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Is limb autotomy really efficient compared to traditional rearing in soft-shell crab (*Scylla olivacea*) production?

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ABSTRACT

There is currently a global high market demand for soft-shell crabs, which are priced much higher than crabs in hard-shell conditions. In order to harvest crabs in soft-shell conditions, they are reared and collected as soon as they molt, and limb autotomy is commonly used to hasten the molting process. In this study, we compared full limb autotomy (the removal of all the appendages except for the swimming legs), partial limb autotomy (the removal of the walking legs), and traditional rearing methods (control) using immature mud crab (*Scylla olivacea*) juveniles. The longest and shortest molt cycle duration was observed in the control group and the full limb autotomy group, respectively. The molting percentage was the highest in the full limb autotomy group (females) and in the partial and full limb autotomy groups (males; no significant difference between the groups). The survival rate was unaffected by limb autotomy. Post-molting, the carapace length (CL) and body weight (BW) increments of both sexes, and the carapace width (CW) increment of males were the highest in the control group. The average BW after molting was the highest in the control group, followed by the partial and then the full limb autotomy group. The average total harvest weight, however, was lowest in the control group, while no significant difference was observed between the limb autotomy treatments, owing to their low molting percentage and resulting weight gain per animal. Thus, although limb autotomy (partial or full) significantly shortened the duration of the molt cycle, it may not be as efficient as traditional rearing methods as it resulted in soft-shell crabs of lower aesthetic value (with missing limbs or uneven limb sizes), body size, and weight, all of which are important criteria for determining crab prices.

1. Introduction

Crabs, as other crustaceans, experience discontinuous growth and they need to molt constantly (shed their old exoskeleton) to increase in size. During molting, the old hard exoskeleton is replaced by a new decalcified, temporary soft exoskeleton. During this brief soft-shell period, the crab takes up water to expand its body size (Hopkins, 1982). Shell hardening commences several hours later and is complete within 24–48 h (Waiho et al., 2015). Soft-shell crabs are harvested during the brief soft-shelled period and they can be frozen immediately for storage. Frozen storage allows soft-shell crabs to be exported easily throughout the world (Hungria et al., 2017).

Mud crabs (genus *Scylla*) are among the few portunid species that are widely exploited for the production of soft-shell crabs, especially in the Indo-West Pacific region where they are found in abundance (Tobias-Quinitio et al., 2015; Fazhan et al., 2017a; Waiho et al., 2018). Compared to other marine portunid species, mud crabs fetch higher prices (Sathiadhas and Najmudeen, 2004) and the production of soft-shell crabs using mud crabs is even more profitable. Additionally, the technological advances and knowledge in larval rearing, juvenile and adult culture, and reproduction are greatest in *Scylla* spp. than in other swimming crabs (Fazhan et al., 2017c; Hungria et al., 2017; Waiho et al., 2017, 2019). This facilitates the cultivation and production of soft-shell crabs from *Scylla* spp.

Crustaceans exhibit autotomy, the reflexive voluntary removal of body parts by the animal caused either by stress or a predator threat

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(Zhang et al., 2018). As limb loss may adversely affect functions such as feeding and mating (Oliveira et al., 2015; Waiho et al., 2015), limb regeneration is urgently required. Crabs can regenerate limbs only via molting, and it has been observed that the molting process is generally hastened by limb loss (Mykles, 2001). Based on this knowledge, limb autotomy is commonly used by many soft-shell crab production farms to induce molting in crabs. Limb autotomy is technically easier to be carried out compared to eyestalk ablation, and the regeneration of limbs is generally faster (Desai and Achuthankutty, 2000). However, there are farmers that still prefer the traditional rearing method, i.e., rearing crabs individually in captivity and harvesting them as soft-shell crabs right after molting, because performing limb autotomy is time consuming and it has a high mortality risk. Additionally, limb autotomy decreases significantly the growth of crab juveniles [internal carapace width (CW) of 20–23 mm] (Quinitio and Estepa, 2011; De la Cruz-Huervana et al., 2019).

There is a knowledge gap on the effect of limb autotomy on the growth of larger immature crabs commonly used by farmers for soft-shell crab production (CW of 65–80 mm). Therefore, this study aimed to determine the efficiency of limb autotomy in crabs, in terms of their body size and weight, molt cycle duration, molting percentage, and survival rate compared to those not subjected to limb autotomy. To this end, we used crabs in the size and weight range of those commonly used by farmers to produce soft-shell crabs. The results of this study could aid farmers in selecting suitable molt induction methods for the production of soft-shell crabs.

