

Original Research Articles

Evaluating the culturing performance of two-skinned mud crabs (*Scylla paramamosain*) using intermolt and mature crabs in various eyestalk ablation and gender conditions

Le H. Vu¹, Ting C. Huang^{2a}, Nguyen V. Bac³, Tien H. Ly⁴, Chau T. Da⁵, Doan X. Diep^{6b}

¹ Faculty of Agriculture and Aquaculture, Bac Lieu University, Bac Lieu Province, Vietnam, ² Department of Aquaculture, National Taiwan Ocean University, Keelung City, Taiwan, ³ Faculty of Agricultural Economics, Ca Mau Community College, Ca Mau Province, Vietnam, ⁴ Bac Lieu University, Bac Lieu Province, Vietnam, ⁵ Group of Applied Research in Advanced Materials for Sustainable Development, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam, ⁶ Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam

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A thorough investigation into the effects of crustacean eyestalk ablation across different genders is crucial for optimizing aquaculture practices. This research was conducted from June to September 2023, with two separate experiments to assess how eyestalk removal and the gender of mud crabs (*Scylla paramamosain*) affected the performance of raising double-skinned crabs using intermolt and mature crabs. Both experiments utilized a completely randomized design with three replications in rectangular tanks (a bottom area of 0.3 m²). Crabs were divided into four eyestalk ablation groups (no ablation, right ablation, left ablation, and bilateral ablation) in experiment 1, while experiment 2 focused on right eyestalk ablation crabs and categorized them into three gender groups (males, females, and mixed-sex). The animals were stocked at a density of 20 individuals m⁻² and were fed trash fish at a rate of 5% of their wet body weight throughout the experiments. The results showed that eyestalk ablation did not significantly affect overall yields. However, it improved the specific growth rate (SGR), the time needed to form double-skinned crabs (duration), and the feed conversion ratio (FCR). The right ablation group showed the most notable improvements. The bilateral ablation group showed improvements in FCR but had the fewest benefits in SGR, duration, and survival rate (SR). Additionally, gender factors had a minor effect on duration, with the shortest time recorded for the female group. Both male and mixed-sex groups showed improvements in FCR but experienced a decline in SR, with a significant reduction in the male group. Based on these findings, it is recommended that right eyestalk ablation in intermolt and mature female mud crabs is the most effective strategy for producing double-skinned crabs under the conditions studied.

INTRODUCTION

Mud crabs (*Scylla spp.*) have high economic potential and are widely farmed in the Asia-Pacific region¹ because their meat contains abundant nutrients and delicate flavor.²⁻⁹ Production of mud crabs ranks third in global seafood total, after shrimp and lobster.¹⁰ Among mud crab species, *S. paramamosain* possesses rapid growth and large size.¹¹ This crab species plays a key role in commercial farming and contributes significantly to farmers' income in the Mekong Delta, Vietnam.^{12,13}

Commercial mud crab products are quite variable. One of the most valuable products is soft-shell crabs—individuals harvested within a few hours of molting with soft new shells. These crabs are popular among consumers as convenient food. They can be eaten whole without needing to remove the shell and can also be returned into various delicious dishes. However, there are challenges in the production of soft-shell mud crabs. Since mud crabs are aggressive predators, they must be kept in individual cages during farming to prevent cannibalism.¹⁴ Additionally, molting in most crustaceans is accompanied by a fasting period,¹⁵ making molting an energy-intensive process that requires a

a Corresponding author, Cheng-Ting Huang, e-mail: cthuang@email.ntou.edu.tw

b Equal contributor

Corresponding author: Doan Xuan Diep, e-mail: doanxuandiep@tdtu.edu.vn.

significant amount of nutrition and minerals; if these needs are not met, death can occur.¹⁶ Molt death syndrome has hindered the growth of the crab-farming industry, including soft-shell crabs.¹⁷

