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The influence of eyestalk ablation on the reproduction of the freshwater *Macrobrachium acanthurus* shrimp in captivity

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ABSTRACT. *Macrobrachium acanthurus* is distributed along the Brazilian coastal rivers and shows potential for aquaculture. This study was carried out to provide information on the reproduction of *M. acanthurus* in captivity and the influence of unilateral eyestalk ablation. A total of 48 females and 24 males were captured in the Sahy river in Mangaratiba, Rio de Janeiro State. The animals were distributed into twelve 20-liter aquariums, each with two males and four females. Two females in each aquarium were ablated. Every day the occurrence of ovigerous females was checked in each aquarium. The U test (Mann-Whitney) showed a significant difference among laying intervals and the number of hatchings between ablated and non ablated females.

Key words: freshwater shrimp, gonadal maturation, spawning, fertility.

RESUMO. Influência da ablação do pedúnculo ocular na reprodução do camarão de água doce *Macrobrachium acanthurus* em cativeiro. *Macrobrachium acanthurus* é uma espécie com viabilidade para o cultivo e ocorre nos rios da costa brasileira. O objetivo deste trabalho foi obter informações sobre o aspecto reprodutivo de *M. acanthurus* em cativeiro e a influência da técnica de ablação unilateral do pedúnculo ocular na reprodução. Foram utilizados no experimento 48 fêmeas e 24 machos coletados no Rio Sahy, Mangaratiba, Estado do Rio de Janeiro. Os animais foram distribuídos na proporção de dois machos para quatro fêmeas em doze aquários com capacidade de 20 litros. Das quatro fêmeas de cada aquário, duas foram abladas unilateralmente. Todos os dias foram verificados a ocorrência de exteriorização dos ovos em cada fêmea. Através do teste U (Mann-Whitney) foi verificada diferença significativa para o intervalo entre as desovas e quanto ao número de desovas entre fêmeas abladas e não abladas.

Palavras-chave: Camarão de água doce, maturação gonadal, desova, fertilidade.

Introduction

Macrobrachium acanthurus Weigmann, 1836 (Decapoda: Palaemonidae) known as 'canela shrimp', 'pitu' or 'freshwater shrimp' is native to the Americas, being found from Georgia, United States, to the southern Brazil and the Western Indies, in freshwater and brackish water. *M. acanthurus* is an omnivorous species, with a varied diet that includes both benthic macrofauna elements and Chara macroalgae associates, such as plant fragments and debris (ALBERTONI et al., 2003).

The freshwater shrimp, *M. acanthurus* presents varied features accrediting it as a potentially viable species for commercial scale cultivation, such as captivity reproduction, easy maintenance, simple feeding, high survival rate, rare disease incidence and good acceptance by the market (NEW, 1995).

The technique of unilateral eyestalk ablation has been applied with success on the gonadal maturation induction of crustacean decapods (PRIMAVERA, 1978), resulting in anticipation of ovigerous condition and an increase in spawning frequency. Unilateral eyestalk ablation is the most effective way to inducing ovarian maturation in Penaeidae shrimp cultivation, especially on hard captive reproduction species. However, fast maturation and latency period reduction between spawnings are usually observed, conflicting results have been reported on the effect of unilateral eyestalk ablation on spawning quality (TAN-FERMIN, 1991).

Unilateral ablation on *M. rosenbergii* presented positive results, such as: anticipation of first spawning; increase in the number of consecutive spawnings; reduction of periods between spawnings; high spawning by survival rate obtainment at the end of the experiment (SANTOS; PINHEIRO, 2000). Okumura and Aida (2001) observed that the duration of ovarian development was reduced on bilaterally ablated *M. rosenbergii* females, indicating the occurrence of

inhibiting function of the eyestalk hormone on vitellogenesis.

The present study had the aim to contribute with the obtainment of data on aspects of *M. acanthurus* captive reproduction, verifying the influence of unilateral ablation on reproduction performance, due to the fact that there is no information on it. Additionally, this species presents viable features for its cultivation and at the continental area of Rio de Janeiro State, a natural population decrease has been noticed, demonstrating a need for the development of cultivation viability techniques.

Material and methods

For the study, 48 adult females and 24 adult males of *M. acanthurus* were captured at the Sahy river, Mangaratiba , southern coast of Rio de Janeiro State (22º56'S; 44º01'W).

For animal capture, a 2.0 mm mesh net was used. It was immersed and dragged along the river margin, close to the vegetation and deeper points, close to the organic material at the bottom. The captured shrimps were transferred to styrofoam boxes, on plastic bags with local water and carried to Biological Marine Station of the Rural Federal University of Rio de Janeiro (EBM/Ufrrj) at Itacuruçá, Rio de Janeiro, State.

