An Acad Bras Cienc (2022) 94(Suppl. 4): e20211294 DOI 10.1590/0001-3765202220211294
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Histological and Histochemical Dynamism of Oogenesis in the Cinnamon River Prawn *Macrobrachium acanthurus* (Caridea: Palaemonidae) Induced by Eyestalk Ablation

MARIA M. RODRIGUES, LAURA S. LÓPEZ GRECO, LUIS CARLOS F. DE ALMEIDA & GIOVANA BERTINI

Abstract: *Macrobrachium acanthurus* is a caridean prawn native to Brazil, and studying techniques to assist in its cultivation is important due to economic interest in it. Eyestalk ablation is commonly used to promote ovarian development and maturation of captive crustaceans, but it can have possible consequences on fertility and brood quality. Histological and histochemical dynamism of oogenesis was analyzed under control (non-ablated females) and unilateral eyestalk ablated females. Females with ovaries in the spent stage of gonadal development were divided into two treatments: unilaterally ablated and non-ablated. A second experiment with the same treatments was conducted using females with mature gonads. Histological and histochemical analyses of the ovaries indicated that the ablation did not affect oogenesis based on ovarian structure, oocyte size, histochemical properties, and atretic oocytes. Ovarian maturation was not accelerated by ablation, either. Survival, nuptial molting, and spawning were also unaffected. From an applied point of view, unilateral eyestalk ablation is not needed in *M. acanthurus* farming because it does not improve reproductive performance.

Key words: Maturation, morphology, ovary, vitellogenesis, X-organ sinus gland complex.

INTRODUCTION

Ovarian maturation is regulated by neurohormones synthesized and released by the X-organ sinus gland complex, which is located in the ocular peduncle in decapods (Pervaiz et al. 2011). The X-organ synthesizes gonadal inhibiting hormone, which is stored and distributed by the sinus gland. Additionally, methyl farnesoate, vertebrate steroidal hormones, and neuropeptides are involved in oocyte growth and vitellogenesis (Subramonian 2011, Li et al. 2017). Some methods to control ovarian maturation in shrimp have been studied, such as induction by hormone therapy, pheromones, and RNA interference (Alfaro-Montoya et al. 2019). Nonetheless, the best known and most commonly used technique for inducing ovarian maturation in shrimp is unilateral eyestalk ablation (Sainz-Hernández et al. 2008, Wen et al. 2015). This technique inhibits the gonadal inhibiting hormone and triggers vitellogenin synthesis, accelerating ovarian growth (Fingerman 1987, Tsukimura 2001). As a result, females become ovigerous earlier, increasing their spawning rate (Aktas & Kumlu 1999).

Research on eyestalk ablation in some *Macrobrachium* freshwater prawns has been increasing due to growing commercial exploitation. The following studies focused on the effects of ablation on growth and gonadal maturation of different species: *M. nobilii*

(Sindhukumari & Pandian 1987), *M. rosenbergii* (Santos & Pinheiro 2000, Okumura & Aida 2001, Revathi et al. 2013, Shailender et al. 2013), *M. lanchesteri* (Varalakshmi & Reddy 2010), *M. dayanum* (Pervaiz et al. 2011), and *M. lamarrei lamarrei* (Hussain et al. 2017). Meanwhile, the use of ablation to induce ovarian development in the Brazil-native prawn *M. acanthurus* remains relatively unknown (Cunha & Oshiro 2010, Rodrigues et al. 2021). This species shows great potential for freshwater farming (Kutty & Valenti 2010), and its geographic distribution ranges from North Carolina in the United States to Rio Grande do Sul in Brazil (Anger 2013, Pileggi et al. 2014). This species is heavily exploited by artisanal fishing for human consumption and for live bait in sport fishing (Bertini & Valenti 2010, Bertini et al. 2021).

Although the need to cultivate endemic species has been discussed since the early 2000s (Kutty et al. 2000), there is no progress on the cultivation of *M. acanthurus*. There have been studies on sperm extraction methods (Costa et al. 2016), semen storage (Costa et al. 2017), supply of inert diet (Rodrigues et al. 2017), and larval survival in different salinities and with different diets (Rodrigues et al. 2018), but there are still no studies on the influence of ablation on ovarian development. Because unilateral eyestalk ablation can accelerate gonadal maturation (Okumura & Aida 2001, Pervaiz et al. 2011, Hussain et al. 2017), it could be a useful tool for cultivating this native species. Hence, this study investigated the influence of the technique on the ovarian development of *M. acanthurus* under laboratory conditions, both in pre- and postspawning females.

MATERIALS AND METHODS

Collection and acclimation of broodstock

Adult females of *M. acanthurus* were collected using a sieve (0.5 m^2, 5 mm mesh size) from the Ribeira de Iguape River (24°64'87"S, 47°51'09"W) in São Paulo, Brazil, in January 2017. The prawns were placed in thermal boxes with water from the collection site and transported to the laboratory, where they were disinfected in formaldehyde (25 ppm) for 30 min (Maciel & Valenti 2014). They were then acclimated in 60 L black polyethylene boxes with freshwater and a constant-aeration water recirculation system for 24 h. To prevent stress, pieces of PVC pipe and artificial aquatic macrophytes were placed in the boxes as shelter. The photoperiod was the same as the field condition (natural photoperiod). Water temperature was kept at 29 ± 1 °C with a heater attached to a thermostat, as previously recommended for *Macrobrachium* species (Habashy & Hassan 2011).

Female selection and eyestalk ablation

Adult females with ovarian development in spent or mature stages and with carapace lengths ranging from 12 to 15 mm were selected, following Bertini et al. (2014). Carapace length was measured as the distance from the orbital sinus to the midpoint of the posterior margin of the carapace. Ovarian developmental stage was characterized macroscopically based on color and size relative to the carapace, following Carvalho & Pereira (1981).

Eyestalk ablation was adapted from the method described for marine shrimp by Primavera (1985). Prior to ablation, anesthetic lidocaine ointment was applied to the base of the eye peduncle. Then, an incision close to the base was made with scissors. The incision site was cauterized with heat using a soldering iron. A mixture of antibiotic ointments (nitrofurazone

and oxytetracycline-polymyxin B, 1:1 ratio) was administered at the cauterized sites. The ablation technique was performed quickly under a stereomicroscope to minimize stress.

Experimental design

Two experiments were designed to analyze the histological and histochemical dynamics of oogenesis as well as the occurrence of nuptial molting and spawning.

In the first experiment, females with spent-stage ovaries (post-release of mature oocytes) (Figures 1a, 1b) were separated into two treatments: unilaterally eyestalk ablated and non-ablated. In the second experiment, females with mature ovaries (Figures 1c, 1d) were submitted to the same two treatments. For each experiment, 112 females (56 eyestalk ablated and 56 non-ablated) were randomly selected, and individually placed them in 1 L freshwater containers with constant aeration and a piece of PVC pipe to provide shelter. Temperature (29 ± 1°C) and photoperiod (12 h light/12 h dark) were controlled in a BOD incubator (Eletrolab® EL 202). Pieces of squid and extruded diet for adult freshwater prawn (30% crude protein, Nutriave®) were offered as food *ad libitum* in the morning. The total volume of water in the containers was renewed daily after feeding. Observations were made regarding molting, spawning oocyte abortion, feeding, gonadal stages, and death.

Changes and abnormalities in ovarian development were monitored on days 1, 3, 5, and 7. On each monitoring day, 14 females out of the 56 females in each treatment group were randomly selected. At the end of each monitoring day, all survivors from the 14 individuals assigned to that day from both treatments were anaesthetized by thermal shock and ovaries were dissected. The seven-day length was determined based on Fukuda et al. (2013), as this period is sufficient for ovarian development from spent to mature.