2. Material and methods

2.1. Animal collection and culture condition

A total of 288 [average CW = 76.4 ± 2.2 mm; average body weight (BW) = 92.5 ± 5.7 g] intermolt immature mud crabs were obtained from local fishermen in Barru, South Sulawesi Province, Indonesia. Only *Scylla olivacea* was used in this study. Mud crabs were identified to the species level based on the work of Keenan et al. (1998). The selected experimental juvenile CW was based on the size at maturity of *S. olivacea* (84–92 mm based on gonadal maturation), which was determined by a previous study by Waiho et al. (2016b). The female maturity status was assessed according to their abdomen shape and coloration (Waiho et al., 2016b), while the male maturity status was assessed according to their abdomen looseness (Waiho et al., 2016a). The selected CW and BW of the experimental crabs used in this study conformed to those of crabs used by commercial farmers for soft-shell crab production, i.e., CW of 65–80 mm and BW of 85–100 g (personal communication). The selection of intermolt juveniles was based on the molt stage classification of *Scylla serrata* by Quinitio and Estepa (2011), with slight modifications based on Sather (1966). Intermolt crabs (C_3) exhibit completely hardened exoskeleton and minimal epidermal separation from the swimming leg cuticle. Crabs that are still in postmolt (soft papery shell and poorly developed setal base), early intermolt (C_1-C_2) (depressible sternites and soft abdominal segments), late intermolt (C_4) (presence of a membranous layer between the hypodermis and the exoskeleton), and premolt (presence of a clear epidermal retracted zone) stages were not used in this study, as limb autotomy during the intermolt stage has been shown to hasten the molting process (McCarthy and Skinner, 1977).

The rearing experiment was carried out in an earthen pond (5000 m^2) at the regency of Barru, South Sulawesi Province, Indonesia from November 2018 to February 2019. Only active immature juveniles without limb injuries and parasites were chosen. The salinity, temperature, pH, and dissolved oxygen (DO) were measured daily using an ATAGO handheld refractometer (Master-S/MillM; ATAGO CO., LTD., portable DO meter (HI9142; Hanna Instruments), respectively. The salinity, temperature, pH, and DO of the water were 32.1 ± 2.5 ppt, 28.6 ± 1.0 °C, 6.9 ± 0.9, and 5.4 ± 1.4 ppm, respectively. No specific treatment was applied to preserve the water quality of the earthen pond, owing to its large size. The earthen pond used in this study was partially connected to the local mangrove forests, and the water exchange rate was approximately 20 % during neap tides and 50 % during spring tides. This pond was one of those used for soft-shell crab (*Scylla* spp.) production in this facility, with an average yield of 0.8–1 tonnes per hectare per month.

In this experiment, we followed the recommendations of the ARRIVE guidelines, and the mud crabs were handled in accordance with the Association for the Study of Animal Behaviour (ASAB) (2012) "*Guidelines for the treatment of animals in behavioural research and teaching*" published in Animal Behaviour 83: 301–309. *Scylla olivacea* is a commercially available mud crab species, thus no special permit was required for its acquisition.

During culture, crabs were individually placed into enclosed rectangular plastic sieves (21 cm length × 15 cm width × 8 cm height) based on a randomized block design, and were fed dried trash fish (3% per body weight) every two days. Daily observations were conducted every 3 h to record molting or mortality cases. A maximum rearing period of 80 days was applied to all treatments.

2.2. Molting experiments

To compare the effect of different molt induction techniques on molting, the intermolt juveniles were randomly subjected into three treatments, i.e., (1) control, (2) full limb autotomy, and (3) partial limb autotomy. Each treatment consisted of three replicates, with 16 male and 16 female intermolt juveniles in each replicate. The CW and carapace length (CL) of all intermolt juveniles were measured using a standard Vernier caliper (to the nearest 0.1 mm), while their BW was measured using a digital weighing balance (to the nearest 0.1 g), before subjecting them to different treatments.