At the lab, a collected material trail was done, selecting animals by sex, based on the secondary sexual features (CARVALHO, 1973), especially by the presence of masculine appendix in males.

Female abdomen aspects were verified to determine their reproduction conditions and, consequently, whether they were ovigerous females or not. In the experiment, females with 35.1 and 65.0 mm total length (TL) variation were used.

The specimens were distributed in 12 aquariums, each with two males and four females, in a total of six animals per aquarium. In each aquarium, two females were ablated and two were not, establishing the following treatments: 1) Not ablated females, and 2) Unilaterally ablated females. Therefore, each treatment presented 24 similar situations (animals), in similar environment conditions.

The eyestalk ablation method used was the one described for marine shrimps by Primavera (1978). The females were taken from the water and softly dried with paper towel, having their eyestalk extracted with a scalpel, close to the base. The incision spot was 'hotly' cauterized with an electric cauterizer and antibiotic pomades were used (Furacin and Terramicin, in 1:1 ratio).

Each female was branded by the ablation and cuts on the uropods for identification.

The aquariums used in the experiment had 20-liter capacity and each had a biological filter (stones, gravel, thick and thin sand) and air pumps. The experiment temperature and light were natural and the animal distribution on the aquariums was done at random.

In order to avoid cannibalism during molting, pieces of PVC pipe (15 x 3 cm Ø), stones and macrophytes such as Vallisneria gigantea, Elodea densa, aquatic Cabomba types were used as aquarium ornaments, creating a refuge for the animals. During the experiment, the animals were fed daily with Tropifish (TF 200) pelletized food for crustaceans, with 36% crude protein and a mixture fresh fish pieces.

Water temperature was recorded daily, on the 12 aquariums and on the containers with ovigerous females, which were individualized using a thermometer. Abiotic factors such as pH, ammonium, nitrite and dissolved oxygen were taken weekly using aquariophilia, consisting of the colorimetric method.

Daily observations recorded each female eggs externalization date, monitoring the embryonic development so the incubation period could be determined. Each ovigerous female, after the third incubation day, was separated and put in a one-liter container with air pumps, until larval outbreaks. After eclosion, fertility was estimated by counting total viable larvae, and the females were returned to the aquarium for a new reproduction phase.

The experimental period lasted eight months, including fall, winter and spring seasons.

The non parametric U test (Mann-Whitney) was used to verify the occurrence of a significant difference between average spawning numbers (NS) and the average gap between the spawnings (IS) by days, between the treatments and between each treatment size classes. The Pearson Correlation (r) analysis was used to examine: temperature influence on incubation time, in which the average temperature was correlated for each incubation period (days) and the total individual length that was correlated with each female average fertility. The 't' test was used to verify the occurrence of significant fertility difference between ablated and non ablated females and also to compare spawning intervals between ablated and non ablated female size classes.

Results and discussion

Sudden temperature variations are harmful to shrimp development, having an indirect effect on the organisms, because of changes in water density and viscosity, concentration of important gases such as oxygen, as well as the effect of toxic substance on shrimp (VALENTI, 1998).

The temperature in all aquariums, pH, nitrite, ammonium and dissolved oxygen in the present study were found at: 24.7ºC mean temperature and mean pH of 7.0 in most aquariums; this was below the average considered ideal, of 29.5ºC and 7.8 respectively, because the experiment was performed during the fall and spring periods, with ambient temperature, without the use of heaters or thermostats. The nitrite and the ammonium were below tolerance limits while the present work was done; 0.48 mg L^{-1} and 1.98 mg L^{-1}, respectively. The dissolved oxygen remained above optimum levels, averaging 5 mg L^{-1} (NEW, 1995).

According to Carvalho (1978) the reproduction cycle of *M. acanthurus* suffered the influence of water temperature and pluviometric regime. Takino et al. (1989) verified that Macrobrachium petronioi and *Macrobrachium birai* reproduction in Rio Branco was influenced by the rise in temperature.

According to Aktas et al. (2003), unilaterally ablated *Penaeus semisulcatus* females, in 20ºC temperature, developed their ovary until the fourth stage, but did not spawn.

Valenti et al. (1986) verified that *M. acanthurus* individual fecundity ranged between 740 and 17,769 eggs (average of 8,929 eggs) for females 60.0 to 135.0 mm long.

For the statistical analysis, 21 ablated and non ablated females were used, because five females that were above or below the three size classes and one female that died before doing any spawning were discarded.

The experimental result for the analyzed reproduction performance parameters, according to the three length classes of ablated and non ablated females are show on Tables 1 and 2, respectively. The examined parameters were: average fertility, constituting the number of hatched eggs per spawning (F); the average number of spawnings (NS) during the whole experimental period; and the average spawning interval (IS), verifying the number of days between one spawning and another.

