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# Unilateral eyestalk ablation improves molting frequency and reproduction in *Macrobrachium amazonicum* females

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### Unilateral eyestalk ablation improves molting frequency and reproduction in *Macrobrachium amazonicum* females

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#### ABSTRACT

This study has investigated the influence of unilateral eyestalk ablation on the survival, molting frequency, fecundity, reproductive output, and fertility of *M. amazonicum* females. We divided 96 females into with ablation and without ablation groups with six replicates. Water quality, growth, survival rate, intermolt period, molts number, and ovigerous females over a 90-day period were monitored. Unilateral eyestalk ablation reduced intermolt period by 30% and increased molts number, ovigerous females, and reproductive output. Our findings indicate that unilateral eyestalk ablation positively influences reproduction in *M. amazonicum* and is appropriate for females of this species having a body length of  $\geq$  70 mm.

**KEYWORDS** 

Ablation; aquaculture; freshwater prawn; molt

### Introduction

Global aquaculture production of the genus *Macrobrachium* Bate, 1868 is increasing rapidly (Silva, Jacobucci, and Mossolin 2017). However, intensive culture technologies for the native Amazonian prawn (*Macrobrachium amazonicum* Heller, 1862) are lacking (Marques and Moraes-Valenti 2012). This species constitutes an important source of protein and income for riverside communities in the Amazon region. Currently, the volume of individuals captured surpasses the renewal capacity of wild populations (Silva, Frédou-Lucena, and Rosa Filho 2007). Therefore, commercial cultivation of *M. amazonicum* represents a means of reducing pressure on stocks and ensuring adequate market supply.

Aquaculture production of *M. amazonicum* depends on the availability of postlarvae. In the Amazon region, this is limited by low availability of ovigerous females throughout the year because breeding occurs only during the dry season (Freire, Marques, and Silva 2012). Low female fertility is an additional limiting factor—females of this species produce up to 5,000 eggs per clutch, whereas those of larger species such as *M. rosenbergii* (Malaysian

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prawn) and *M. carcinus* (pitú) can produce 50,000 eggs per clutch and 240,000 eggs per clutch respectively (Lara and Wehrtmann 2009; Lima et al. 2014). Therefore, it is necessary to increase the production and productivity of *M. amazonicum* by reducing the period of reproductive development.

The reproduction of decapod crustaceans may be induced by eyestalk ablation, which involves removal of the X-organ and sinus gland (Santiago 1977). By removing the organs that synthesize gonad-inhibiting and molt-inhibiting hormones, eyestalk ablation leads to induction of gonadal maturation, reduction of the spawning cycle, an increase in the number of molting cycles, and a reduction in the intermolt period (Babu, Shailender, and Krishna 2013; Nagaraju 2011; Pervaiz, Jhon, and Sikdar-Bar 2011); hence, the technique is of economic benefit to producers. Since the 1990s, eyestalk ablation has been widely used in the cultivation of marine shrimp, particularly species having limited reproduction in captivity.

The eyestalk ablation has been used to induce gonadal maturation, stimulate the ovigerous state, increase the spawning frequency, and reduce the intermolt period in *Macrobrachium* species (Asusena et al. 2012; Cunha and Oshiro 2010; Pervaiz, Jhon, and Sikdar-Bar 2011; Santos and Pinheiro 2000). To the best of our knowledge, however, there are no previous studies regarding the use of eyestalk ablation in *M. amazonicum*. Thus, we investigated the influence of unilateral eyestalk ablation on the survival, growth, molting frequency, fecundity, reproductive output, and fertility of *M. amazonicum* females. We predicted that unilateral eyestalk ablation would induce sexual maturation and shorten the reproductive cycle.

### **Materials and methods**

### Animals and experimental conditions

We conducted our experiment in the Larviculture Laboratory of Embrapa Amapá (0°0'50.07"S and 51°5"8.58"W), Macapá, Amapá state (Brazil). We used 24 males of the Cinnamon Claw morphotype and 96 *M. amazonicum* females in the intermolt period. We selected individuals by inspecting the outer distal margin of the exopod of the uropod, as suggested by Drach and Tchernigovtzeff (1967). The animals were cultured in 12 black tanks (70 L) using a recirculation system with biological filtration and constant aeration. In each tank, we established a female:male ratio of 4:1. The animals were fed twice daily with commercial pelleted prawn feed (35% crude protein; daily supply of 10% of the biomass in each tank) for 90 days. We observed each female twice daily (08:00 and 17:00) and recorded the occurrence of molting and number of ovigerous females. Females with mature gonads were characterized by an orange color that was macroscopically visible through the

carapace. In these females, copulation generally occurred 24-48 h after molting.

We monitored temperature (27.06  $\pm$  0.31°C), pH (7.51  $\pm$  0.09), electrical conductivity (0.519  $\pm$  0.003 µS cm<sup>-1</sup>), and dissolved oxygen (7.17  $\pm$  0.01 mg L<sup>-1</sup>) of water daily using a multiparameter gauge (Horiba, Model U-52G, Tokyo, Japan). We measured ammonia levels (0.12  $\pm$  0.07 mg L<sup>-1</sup>) every 3 days using a photometer (Hanna, model HI 96715C, Romania).

