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Effect of two diets on the fecundity of ablated and non-ablated female of *Macrobrachium amazonicum*

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ABSTRACT. *Macrobrachium amazonicum* is a species of freshwater shrimp of great importance for aquaculture and to know its reproductive potential, through the study of its fertility is necessary. Therefore, this study aimed to evaluate the effect of two different diets on the reproductive performance of the species, whether or not adopting the ablation procedure. The experiment was carried out in a completely randomized design in a 2 x 2 factorial arrangement, with two types of feed (inert and fresh) and two conditions (ablated and non-ablated females), using six replicates per treatment. After 10 days of spawning, the eggs were removed and counted in their entirety. For each female, three spawns were obtained, totaling 72 samples. The fertility rate, the interval between spawning and the weight gain of the females were analyzed. There was no interaction between the studied factors, except for weight gain, in which ablated females that received fresh feed gained up to three times more weight than non-ablated females. Females that received fresh feed obtained higher fertility (1,373.9 eggs) than those that received only inert feed (1,084.1 eggs). The interval between spawns was 1.6 days shorter in ablated females.

Keywords: ablation; nutrition; spawning; shrimp.

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Introduction

Macrobrachium amazonicum is a freshwater shrimp which has a great economic importance to the riverside communities being explored for the artisanal fishing and commercialized at markets and fairs in the municipalities of the north region of Brazil (Bentes et al., 2011; Souza, Florentino, & Piñeyro, 2014; Silva et al., 2017).

This shrimp have continuous reproduction type, in the lakes of the central Amazon with predominance of ovigerous females in three seasons of the year: in the middle of the draining, in the dry and in the middle of the flooding. The populations that live in the coastal rivers need the brackish water from the estuary to complete the larval development, while the ones which live in the rivers and lakes from central and western Amazon have the entire life cycle in the fresh water (Magalhães, 1985; Bentes et al., 2011; Pantaleão, Carvalho-Batista, Teodoro, & Costa, 2018).

The specie has aroused interest in the aquaculture due to the important characteristics of the cultivation such as: fast growing, rusticity, disease resistance, maturation and larvae production methods are simpler and easy captivity maintenance (Moraes-Riodades & Valenti, 2002; Silva, Frédou, & Filho, 2007).

The knowledge of fecundity of shrimp from genus *Macrobrachium* is important to assess the potential use of this specie in the commercial aquaculture and contribute to the reproduction potential estimate, whether in captivity or in the natural population. In addition, this fecundity knowledge can be used to determine the minimum number of adults needed to keep the recruitment and surviving rates until the adult phase (Valenti, Mello, & Lobão, 1989). According to Corey and Reid (1991), the fecundity of these shrimps has shown a large plastic variety inter and intra specific, and among the species *M. rosenbergii* and *M. carcinus* can be found the highest rates able to incubate between 80,000 and 100,000 eggs (Ismael & New, 2000).

Unlike the bigger size species, *M. amazonicum* can have its life cycle limited to fresh water frequently presenting hatch, compensating the low fecundity (Magalhães, 1985; Scaico, 1992). In laboratory conditions, Coelho, Ramos-Porto, Barreto, and Costa (1982) reported spawning up till 6,000 eggs and Valenti, Mello, and

Lobão (1986) found fecundity varying between 178 to 1,344 eggs. In natural conditions, Scaico (1992) analyzed females from Brazilian Northeast weirs, with a length between 35 to 71 mm, and determined a fecundity varying from 148 to 1,128 eggs. Da Silva, Sampaio, and Santos (2004) reported a fecundity of 2,193 eggs in females collected in Rio Jaguaribe, Ceará State. It is important to highlight that not all the incubated eggs from a female are viable, and sometimes the fertility can be less than the produced eggs.