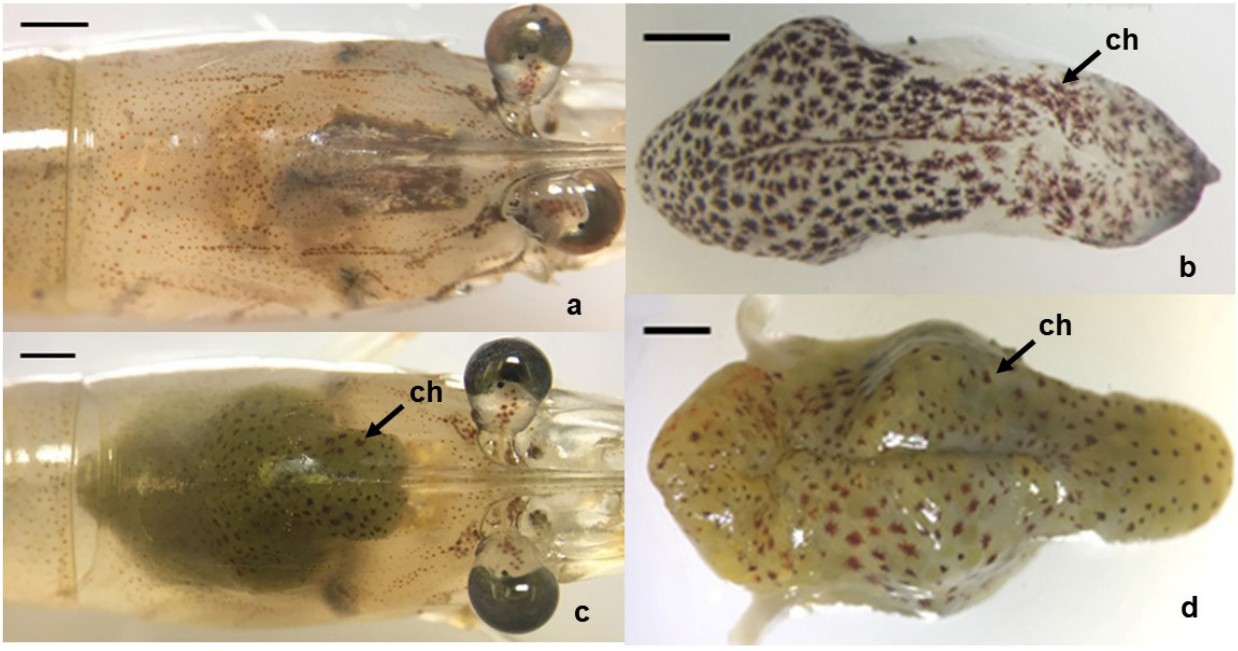

Figure 1. ***Macrobrachium acanthurus.*** **a) Dorsal view of the female cephalothorax with the ovaries in spent developmental stage (after spawning); b) Spent ovaries dissected; c) Dorsal view of female cephalothorax with ovaries in the mature stage; d) Mature ovaries dissected. Scale bar = 2 mm. Chromatophores - ch.**

Microscopic analysis

For histological and histochemical analyses, seven females/treatment/day/experiment were used. Gonads were fixed in 10% buffered formaldehyde (NaOH, pH 7.4) for 24 h and transferred to 70% alcohol. They were then dehydrated using an ascending sequence of 70 to 95% ethyl alcohol and embedded in methacrylate (Leica Historesin®, Nussloch/Heidelberg, Baden-Württemberg, Germany). The polymerized blocks were sectioned into 5 µm thick slices using a Leica RM® microtome. Slides were stained with hematoxylin and eosin or submitted to histochemical techniques following Junqueira & Junqueira (1983). Slides were stained with bromophenol blue to detect proteins, periodic acid–Schiff-stained (PAS) to detect neutral polysaccharides, and von Kossa (safranin) counterstained to detect calcium. After staining and drying, slides were mounted in Canada balsam. Digitization and image analysis were performed using a Leica DM2500® photomicroscope.

Classification of the oocyte development stage followed Meeratana & Sobhon (2007), Ravi et al. (2013), Sharifian et al. (2015), Souza et al. (2017). The largest cell diameter of 20 oocytes per treatment was measured in the following developmental stages: oogonia (Og), previtellogenic (Pv), in vitellogenesis (Iv), and mature (Mt). Atretic oocytes (At) were identified but not measured, as they lost cell boundaries. Measurements were performed using Leica LAS EZ 3.0.0 software.

For each developmental stage, the area occupied by oocytes in relation to the total area of the ovaries was measured using Image Pro-Plus® software (Trial version). Measurement was made by dividing the oocytes into three groups: oogonia and previtellogenic (Og + Pv), in vitellogenesis and mature (Iv + Mt), and atretic (At). The percentage of the area of each group was estimated for the ovaries of the females from different treatments.

The number of oocytes per developmental stage per sample was determined visually using a representative region on the slide containing at least 50 germ cells. The oocytes per stage present in the sampled regions were individually counted, and these numbers were converted to a percentage for each treatment. For this calculation, oocyte stages were grouped as oogonia (Og), previtellogenic (Pv), and in vitellogenesis and mature (Iv + Mt).

Statistical analysis

Data exploration was performed following Zuur et al. (2010). The effect of eyestalk ablation on the diameter (µm), area (%), and number (%) of oocytes at different developmental stages was analyzed by fitting linear mixed-effects models (LME), with treatment (ablated versus non-ablated) and day (1, 3, 5, or 7) as fixed factors, and the individual as a random factor.

A generalized linear model (GLM) with binomial distribution and log link function to analyze the effects of eyestalk ablation on survival, nuptial molting, and spawning. This model was chosen according to the Akaike information criterion (Akaike 1974) because it presented the lowest value within the tested models.

Statistical analysis was performed using the nlme package (Pinheiro et al. 2017) in R software (R Core Team 2016). Statistical significance was set at $\alpha \leq 0.05$. When a hypothesis of equality between the results was rejected, Bonferroni correction was applied.

RESULTS

Ovarian development

Macroscopic and histological analyses revealed no difference between the ovaries of ablated

and non-ablated *M. acanthurus* females. The proliferation zone was in the middle of the ovaries, in which the germinal layer of the oogonia lies (Figures 2a-e). Follicular cells surround clusters of oogonia and oocytes in early stages; as the oocytes grow, follicular cells start surrounding each oocyte, originating the ovarian follicles (Figures 2b, 2c, 2d, 2f). As ovarian maturation advances, the follicles move to the periphery of the ovary, and the lobes expand into the hemocellic space.

The spent stage of the ovaries is observed shortly after spawning. At this time, oocytes are dispersed and the ovary is flaccid, presenting a great amount of connective tissue. The ovary is mostly composed of previtellogenic and early vitellogenesis oocytes. The germ zone is easily located, with large groups of oogonia. Around the oocytes, follicular cells with an ovoid shape can be observed, and there are no oocytes in the final vitellogenesis (Figures 2a, 2b).

The mature ovary is mainly composed of vitellogenic oocytes. The germinal epithelium is reduced, the follicular cells are flattened, and the germinal zone is hardly ever found in histological sections. When present, the germinal zone is compressed by mature oocytes. No oocyte was found in early vitellogenesis (Figures 2e, 2f).

Ovaries presented oocytes in different numbers and at different maturation stages, varying according to gonadal development (Figures 2a-f). Oocyte classification was based on size, location, cytoplasmic appearance, nucleus visualization, chromatin pattern, and accumulation of yolk granules and lipid droplets in the cytoplasm. Therefore, oocytes were categorized as follows:

1) Oogonia (Og)—primary germ cells in the middle of the ovaries with a mean diameter of 15.94 ± 2.51 µm. They presented a homogeneous and barely evident cytoplasm, a basophilic spherical nucleus that occupied most of the cell, chromatin in varying degrees of condensation, and no evident nucleoli (Figures 2b-d).
2) Previtellogenic oocytes (Pv)—cells larger than the oogonia with a round shape and mean diameter of 70.89 ± 32.39 µm. These were found externally from the oogonia within the ovary. The cytoplasm was basophilic and larger than that of the oogonia. The nucleus was central, less basophilic than the cytoplasm, and with more dispersed chromatin. These oocytes were surrounded by round follicular cells (Figures 2b, 2c).
3) Oocytes in vitellogenesis (Iv)—larger than previtellogenic oocytes, with a mean diameter of 205.84 ± 54.15 µm. These oocytes were distinguished by the presence of yolk granules and lipid droplets mainly distributed in the periphery of the acidophilic cytoplasm. The basophil nucleus and nucleoli were still visible. There were greater numbers of flattened follicular cells around each oocyte (Figures 2c, 2d).
4) Mature oocytes (Mt)—cells in the maximum stage of maturation, having hexahedral shape and average diameters of 510.52 ± 90.75 µm. The cytoplasm of these oocytes was intensely acidophilic. Many lipid and yolk vesicles were distributed throughout the cytoplasm. Visualization of the nucleus was usually difficult due to a great accumulation of dense vitellogenic granules in the cytoplasm. At this stage, the oocytes were surrounded by elongated and flattened follicular cells (Figures 2e, 2f).