### Unilateral eyestalk ablation of M. amazonicum females

We performed unilateral eyestalk ablation on 3-month-old *M. amazonicum* females (body length, 74.0  $\pm$  4.3 mm; body weight, 3.63  $\pm$  0.65 g). The ablation procedure comprised removal of the right peduncle following Primavera (1985), with minor modifications. These modifications involved the use of an anesthetic (xylocaine), followed by hot cauterization with an electric cautery. Each female was subsequently marked to facilitate assessment of the number of molts. The females were divided into two groups: those with unilateral eyestalk ablation (treatment group) and those without unilateral eyestalk ablation (control group). We used six treatment and six control replicates, each with eight females and two males. The males did not undergo unilateral eyestalk ablation. In each tank, we placed 10 segments of PVC pipe (diameter 40 mm; length 100 mm) to provide shelter. We used a recirculation aquaculture system with adequate aeration, a biofilter, and a 12 h photoperiod. We measured the body weight (g) and body length (mm) of each female every 30 days after ablation. In addition, we measured the body length and body weight of each female before and after each molt. As each animal had been marked with one color, after every molt, it was possible to identify which animal had undergone molt. The body weight was measured using a semianalytical scale (Marte, model BL 320H), and the body standard length (from the end of the rostrum to the end of the telson) was measured using a digital pachymeter (Starrett). We conducted our study in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA).

### Growth and biometric parameters of *M. amazonicum* females

Growth was calculated as a mass-length relationship based on the body length and body weight measurements of each live animal at the start and end of the experiment using the equation  $W = aL^b$ , where W is the total weight (g), L is the standard length (mm), and a and b are constants estimated using linear regression of the transformed equation as follows:  $W = loga + b \ge logL$ . We used the t-test to evaluate whether b = 3 (Le Cren 1951). Based on these data, we determined the relative condition factor (Kn) as the total weight observed  $(W_t)$  divided by the expected weight  $(W_e)$ .

By daily inspection of all animals, we assessed the number of molts by recording the intermolt period and the number of ovigerous females. All animals were marked with 10 assorted color ethylene-vinyl acetate sheets clippings (3 mm x 3 mm) that were fixed on its second abdominal segment (by use of instant glue). There was care to secure the animal's movement. We used the Pearson correlation coefficient (r) to determine the correlations between body length and body weight before and after each molt, between body length and intermolt period, between body length and animal fecundity, and between body length and fertility.

### Fecundity, reproductive output, and fertility of M. amazonicum females

We determined the fecundity by counting the number of eggs extracted from 20 ablated ovigerous females and 11 nonablated ovigerous females. After biometry, all females were returned to the source tanks. We calculated the reproductive output (RO) for each female using the equation described by Clarke, Hopkins, and Nilssen (1991): RO = WO/W, where WO = the total wet weight of eggs and W = the wet mass of the same female without eggs. We determined the fertility by using a different group of 14 ablated females and 13 nonablated females. These females were isolated in 2 L hatching tanks after biometry, maintained in the hatching tanks until larval hatching, and then returned to the original tanks. We determined the fertility by collecting 20 samples and calculating the arithmetic mean of the number of larvae present in 2 mL of culture water. The means were then extrapolated to the total volume of the container containing the larvae.

### Statistical analyses

Data were initially evaluated for normality and homoscedasticity pattern using the Shapiro-Wilk and Bartlett tests respectively. When data followed a normal and homoscedastic distribution, we used the *t*-test to compare treatments (ablated and nonablated) (Zar 2010). All statistical analyses were performed using R software (R Core Team 2013).

### Results

## Influence of unilateral eyestalk ablation on growth of *M. amazonicum* females

The mean body length (80.5 ± 2.8 mm vs. 78.5 ± 4.8 mm; t = -1.12, P = 0.26) and mean body weight (5.2 ± 0.4 g vs. 5.7 ± 0.6 g; t = -0.121, P = 0.22) did

not differed significantly between ablated and nonablated females. Similarly, the numerical increases in body length and body weight did not differ significantly between ablated and nonablated females (7.2  $\pm$  1.2 mm vs. 6.9  $\pm$  2.6 mm; t = 0.728, P = 0.47). However, the results revealed that the body weight increase was 13% higher in ablated females than in nonablated females (t = 5.52, P = 0.04). The estimated daily growth rates did not differ significantly between ablated and nonablated females ( $0.02 \pm 0.006$  mm vs. 0.019  $\pm$  0.006 mm; t = 1.03, P = 0.32).

The equations describing the body weight (W) and body length (L) of ablated and nonablated females show similar negative allometric growth in the treatment and control groups. Thus, there was a greater increase in body weight than in body length; moreover, the ablation procedure did not influence the type of growth.