Table 1. Total Length class (TL), fertility (F), number of spawnings (NS), spawning interval (IS) (days) and number of animals (N), for ablated *Macrobrachim acanthurus* females maintained in captivity during the experimental period (averages and standard deviation).

Total Length Class (TL)	F	NS	IS	N
35.1 - 45.0	388.7 ± 47.9	3.3 ± 2.1	3.3 ± 0.4	8
45.1 - 55.0	531.0 ± 136.5	4.6 ±1.8	3.2 ± 1.2	9
55.1 - 65.0	743.2 ± 174.2	2.4 ±1.3	4.5 ± 0.7	4
Total	554.4 ± 177.2	3.8 ± 1.9	3.4 ± 1.1	21

The average fertility in this study varied from 388.7 to 743.2 larvae on ablated females and from 374.5 to 756.7 larvae on non ablated females (Table 1 and 2).

Table 2. Total Length class (TL), fertility (F), number of spawnings (NS), spawning interval (IS) (days) and number of animals (N), for non ablated *Macrobrachium acanthurus* females maintained in captivity during the experimental period (averages and standard deviation).

Total Length Class (TL)	F	NS	IS	N
35,1 - 45,0	374,5 ± 114,8	2,8 ± 1,5	4,5 ± 1,3	4
45,1 - 55,0	612,3 ± 221,3	3,2 ± 2,2	4,6 ± 1,6	12
55,1 - 65,0	756,7 ± 160,9	4,5 ± 2,5	3,8 ± 1,1	5
Total	549,1 ± 225,1	3,3 ± 2,0	4,5 ± 1,3	21

The fertility values found for *M. acanthurus* females in this study were in the range found by Valenti et al. (1986), which varied from 525 to 6,543 larvae, for females of 50.0 to 110.0 mm total length.

The Pearson correlation (r) demonstrated the occurrence of a direct dependence relationship between the number of larvae produced by *M. acanthurus* ablated females ($r = 0.73$; $p \leq 0.01$) as well as non ablated females ($r = 0.70$; $p \leq 0.01$), with length (TL) showing that fertility increases according to the female's size (Figures 1 and 2).

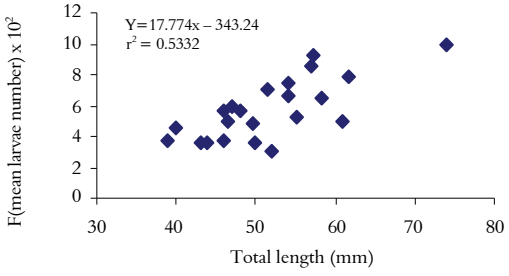

Figure 1. Relationship between the mean number of larvae (F) and length (TL) of ablated *M. acanthurus* female in captivity.

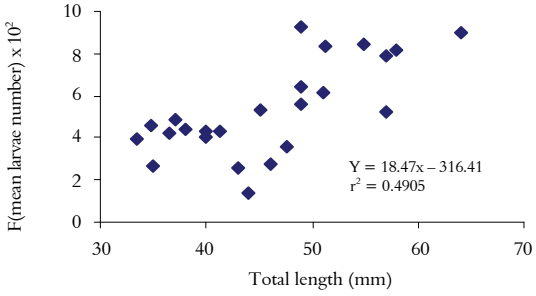

Figure 2. Relationship between the mean number of larvae (F) and length (TL) of non ablated *M. acanthurus* female in captivity.

The U test (Mann-Whitney) comparing both treatments fertilities and the three classes, did not demonstrate a significant difference ($U = 214.50$; $p = 0.44$) (Table. 3).

The *M. acanthurus* individual spawn number, in this study, varied from 1 to 7, on ablated females as well as on non ablated females. For a total of 21 females, 80 spawns were observed for ablated females and 69 spawns for non ablated females.

The U test demonstrated there was no significant difference for the number of spawns between the two treatments, and also when the three size classes for ablated and non ablated females are compared (Table 3).

Table 3. Statistical analysis result comparing reproduction performance parameters, with regard to the length classes of ablated and non ablated females (fertility, number of spawns and interval between spawns) on *Macrobrachium acanthurus* in captivity. ⋆ = significant difference ($p < 0.05$).

Total Length Class (TL)	Non Ablated x Ablated		
	Fertility	Number of spawns	Spawn interval
35.1 – 45.0	U = 13.0	U = 13.0	t = 1.5959
	p = 0.3052	p = 0.3052	p =0.0808
45.1 – 55.0	U = 43.0	U = 33.0	t = 1.8843
	p = 0.2172	p = 0.0678	p =0.0402⋆
55.1 – 65.0	U = 9.0	U = 4.0	t = 0.6101
	p = 0.4032	p = 0.0708	p =0.3019
Total	U = 214.5	U = 184.00	U = 51.0
	p = 0.44	p = 0.1793	P = 0.0184⋆

This result does not agree with the one found by Santos and Pinheiro (2000), which showed, for unilaterally ablated *M. rosenbergii* females, a significant increase of consecutive spawns number. Possibly this disagreement is due to ambient temperature influence, physiological conditions of the females, year season and latitude, which regulate the eyestalk endocrine system hormonal mechanism on decapods, acting on gonadal development (COELHO et al., 1982).