In addition to these developmental stages, an atretic stage was also observed. Oocytes at this stage were reabsorbed, losing their plasmatic membranes (Figure 3a). The oocytes were acidophilic, with indistinguishable nuclei and intensely vacuolated cytoplasm with many

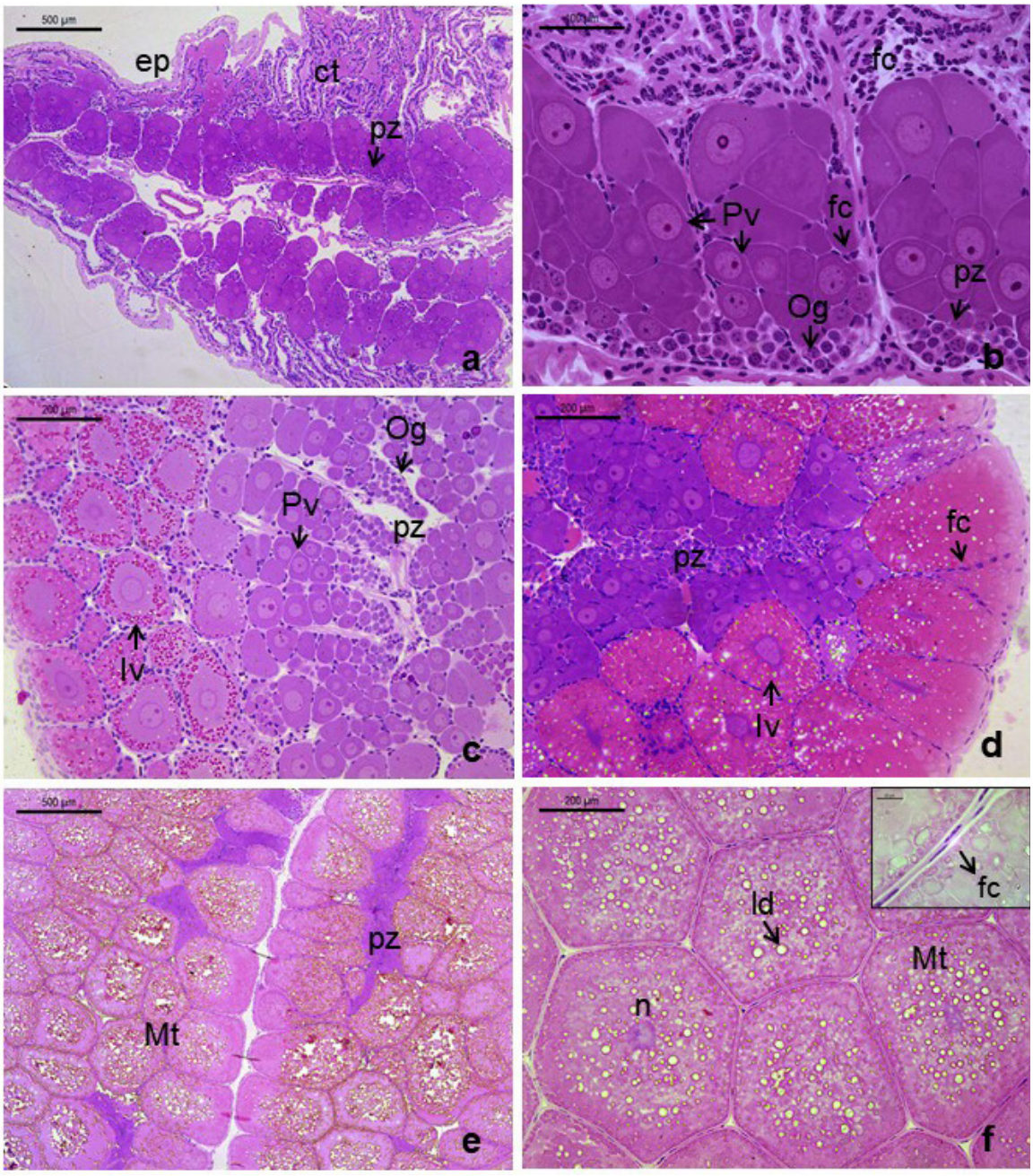

Figure 2. Histological sections of the ovaries of *Macrobrachium acanthurus*. a) Spent ovaries, note the disorganized ovary with ovarian epithelial tissue with much connective tissue and most oocytes in early vitellogenesis; b) Detail of spent oocyte; c) In maturation ovaries, evolution of the oocytes from inside out, in the middle region is the proliferative zone (oogonia and previtellogenic oocytes); there are oocytes in vitellogenesis concentrating yolk granules in the peripheral region; d) Detail of a in maturation gonad showing follicular cells involving oocytes in vitellogenesis that have large accumulation of yolk granules and a still visible nucleus; e) Mature ovaries, showing a small zone of proliferation and predominance of oocytes in final vitellogenesis (Developed); f) Mature ovaries, note that the mature oocytes are hexaedra-shaped with small nucleus and many lipid droplets, and are surrounded by flat-shaped follicular cells. Staining: Hematoxylin-eosin; Connective tissue - ct; epithelial tissue - ep; follicular cells - fc; oocyte in vitellogenesis - lv; mature oocytes- Mt; lipid droplet- ld; nucleus - n; oogonia - Og; previtellogenic oocyte - Pv; proliferative zone - pz. 4x (a, e), 10x (c, d, f), 20x (b), 100x (insert).

lipid droplets. Sometimes, there were areas of homogeneous cytoplasm on the periphery. Follicular cells, although present, did not continuously surround these oocytes. The atretic stage was mainly found in females with spent or mature gonads. In spent females, atretic oocytes were found in lesser quantities due to possible reabsorption of some mature oocytes. In mature females, atretic oocytes were seen in individuals that had undergone ecdysis without releasing oocytes. These oocytes often occupied almost the entire volume of the ovaries.

By tracking the development of oocytes from newly laid eggs, no morphological difference was observed between the ovaries of ablated and non-ablated females (Figures 3a-3g). The ovaries presented rapid reorganization. Approximately five days after spawning, they presented many oocytes in vitellogenesis (Iv) with yolk granules and lipid droplets (Figure