Another unique offering from mud crabs, double-shelled/double-skinned crabs, has been produced and marketed in upscale restaurants in Vietnam and several other countries, including China, the United States, and various European nations. The term “double-skinned crabs” refers to crabs just before the molting, with a new soft shell forming beneath the old one, giving them the appearance of having two layers of skin.¹⁸ While the nutritional profile of double-skinned crabs has yet to be extensively studied, it is well-established that they reach their peak texture and nutrition during this phase. This is because, before molting, crabs store significant amounts of energy and nutrients to fuel the molting process and rapid post-molt growth.^{11,19,20} As a result, double-skinned crabs have firm, plump bodies with rich, meaty flesh and minimal water content. Moreover, combining a hard outer shell and soft inner skin intensifies their distinctive flavor profile. In Vietnam, while the common mud crab price ranges from 200,000 to 250,000 VND for a kilogram, double-skinned crabs can be priced as high as 700,000 to 800,000 Vietnamese Dong for one.

The eyestalk ablation of unilateral or bilateral in crustaceans is commonly used to enhance growth, ovarian development, and spawning because the eyestalk contains a crucial neuroendocrine center responsible for producing and releasing molt and gonad-inhibiting hormones,²¹ in which eyespots are essential photoreceptors and play a significant role in various biological processes, including reproduction, growth, and development.²² However, the impacts of unilateral or bilateral eyestalk ablation on physiological responses, growth, and reproductive performance may differ meaningfully depending on the crustacean species, phases in the life cycle, and the specific procedure conditions (laboratory conditions) of the practice.^{23,24} Therefore, further research is essential to find the best approach for each production situation.

In another aspect, sexual dimorphism is widely observed in the animal kingdom, including among crustaceans.²⁵⁻²⁷ The pronounced sexual differences in morphology and physiology are referred to as secondary sexual traits,^{22,28} which enable each sex to adapt more effectively to their specific life conditions.^{29,30} The mud crab displays significant sexual dimorphism in body size, growth rate, and abdominal morphology.^{31,32} In wild mud crabs, males grow faster than females in weight, with the growth rate of males being 0.93/year, while the growth rate of females was 0.69/year.³²

Currently, the production of double-skinned crabs in the market is limited because of their burrowing behavior during molting, making them scarce to encounter in the wild.³³ Additionally, available research on cultivation techniques for these crabs is rare. As interest in double-skinned mud crab products continues to grow, their commercial production can only become viable with the development of effective cultivation techniques. Based on previous findings, this study aims to evaluate the effectiveness of culturing inter-

molt and mature mud crabs (*S. paramamosain*) during the stage when they develop double-skinned crabs under four different eyestalk ablation conditions (non-ablation, right ablation, left ablation, and bilateral ablation). Additionally, the study explores the effects of three gender conditions across three groups (males, females, and mixed-sex). This approach can help improve farming techniques, enhance yields, and expand the market for these mud crabs.

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

Intermolt and mature mud crabs, with an average weight range of 15.4–15.8 g, were collected from a local extensive mud crab farm. The crabs were then transported to the aquaculture experimental hatchery at the Community College of Ca-Mau Province in South Vietnam, using Styrofoam coolers containing water with a salinity of 25‰ and constant aeration to maintain a dissolved oxygen concentration of 5–6 mg L⁻¹. Upon arrival, the crabs were acclimated to the experimental conditions for one week in 1 m³ composite tanks with aeration. During the acclimation period, they were fed trash fish (the same feed used in both experiments) once a day. Uneaten feed and feces were removed daily at 9:00 PM to maintain water quality.

In Experiment 1, unilateral or bilateral eyestalk ablation was performed on acclimated, healthy crabs. The crabs were first cleaned with running tap water, dried, and then swabbed with 70% alcohol. Using sterilized surgical scissors, the eyestalks were carefully ablated at the base. The wounds were promptly cauterized with a blunt, red-hot needle to prevent bleeding.³⁴ Following the ablation, the crabs were divided into four groups (none ablation, right ablation, left ablation, and bilateral ablation) and placed into the aquarium, where the water was continuously aerated.

For experiment 2, the healthy, acclimated, and right eyestalk-ablated crabs were sorted into three gender groups of males, females, and mixed-sex (both males and females). The gender determination of the crabs was performed by observation of their abdominal flap shape. The male crabs had narrower and triangular abdominal flaps (V-shape); meanwhile, the abdominal flaps of the female crabs were wider, globular (U-shape), and darkened in color³⁵ (Figure 1). The crabs after eyestalk ablation were nurtured to have health stability for one week before the experimental period.