The technic of eyestalk ablation consists in the extirpation of one or both stalks. The stalk houses neurosecretory cells that form the organ X/sinus gland complex (OX/SG) which is responsible for the production, storage and distribution of regulatory hormones of ecdysis and gametogenesis (Corrêa, Matos, Gomes, Santos, & Amaral, 1996). With the eyestalk ablation cease the synthesis of these neurohormones, promoting a hormonal imbalance with direct changes on such process. The organ-X synthetizes the Gonadal Inhibitor Hormone (GIH) which is stored and distributed by the sinus gland. With the removal of the eyestalk the GIH producing source is eliminated, allowing a higher concentration of the Gonadal Stimulating Hormone (GSH), produced by organ-Y at the cephalothorax, hence stimulating the gonadal maturation (Pillai, Sahoo, Sahu, Vijaykumar, & Sahu, 2010; Wen et al., 2015).

According to Primavera (1979), the eyestalk ablation must be done in the inter ecdysis stage in order to ensue gonadal maturation, because otherwise it is seen the activation of the ecdysis process and an increase of the latency period to the next maturation. This fact is a reflex of an antagonism between the growing process and the reproduction in the Decapods crustaceans, which compete for the same energetic source stored in the hepatopancreas (Kyomo, 1988).

In studies involving the eyestalk ablation in freshwater shrimps, it can be highlighted those carried out by Santos and Pinheiro (2000) and Pillai et al. (2010) in *M. rosenbergii*, by Cunha and Oshiro (2010) in *M. acanthurus*, by Pervaiz, Jhon, and Sikdar-Bar (2011) in *M. dayanum*, by Asusena, Carlos, Arturo, and Genaro (2012) in *M. americanum* and by Bastos, Lima, and Tavares-Dias (2018) in *M. amazonicum*. According to these authors, unilateral eyestalk ablation was an efficient treatment to the reproduction of the species, presenting advantages such as: effect on male and female growth; anticipation of first spawning; increase of consecutive spawning; reduction of the period between spawning; achievement of high rate of spawning per surviving at the end of the experiment. However, there is no record on the literature related to the effect of feeding at the fecundity of ablated females of *M. amazonicum* in captivity. The knowledge of this information constitutes an important tool for the farming of the specie, serving as a component to the management of its cultivation.

The aim of this study was to evaluate the effect of two diets on the reproductive performance of females of *M. amazonicum* ablated or not.

Material and methods

Animals and place

Macrobrachium amazonicum shrimps were collected (authorized 60993-3 by ICMBio/MMA) on the margins of the lakes Macurany and Parananema, both located at the municipally of Parintins on the Amazonas State, Brazil, transported alive to the lab of Social Sciences, Education and Animal Science Institute of the Federal University of Amazon, where they were sexed (according to the morphology of the second pair of pleopods) and acclimatized for 10 days in tanks of 1000 L.

To the execution of the experiment, 36 animals were used, 12 males and 24 adult females, at the proportion male: female of 1:2, distributed in 12 tanks with capacity of 20 L of water, with constant aeration and artificial shelters. Temperature, pH and dissolved oxygen with digital thermometer, pH meter and oximeter were daily monitored, respectively. The total ammonia was measured weekly, with a colorimeter kit. The experiment lasted six months.

Design and experimental treatments

For the composition of the experimental diets, the fresh feed was made up of a blending of regional fish's muscles (Tambaqui (*Colossoma macropomum*), Surubim (*Pseudoplatystoma corruscans*) and Tucunaré (*Cichla ocellaris*) and squid (1:1; with 16% of crude protein and 2% of fat), while the inert feed was made up of a commercial fish feed with 45% of crude protein and 8% of fat. The squid was introduced in the fresh feed due to the high contend of polyunsaturated fat acids, especially EPA and DHA (Sikorski & Kolodziejska, 1986), essential in the crustacean reproduction. The animals were feed twice a day at 8 and 18h, at a rate of 10% of live weight and the rest were siphoned daily when the volume of water was partially changed (approximately 20%).

The ablation was done in only one female per tank, by the eyestalk extirpation, as described by Primavera (1978) for saltwater shrimps. The females were removed from the water and dried lightly with a paper towel, then an eyestalk removed with a scalpel, close to the base. The place of the incision was cauterized with an electric cauterizer and administered a mix of antibiotic ointments at the place (Furacin (Nitrofurazone) and Terramicina (Oxytetracycline) at the proportion of 1:1) as described by Cunha and Oshiro (2010). Males were not ablated.