We found no significant differences in the mean relative condition factor (Kn) between ablated and nonablated females (0.99  $\pm$  0.02 vs. 1.00  $\pm$  0.02; t = -0.260, P = 0.79). Similarly, the standard value of Kn (1.00) did not differ significantly between the treatment and control groups. Thus, the ablation procedure did not influence the body condition of the experimental animals.

### Influence of unilateral eyestalk ablation on number of molts and intermolt period in *M. amazonicum* females

We observed 354 molts during the experiment—200 in ablated females and 154 in nonablated females. The mean number of molts per female differed significantly between ablated females and nonablated females  $(4.2 \pm 2.2 \text{ vs. } 3.20 \pm 1.4; t = 2.59, P = 0.01)$  (Figure 1A). In the 60-70 mm body length class, we observed 133 (67%) molts in ablated females and 97 (63%) molts in nonablated females. The mean intermolt period differed significantly between the control and treatment groups (t = -7.09, P < 0.001) and was approximately 6 days shorter in ablated females than in nonablated females (Figure 1B). In ablated females, the intermolt period during the first 30 days of cultivation did not differ between the length classes; thereafter, the intermolt period increased with increasing body length (Table 1). In contrast, in nonablated females, the intermolt period increased with increasing body length but did not differ between the different length classes. The intermolt period was positively correlated with body length (r = 0.97, P = 0.001) and body weight (r = 0.87, P = 0.001) in both ablated and nonablated females.



**Figure 1.** Influence of ablation on molting frequency in *Macrobrachium amazonicum* females. Ablated and nonablated females were cultivated for 90 days. (A) number of molts; (B) intermolt period. The bars represent the mean, and the error bars represent SD. Bars superscripted by different letters differ significantly according to the *t*-test (P < 0.05).

### Influence of unilateral eyestalk ablation on reproductive output, fecundity, and fertility in *M. amazonicum* females

The frequency of occurrence of ablated ovigerous females was higher than the number of nonablated ovigerous females  $(1.22 \pm 0.97 \text{ vs. } 0.83 \pm 0.97; t = 1.99, P = 0.04)$  (Figure 2).

The reproductive output was positively correlated with the body length in ablated and nonablated females (r = 0.8213, P < 0.001 and r = 0.8864, P < 0.001 respectively); thus, the reproductive output is influenced by the body length and increases as the animals grow (Figure 3A). In addition, the reproductive output was significantly higher in ablated females than in nonablated females (6.17 ± 2.06 vs. 4.12 ± 0.95; t = 4.07, P < 0.001); thus, the ablation procedure positively influenced the reproductive output of *M. amazonicum* females (Figure 3B).

	Intermolt period						
Length class	30 days		60 days		90 days		
(mm)	Treatment	Control	Treatment	Control	Treatment	Control	
60–65	$14.0 \pm 0.7aA$	$14.0 \pm 0.3aA$	—	—	—	_	
	(n = 7)	(n = 4)					
66–70	$14.3 \pm 1.2aA$	$18.5\pm3.2bB$	$12.7 \pm 2.1aA$	15.8 ± 1.1bA	$10.0 \pm 1.2aA$	18.5 ± 1.1bA	
	(n = 11)	(n = 9)	(n = 8)	(n = 8)	(n = 3)	(n = 1)	
71–75	$13.6 \pm 2.3aA$	$15.2 \pm 0.7 bA$	$12.4 \pm 3.5aA$	$18.2\pm0.4bB$	$11.7 \pm 0.5aA$	17.0 ± 2.2bA	
	(n = 12)	(n = 10)	(n = 10)	(n = 7)	(n = 5)	(n = 5)	
76–80	$13.2 \pm 2.7aA$	$17.0 \pm 1.4 \text{bB}$	$10.5 \pm 2.1aA$	$19.3 \pm 0.4 \text{bB}$	$14.0\pm1.0aB$	18.1 ± 1.0bA	
	(n = 7)	(n = 11)	(n = 10)	(n = 7)	(n = 8)	(n = 12)	
81–85	14.7 ± 2.4aA	18.7 ± 3.9bB	$11.7 \pm 1.7 aA$	$21.0\pm0.8bB$	$16.8 \pm 0.4aB$	19.0 ± 1.2bA	
	(n = 6)	(n = 6)	(n = 8)	(n = 10)	(n = 4)	(n = 10)	
86–90	$15.0 \pm 3.0aA$	$18.5 \pm 1.7 \text{bB}$	$12.7 \pm 2.4aA$	$23.0\pm0.4bB$	$18.5 \pm 0.5aA$	$21.8 \pm 0.8 \text{bB}$	
	(n = 3)	(n = 4)	(n = 4)	(n = 3)	(n = 4)	(n = 5)	
91–95	15.0	—	$15.7 \pm 4.1B$	25.0bB	—	—	
	(n = 1)		(n = 2)	(n = 3)			
Average	13.9 ± 7.5a	17.5 ± 3.0b	12.7 ± 3.1a	19.2 ± 3.0b	13.6 ± 2.6a	18.9 ± 1.8b	

 Table 1. Length of the intermolt period in different body length classes after cultivation for

 30 days, 60 days, and 90 days in ablated and nonablated Macrobrachium amazonicum females.