The water with 25‰ salinity used in the experiments was prepared from two water sources: brine water (80‰) and tap-fresh water (0‰). The mixed water was filtered via an activated carbon block filter core with a 1 µm diameter (H-F2510AC Model, AMI Brand, made in the USA); after that, it flowed through a UV-C lamp system before entering the containment tanks to remove heavy metals using EDTA (10 mg L⁻¹). The water after treatment was maintained at an alkalinity level of 100–120 mg CaCO₃ L⁻¹ using NaHCO₃ before entering the culture tanks.



Figure 1. Different sexes of crab. Photos taken from the present study.

a) Male crab with a V-shaped carapace, (b) female crab with a U-shaped carapace.

In both experiments, the 1 m³ round composite tanks were used as water treatment tanks, and the rectangular plastic tanks (with a bottom area of 0.3 m²) were used as culture tanks.

EXPERIMENTAL DESIGN

This study was conducted from June to September 2023 at the Aquaculture Experimental Hatchery of the Community College of Ca Mau Province in South Vietnam. It consisted of two independent indoor experiments, each with a completely randomized design and three replications. The objective was to evaluate the effectiveness of culturing intermolt and mature mud crabs (*S. paramamosain*) during the formation of double-skinned crabs in four eyestalk ablation conditions (non-ablation, right ablation, left ablation, and bilateral ablation), along with three gender conditions for the right-eyestalk-ablated crabs (males, females, and mixed-sex). The experimental details are as follows:

EXPERIMENT 1: COMPARING THE GROWTH AND SURVIVAL RATES OF MUD CRABS CULTURED UNDER VARIOUS EYESTALK ABLATION CONDITIONS

This experiment was conducted from June to July 2023 in 12 culture tanks. The water salinity in the tanks was maintained at 25‰. The culture tanks were connected to a recirculating biological filter system, which provided a daily water-recirculating flow rate of 500%. Four groups of eyestalk-ablated mud crabs (none ablation, right ablation, left ablation, and bilateral ablation) were randomly stocked into the tanks at a density of 20 ind. m⁻² (6 ind. tank⁻¹). Prior to stocking, individuals were tagged with a serial number on their claws for identification during monitoring. Throughout the culturing period, the crabs were fed daily fresh trash fish at 19:00. The feeding regime was equal to 5% of the crab's body weight and adjusted based on their bait status. The culture tanks were continuously lightly aerated, with no water changes during the experimental period. Waste material was siphoned daily from the tanks at 21:00 while the aeration system was turned off to allow sediment to settle at the bottom of the tanks.

EXPERIMENT 2: COMPARING THE GROWTH AND SURVIVAL RATES OF MUD CRABS CULTURED UNDER VARIOUS GENDER CONDITIONS

In this experiment, three gender groups of right-eyestalk-ablated mud crabs (males, females, and mixed-sex) were randomly assigned to nine culture tanks to monitor their growth and survival rates. The experimental conditions, as well as the care and management practices, were consistent with those used in Experiment 1. The experiment ran from August to September 2023.

DATA COLLECTION AND CALCULATION

The water quality parameters in the culture tanks were monitored just before and during the culture period. Temperature (using a DYS-DMT meter, DMT-20 Model, DYS Band, made in Korea) and pH (using a pH tester, HI98103 Model, HANNA) were measured twice daily at 7:00 and 14:00 using a DYS-DMT meter (DMT-20 Model, DYS Band, made in Korea). In addition, total ammonia nitrogen (TAN) and nitrite (N-NO₂⁻) concentrations were tested every three days at 7:00 AM using test kits (GH test kit-Germany Model, Sera Band, made in Germany).

Duration was monitored daily through visual observation using an LED light. The duration was recorded from when the mud crabs were stocked in the culturing tanks until a dark red outline appeared along the edges of their swimming legs and cracks formed on the sides of their carapaces (Figure 2).