Measurements

All the animals were measured for the cephalothorax length (CL), measurement done from the base of the rostrum to the final of the cephalothorax, and the total length (TL), measurement done from the base of the rostrum to the final of the telson, with a digital pachymeter. A digital scale (0,001 g) was used to obtain the initial and final weight.

The females were observed daily and followed the same feeding schedule until the spawning, characterized by the presence of adhered eggs to the abdominal pleopods. From this moment 10 days of incubation were counted, then all the eggs were removed with a clamp to verify fecundity, and following the females were returned to the tank in order to restart the reproductive cycle.

According to Bertini and Baeza (2014) and Rodrigues, López-Greco, Almeida, and Bertini (2021) the females lost the eggs during the incubation period (12 to 18 days), what can be explained by the increase of the embryonic volume during the incubation, that can lead to a restriction of physical space and agglomeration of eggs at the abdominal chamber. To verify the real fecundity in each female, a 10-day period was established taking into account the time for the incubation of the eggs of *M. amazonicum*.

From each female three spawning were counted, with a total of 72 spawning. After the collection of the eggs, fixed in 70% alcohol, for posterior total counting with a manual counter. The interval between spawning was analyzed to verify if there was an interference of the ablation in this period.

The average fecundity was determined by the equation: $Fec = (n_y + \cdots + n_z)/w$, where n corresponds to the quantitative of spawning and *w* to the total number of spawning, according to the treatment. The weight gain was determined by the equation: $WG = \left(\left(FW - \frac{IW}{IW}\right)\right) * 100$, where FW is the final weight and IW the initial weight.

Statistical analyses

The experiment was accomplished in a completely randomized design in a 2 x 2 factorial arrangement, with two types of feed (inert and fresh), and two conditions (ablated and non-ablated females), with six replicates per experimental unit, following the model:

$$Y_{ijk} = m + a_i + b_i + (ab)_{ij} + e_{ijk},$$

being,

Y_{ijk} the observed value of the variable in the study;
m = general average of the experiment;
a_i = feed effect (A);
b_i = ablation effect (B),
$(ab)_{ij}$ = effect of the interaction between A and B;
e_{ijk} = experimental error.

Data were analyzed by two-way analysis of variance with the statistical program SISVAR (Ferreira, 2011), when the effect of the averages was significative by the test F it was evaluated by the Tukey test with 5% of significance. In order to verify the relation between the variables fecundity and the cephalothorax and total length and the weight gain were carried out the analysis of Pearson's correlation coefficient (r).

Results and discussion

M. amazonicum has a great trophic plasticity, showing high adaptability to different environment conditions, once the biotic parameters analyzed, among that considered satisfactory for the specie, having no interference in its productivity in captivity (Preto, Kimpara, Moraes-Valenti, Rosa, & Valenti, 2011; Meireles, Valenti, & Mantelatto, 2013). The parameters of water quality did not differ among the treatments (p > 0,05), where the average temperature was 27.5±0.92°C, dissolved oxygen was 6.8±0.04 mg L^{-1}, pH was 7.3±0.23 and for the total ammonia concentration it was 0.003±0.001 mg L^{-1}.

Significant effects on the fecundity, spawning interval and the weight gain of the ablated and non-ablated females were observed (Table 1), where only for the variable weight gain an interaction between the treatments (p=0.0097). The fecundity was affected for the factor feed (p=0.0226), where the females that were fed with fresh feed had the higher fecundity (1410.3 eggs) in relation to the females fed with inert feed (1047.7 eggs). On the other hand, the ablation of the females influenced the spawning interval (p=0,001), in a way that the females that were ablated had the smaller interval (13.0 days) whereas the females that were not eyestalk ablated had 14.6 days of interval (Figure 1).

Table 1. Reproductive and zootechnical parameters of de *Macrobrachium amazonicum* females ablated or not, receiving fresh or inert feed.