The intermolt juveniles of the control group (not subjected to autotomy) were individually placed into an enclosed rectangular plastic sieve before being immersed close to the water surface of the rearing pond. In the second treatment, 96 crabs were subjected to full limb autotomy (the removal of all appendages including large chelipeds), except for their swimming legs. The last treatment group involved the bilateral removal of pereiopods (second to fourth walking legs), but with the chelipeds and swimming legs still intact. To ensure that feeding would not be interrupted owing to the lack of appendages in the full limb autotomy group, trash fish pieces were placed directly in front of the mouth opening of each crab.

Once molting was observed, a crab was removed from the enclosure and its CW, CL, and BW were measured. The measurements were taken approximately 1 h after shell release, when the new shell had been stretched perfectly but the spines were still soft.

2.3. Data analyses

All data analyses were conducted in Microsoft Excel (ver. 16.27) and IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, NY, USA). As the homogeneity of variance was violated ($P < 0.05$), the relative increase of the CL_{female}, CW_{female}, BW_{male}, and BW_{female} as well as the molt cycle duration (days until next molting) of males and females was analyzed using one-way analysis of variance (ANOVA) with Welch's correction (Welch, 1947). A subsequent post hoc (Games-Howell) test was conducted to detect potential differences among the treatments (Kirk, 1995). The BW changes of the crabs in the partial and full limb autotomy treatments were based on the difference between the

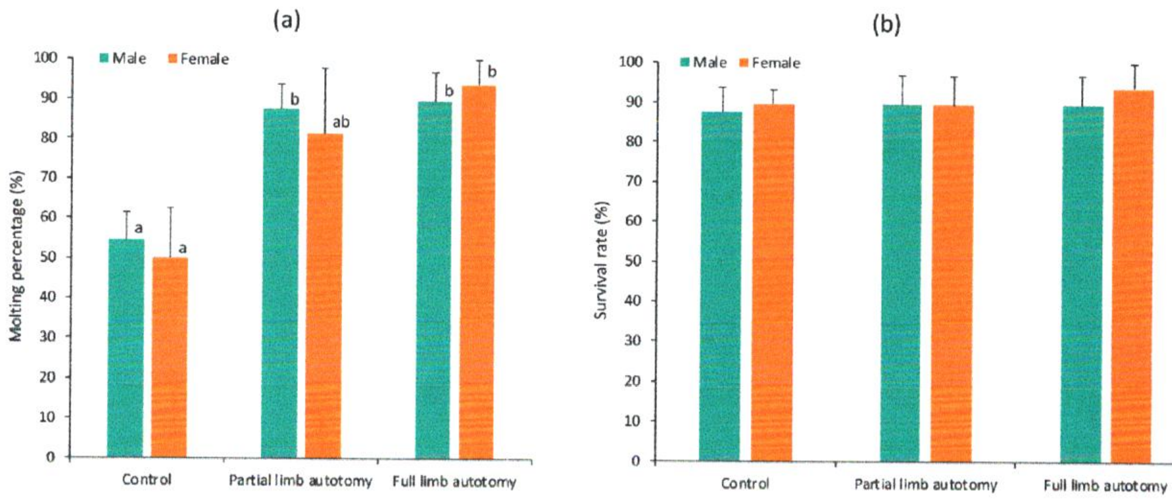


Fig. 1. The (a) molting percentage and (b) survival rate of *Scylla olivacea* under different treatments. Different lowercase letters indicate significant difference among treatments according to sex ($P < 0.05$). There was no significant difference in survival rate among treatments. Error bar represents standard deviation.

normal one-way ANOVA and a subsequent post hoc Tukey's test were used to determine differences among treatments. A one-way ANOVA, a subsequent Tukey's test, and paired *t*-tests were also conducted on the initial BW and final BW of crabs in all treatments, as all BW data were normally distributed (Kolmogorov-Smirnov; all $P > 0.1$).

3. Results

All treatments were terminated after 80 days. Molting activities occurred during the rearing period were recorded and compared among treatments.