The survival rate was assessed at the end of each experiment. Additionally, the wet weights of the crabs were determined both at the beginning and the end of the experiments using an electronic balance with an accuracy of 0.01 mg after blotting the crabs on filter paper. Furthermore, weight gain (WG), daily weight gain (DWG), SGR, SR, FCR, and yield were calculated using the following equations:

- $WG(g) = \text{final weight} - \text{initial weight}$
- $DWG(g \text{ day}^{-1}) = \text{final weight} - \text{initial weight} / \text{duration}$
- $SGR (\% \text{ day}^{-1}) = [(\ln \text{ final weight}) - (\ln \text{ initial weight})] / \text{duration} \times 100$
- $FCR = \text{consumed feed (g)} / \text{weight gain (g)}$
- $SR (\%) = (\text{final crab number} / \text{initial crab number}) \times 100$
- $\text{Yield (kg m}^{-2}\text{)} = \text{crab biomass} / \text{culture area}$

DATA ANALYSIS

The data in this study were analyzed using a one-way analysis of variance (ANOVA). Mean differences were determined with Duncan's test at a significance level of $p < 0.05$. Homogeneity of variance was assessed using Levine's test, and percentage data were arcsine-transformed before analysis. Statistical analysis was performed using SPSS software for Windows, version 20.0.



Figure 2. Signs to determine double-skinned crab status. Photos taken from the present study.

a) dark red outline appeared along the edges of the swimming legs.
b) cracks formed on the side of the carapace.

Table 1. Water quality parameters during experiment periods.

Groups	Temperature (°C)		pH		NO ₂ ⁻ (mg L ⁻¹)	TAN (mg L ⁻¹)
	7:00	14:00	7:00	14:00		
None ablation	29.1±0.2	30.1±0.2	7.8±0.1	7.8±0.1	0.001±0.000	0.01±0.00
Right ablation	29.1±0.2	30.1±0.2	8.0±0.0	8.0±0.1	0.001±0.000	0.01±0.00
Left ablation	29.1±0.2	30.1±0.2	8.0±0.0	8.0±0.0	0.00±0.0000	0.01±0.00
Bilateral ablation	29.1±0.2	30.1±0.2	8.0±0.0	8.0±0.0	0.001±0.000	0.01±0.00
Males	27.8±0.1	29.4±1.1	7.9±0.2	7.9±0.2	0.001±0.000	0.26±0.05
Females	27.8±0.1	29.3±1.2	7.7±0.2	7.7±0.2	0.00±0.0000	0.31±0.09
mixed-sex	27.7±0.6	29.6±1.2	7.8±0.2	7.8±0.2	0.001±0.000	0.29±0.08

Values are presented as mean±SD.

RESULTS

WATER QUALITY PARAMETERS

Throughout the culturing periods, the water quality parameters in both experiments showed minor differences and remained stable. Temperatures fluctuated between 27.7 and 29.1°C in the morning and 29.3 and 30.1°C in the afternoon for both experiments. The diurnal pH variations were also light, ranging from 7.7 to 8.0 in the morning and afternoon. Additionally, the nitrite (N-NO₂⁻) and TAN (N-NH₄⁺) concentrations were 0.001 mg L⁻¹ and 0.01 mg L⁻¹, respectively, in experiment 1, while in experiment 2, they were 0.001 mg L⁻¹ and 0.26–0.31 mg L⁻¹, respectively (Table 1).

CULTURING PERFORMANCE UNDER VARIOUS EYESTALK ABLATION CONDITIONS

GROWTH PERFORMANCE

Eyestalk ablation had no significant impact on the final mean weight, WG, DWG, or yield of the experimental crabs ($p > 0.05$). However, SGR was highest in the group with right ablation (0.65% day⁻¹), followed by those with left ablation (0.58% day⁻¹), bilateral ablation (0.54% day⁻¹), and non-ablation (0.48% day⁻¹). Notably, the SGR in the right

ablation group was significantly greater than that of the non-ablation group ($p < 0.05$). The findings indicated the positive effect of eyestalk ablation on the SGR of mud crabs, and right eyestalk ablation made the strongest enhancement in growth rates (Table 2).