All data are expressed as mean  $\pm$  SD. Means followed by the same letter do not differ significantly according to the *t*-test (P > 0.05). Upper and lower case letters represent within and between column tests respectively. Dashes denote no females in this class.



**Figure 2.** Influence of ablation on the number of ovigerous *Macrobrachium amazonicum* females. Ablated and nonablated females were cultivated for 90 days. Bars superscripted by the same letter did not differ significantly according to the *t*-test (P < 0.05).

We found no difference in fecundity between ablated and nonablated females (792.3  $\pm$  276.9 vs. 850.44  $\pm$  199.78; t = 0.644, P = 0.522). On the other hand, we determined a positive correlation between body length and number of eggs produced in both ablated and nonablated females (r = 0.95, P = 0.01 and r = 0.99, P < 0.01 respectively); thus, regardless of the ablation procedure, fecundity increased as females grew longer (Figure 4).

We observed no significant differences in fertility between ablated and nonablated females (662.1  $\pm$  205 vs. 859.8  $\pm$  237; t = 5.7, P = 0.52). However,



**Figure 3.** Influence of ablation on reproductive output in *Macrobrachium amazonicum* females. Ablated and nonablated females were cultivated for 90 days. (A) Correlation between reproductive output and body length; (B) mean reproductive output. Dotted line for ablated and dashed line for nonablated females. The bars represent the mean, and the error bars represent SD. Means followed by the same letter did not differ significantly according to the *t*-test (P > 0.05).

we found positive correlations between body length and number of larvae hatched in both ablated and nonablated females (r = 0.85, P = 0.001 and r = 0.8, P = 0.01 respectively). Thus, regardless of the ablation procedure, fertility increased as females became longer.

### Influence of unilateral eyestalk ablation on survival of M. *amazonicum* females

At the end of the experiment, we recorded the death of 20 ablated and 10 nonablated females (Table 2). In ablated females, 70% of the observed mortality



**Figure 4.** Influence of ablation on the relationship between fecundity and body length in *Macrobrachium amazonicum* females. Ablated and nonablated females were cultivated for 90 days. The correlations are based on 26 ablated females and 16 nonablated females. Dotted line for ablated and dashed line for nonablated females.

 
 Table 2. Percentage of dead individuals in different body length classes in ablated and nonablated Macrobrachium amazonicum females.

	Dead individuals					
Length class (mm)	Ablated $(n = 20)$	%	Nonablated ( $n = 10$ )	%		
60–65	8	40	1	10		
66–70	2	10	2	20		
71–75	4	20	4	40		
76–80	1	5	1	10		
81–85	3	15	2	20		
86–90	1	5	0	0		
91–95	1	5	0	0		

after molting occurred among individuals with a body length of 60–75 mm, and the highest mortality rate was recorded after the third molt. In contrast, in nonablated females, we noted no specific period of high mortality.

### Discussion

Throughout the present study, the water quality parameters remained within the recommended standards for cultivation of *M. amazonicum* (Aya-Baquero & Velasco-Santamaría 2013; Moraes-Riodades and Valenti 2001; Preto et al. 2011). Thus, the management and recirculation aquaculture system used efficiently maintained the water quality. We did not control the temperature during the experiment; hence, the minimal variation in this parameter may be explained by the proximity of the study region to the equator (Souza and Cunha 2010). Silva, Sampaio, and Santos (2004) reported that the temperature of natural environments influences the frequency of occurrence of ovigerous *M. amazonicum* females. We observed no significant temperature differences between the control and treatment groups; thus, the observed differences in reproductive behavior of *M. amazonicum* females may be derived from the ablation technique.

Ablated and nonablated *M. amazonicum* females showed negative allometric growth. These results are in accordance with those previously reported for this species captured in the estuarine region of the eastern Amazon (Moraes-Riodades and Valenti 2002; Silva, Frédou-Lucena, and Rosa Filho 2007; Sousa, Bentes, and Martinelli-Lemos 2014). In our present study, ablated females had negative allometric growth throughout the cultivation period and underwent a larger number of molts. However, the growth of ablated and nonablated *M. amazonicum* females was lower than previously reported by Lobão, Rojas, and Valenti (1986) for this same species.