Studies done on the Caeté river, Pará State, with *M. acanthurus* showed that this species reproduction happens between January and June (QUADROS, 2002) and on Ribeira do Iguape River, São Paulo State in December and January (VALENTI et al., 1986).

In this research study, ablated females present an interval of spawns varying from 1 to 8 days, an average of 3.4 ± 1.1 days, while non ablated females vary from 2 to 8 days, an average of 4.5 ± 1.3 days. The non parametric U test (Mann-Whitney) demonstrated the existence of a significant difference for the gap between the spawns for the ablated and non ablated females ($U = 51.0$; $p = 0.0184$). When the 't' test (Student) was applied, for the three size classes, comparing both treatments, a difference was noticed in the intermediate class, with 45.1 to 55.0 mm of total length ($t = 1.8843$; $p = 0.0402$) (Table 3).

This result confirms the one observed for Penaeus schmitti ablated females, which took less time for ovary maturation if compared to non ablated females (NASCIMENTO et al., 1991). Tan-Fermin (1991) also concluded that the early ovary maturation, right after the spawn, was a direct result of the Penaeus monodon eyestalk ablation. Santos and Pinheiro (2000) also guaranteed that unilateral eyestalk ablation was the efficient treatment for *M. rosenbergii*, presenting a reduction in the interval between spawns.

In the present work, a new maturation cycle right after the spawn was observed, confirming the study mentioning unilateral ablation as the best way of promoting gonadal maturation by endocrine imbalance relating it to the reduction of the Gonadal Inhibitor Hormone (GIH) in the hemolymph on ablated females (BROWDY; SAMOCHA, 1985).

In the present study, the ablation technique had a important role for ovary development and spawning with time reduction between spawns. Aktas et al. (2003) verified on *Penaeus semisulcatus* that just having favorable temperature conditions was not enough for maturation and captive spawning in the winter period, showing the need for ablation or temperature fluctuation as stimulants for a greater induction effect on the ovary development and spawn.

The average period for incubation of ovigerous *M. acanthurus* females in this research was 18 days, varying from 13 to 32 days. Carvalho (1978), studying this species, noticed the egg incubation period was 19 to 21 days.

The Pearson Correlation (r) demonstrated that there was no dependent relationship between incubation period (days) and the water temperature on ablated females ($r = -0.16$; $p > 0.05$), meaning the incubation period was not influenced by the rise in water temperature (Figure 3A). However, the non ablated females showed a dependent relationship between the incubation period (days) and water temperature ($r = -0.16$; $p > 0.05$), demonstrating that the incubation period was influenced by the rise in water temperature (Figure 3B).

This research study presented results demonstrating that the duration of egg incubation period can be influenced by water temperature variation, as well as the results found by Bond and Buckup (1982), which verified non ablated *M. borellii* and *M. potiuna* females.

It can be concluded that the unilateral eyestalk ablation applied on *Macrobrachium acanthurus* captive females demonstrated influence on spawning gaps, decreasing the time period between spawns on ablated females. However, incubation period did not show a dependent relationship with water temperature on ablated females.

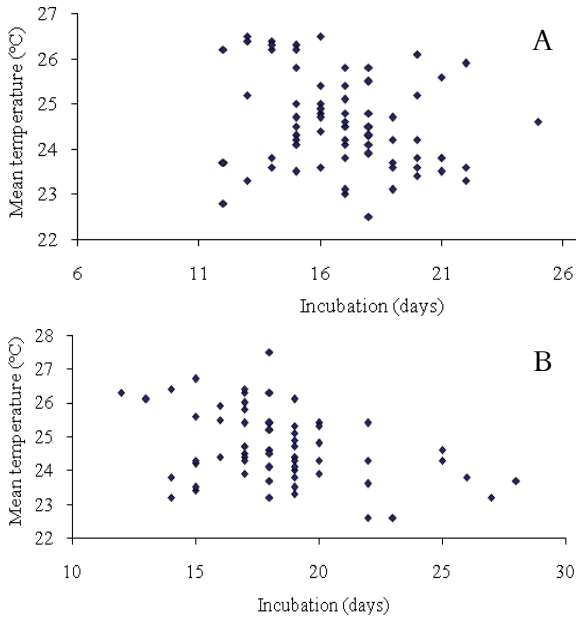

Figure 3. Relationship between the incubation period (A) on ablated (B) non ablated *M. acanthurus* females and the mean water temperature on captivity animals.

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