Durations for the right and left ablation groups were 32.5 days and 33.4 days, respectively, with no significant difference ($p > 0.05$); however, both durations were significantly shorter than those in the bilateral ablation group (35.6 days) and the no-ablation group (39.6 days) ($p < 0.05$). Additionally, duration in the bilateral ablation group was significantly shorter than in the no-ablation group ($p < 0.05$) (Table 2).

SURVIVAL RATE AND FEED CONVERSION RATIO (FCR)

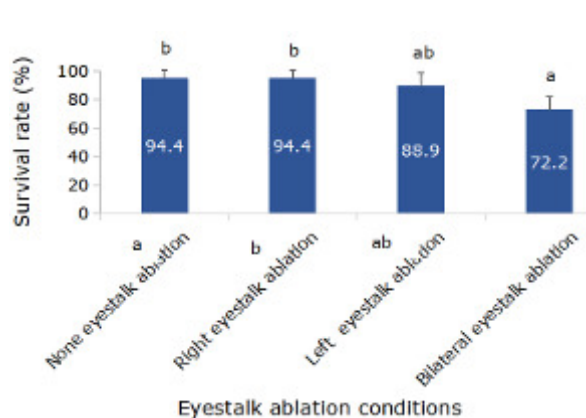
SR ranged from 72.2% to 94.4%, with the highest values observed in the non-ablation and right ablation groups (94.4%), followed by the left ablation group (88.9%), and the lowest in the bilateral ablation group (72.2%). Additionally, a significant decrease in SR was observed in the bilateral ablation group compared to the non-ablation and right ablation groups ($p < 0.05$) (Figure 3).

There were slight improvements in FCR for groups of unilateral ablations (left or right), while a significant improvement was observed in crabs with bilateral ablation (p

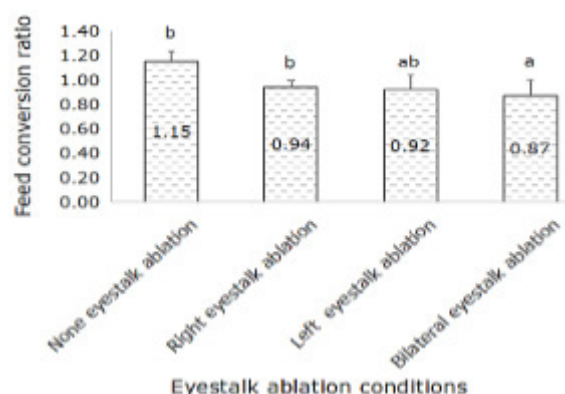
Table 2. The final mean weight, weight gain, daily weight gain (DWG), specific growth rate (SGR), yield, and the time required to form double-skinned crabs (duration) of *S. paramamosain* cultured under various eyestalk ablation conditions.

Parameters	Groups			
	None ablation	Right ablation	Left ablation	Bilateral ablation
Initial mean weight (g)	163±13.9 ^a	156±11.8 ^a	163±14.1 ^a	164±12.7 ^a
Final mean weight (g)	198±19.4 ^a	191±14.4 ^a	198±13.7 ^a	198±16.8 ^a
Weight gain (g)	34.2±9.8 ^a	36.3±9.2 ^a	34.8±10.3 ^a	35.5±10.2 ^a
DWG (g day ⁻¹)	0.86±0.24 ^a	1.12±0.29 ^a	1.04±0.30 ^a	0.98±0.29 ^a
SGR (% day ⁻¹)	0.48±0.12 ^a	0.65±0.16 ^b	0.58±0.17 ^{ab}	0.54±0.15 ^{ab}
Yield (kg m ⁻²)	3.74±0.38 ^a	3.72±0.25 ^a	3.52±0.49 ^a	2.85±0.58 ^a
Duration (days)	39.6±2.7 ^c	32.5±3.3 ^a	33.4±2.7 ^a	35.6±1.3 ^b

Values are presented as mean±SD. Values with different superscript letters (a, b) in the same rows show a significant difference ($p < 0.05$).

**Figure 3. Survival rate (SR) of *S. paramamosain* cultured under various eyestalk ablation conditions**

Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference ($p < 0.05$).

**Figure 4. Feed conversion ratio (FCR) of *S. paramamosain* cultured under various eyestalk ablation conditions.**

Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference ($p < 0.05$).