The body weight to body length ratio is an important tool used in fishery biology to assess the relative condition factor of a population (Deekae and Abowei 2010). The relative condition factor is a quantitative indicator of the body condition in response to food, reproduction, and environmental factors (Deekae and Abowei 2010; Le Cren 1951). In our present study, the relative condition factor in ablated and nonablated M. amazonicum females was close to the reference Kr value of 1.00) (Le Cren 1951). The increases in body weight and body length were approximately 13% and 1% higher respectively in ablated females than in nonablated females; however, the relative condition factor did not differ significantly between the treatment and control groups. Increased body weight and body length of ablated females may be explained by a decrease in the feed-inhibitory factor, leading to changes in dietary patterns and accumulation of reserves. These changes frequently occur in crustaceans subjected to ablation-for example, Penaeus orientalis (Arnstein and Beard 1975), P. monodon (Santiago 1977; Shailender et al. 2013), M. rosenbergii (Santos and Pinheiro 2000; Shailender et al. 2013), M. malcolmsonii (Soundarapandian 2008), M. lanchesteri (Varalakshmi and Reddy 2010), and M. acanthurus (Cunha and Oshiro 2010). Thus, ablation may accelerate the metabolic rate and increase food consumption. The physiological processes involved in molting are regulated by hormonal interactions, and hormonal regulation depends on different stimuli in the central nervous system.

In general, the length of the crustacean molting cycle increases as growth occurs (Asusena et al. 2012; Lobão, Rojas, and Valenti 1986; Santos and Pinheiro 2000). Reproduction (consisting of copulation and spawning) in *Macrobrachium* species and other caridean prawns is preceded by molting. These interrelated processes of reproduction and molting generate considerable energy demand (Hartnoll and Bryant 1990). Several studies have shown that unilateral eyestalk ablation influences not only the molting cycle but also the reproductive cycle of crustaceans. Gonad-inhibiting and molt-inhibiting hormones are associated with the eyestalk. Therefore, eyestalk removal can

be used to stimulate the first spawning, increase the number of molts and consecutive spawns, decrease the intermolt and interspawn periods, and increase the gonadosomatic indexes and size of the androgenic gland (Okumura and Aida 2001; Santos and Pinheiro 2000; Uawisetwathana et al. 2011; Varalakshmi and Reddy 2010). In this current study, unilateral eyestalk ablation led to a 30% increase in the number of ovigerous females and a 13% increase in the number of molts. However, in species such as *M. americanum*, which has asynchronous molting and reproduction, these effects have been shown not to occur (Asusena et al. 2012). In the present study, unilateral eyestalk ablation reduced the intermolt period in *M. amazonicum* females by approximately 6 days.

We determined no significant differences in fecundity and fertility rates between ablated and nonablated M. amazonicum females. However, within the 71–75 mm body length class, ablated females produced more eggs than nonablated females. Tan-Fermin (1991) used histological techniques to demonstrate that unilateral eyestalk ablation influences the number and size of oocytes in ovaries of P. monodon females. Varalakshmi and Reddy (2010) reported higher productivity in ablated females than in nonablated females. The mean fertility determined for ablated and nonablated M. ama*zonicum* females (662  $\pm$  205 and 859  $\pm$  237 respectively) in the present study are higher than those reported in several previous studies of the same species -for example, Guest (1979) observed 565 larvae/female, while Scaico (1992) found 434 larvae/female, and Ava-Baquero & Velasco-Santamaría (2013) observed 287 larvae/female). On the other hand, the values observed in the present study are lower than the value reported by Silva, Sampaio, and Santos (2004), who obtained 925 larvae/female. These previous studies were conducted in different regions; hence, the observed discrepancies may be derived from environmental variations. Alternatively, they may be explained by low fecundity in females of the same body size (Scaico 1992).

The reproductive output of ablated *M. amazonicum* females was higher than that of nonablated females. The reproductive output values determined in the present study are in accordance with the findings reported for *M. amazonicum* in the state of Pará (RO = 0.04-0.21; Meireles, Valenti, and Mantelatto 2013) and in the state of Amapá (RO = 0.05-0.22)(Lima et al. 2014), as well as for *M. carcinus* in Costa Rica (RO = 0.04-0.21; Lara and Wehrtmann 2009). Our present findings imply that unilateral eyestalk ablation is an efficient technique for increasing the reproductive output of *M. amazonicum* females.

The fecundity and fertility of *M. amazonicum* increased with increasing body length, regardless of the ablation procedure. These results are in accordance with those previously reported for populations in natural environments (Lobão, Rojas, and Valenti 1986; Scaico 1992; Aya-Baquero & Velasco-Santamaría 2013).

The survival of prawns cultured in captivity may be reduced by increased aggression among adult animals during the molting cycle (Hayd, Anger, and Valenti 2008). Therefore, the use of substrates to provide shelter after molting is essential (Nakayama, Wasielesky, and Cavalli 2009; Ward 1992). In the present study, we provided shelters for both the treatment and control groups; hence, the differences in survival rates between ablated and nonablated females may have been derived from other factors—for example, female condition after each molt or competition. Thus, we found no evidence suggesting that the ablation procedure directly influenced survival (e.g., via infection or weakening).