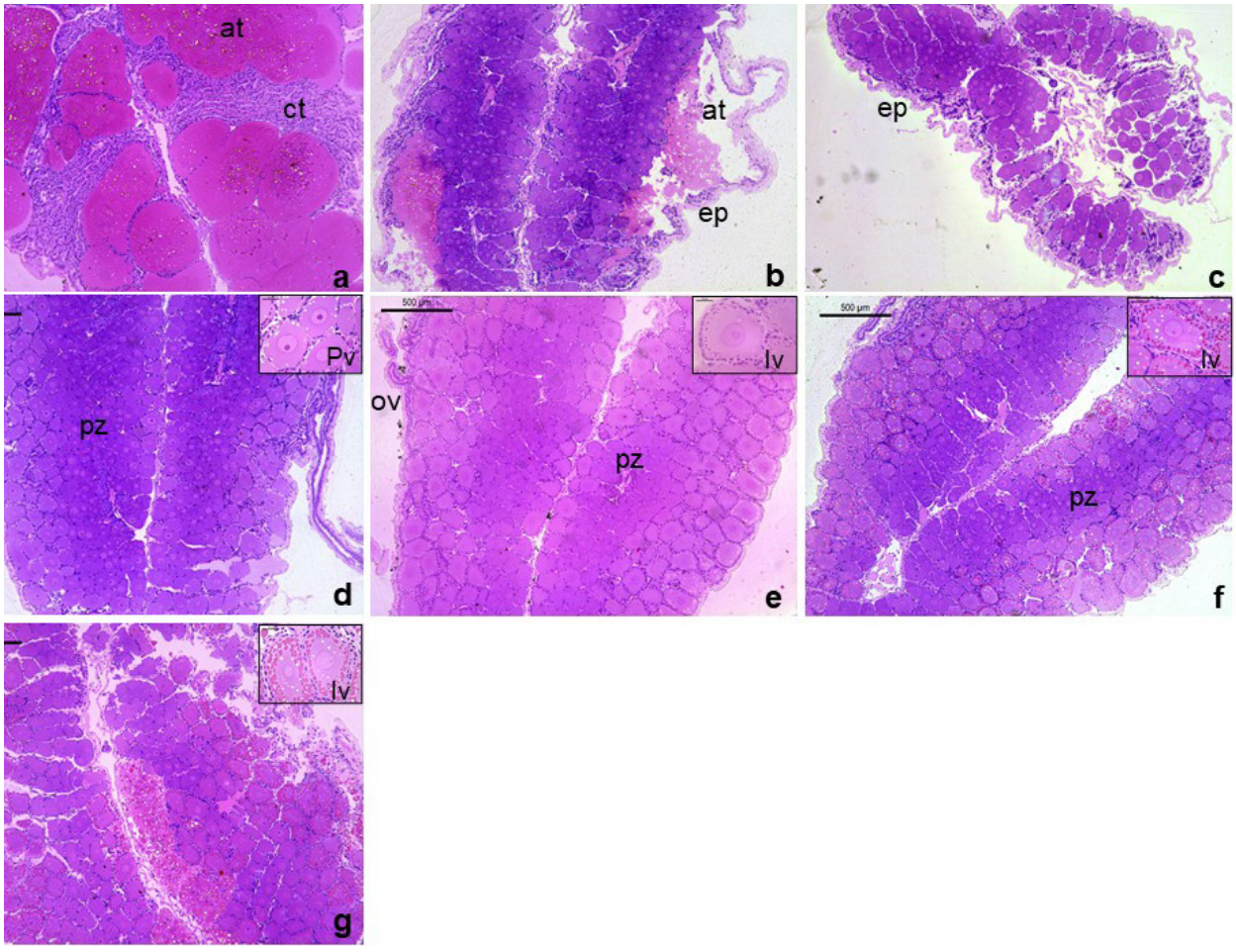

Figure 3. Oocyte development of *Macrobrachium acanthurus* from oocyte deposition. a) Day of spawning, observe the atretic oocytes and much connective tissue; b) 1 day after spawning, still disorganized "loose" ovary with few atretic oocytes and most oocytes in early vitellogenesis; c) 2 days after spawning, atretic oocytes are no longer present; d) 3 days after spawning, well-organized ovary, beginning lipid storage in the periphery of previtellogenic oocytes; e) 4 days after spawning, oocytes in vitellogenesis initiating a fine peripheral yolk granulation; f) 5 days after spawning, many oocytes in vitellogenesis; g) 6 days after spawning, oocytes in vitellogenesis presenting larger amount of yolk granules. Staining: Hematoxylin-eosin (H-E). Atretic oocytes – at; connective tissue - ct; epithelial tissue - ep; oocyte in vitellogenesis - Iv; oviduct- ov; previtellogenic oocyte - Pv; proliferative zone - pz. 4x (a-g), 40x (inserts).

3f). Regardless of ablation, females who started the experiment with spent ovaries (Experiment 1) presented mature ovaries after seven days. Females that started the experiment with mature ovaries (Experiment 2) spawned (oocytes were unfertilized), aborted, and started new maturation cycles within the seven-day experimental duration. After this period, most of them presented ovaries in maturation.

Average oocyte diameter at each stage did not significantly differ among ablated and non-ablated females in either experiment throughout the time ($p > 0.05$) (Figure 4).

Regarding the percentage of the area and the number of oocytes, LME results showed no significant difference for the interaction treatment*day*oocyte stage between Experiments 1 and 2 ($p > 0.05$). On the other hand, significant differences in oocyte stage were present when some factors were analyzed separately ($p < 0.05$) (Tables I and II). In Experiment 1, a significant difference in the percentage of the area was found for the day*oocyte stage interaction ($p < 0.05$) (Table I), indicating a decrease of the area occupied by Og + Pv oocytes and an increase in the Iv + Mt oocytes over time (Figure 5). In Experiment 2, was found a difference in the day*oocyte stage interaction for both the area and number of oocytes ($p < 0.05$) (Tables I and II). In this case, it was evident that the area of Iv + Mt oocytes decreased over time, and the area occupied by At ones increased. The oocyte number per developmental stage followed the same trend: the number of Iv + Mt oocytes decreased, while the number of Pv oocytes increased (Figure 6).

Histochemical analysis revealed no difference between treatments of either experiment. All stages of oocyte development appear to have had the same distribution

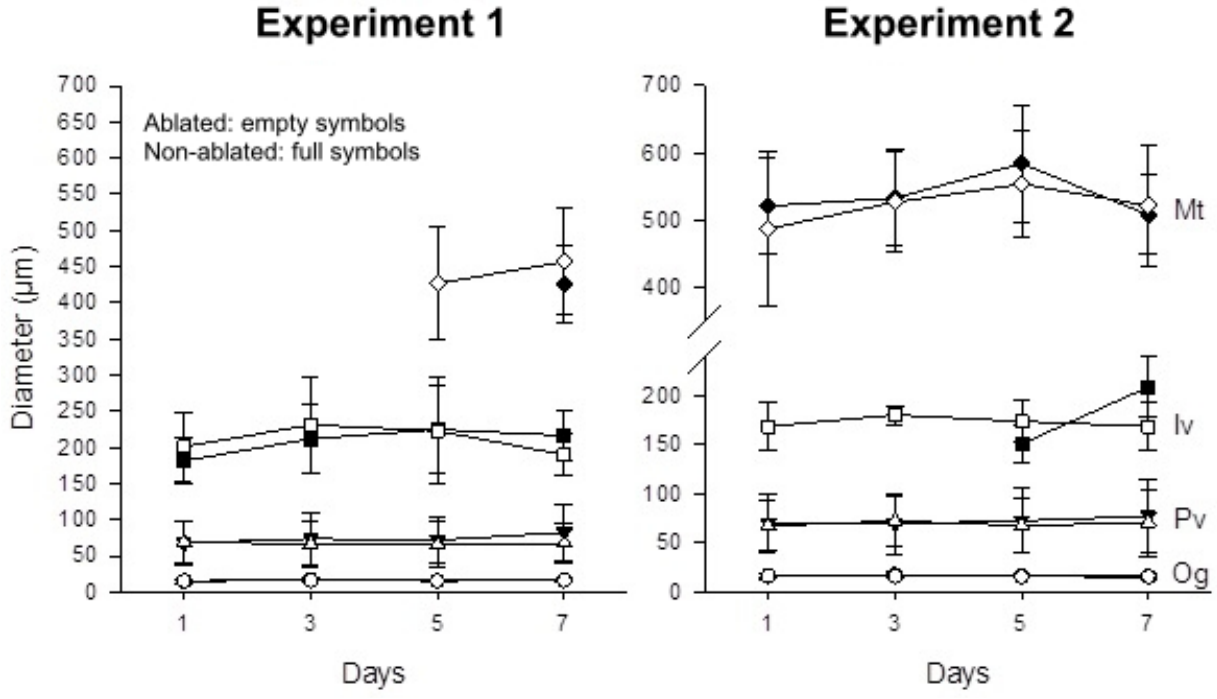

Figure 4. Mean diameter of oocyte per developmental stage in the ovaries of *Macrobrachium acanthurus* females in two experiments after 1, 3, 5, or 7 days. Oocyte in vitellogenesis - Iv; mature oocyte - Mt; oogonia - Og; previtellogenic oocyte - Pv. There was no significant difference ($p > 0.05$) between ablated and non-ablated females.

of proteins, polysaccharides, and calcium regardless of ablation.

Iv oocytes showed more pronounced staining in relation to the Og and Pv oocytes in the detection of proteins and neutral polysaccharides (Figure 7a-d; Table III). The peripheral region of the cytoplasm of Pv oocytes was less reactive to PAS when compared to the central region, which was moderately positive (Figure 7b). In respect to protein, Pv oocyte cytoplasm staining was more uniform, although more reactive fine granulation was observed in the cytoplasm near the nucleus. An intense reaction in the periphery of the cytoplasm among Iv oocytes was observed (Figure 7d). The cytoplasm of follicular cells did not react to polysaccharide staining, and protein detection staining resulted in poor staining. The Og group had no reaction to neutral polysaccharides, and showed poor protein positivity (Table III). Regarding calcium detection, Mt oocytes presented weaker staining compared to Pv oocytes. Iv oocytes presented moderate positive staining. The nuclei of the Og and follicular cells showed a considerable amount of calcium (Figures 7e, 7f).

Survival, nuptial molt, and spawning

Survival rates between ablated and non-ablated females were similar in both experiments, with no significant difference between treatments ($p > 0.05$). In both treatments of Experiment 1, the overall survival rate was 100% for most analyzed days, except for ablated females analyzed on Day 5, which presented a 92.9% survival rate (Table IV). In Experiment 2, the survival rates for both treatments did not differ among monitoring days. Nonetheless, there was greater mortality among females analyzed on day 7, especially after nuptial molting. Overall survival was 85.7% in non-ablated and 57.1% in ablated individuals (Table IV).

