteristics used in our study correspond to those studies.

The cell types found in the ovaries of *M. amazonicum* corroborate those found by K. Ribeiro (unpubl. data) and Ferreira *et al.* (2012), with the exception of atresic oocyte and postovulatory follicle not described in these previous studies. Similarly, our study corroborates the research of Chaves & Magalhães (1993) who examined the cells found in females from a lake in the state of Amazonas (Brazil), and also described the presence of postovulatory follicles indicating their occurrence across different populations of the species. As found in this study, Chaves & Magalhães (1993) and K. Ribeiro (unpubl. data) reported the stages of development of the reproductive cells, reporting the development from the center to the periphery of the gonad, confirming the uniformity of the reproductive features in the species.

In the germination zone, the characteristics observed in the OO corroborate those found in the existing literature: the scarce cytoplasm and presence of granulations in the nucleus (Chaves & Magalhães, 1993; K. Ribeiro, unpubl. data), a fact also observed in the OO illustration for *Macrobrachium olfersii* (Wiegmann, 1836) collected from a river in the state of São Paulo (Brazil) in the study by Mossolin & Bueno (2002). Moreover, K. Ribeiro (unpubl. data) also reported dispersed chromatin and the presence of a nucleolus. Such descriptions demonstrate consistent similarity of the reproductive process in multiple populations of *M. amazonicum* and between species of the genus, even across different environments. As for the PVOs, our findings corroborate those of K. Ribeiro (unpubl. data), who reported the existence of a second nucleolus, while Chaves & Magalhães (1993) mentioned the presence of only one nucleolus. This fact is explained by the process of histological cut of a cell that usually does not bisect all the organelles and, therefore, these may not be visualized even if present. Although Chaves & Magalhães (1993) and K. Ribeiro (unpubl. data) have reported more discrete granulations at this cellular stage, a marked presence of these granules could still be noticed in our study. This fact is related to the compacting of chromatin, which is more easily visible when dense.

In the maturation zone, K. Ribeiro (unpubl. data) reported VOs sometimes exhibit a second nucleolus, which was not found in our study. Chaves & Magalhães (1993) also described the presence of a single nucleolus at this cellular stage; however, the ultrastructural analysis performed by K. Ribeiro (unpubl. data) may have been better suited for examining characteristics of these cells and explaining the finding of this second organelle. As for MOs, as found in the present study, Chaves & Magalhães (1993) reported this phase with larger cells, intensely acidophilic cytoplasm and a rarely sectioned nucleus. According to the authors, the nucleus volume is smaller at this stage compared to the cell volume. These findings reaffirm the similarity of the characteristics of ovarian development across populations of *M. amazonicum*. Considering the similarity of results, whose differences are considered only methodological, this process is demonstrated to be standard for the species. However, the process speed may vary across different populations, since the gonadal maturation seems to be related to environmental factors (Collart, 1991). Therefore, metabolism can be influenced by these factors. Araujo & Valenti (2011) found greater larval development of *M. amazonicum* under high luminosity tested in laboratory. Also, according to Martins *et al.* (2006), the feeding of females is important condition for the reproductive process due to the yolk formation related to the individuals' diet.

Tab. I. Relation of correspondence between macroscopic stage classification of ovarian development of *Macrobrachium amazonicum* (Heller, 1862) by different authors in different locations (un, unknown; *, stage of reorganized ovaries in a new reproductive cycle, which passes by the 6th stage of the present study, and is therefore presented in this section).

Studies			
Present study	K. Ribeiro (unpubl. data)	Lucena-Frédou *et al.* (2010)	Ferreira *et al.* (2012)
Places			
Reservoir Pernambuco State, Brazil	Prawn nurseries São Paulo State, Brazil	Island Pará State, Brazil	Natural environment Pará State, Brazil
Stages			
1st (not developed)	1st	1st	1st
2nd and 3rd (in initial and advanced development)	2nd to the 4th	2nd	2nd
4th (developed)	5th	3rd	3rd
5th (spawned in maturation in ovigerous females)	un	4th	4th
6th (spawned)	un	5th	5th*

Porto & Santos (1996) found a discontinuous (reproduction) spawning period for the shrimp *Farfantepenaeus subtilis* (Pérez-Farfante, 1967) collected from an island in the state of Maranhão (Brazil), and two separate spawning events suggesting biannual total spawning. *Macrobrachium amazonicum* has continuous reproduction (see Collart, 1991; Bialetzki *et al.*, 1997; Lucena-Frédou *et al.*, 2010; Freire *et al.*, 2012; Bentes *et al.*, 2016; Taddei *et al.*, 2017) confirming the parceled spawning for this species. Sampaio *et al.* (2007), when examining *M. amazonicum* collected from a river in the state of Ceará (Brazil), not only found continuous reproduction but also ovigerous females with gonads in different developmental stages. This demonstrates a continuous process of ovary maturation even after spawning, which is a condition for parceled spawning and supports our results. Similar observations were made by Mossolin & Bueno (2002) for *M. olfersii*, concluding that spawning for this species must be parceled. Ferreira *et al.* (2012) found four cell types (OO, PVO and both early and advanced phase VO) in all stages of gonadal development (Tab. I) with the exception of the 3rd stage in which MO has predominated. This demonstrates the existence of more than two oocyte clutches and corroborates the finding of parceled spawning. Despite K. Ribeiro (unpubl. data) having described the existence of total spawning for *M. amazonicum*, such study has no evidence supporting this assertion. In view of all the evidence showed in this section, the parceled spawning is undeniable for this species. The reproductive mechanism of *M. amazonicum* with constant generation of individuals makes it a potential species for aquaculture and allows decreases its risk of extinction.

Vazzoler (1996) explains that the oocytes mature synchronously in each batch and, as more developed batches become mature, they are eliminated. Chaves & Magalhães (1993) found MOs located further from the center than all oocytes when present in the ovaries. As in the present study, K. Ribeiro (unpubl. data) and Ferreira *et al.* (2012) described mature ovaries as exhibiting MO and germinative zone cells. Mature ovaries of individuals with parceled spawning exhibit germination zone, VO and MO, when will perform the first spawning of a given reproductive cycle. The predominance of MO in the ovaries (Fig. 21) (cells from the class of 200 µm) with cells from the reserve stock indicates these MOs are part of the last clutch of that reproductive cycle.

In conclusion, the ovaries of *M. amazonicum* and their reproductive cells mature in stages indicated by gradually increasing volume and the presence of nutritive compounds, are favorable to gonadal development. The ovarian development is demonstrated to have a pattern for the species regardless the inhabiting region. The ovaries showed continuous maturation dynamic, in which even after spawning, the gonad retains developing cells, arranging themselves in anticipation of the next spawning event. The presence of more than two oocyte clutches in the ovaries of *M. amazonicum* indicates parceled spawning. In spite of the several studies on *M. amazonicum*, this is the first record on the spawning type of the species. We emphasize the method used for spawning type as a convenient procedure for this analysis. Our findings are important asset for understanding the *M. amazonicum* biology by providing basis for reproductive studies that will contribute to rational means of commercial exploitation of the species and to promote conservation efforts in its natural environment.

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