The influence of the ablation on survival may be immediate or long-term (Lumare 1979; Pervaiz, Jhon, and Sikdar-Bar 2011; Primavera 1985; Varalakshmi and Reddy 2010; Wen et al. 2015). In some cases, mortality occurs immediately after ablation owing to the loss of hemolymph or presence of infection (Santos and Pinheiro 2000). In some species such as P. monodon (Vicente, Valdez, and Valdez 1979), P. plebejus (Kelemec and Smith 1980), and M. americanum (Asusena et al. 2012), ablation does not influence survival. Other probable causes of mortality in females include environmental handling nutritional deficiency, stresses, spawning, (Primavera 1985; Pudadera, Primavera, and Young 1980), and the presence of pathogens in the culture (Chen and Chen 2003). In our study, the survival rate was higher in nonablated females than in ablated females. However, the observed differences in survival rate were not statistically significant (P = 0.12). In general, deaths occurred after molts, when the animals were more susceptible to attack by other individuals. No pathogens were observed in the culture, and no deaths occurred in immediate response to the ablation. Therefore, we do not believe that the mortality was caused by the ablation procedure itself. However, it is still possible that it indirectly influenced survival via physiological changes.

### Conclusions

We have shown that the number of molts, length of the intermolt period, number of ovigerous females, and reproductive output of *M. amazonicum* are positively influenced by unilateral eyestalk ablation. However, for efficacy of this technique, the females of *M. amazonicum* must be  $\geq$  3 months old and have present body weight of  $\geq$  5 g and length of  $\geq$  70 mm. Therefore, we propose unilateral eyestalk ablation as a technique for increasing the production of *M. amazonicum* in aquaculture.

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#### **Disclosure statement**

No potential conflict of interest was reported by the authors.

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#### References

- Arnstein, D. R., and T. W. Beard. 1975. Induced maturation of the prawn *Penaeus orientalis Kishinouye* in the laboratory by means of eyestalk removal. *Aquaculture* 5:411–12. doi:10.1016/0044-8486(75)90060-5.
- Asusena, A. C. J., S. H. J. Carlos, F. C. J. Arturo, and D. P. Genaro. 2012. The effects of eyestalk ablation on the reproductive and immune function of female *Macrobrachium americanum*. *Journal of Aquaculture Research & Development* 3:156. doi:10.4172/2155-9546.1000156.
- Aya-Baquero, E., and Y. Velasco-Santamaría. 2013. Fecundidad y fertilidad de Macrobrachium amazonicum (Heller, 1862) (Decápoda, Palaemonidae) del Piedemonte Llanero Colombiano. Revista MVZ Crdoba 18:3773–80. doi:10.21897/rmvz.147.
- Babu, S. C., M. Shailender, and P. Krishna. 2013. Comparative study of spawning success in the giant freshwater prawn, *Macrobrachium rosenbergii* broodstock by unilateral and bilateral eyestalk ablation methods. *International Research Journal of Biological Sciences* 3:88–93.
- Chen, S. M., and J. C. Chen. 2003. Effects of pH on survival, growth, molting and feeding of giant freshwater prawn . *Macrobrachium rosenbergii*.*Aquaculture* 218:613–23. doi:10.1016/S0044-8486(02)00265-X.
- Clarke, A., C. Hopkins, and M. Nilssen. 1991. Egg size and reproductive output in the deepwater prawn *Pandalus borealis* Krøyer, 1838. *Functional Ecology* 5:724–30. doi:10.2307/2389534.
- Core Team, R., 2013. R Core Team. R A Lang. Environ. Stat. Comput. R Found. Stat. Comput. Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