Only females from Experiment 2 underwent nuptial molting followed by spawning, as they had started the experiment with mature gonads. However, there was no statistical difference in the number of ecdysis and spawning events between non-ablated and ablated females ($p > 0.05$) (Table IV). No female analyzed on Day

Table I. Area (%) occupied by the different developmental oocytes stages in the ovaries of *Macrobrachium acanthurus* both in ablated/non-ablated females assigned on different monitoring days (1, 3, 5 and 7) of experiments 1 and 2 (F- and *p*-values).

Variables	Experiment 1		Experiment 2	
	F	***p***	***F***	***p***
Treatment	.000	1.000	.000	1.000
Day	.000	1.000	.000	1.000
Oocyte stage	39.172	.000	.000	.000
Treatment*day	.000	1.000	.000	1.000
Treatment*oocyte stage	.570	.567	.000	.403
Day*oocyte stage	3.707	.002	.000	.000
Treatment*day*oocyte stage	.529	.786	.000	.074

Table II. Number of oocytes (%) per developmental stage in the ovaries of *Macrobrachium acanthurus* both in ablated/non-ablated females assigned on different monitoring days (1, 3, 5 and 7) of experiments 1 and 2 (F- and *p*-values).

Variables	Experiment 1		Experiment 2	
	F	*p*	*F*	*p*
Treatment	.000	1.000	.000	1.000
Day	.000	1.000	.000	1.000
Oocyte stage	29.868	.000	45.314	.000
Treatment * Day	.000	1.000	.000	1.000
Treatment * oocyte stage	3.721	.027	.550	.578
Day * oocyte stage	1.805	.102	6.703	.000
Treatment * day * oocyte stage	.440	.851	.598	.732

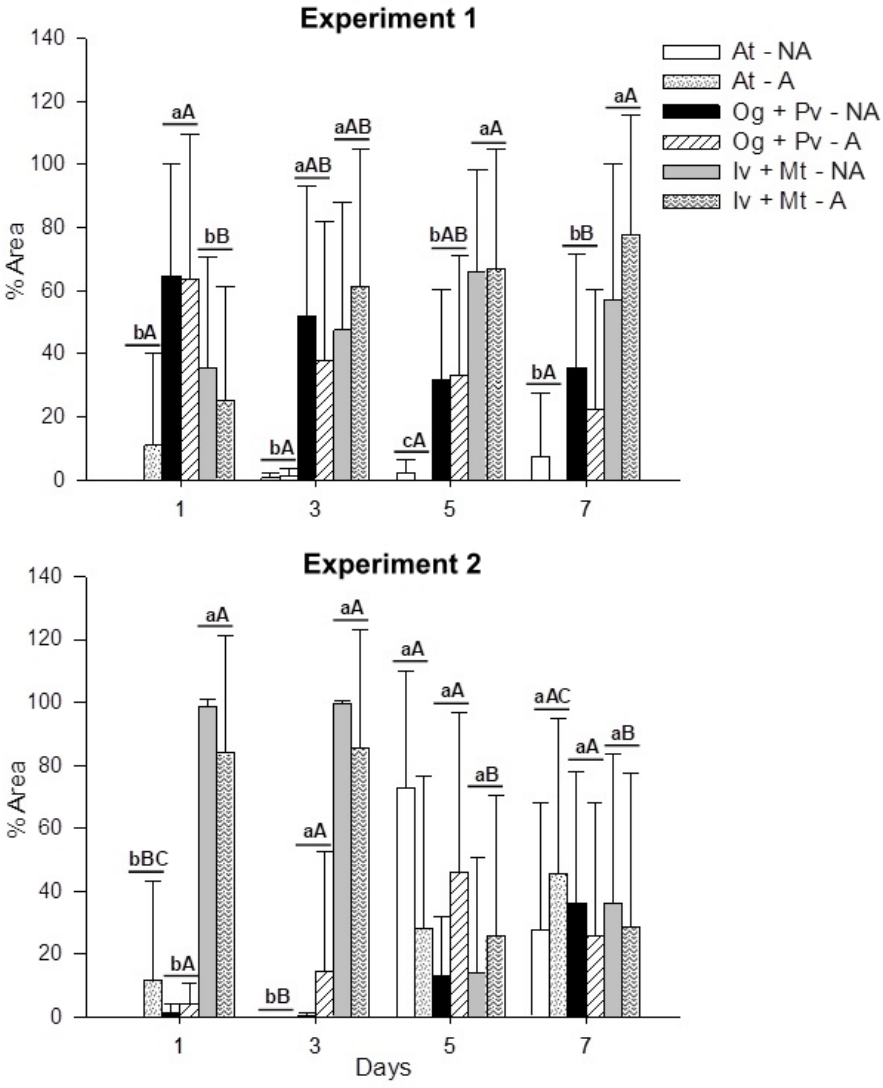

Figure 5. Percentage of area occupied by different developmental stages of oocytes of *Macrobrachium acanthurus* in experiments 1 and 2. Atretic oocyte - At; oocyte in vitellogenesis - Iv; mature oocyte - Mt; oogonia - Og; previtellogenic oocyte - Pv. A- Ablated and NA- Non-ablated. Letters refer to the result of the unfolding interaction day*oocyte stage. Bars with the same letter do not differ statistically ($p > 0.05$). Lowercase letters indicate comparison among stages within each day and uppercase indicate comparison of each stage among days.

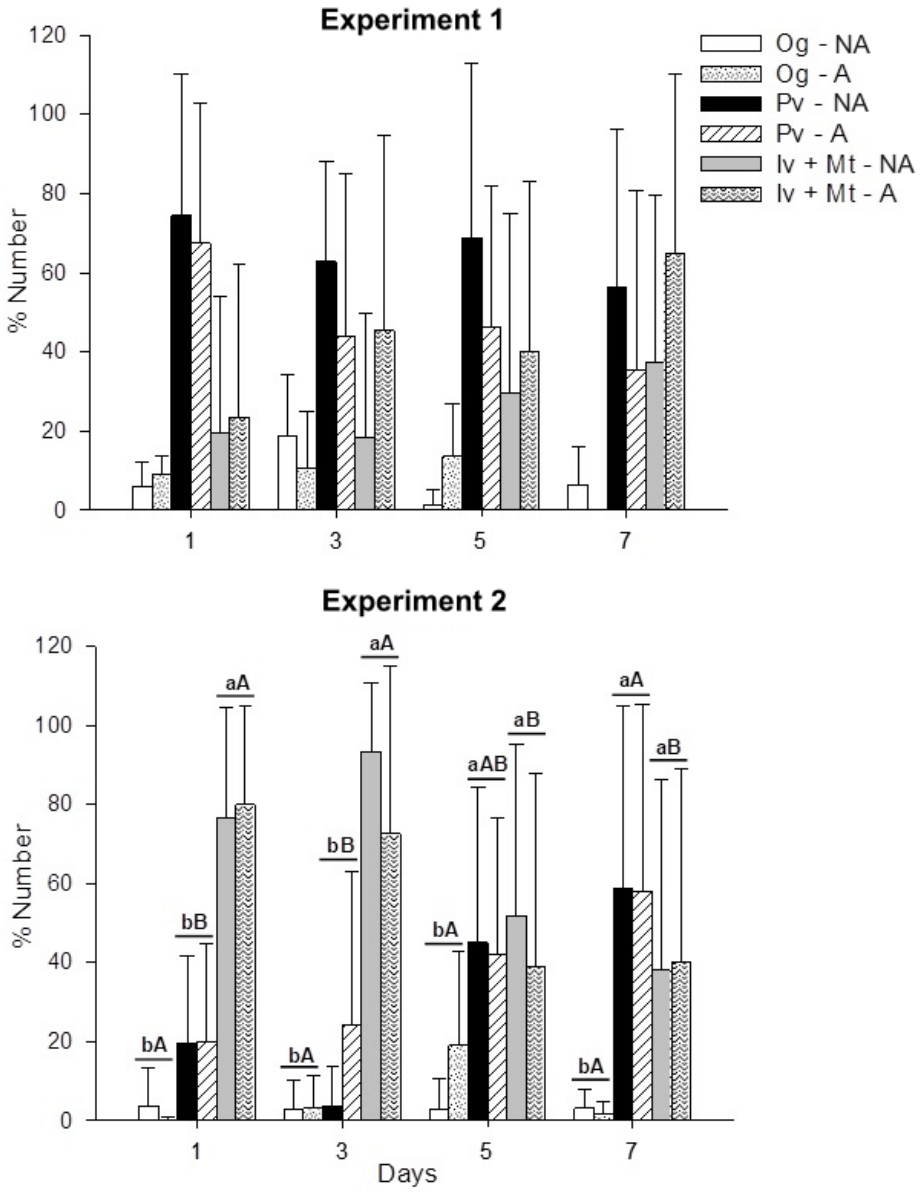

Figure 6. Percentage of number of oocytes per developmental stage in ovaries of *Macrobrachium acanthurus* in experiments 1 and 2. Oocyte in vitellogenesis - Iv; mature oocyte - Mt; oogonia - Og; previtellogenic oocyte - Pv. A- Ablated and NA- Non-ablated. Letters refer to the result of the unfolding interaction day*oocyte stage. Bars with by the same letter do not differ statistically ($p > 0.05$). Lowercase letters indicate comparison of different stages within each day and uppercase letters indicate comparison of stage among days.