< 0.05). In comparison, the FCR values rose with the following trends: bilateral ablation (0.87), left ablation (0.92), right ablation (0.94), and none ablation (1.15) (Figure 4).

CULTURING PERFORMANCE UNDER VARIOUS GENDER CONDITIONS

GROWTH PERFORMANCE

Gender conditions did not have a significant impact on growth performance, yield, or duration ($p > 0.05$), with values varying within the following ranges: final mean weight (178.1–181.2 g), WG (19.7–21.3 g), DWG (0.53–0.57 g day⁻¹), SGR (0.32–0.33% day⁻¹), yield (2.58–3.42 kg m⁻²), and duration (36.9–40.5 days). However, the duration in the male group was slightly longer than in the female and mixed-sex groups (Table 3).

SURVIVAL RATE (SR) AND FEED CONVERSION RATIO (FCR)

SR was observed in the female group (94.4%), followed by the mixed-sex group (77.8%), while the lowest SR was found in the male group (66.7%). The SR in the female group (94.4%) was very high, and ANOVA analysis indicated a significant reduction in survival rates for the male group compared to the female group ($p < 0.05$) (Figure 5).

FCR increased in the order of female, mixed-sex, and male groups, with respective values of 1.15±0.10, 0.87±0.05, and 0.76±0.11. In addition, a significant difference in FCR was observed between the two groups of males and females ($p < 0.05$) (Figure 6).

DISCUSSION

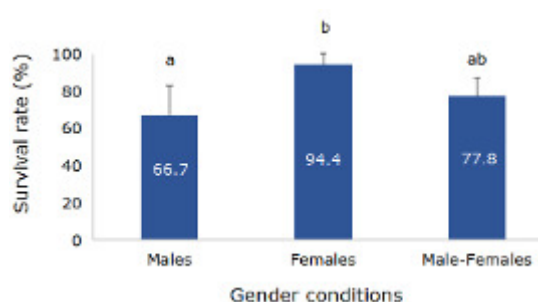
WATER QUALITY PARAMETERS

The water quality parameters remained stable throughout the experimental period and were within the optimal range for mud crabs. These included temperature (27.7 to 30.1 °C), pH (7.7–8.0), nitrite (stable at 0.001 mg L⁻¹), and TAN (0.01–0.31 mg L⁻¹) (Table 1). Previous studies have recommended suitable water quality conditions for culturing mud crabs during both their larval and adult stages, such as a temperature of 28–30 °C,³⁶ a pH of 7.5–8.5,³⁷ TAN levels

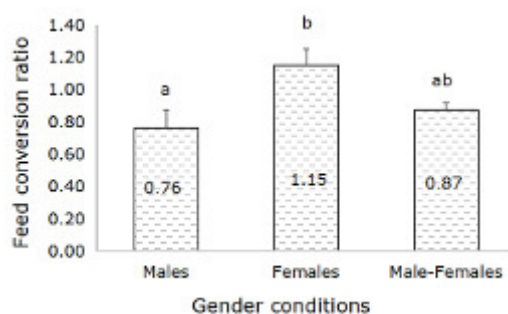
Table 3. The final mean weight, weight gain, daily weight gain (DWG), specific growth rate (SGR), yield, and the time required to form double-skinned crabs (duration) of *S. paramamosain* cultured under various gender conditions.

Parameters	Groups		
	Males	females	mixed-sex
Initial mean weight (g)	157.6±3.34 ^a	162.1±3.27 ^a	162.1±3.27 ^a
Final mean weight (g)	178.1±7.75 ^a	181.2±3.91 ^a	181.2±3.91 ^a
Weight gain (g)	21.3±3.94 ^a	19.7±2.30 ^a	19.7±2.30 ^a
DWG (g day ⁻¹)	0.53±0.07 ^a	0.57±0.06 ^a	0.57±0.06 ^a
SGR (% day ⁻¹)	0.32±0.04 ^a	0.33±0.04 ^a	0.33±0.04 ^a
Yield (kg m ⁻²)	2.58±0.42 ^a	3.42±0.30 ^a	3.42±0.30 ^a
Duration (days)	40.5±1.93 ^b	36.9±1.40 ^{ab}	36.9±1.40 ^{ab}

Values are presented as mean±SD. Values with different superscript letters (a, b) in the same rows show a significant difference ($p < 0.05$).