- 350 👄 A. M. BASTOS ET AL.
- Cunha, C. H., and L. M. Y. Oshiro. 2010. The influence of eyestalk ablation on the reproduction of the freshwater *Macrobrachium acanthurus* shrimp in captivity. *Acta Scientiarum. Biological Sciences* 32:217–21. doi:10.4025/actascibiolsci.v32i3.3911.
- Deekae, S. N., and J. S. N. Abowei. 2010. *Macrobrachium macrobrachion* (Herklots, 1851) length-weight relationship and Fulton's condition factor in Luubara creek, Ogoni Land, Niger Delta, Nigeria. *International Journal of Animal and Veterinary Advances* 2:155–62.
- Drach, P., and C. Tchernigovtzeff. 1967. Sur la méthode de détermination des stades d'intermue et son application générale aux crustacés. *Vie Milieu* 18:595–610.
- Freire, J. L., C. B. Marques, and B. B. Silva. 2012. Estrutura populacional e biologia reprodutiva do camarão-da-Amazônia *Macrobrachium amazonicum* (Heller, 1862) (Decapoda: Palaemonidae) em um estuário da região nordeste do Pará, Brasil. *Brazilian Journal of Aquatic Science and Technology* 16:65–76. doi:10.14210/bjast.v16n2.p65-76.
- Guest, W. C. 1979. Laboratory life history of the palaemonid shrimp *Macrobrachium ama*zonicum (Heller) (Decapoda, Palaemonidae). Crustaceana 37:141–52. doi:10.1163/ 156854079X00979.
- Hartnoll, R. G., and A. D. Bryant. 1990. Size-frequency distributions in decapod Crustacea: The quick, the dead, and the cast-offs. *Journal of Crustacean Biology* 10:14–19. doi:10.2307/1548665.
- Hayd, A. L., K. Anger, and W. C. Valenti. 2008. The moulting cycle of larval Amazon river prawn *Macrobrachium amazonicum* reared in the laboratory. *Nauplius* 16:55–63.
- Kelemec, J. A., and I. R. Smith. 1980. Induced ovarian development and spawning of *Penaeus plebejus* in a recirculating laboratory tank after unilateral eyestalk enucleation. *Aquaculture* 21:55–62. doi:10.1016/0044-8486(80)90125-8.
- Lara, L. R., and I. S. Wehrtmann. 2009. Reproductive biology of the freshwater shrimp Macrobrachium carcinus (L.) (Decapoda: Palaemonidae) from Costa Rica, Central America. Journal of Crustacean Biology 29:343–49. doi:10.1651/08-3109.1.
- Le Cren, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and conditions in the perch (*Perca fluviatilis*). *Journal of Animal Ecology* 20:201–19. doi:10.2307/1540.
- Lima, J. F., L. A. M. Silva, T. C. Silva, J. S. Garcia, I. S. Pereira, and K. D. S. Amaral. 2014. Reproductive aspects of *Macrobrachium amazonicum* (Decapoda: Palaemonidae) in the State of Amapá, Amazon river mouth. *Acta Amazonica* 44:245–54. doi:10.1590/S0044-59672014000200010.
- Lobão, V. L., N. E. T. Rojas, and W. C. Valenti. 1986. Fecundidade e fertilidade de Macrobrachium amazonicum (Heller, 1862) (Crustacea, Decapoda) em laboratório. Boletim Do Instituto De Pesca 13:15-20.
- Lumare, F. 1979. Reproduction of *Peneaus kerathurus* using eyestalk ablation. *Aquaculture* 18:203–14. doi:10.1016/0044-8486(79)90012-7.
- Marques, H. L. A., and P. M. C. Moraes-Valenti. 2012. Current status and prospects of farming the giant river prawn (*Macrobrachium rosenbergii* (De Man 1879) and the Amazon river prawn *Macrobrachium amazonicum* (Heller 1862) in Brazil. *Aquaculture Research* 43:984–92. doi:10.1111/j.1365-2109.2011.03032.x.
- Meireles, A. L., W. C. Valenti, and F. L. Mantelatto. 2013. Reproductive variability of the Amazon river prawn, *Macrobrachium amazonicum* (Caridea, Palaemonidae): Influence of life cycle on egg production. *Latin American Journal of Aquatic Research* 41:718–31. doi:10.3856/vol41-issue4-fulltext-8.
- Moraes-Riodades, P. M. C., and W. C. Valenti. 2001. Freshwater prawn farming in Brazilian Amazonia shows potential for economic, social development. *Global Aquaculture Advocate* 4:73–74.