1 spawned, while 35.7% of ablated females and 71.4% of non-ablated females analyzed on Day 7 spawned (Table IV).

DISCUSSION

The impact of unilateral eyestalk ablation in the ovarian development of females of the Brazilian prawn *M. acanthurus* was analyzed for the first time. The changes observed in the ovaries were related to area and number of oocytes per stage of development over time. Females with spent ovaries (Experiment 1) reduced the number of oocytes in primary vitellogenesis and increased them in secondary vitellogenesis over time. Females with mature ovaries (Experiment 2) underwent nuptial molt and spawning, increased the amount of atretic oocytes, and reduced the number of oocytes in secondary vitellogenesis. Comparison of ovarian development between spent and mature stages has been previously described for this species

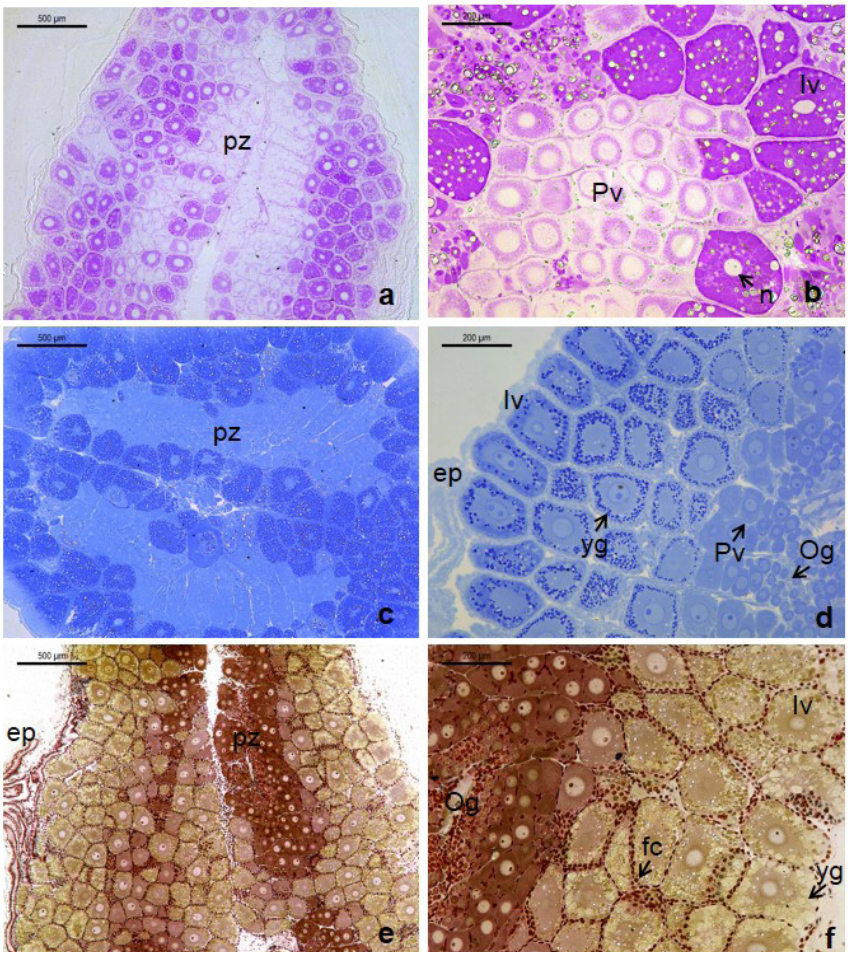

Figure 7. Histological sections of in maturation ovaries of *Macrobrachium acanthurus* submitted to different histochemical staining techniques: periodic acid-Schiff (PAS) (a-b), bromophenol blue (c-d), and von Kossa (e-f). a, c) Ovaries showing a central proliferation zone poorly reactive to the neutral polysaccharide and protein test, respectively; b) Highly reactive oocytes in vitellogenesis and previtellogenic oocytes weakly positive for detection of polysaccharides; d) Yolk granules present in the cytoplasm of oocytes in vitellogenesis with intense marking; e) Proliferative zone strongly stained for calcium detection; f) Less stained oocytes in vitellogenesis presenting yolk granules with less calcium and nucleus of intensely stained oogonia. Epithelial tissue - ep; follicular cells - fc; oocyte in vitellogenesis - lv; nucleus - n; oogonia - Og; previtellogenic oocyte - Pv; proliferative zone - pz; yolk granule - yg. 4x (a, c, e), 10x (b, d, f).

Table III. Histochemical analysis of the germ cells (oogonia and oocytes) of *Macrobrachium acanthurus*.

	Cytoplasmic granules			
Germ Cell	**Protein**	**Polysaccharide**	**Lipid**	**Calcium**
Oogonia	+	-	-	++
Previtellogenic	+	+	+	++/+++
In vitellogenesis	++/+++	+++	++	++
Mature	+++	+++	+++	+

(–) negative; (+) weakly positive; (++) moderately positive; (+++) strongly positive.

(Carvalho & Pereira 1981) as following a general pattern found in other *Macrobrachium* species (Chaves & Magalhães 1993, Meeratana & Sobhon 2007).

The ovarian characteristics found for *M. acanthurus* were similar to those described by Carvalho & Pereira (1981). Nonetheless, Carvalho & Pereira (1981) stated that *M. acanthurus* females have ovaries with full maturity and partial spawning. In the present study, there were no or few oocytes in secondary vitellogenesis in newly spent ovaries. The remaining oocytes in secondary vitellogenesis were not subsequently spawned. Instead, they underwent atresia and were reabsorbed. There was no evidence

Table IV. Means of the variables analyzed in females of *Macrobrachium acanthurus* both in ablated/non-ablated females assigned on different monitoring days (1, 3, 5 and 7) of experiments 1 and 2.

% Survival (Experiment 1 and 2)				
	Days			
Treatments	1	3	5	7
Experiment 1				
Ablated	100.0	100.0	92.9	100.0
Non-ablated	100.0	100.0	100.0	100.0
Experiment 2				
Ablated	100.0	85.7	78.6	57.1
Non-ablated	100.0	85.7	78.6	85.7
% Moult (Experiment 2)				
	Days			
Treatments	1	3	5	7
Ablated	0.0	35.7	92.9	92.9
Non-ablated	7.1	57.1	100.0	100.0
% Spawning (Experiment 2)				
	Days			
Treatments	1	3	5	7
Ablated	0.0	14.3	42.9	35.7
Non-ablated	0.0	14.3	57.1	71.4

of partial spawning in this population of *M. acanthurus*.

Eyestalk ablation in *M. acanthurus* did not induce an acceleration of ovarian maturation in this study. Hence, our results are contrary to those of previous studies on eyestalk ablation in freshwater prawn farming. This technique has been proven efficient for gonadal maturation, anticipation of spawning, and reduction of the interval between spawning in *M. rosenbergii* (Santos & Pinheiro 2000) and *M. amazonicum* (Bastos et al. 2018), and induction of growth and spawning in *M. rosenbergii* (Shailender et al. 2013). Also, an increase in ovarian indices of ablated (over non-ablated) females of *Macrobrachium dayanum* and *M. lamarrei lamarrei* have been recorded (Pervaiz et al. 2011, Hussain et al. 2017).

The rapid ovarian maturation observed in *M. acanthurus* in this study may have also contributed to the lower efficiency of unilateral eyestalk ablation. Regardless of ablation, the ovaries reorganized only five days after spawning, presenting many oocytes in advanced vitellogenesis. This shows that *M. acanthurus* is able to release larvae, mate, and spawn in a period as short as a few days.