3.1. Molt cycle duration, molting percentage, and survival rate

The molting percentages differed significantly among the treatments in males (ANOVA; $F_{2,6} = 18.17$, $P = 0.003$) and females (ANOVA; $F_{2,6} = 8.18$, $P = 0.019$) (Fig. 1a). In males, the molting percentage was the lowest in the control group (average = 54.17 ± 7.22 %) (Tukey's test; $P = 0.004$), while there was no significant difference between the two autotomy treatment groups ($average_{partial\ limb\ autotomy}$ = 87.50 ± 6.25 %; $average_{full\ limb\ autotomy}$ = 89.58 ± 7.22 %) (Tukey's test; $P = 0.891$). Female crabs subjected to full limb autotomy had a higher molting percentage than the control group ($average_{full\ limb\ autotomy}$ = 93.75 ± 6.25 %; $average_{control}$ = 50.00 ± 12.50 %) (Tukey's test; $P = 0.017$). The molting percentage of crabs subjected to partial limb autotomy (average = 81.25 ± 16.54 %) did not differ significantly from that of the other two groups (Tukey's test; control versus partial limb autotomy, $P = 0.103$; full limb autotomy versus partial limb autotomy, $P = 0.349$). The survival rate did not vary significantly among treatments (ANOVA; $F_{2,15} = 0.77$, $P = 0.483$) (Fig. 1b). In all three treatments, crab mortality occurred during the intermolt stage, probably due to natural causes.

The molt cycle duration was significantly different among treatments in both males (ANOVA; $F_{2,52.43} = 115.81$, $P < 0.001$) and females (ANOVA; $F_{2,43.18} = 78.37$, $P < 0.001$) (Table 1). In both sexes, the control groups exhibited the longest molt cycle duration, followed by the partial limb autotomy treatments. The full limb autotomy groups had the shortest molt cycle duration (Games-Howell test; all $P \leq 0.001$) (Fig. 2).

3.2. Body size (CL, CW) and BW increment, average BW and total harvest weight after molting

(ANOVA; CL_{female}: $F_{2,52.92} = 36.45$, $P < 0.001$; CW_{female}: $F_{2,53.21} = 27.86$, $P < 0.001$) were significantly different among treatments (Table 1). In males, the CL and CW increment of the control group was the highest (CL increment = 7.7 ± 1.9 mm; CW = 11.3 ± 2.6), followed by the partial limb autotomy and the full limb autotomy treatments (Tukey's test; all $P \leq 0.001$). In females, however, only the CL increment showed a similar trend [CL increment: control (8.0 ± 2.1) > partial limb autotomy (6.4 ± 1.5) > full limb autotomy (4.5 ± 1.3)]. The CW increment of the control group was not significantly different from that of the partial limb autotomy group (Games-Howell test; $P = 0.51$), although the CW increments of both groups were higher than that of the full limb autotomy group (Games-Howell test; $P < 0.001$).

There were significant differences in the BW increment of males (ANOVA; $F_{2,54.28} = 131.33$, $P < 0.001$) and females (ANOVA; $F_{2,47.97} = 130.18$, $P < 0.001$) among the different treatments after molting (Table 1). Both sexes showed similar trends, i.e., the BW increment was the lowest in the full limb autotomy groups, it was significantly higher in the partial limb autotomy groups, and the highest in unmutilated (control) crabs (Games-Howell test; all $P \leq 0.001$). It is important to note that the average reduction in the BW of males and females after the initial removal of only the pereiopods (partial limb autotomy) was 8.8 ± 1.6 g and 7.9 ± 1.2 g, respectively. On the contrary, the removal of both chelipeds and pereiopods (full limb autotomy) resulted in a weight reduction of 32.0 ± 2.0 g and 28.8 ± 2.3 g in males and females, respectively. Based on the results of the paired *t*-test, all treatments resulted in increased BW after molting (all $P < 0.001$) (Fig. 3a). There was no significant difference in the average BW (ANOVA; $F_{2,137.25} = 0.262$, $P = 0.770$) or the average total harvest weight (ANOVA; $F_{2,6} = 0.018$, $P = 0.982$) before molting among the treatments. However, both partial and full limb autotomy adversely affected the final BW after molting (ANOVA; $F_{2,115.94} = 147.11$, $P < 0.001$). The highest average BW was observed in the control group, followed by the partial limb autotomy and full limb autotomy groups (Fig. 3a). Most of the crabs subjected to partial or full limb autotomy either could not regenerate their limbs in time prior to molting (Fig. 4a) or the regenerated limbs were smaller in size, thus resulting in the lower average BW (Fig. 4b, c). However, the average total harvest weight was the lowest in the control group (2,146.3 ± 142.9 g), whereas no significant difference was observed between the partial (3,058.7 ± 401.7 g) and the full limb autotomy treatments (2,836.2 ± 70.7 g) (ANOVA; $F_{2,6} = 10.904$, $P = 0.01$) (Fig. 3b).