- Moraes-Riodades, P. M. C., and W. C. Valenti. 2002. Crescimento relativo do camarão canela Macrobrachium amazonicum (Heller) (Crustacea, Decapoda, Palaemonidae) em viveiros. Revista Brasileira De Zoologia 19:1181–214. doi:10.1590/S0101-81752002000400023.
- Nagaraju, G. P. C. 2011. Reproductive regulators in decapod crustaceans: An overview. *Journal of Experimental Biology* 214:3-16. doi:10.1242/jeb.047183.
- Nakayama, C. L., J. W. Wasielesky, and R. O. Cavalli. 2009. Avaliação do desempenho reprodutivo do camarão-rosa Farfantepenaeus paulensis (Pérez-Farfante, 1967) em tanques com diferentes profundidades de água. Boletim Do Instituto De Pesca 35:83–89.
- Okumura, T., and K. Aida. 2001. Effects of bilateral eyestalk ablation on molting and ovarian development in the giant freshwater prawn. *Macrobrachium Rosenbergii. Fisheries Science* 67:1125–35. doi:10.1046/j.1444-2906.2001.00370.x.
- Pervaiz, P. A., S. M. Jhon, and M. Sikdar-Bar. 2011. Studies on the effect of unilateral eyestalk ablation in maturation of gonads of a freshwater prawn *Macrobrachium dayanum*. World Journal of Zoology 6:159–63.
- Preto, B. L., J. M. Kimpara, P. Moraes-Valenti, F. R. T. Rosa, and W. C. Valenti. 2011. Production strategies for short term grow-out of the Amazon river prawn *Macrobrachium amazonicum* (Heller 1862) in ponds. *Pan-American Journal of Aquatic Sciences* 6:1–8.
- Primavera, J. H. 1985. A review of maturation and reproduction in closed thelycum penaeids. In Proceedings of the First International Conference on the Culture of Penaeid Prawns/ Shrimps, 4-7 December 1984, Y. Taki, J. H. Primavera, and J. A. Llobrera. Eds., 47–64. Iloilo City, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.
- Pudadera, R. A., J. H. Primavera, and A. T. G. Young. 1980. Effects of different sex ratios on maturation, fecundity and hatching rates of ablated *Penaeus monodon* wild stock. *Fisheries Research Journal of the Philippines* 5:1–6.
- Santiago, A. C. Jr. 1977. Successful spawning of cultured *Peneaus monodon* Fabricius after eyestalk ablation. *Aquaculture* 11:185–96. doi:10.1016/0044-8486(77)90111-9.
- Santos, M. J. M., and M. A. A. Pinheiro. 2000. Ablação ocular no camarão Macrobrachium rosenbergii (De Man) (Crustacea, Decapoda, Palaemonidae): Efeitos sobre a reprodução, pigmentação epidérmica e atividade alimentar. Revista Brasileira De Zoologia 17:667–80. doi:10.1590/S0101-81752000000300012.
- Scaico, M. A. 1992. Fecundidade e fertilidade de *Macrobrachium amazonicum* (Crustacea: Decapoda) de um açude do nordeste brasileiro. *Boletim Do Instituto De Pesca* 19:89–96.
- Shailender, M., D. Amarnath, B. Kishor, and S. Babu. 2013. Effect of unilateral eyestalk ablation in reproductive cycle of *Penaeus monodon* (Fabricius) after spawning under laboratory conditions. *International Journal of Chemical and Life Sciences* 2:1121–25.
- Silva, M., F. Frédou-Lucena, and J. Rosa Filho. 2007. Estudo do crescimento do camarão Macrobrachium amazonicum (Heller, 1862) da ilha de Combú, Belém, estado do Pará. Amazônia: Ciência & Desenvolvimento 2:85-104.
- Silva, R. C., G. B. Jacobucci, and E. C. Mossolin. 2017. Reproductive biology of Macrobrachium amazonicum (Heller, 1862) (Decapoda: Palaemonidae) in a reservoir situated in Minas Gerais State, southeastern Brazil. *Latin American Journal of Aquatic Research* 45(4):776–86. doi:10.3856/vol45-issue4-fulltext-13.
- Silva, R. R., C. M. S. Sampaio, and J. A. Santos. 2004. Fecundity and fertility of Macrobrachium amazonicum (Crustacea, Palaemonidae). Brazilian Journal of Biology 64:489–500. doi:10.1590/S1519-69842004000300012.
- Soundarapandian, P. 2008. Effect of unilateral eyestalk ablation and diets on the growth of freshwater prawn juveniles of *Macrobrachium malcolmsonii* (H. Milne Edwards). *Journal of Fisheries and Aquatic Science* 3:47–53. doi:10.3923/jfas.2008.47.53.

- 352 👄 A. M. BASTOS ET AL.
- Sousa, P., B. Bentes, and J. M. Martinelli-Lemos. 2014. Population structure and relative growth of the Amazon shrimp *Macrobrachium amazonicum* (Heller, 1862) (Decapoda: Palaemonidae) on two islands in the fluvial-estuarine plain of the Brazilian Amazon. *Nuplius* 22:13-20. doi:10.1590/S0104-64972014000100002.
- Souza, E. B., and A. C. Cunha. 2010. Climatologia de precipitação no Amapá: Mecanismos climáticos de grande escala. In *Tempo, clima e recursos hídricos: Resultados do projeto REMETAP no Estado do Amapá*, ed by A. C. Cunha, E. B. Souza, and H. F. A. Cunha, 177– 95. IEAP, Macapá.
- Tan-Fermin, J. D. 1991. Effects of unilateral eyestalk ablation on ovarian histology and oocyte size frequency of wild and pond-reared *Penaeus monodon* (Fabricius) broodstock. *Aquaculture* 93:77–86. doi:10.1016/0044-8486(91)90206-M.
- Uawisetwathana, U., R. Leelatanawit, A. Klanchui, J. Prommoon, S. Klinbunga, and N. Karoonuthaisiri. 2011. Insights into eyestalk ablation mechanism to induce ovarian maturation in the black tiger shrimp. *PLoS One* 6. doi:10.1371/journal.pone.0024427.
- Varalakshmi, K. N., and R. Reddy. 2010. Effects of eyestalk ablations on growth and ovarian maturation of the freshwater prawn *Macrobrachium lanchesteri* (de Man). *Turkish Journal* of Fisheries and Aquatic Sciences 10:403–10. doi:10.4194/trjfas.2010.0314.
- Vicente, H. J., F. M. Valdez, and L. S. Valdez. 1979. Land-based maturation and spawning of Penaeus monodon Fabricius in MSU-IFRD and its future research aspects, 115–23. Mindanao State Univ. Institute Fish Research and Development of Techical Reports, Philippines.
- Ward, J. V. 1992. Aquatic insect ecology. New York: John Wiley & Sons Inc.
- Wen, W., L. Qiu, Q. Yang, J. Huang, and F. Zhou. 2015. Effect of eyestalk ablation on ovarian maturation and spawning in green tiger shrimp *Penaeus semisulcatus* (De Haan 1844). *Indian Journal of Fisheries* 62:141–45.
- Zar, J. H. 2010. Biostatistical analysis. New Jersey, USA: Prentice Hall.