Mean Fecundity (eggs)

Female status	Feed		Mean	CV (%)[1]	Probability[2]		
	Fresh	Inert			Fe	Ab	Fe x Ab
Ablated	1589.4	1158.4	1373.9	53.7	0.0226	0.0665	0.6611
Not ablated	1231.1	937.0	1084.1				
Mean	141.3^{a}	1047.7^{b}					

Mean interval between spawning (Days)

Female status	Feed		Mean	CV(%)[1]	Probability[2]		
	Fresh	Inert			Fe	Ab	Fe x Ab
Ablated	13.1	12.8	13.0^{A}	6.1	0.4917	<0.001	0.0900
Not ablated	14.3	14.8	14.6^{B}				
Mean	13.7	13.8					

Females' cephalothorax length (mm)

Female status	Feed		Mean	CV(%)[1]	Probability[2]		
	Fresh	Inert			Fe	Ab	Fe x Ab
Ablated	15.6	15.6	15.6	1.7	0.4549	0.3792	0.3055
Not ablated	15.8	15.6	15.7				
Mean	15.7	15.6					

Initial weight (g)

Female status	Feed		Mean	CV(%)[1]	Probability[2]		
	Fresh	Inert			Fe	Ab	Fe x Ab
Ablated	2.8	2,8	2.8	10.3	0.7644	0.1109	0.9353
Not ablated	3.0	2.9	3.0				
Mean	2.9	2.8					

Weight gain (%)

Female status	Feed		Mean	CV(%)[1]	Probability[2]		
	Fresh	Inert			Fe	Ab	Fe x Ab
Ablated	4.7^{aA}	1.7^{bA}	3.2	63.3	0.0966	0.0720	0.0097
Not ablated	1.5^{aB}	2.2^{aA}	1.8				
Mean	3.1	1.9					

[1]Coefficient of variation; [2]Fe=Feed; Ab= ablation; Means with upper and lower case letters in the columns are for feed, and in the rows, for ablation, significantly differs at 5% of probability by the Tukey test.

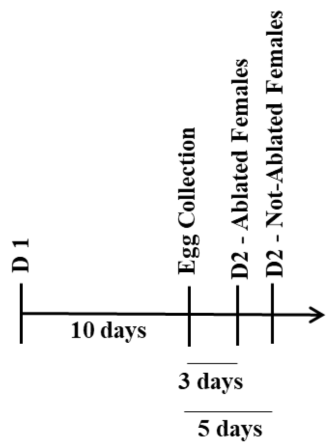

Figure 1. Demonstrative scheme of the spawning interval (D1 and D2) of ablated and non-ablated females.

The results of this study disagree with the ones reported by Bastos et al. (2018) for the same species and with Rodrigues et al. (2021) for M. *acanthurus*. However, it corroborates with those reported by Varalakshmi and Reddy (2010), which described greater quantity of produced eggs in ablated females of *M. lanchesteri*. According to Martins, Rosa, Rivero, Nazari, and Müller (2006), the variation observed in the fecundity of *Macrobrachium* can be assigned to different physiological conditions, female's age, season of the year and food offer.

The feeding is of fundamental importance to the maturation process, mainly when rearing in small tanks, where the animals cannot explore an environmental diversity in order to supply its daily requirements. A proper diet is identified as crucial factor to the shrimp's sexual maturity and reproduction in captivity. When the diet is unbalanced or incomplete can cause low reproductive performance or even stop the reproduction (Wouters, Lavens, Nieto, & Sorgeloos, 2001).

In relation to the weight gain, analyzing each factor separately, it was observed that ablated females receiving fresh feed had higher weight gain in comparison to the other combinations. The statistical tests indicated a positive correlation between fecundity and the total length ($r=0.6351$, $p < 0.0001$). The fecundity did not show correlation with the cephalothorax length ($r=0.1045$, $p=0.1233$) and weight gain ($r=0.0907$, $p=1526$).

Although research have been done, few are the information about nutritional requirement of M. *amazonicum* specie with a potential in aquaculture, and such information are fundamentals to the feed formulation used in the freshwater shrimp farm in order to enable the maximum productive and reproductive expression (New, Valenti, & Tidwel, 2010). According to Pezzato et al. (2003) reports regarding the qualitative and quantitative aspect of protein, energy requirement and the relation energy: protein, for the maximum productive response, are essential to the development of efficient diets, because the energy must be supplemented in sufficient quantities, once the protein is utilized almost exclusively to the tissue synthesis and the main energetic source should come from carbohydrates and lipids.