Although eyestalk ablation results in high mortality in shrimp and other decapods (Koshio et al. 1992, Sainz-Hernández et al. 2008, Liu et al. 2014), and in most cases mortality increases after ecdysis while animals are soft, no significant differences were observed among treatments in this study. Ablated *Penaeus monodon* females had poorer survival compared to non-ablated females. The main cause of mortality of this species was the stress of molting and cannibalism (Makinouchi et al. 1995). In our study, females were isolated, preventing cannibalism.

Histochemical analyses of the ovaries revealed that unilateral ablation did not significantly influence the accumulation of nutrients in the ovaries. Although eyestalk hormones regulate carbohydrate, nitrogen, and lipid metabolism in crustaceans (Highnam & Hill 1977), the growth of *M. acanthurus* oocytes seemed not to be impaired by the removal of a single eyestalk. This technique was likely not enough to disrupt vitellogenesis in this species.

The difference in protein and polysaccharide distribution in oocytes of *M. acanthurus* indicates that protein synthesis is initially endogenous (in the oocyte itself) and later exogenous. Similar results were reported for *M. rosenbergii* (Meeratana & Sobhon 2007), *Exhippolysmata oplophoroides* (Braga et al. 2016), *Callichirus major* (Souza et al. 2017).

Greater calcium accumulation was noticed in previtellogenic oocytes, with a decrease as they transitioned to vitellogenesis. Similar results were reported for *E. oplophoroides* (Braga et al. 2016). According to Braga et al. (2016), this stage of oocyte development is a temporary storage site for calcium, which may be mobilized with the progressive development of oocytes to constitute the chorion in final vitellogenic oocytes and the future cuticle of the embryo.

Overall, no difference was found in either ovarian maturation or histochemical oocyte quality between non-ablated and ablated females. No distinction in survival, maturation, molting, or spawning separated *M. acanthurus* from other marine and freshwater prawn species. Since the expected effect was not confirmed, *M. acanthurus* could be a suitable model to explore the physiological and hormonal changes associated with ovarian maturation after eyestalk ablation. Other physiological parameters such as levels of vitellogenin, ecdisteroids, or neuropeptides in hemolymph could be used in future investigations. From an applied point of view, unilateral eyestalk ablation is not needed in *M. acanthurus* farming. Finally, our results showed quick cellular reorganization in the ovaries after spawning and a short period between spawns for this species.

Acknowledgments

The authors are grateful to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for financing part of the work (#13/06457-2). We thank Dr. Erika Takagi Nunes and the Animal Morphology Laboratory of the Universidade do Espírito Santo (UFES), for the technical support for histology and Dr. Daniela Eliana Sganga (IBBEA, CONICET-UBA) for assistance in statistical analysis.

REFERENCES

ALFARO-MONTOYA J, BRAGA A & UMAÑA-CASTRO R. 2019. Research frontiers in penaeid shrimp reproduction: Future trends to improve commercial production. Aquaculture 503: 70-87.

AKAIKE H. 1974. A New look at the statistical model identification. IEEE Trans Automat Contr 19: 716-723.

AKTAS M & KUMLU M. 1999. Gonadal maturation and spawning of *Penaeus semisulcatus* (Penaeidae: Decapoda). Turk Zool Derg 23: 61-66.

ANGER K. 2013. Neotropical *Macrobrachium* (Caridea: Palaemonidae): on the biology, origin, and radiation of freshwater-invading shrimp. J Crustac Biol 33: 151-183.

BASTOS AM, LIMA JF & TAVARES-DIAS M. 2018. Unilateral eyestalk ablation improves molting frequency and reproduction in *Macrobrachium amazonicum* females. J Appl Aquac 30: 337-352.

BERTINI G, BAEZA JA & PEREZ E. 2014. A test of large-scale reproductive migration in females of the amphidromous shrimp *Macrobrachium acanthurus* (Caridea: Palaemonidae) from south-eastern Brazil. Mar Freshw Res 65: 81-93.

BERTINI G, RODRIGUES MM, IZUMI KK & IZUMI KS. 2021. Camarões-de-Água-Doce do Vale do Ribeira: Riqueza, Importância Ecológica e Econômica, p. 69-111. In: Cunha-Lignon M, Bertini G & Montealegre-Quijano S (Eds). Manguezais, camarões-de-água-doce e manjuba-de-iguape: patrimônios natural e cultural do Vale do Ribeira e Litoral Sul do Estado de São Paulo. Registro, Unesp.

BERTINI G & VALENTI WC. 2010. Importância econômica dos camarões de água doce, p. 155-170. In: Silva RB & Ming LC (Eds). Relatos de pesquisas e outras experiências vividas no Vale do Ribeira. Jaboticabal, FUNEP.

BRAGA AA, NUNES ET, LÓPEZ-GRECO LS, CAMARGO-MATHIAS MI & FRANSOZO V. 2016. Histological and histochemical features of the oogenesis in the simultaneous protandric hermaphrodite shrimp *Exhippolysmata oplophoroides* (Decapoda: Caridea). Micron 88: 60-67.

CARVALHO HA & PEREIRA MCG. 1981. Descrição dos estádios ovarianos de *Macrobrachium acanthurus* (Wiegmann 1836) (Crustacea, Palaemonidae) durante o ciclo reprodutivo. Ciênc Cult 33: 1353-1358.

CHAVES PTC & MAGALHÃES CO. 1993. O desenvolvimento ovocitário em *Macrobrachium amazonicum* (Heller, 1862) (Crustacea: Decapoda: Palaemonidae), camarão dulcícula da região amazônica. Acta Amazon 23: 17-23.

COSTA TV, YOSHII-OSHIRO LM, LÓPEZ-GRECO LS, MELO EP, MATTOS LA & BAMBOZZI-FERNANDES A. 2016. Determinación del voltaje y el tamaño del animal óptimos para la extracción de espermatóforos en el camarón de agua dulce *Macrobrachium acanthurus* (Palaemonidae). Lat Am J Aquat Res 44: 422-428.

COSTA TV, YOSHII-OSHIRO LM, MATTOS LA, LÓPEZ-GRECO LS, MELO EP & FLOR HR. 2017. Toxicity, freezing and cold storage test of native species semen. Bol Inst Pesca 43: 334-346.

CUNHA CH & OSHIRO LMY. 2010. The influence of eyestalk ablation on the reproduction of the freshwater *Macrobrachium acanthurus* shrimp in captivity. Acta Sci Biol Sci 32: 217-221.

FINGERMAN M. 1987. The endocrine mechanisms of Crustaceans. J Crustac Biol 7: 1-24.

FUKUDA B, BERTINI G, SANTOS DC, BRAGA ACA & NUNES ET. 2013. Efeito da ablação do pedúnculo ocular no desenvolvimento ovariano de *Macrobrachium acanthurus* (Palaemonidae). IV Congresso Brasileiro de Aquicultura de Espécies Nativas. Belém, Pará, Brasil.

HABASHY MM & HASSAN MMS. 2011. Effects of temperature and salinity on growth and reproduction of the freshwater prawn, *Macrobrachium rosenbergii* (Crustacea Decapoda) in Egypt. Int J Environ Sci Educ 1: 83-90.

HIGHNAM HC & HILL L. 1977. The comparative endocrinology of the invertebrates. 2nd ed., Edward Arnold Publishers, England, 368 p.

HUSSAIN S, YADAV P, MANOHAR S & PARMAR P. 2017. Effect of unilateral eyestalk ablation on ovarian maturation of female freshwater prawn, *Macrobrachium lamarrei* {H. Milne Edwards, 1837}. Int J Fish Aquat Stud 5: 178-181.

JUNQUEIRA LCU & JUNQUEIRA LMMS. 1983. Técnicas básicas de citologia e histologia. 1ª ed., Santos, São Paulo, 123 p.

KOSHIO S, TESHIMA S & KANAZAWA A. 1992. Effects of unilateral eyestalk ablation and feeding frequencies on growth, survival and body compositions of the juvenile freshwater prawn, *Macrobrachium rosenbergii*. Nippon Suisan Gakk 58: 1419-1425.

KUTTY MN, HERMAN F & MENN HL. 2000. Culture of other prawn species, p. 393-410. In: New MB and Valenti WC (Eds), Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*. Wiley-Blackwell, Oxford.

KUTTY MN & VALENTI WC. 2010. Culture of other freshwater prawn species, p. 502-523. In: New MB, Valenti WC, Tidwell JH, D'Abramo LR and Kutty MN (Eds), Freshwater prawns: biology and farming, vol. 1, Wiley-Blackwell, Oxford.