**Figure 5. Survival rate (SR) of *S. paramamosain* cultured under various gender conditions.**

Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference ($p < 0.05$).

**Figure 6. Feed conversion ratio (FCR) of *S. paramamosain* cultured under various gender conditions.**

Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference ($p < 0.05$).

below 3 mg L⁻¹,³⁸ and NO₂⁻ concentrations under 10 mg L⁻¹.³⁸

CULTURING PERFORMANCE UNDER VARIOUS EYESTALK ABLATION CONDITIONS

Eyestalk ablation in crustaceans offers several benefits, making it common in aquaculture, particularly in regulating molting and promoting growth.³⁹ The procedure targets

removing the X organ-sinus gland, which secretes the molting-inhibiting hormone (MIH) or growth-inhibiting hormone (GIH).^{40,41} This leads to earlier or more frequent molting, resulting in faster growth and earlier harvesting of farmed crustaceans.⁴² Eyestalk ablation has been successfully applied across various crustacean species, including several types of crabs.^{41,43,44}

The current study assessed the culturing performance of double-skinned crabs (just before molting) using intermolt and mature *S. paramamosain* under different eyestalk ablation conditions. It was found that the eyestalk ablation groups (unilateral or bilateral) showed improved SGR and shorter durations compared to the none ablation group. The improvements in SGR and duration were ranked in the following order: right ablation, left ablation, bilateral ablation, and none ablation. Notably, the right eyestalk ablation group exhibited a significantly higher SGR than the none ablation group, and the duration in both the right and left ablation groups was significantly shorter than in the bilateral ablation and none ablation groups (Table 2). Additionally, the eyestalk ablation conditions negatively affected survival rates, with a decrease in survival observed in all ablation groups (both unilateral and bilateral), particularly a significant reduction in the bilateral ablation group (Figure 3).

Similar results have been observed in other decapods.⁴⁵⁻⁴⁷ Shrimp species like *Penaeus californiensis*⁴⁵ and *Metapenaeus monoceros*⁴⁸ documented faster molting cycles and improved growth after unilateral or bilateral eyestalk ablation. However, bilateral ablation resulted in reduced molting and growth performance compared to unilateral ablation. For example, *M. monoceros* with unilateral ablation had two to three times higher production rates than intact animals, whereas those with bilateral ablation showed a negative production rate.⁴⁸ In addition, crabs *Chasmagnathus granulatus* and shrimp *Penaeus vannamei* showed that right eyestalk ablation led to greater molting efficiency than left eyestalk ablation.⁴⁹

Crustaceans experience tissue damage during eyestalk ablation, leading to the stress.⁵⁰ Further, eyestalk ablation limits key hormones essential for normal physiology, causing a change in some biological functions and negatively

affecting the animal's well-being.⁵¹⁻⁵⁴ In particular, bilateral eyestalk ablation can impair vision and feeding rates, hindering growth and development, and reducing survival capacity. Shrimp *Metapenaeus dobsoni* with bilaterally ablated eyestalks showed higher mortality compared to those with unilateral or no ablation.³⁹ These findings may help explain why the molting, growth, and survival rates of crabs with bilateral eyestalk ablation were lower than those of crabs with unilateral ablation in the current study.

Ocular independence existed in the animal kingdom, such as teleost fish,⁵⁵⁻⁵⁷ reptiles,⁵⁶ and crustaceans.^{58,59} The degree of independence between an animal's eyes can vary depending on the species and the specific task. Right eyestalk ablation may have more pronounced effects on molting and growth than left eyestalk ablation. This may be due to the asymmetrical hormonal regulation between the eyes, with the right eye playing a more significant role in controlling MIH and other growth-related hormones.⁶⁰ The current study did not explore the mechanisms behind the differing effects of left and right eyestalk ablation on growth and molting in *S. paramamosain*. Nevertheless, our findings align with previous studies, showing that right-eyestalk ablation has a more pronounced effect on growth and molting during the period they are cultured into double-skinned crabs.