According to Barbieri, Bondioli, Melo, and Henriques (2016) protein is one of the most important nutrient for the aquatic organisms, being indispensable the determination of its best level in the diets in order to promote the maximum animal performance and productivity. It also leads to a better health and development, as well as fostering greater profitability in the production, without waste. According to the same authors, the deficit of this nutrient can lead to a lower performance and development of the shrimp. The excess can, besides damaging the physiology and the performance, cause environmental pollution affecting in a negative way the development of these animals. According to Gastelú, Oliveira, Brito, Galvez, and Moreira (2011), in carried out tests with *M. acanthurus*, the lipidic feed was more efficient for the ecdysis and ovarian maturation than the protein feed.

Among the main compounds in the diet there are the fats, mainly the linolenic fatty acids, cholesterol and its derivates. According to Wouters et al. (2001), it is necessary the adequate storage of the nutrients for the beginning of reproduction in shrimp, besides that, diets rich in lipids are important for the gonadal maturation. In crustacean, females can transfer up to 60% of its lipidic reserves to the eggs, which suggests that the metabolism of lipids are extremely dependent of parental reserves. There is more and more evidence that the lipid accumulation at the ovaries comes directly from the diet (Rosa, Calado, Narciso, & Nunes, 2007; Hernández-Abad, Hernández-Hernández, & Fernández-Araiza, 2018). However, according with Ribeiro, Franceschini-Vicentini, Papa, New, and Valenti (2012), the level of the lipids in the diet can be lower if the levels of essential fatty acids are provided, affecting, among other factors, the fecundity.

In a study conducted by Cavalli, Montakan, Lavens, and Sorgeloos (2001) with *M. rosenbergii* it was reported that the total level of lipids in the ovary of the female increase as the maturation progress, being the lipids requirement for the ovarian development dependent of the immediate ingestion of this nutrient in the diet, as in general, crustaceans have the limited ability on biosynthesis of phospholipids. Xu, Ji, Castell, and O'Dor (1994) indicated that the dietary levels of EPA and DHA affected positively the fecundity and the hatchability of the eggs in *Penaeus chinensis*.

The great weight gain observed here on the ablated females submitted to the fresh feed can be explained by a decrease of the inhibitor factor of feeding, leading to changes in the dietary pattern and accumulation of reserves, accelerating the metabolic rate and increasing the feed intake (Santos & Pinheiro, 2000; Cunha & Oshiro, 2010; Pillai et al., 2010; Shailender, Amarnath, Kishor, & Babu, 2013; Bastos et al., 2018).

The results obtained for the interval between spawning in the present study corroborate with the ones carried out by Santos and Pinheiro (2000) and Bastos et al. (2018), that also stated that the unilateral ablation technic was an efficient treatment to make shorter the time between spawning. Studies performed by Browdy and Samocha (1985), also mentioned that the unilateral ablation is the best way to promote the gonadal maturation by endocrine imbalance.

According to Da Silva et al. (2004), there is a strong relation between the fecundity and the size of the female shrimp of the genus *Macrobrachium*. This relation was highlighted by many authors to *M. amazonicum* (Scaico, 1992; Da Silva et al., 2004; Lucena-Frédou, Rosa Filho, Silva, & Azevedo, 2010; Lima et al., 2014; Bastos et al., 2018; Pantaleão et al., 2018), corroborating with the results of this study.

Conclusion

The supplying of the fresh feed had a significant influence on the increase of produced eggs, while the unilateral ablation in the conditions tested in this study, did not influence the *M. amazonicum* fecundity. Thus, a balanced diet may be more efficient to increase the reproductive performance of *M. amazonicum* than the ablation technique.

The ablation decreases the spawning interval, and the interaction of this condition with the feed resulted in a higher weight gain of the females. However, more studies need to be done to evaluate the effects of ablation in different types of feed, notably regarding the quality of the eggs produced and larvae, aiming at increasing the productivity of this shrimp in captivity.

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