LI L, PAN L, HU D, LIU D & LIU M. 2017. The effect of bilateral eyestalk ablation on signal transduction pathways of ion regulation of *Litopenaeus vannamei*. J World Aquac Soc 48: 145-155.

LIU S, GONG S, LI J & HUANG W. 2014. Inducing synchronous ovarian maturation in the crayfish, *Procambarus clarkii*, via eyestalk interventional injection as compared with eyestalk ablation and combined injection of serotonin and domperidone. Aquac Res 45: 1402-1414.

MACIEL CR & VALENTI WC. 2014. Effect of tank colour on larval performance of the Amazon River prawn *Macrobrachium amazonicum*. Aquac Res 45: 1041-1050.

MAKINOUCHI S, SUGAMA K, RUCHIMAT T, TRIDJOKO, SUTARMAT T & LANTE S. 1995. Effects of eyestalk ablation on maturation, spawning, hatching, molting and growth of precocious pond reared *Penaeus monodon*. Suisanzoshoru 43: 103-108.

MEERATANA P & SOBHON P. 2007. Classification of differentiating oocytes during ovarian cycle in the giant freshwater prawn, *Macrobrachium rosenbergii* de man. Aquaculture 270: 249-258.

OKUMURA T & AIDA K. 2001. Effects of bilateral eyestalk ablation on molting and ovarian development in the giant freshwater prawn, *M. rosenbergii*. Fish Sci 67: 1125-1135.

PERVAIZ PA, JHON SM, SIKDAR-BAR M, KHAN HA & WANI AA. 2011. Studies on the effect of unilateral eyestalk ablation in maturation of gonads of a freshwater prawn *Macrobrachium dayanum*. World J Zoo 6: 159-163.

PILEGGI LG, ROSSI N, WEHRTMANN IS & MANTELATTO FL. 2014. Molecular perspective on the American transisthmian species of *Macrobrachium* (Caridea, Palaemonidae). ZooKeys 457: 109-131.

PINHEIRO J, BATES D, DEBROY S, SARKAR D & R CORE TEAM. 2017. nlme: linear and nonlinear mixed effects models. R package.

PRIMAVERA JH. 1985. A review of maturation and reproduction in closed thelycum penaeids, p. 47-64. In: Taki Y, Primavera JH and Llobrera JA (Eds), Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps. Iloilo City, Philippines, SAEFDEC Aquaculture Department, Southeast Asian Fisheries Development Center.

R CORE TEAM. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RAVI R, MANISSERI MK & SANIL NK. 2013. Ovarian maturation and oogenesis in the blue swimmer crab, *Portunus pelagicus* (Decapoda: Portunidae). Acta Zool 94: 291-299.

REVATHI P, IYAPPARAJ P, VASANTHI LA, JEYANTHI S, SANKARALINGAM S, RAMASUBBURAYAN R, PRAKASH S & KRISHNAN M. 2013. Impact of eyestalk ablation on the androgenic gland activity in the freshwater prawn *Macrobrachium rosenbergii* (De Man). World J Fish & Marine Sci 5: 373-381.

RODRIGUES MM, ALMEIDA LCF & BERTINI G. 2018. Survival rate of *Macrobrachium acanthurus* (Caridea: Palaemonidae) larvae in laboratory conditions under different salinities and diets. Panam J Aquat Sci 13: 121-130.

RODRIGUES MM, LÓPEZ-GRECO LS, ALMEIDA LCF & BERTINI G. 2021. Reproductive performance of *Macrobrachium acanthurus* (Crustacea, Palaemonidae) females subjected to unilateral eyestalk ablation. Acta Zool 00: 1-9.

RODRIGUES RA, VETORELLI MP & ARAÚJO PFR. 2017. Regime alimentar na larvicultura de *Macrobrachium acanthurus* (Wiegmann, 1836) em sistema aberto. Rev Bras Eng Pesca 10: 17-30.

SAINZ-HERNÁNDEZ JC, RACOTTA IS, DUMAS S & HERNÁNDEZ-LÓPEZ J. 2008. Effect of unilateral and bilateral eyestalk ablation in *Litopenaeus vannamei* male and female on several metabolic and immunologic variables. Aquaculture 283: 188-193.

SANTOS MJM & PINHEIRO MAA. 2000. Ablação ocular no camarão *Macrobrachium rosenbergii* (De Man) (Crustacea, Decapoda, Palaemonidae): efeitos sobre a reprodução, pigmentação epidérmica e atividade alimentar. Rev Bras Zool 17: 667-680.

SINDHUKUMARI S & PANDIAN TJ. 1987. Effects of unilateral eyestalk ablation on moulting, growth, reproduction and energy budget of *Macrobrachium nobilii*. Asian Fish Sci 1: 1-17.

SHAILENDER M, AMARNATH D, KISHOR B & SURESH BCH. 2013. Effect of unilateral eyestalk ablation (UEA) on the reproductive success of giant fresh water prawn, *Macrobrachium rosenbergii* (De Man) in captivity. Int J Chem Lifesci 2: 1112-1120.

SHARIFIAN S, KAMRANI E, SAFAIE M & SHARIFIAN S. 2015. Oogenesis and ovarian development in the freshwater crab *Sodhiana iranica* (Decapoda: Gecarcinuaidae) from the south of Iran. Tissue Cell 47: 213-220.

SOUZA TL, BRAGA AA, LÓPEZ-GRECO LS & NUNES ET. 2017. Dynamics of oogenesis in ghost shrimp *Callichirus major* (Crustacea: Axiidea): a morphofunctional and histochemical study. Acta Histochem 119: 769-777.

SUBRAMONIAN T. 2011. Mechanisms and control of vitellogenesis in crustaceans. Fish Sci 77: 1-21.

TSUKIMURA B. 2001. Crustacean vitellogenesis: its role in oocyte development. Am Zool 41: 465-476.

VARALAKSHMI KN & REDDY R. 2010. Effects of eyestalk ablations on growth and ovarian maturation of the freshwater prawn *Macrobrachium lanchesteri* (de Man). Turkish J Fish Aquat Sci 10: 403-410.

WEN W, YANG Q, MA Z, JIANG S, QIU L, HUANG J & QIN JG. 2015. Comparison of ovarian maturation and spawning after unilateral eyestalk ablation of wild-caught and pond-reared *Penaeus monodon*. Span J Agric Res 13: 1-6.

ZUUR AF, IENO EN & ELPHICK CS. 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1: 3-14.

How to cite

RODRIGUES MM, LÓPEZ GRECO LS, ALMEIDA LCF & BERTINI G. 2022. Histological and Histochemical Dynamism of Oogenesis in the Cinnamon River Prawn *Macrobrachium acanthurus* (Caridea: Palaemonidae) Induced by Eyestalk Ablation. An Acad Bras Cienc 94: e20211294. DOI 10.1590/0001-3765202220211294.

Manuscript received on September 23, 2021
accepted for publication on April 4, 2022

MARIA M. RODRIGUES[1,3]
https://orcid.org/0000-0001-7309-3891

LAURA S. LÓPEZ GRECO[2]
https://orcid.org/0000-0003-2921-497X

LUIS CARLOS F. DE ALMEIDA[3]
https://orcid.org/0000-0003-3063-1906

GIOVANA BERTINI[3]
https://orcid.org/0000-0003-4489-0351

[1]Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo – IFES, Rua Augusto Costa de Oliveira, 660, 29285-000 Piúma, ES, Brazil

[2]Universidad de Buenos Aires, CONICET, Instituto de Biodiversidad y Biologia Experimental y Aplicada (IBBEA), Facultad de Ciencias Exactas y Naturales, Departamento de Biodiversidad y Biología Experimental, Laboratorio de Biología de la Reproducción y el Crecimiento de Crustáceos Decápodos, Ciudad de Buenos Aires, 1428, Argentina

[3]Universidade Estadual Paulista /UNESP. Laboratório de Biologia e Cultivo de Crustáceos (LABCRUST), Av. Nelson Brihi Badur, 430, Vila Tupy, 11900-000 Registro, SP, Brazil

Correspondence to: **Maria Maschio Rodrigues**
E-mail: maria.maschio@ifes.edu.br

Author contributions

Maria Maschio Rodrigues: - participated in Conceptualization; Data curation; Formal analysis; Visualization; Writing – original draft; Writing – review & editing. Laura S. López Greco - participated in Conceptualization; Formal analysis; Writing – review & editing. Luis Carlos Ferreira de Almeida – participated in Formal analysis (statistics). Giovana Bertini - participated in Conceptualization; Formal analysis; Visualization; Writing – review & editing; Resources.