Freshwater prawns (*Macrobrachium lancesteri*) showed that their unilateral or bilateral eyestalk ablation notably increased the amount of food energy converted into the tissue.⁶¹ The growth of bilaterally eyestalk-ablated prawns was significantly higher than that of unilaterally eyestalk-ablated prawns found in prawns *Homarus americanus*⁶² and *Macrobrachium lancesteri*.⁶¹ An improvement in feed conversion efficiency (FCE) in bilaterally ablated shrimp (*M. dobsoni*) compared to those with unilateral or non-ablation.³⁹ This information supported our findings, which showed only a slight improvement in FCR for crabs with unilateral eyestalk ablation (either left or right), while a significant improvement was observed in crabs with bilateral ablation (Figure 4).

CULTURING PERFORMANCE UNDER VARIOUS GENDER CONDITIONS

The data from Table 3 indicate that growth performance, yield, and duration were not significantly influenced by gender. However, the duration for males tended to be longer than that for the mixed-sex and female groups, in that order. Additionally, the highest SR was observed in females, but it showed a significant reduction in males (Figure 5). In contrast, FCR improved the most in males and the least in females (Figure 6).

Studies have shown that eyestalk-ablated females generally exhibit higher survival rates than males.^{45,63} For example, ablated females in *P. vannamei* had a survival rate of 51.7%, compared to 39.8% for males.⁶³ This may be due to females allocating more energy to growth instead of reproduction⁴⁵ or hormonal imbalances.⁴⁸ In *P. notialis*, males showed greater physiological effects from ablation, including increased metabolic rate.⁶⁴ Other studies also show that females may respond differently to eyestalk ablation

in terms of physiological factors like glucose, triglycerides, and protein concentrations.⁴⁵ Our study mirrors these findings, with females exhibiting shorter durations and higher survival rates than males.

While the effects of eyestalk ablation vary by species and physiological state,⁶⁵⁻⁶⁷ energy demands associated with ablation differ by sex.^{44,64} In *P. notialis*, females showed greater energy conversion efficiency post-ablation compared to males.⁶⁴ Conversely, in *Callinectes arcuatus*, females exhibited higher activity levels after ablation, leading to greater food consumption and a higher FCR than males,⁴⁴ consistent with our findings where FCR was higher in female groups.

CONCLUSIONS

The present study demonstrated that while eyestalk ablation did not significantly affect overall yields, it resulted in improvements in SGR, duration, and FCR, with the most notable gains observed in the right ablation group; meanwhile, the bilateral ablation group showed an improvement in FCR but achieved the least benefits in terms of SGR, duration, and SR. Besides, gender factors had a minor influence on duration, with females exhibiting the shortest time. Both male and mixed-sex groups showed improvements in FCR but experienced a decline in SR, with a significant decrease in the male group. Based on these findings, right eyestalk ablation in intermolt and mature female mud crabs (*S. paramamosain*) is recommended as the most effective strategy for producing double-skinned crabs under the conditions studied. Further research on different feed types and feeding frequencies could further improve the efficiency of double-skinned crab production.

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AUTHORS' CONTRIBUTION

Conceptualization: Le H. Vu (Equal), Ting C. Huang (Equal), Tien H. Ly (Equal). Writing – original draft: Le H. Vu (Equal), Nguyen V. Bac (Equal), Doan X. Diep (Equal). Investigation: Le H. Vu (Equal), Nguyen V. Bac (Equal), Chau T. Da (Equal). Writing – review & editing: Ting C. Huang (Equal), Doan X. Diep (Equal). Supervision: Ting C. Huang (Equal), Doan X. Diep (Equal). Methodology: Nguyen V. Bac (Equal), Tien H. Ly (Equal), Chau T. Da (Equal). Formal Analysis: Tien H. Ly (Equal), Chau T. Da (Equal).

COMPETING OF INTEREST – COPE

No competing interests were disclosed

ETHICAL CONDUCT APPROVAL – IACUC

The animal experiments complied with relevant national and international guidelines. Only the mud crabs underwent eyestalk ablation and were photographed during the experiment, ensuring no harm was caused. After the experiments, the crabs were returned to the commercial storage tanks.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available on reasonable request.

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