Table 1
The average and range of increment of CL, CW, BW and days-to-molt duration of *Scylla olivacea*.

Parameter		Sex	Control	Partial limb autotomy	Full limb autotomy
CL increment (mm)	Mean	M	7.7 ± 1.9[a]	6.0 ± 2.1[b]	6.0 ± 1.5[c]
		F	8.0 ± 2.1[a]	6.4 ± 1.5[b]	4.5 ± 1.3[c]
	Range	M	2.4 – 11.1	1.6 – 11.2	1.4 – 9.6
		F	5.1 – 12.8	5.1 – 12.8	1.6 – 8.1
CW increment (mm)	Mean	M	11.3 ± 2.6[a]	9.0 ± 1.9[b]	7.3 ± 2.8[c]
		F	10.5 ± 2.8[a]	8.9 ± 1.9[a]	6.6 ± 2.7[b]
	Range	M	5.1 – 16.0	2.3 – 11.8	−3.8–13.1
		F	4.0 – 16.3	4 – 16.3	1.4 – 11.3
BW increment (g)	Mean	M	36.1 ± 12.1[a]	26.4 ± 5.8[b]	3.3 ± 8.4[c]
		F	37.6 ± 13.7[a]	24.5 ± 4.0[b]	4.8 ± 7.8[c]
	Range	M	18.4 – 62.1	10 – 36.2	−28.6–19.5
		F	10.0 – 64.3	10 – 64.3	−29.7–18.6
Days-to-molt (day)	Mean	M	59.31 ± 12.43[a]	28.07 ± 11.71[b]	19.81 ± 5.87[c]
		F	57.04 ± 15.28[a]	26.51 ± 8.00[b]	19.58 ± 3.45[c]
	Range	M	26 – 80	1 – 49	13 – 53
		F	23 – 79	23 – 79	11 – 28

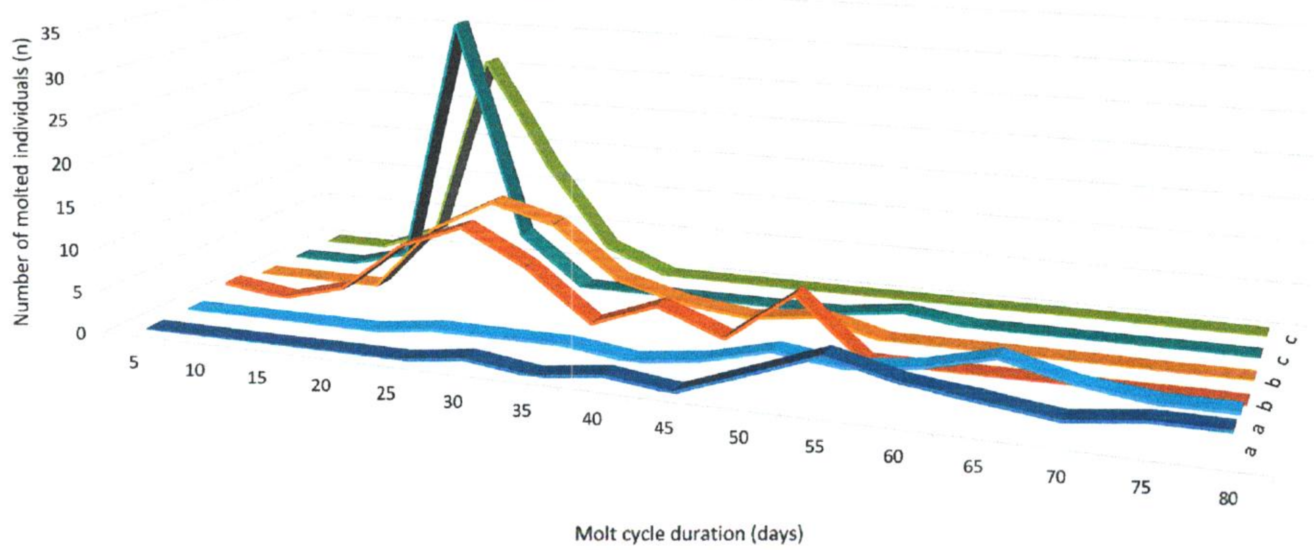
Note: CL = carapace length, CW = carapace width, BW = body weight, M = male, F = female. Different lowercase superscript letters indicate significant difference among treatments according to sex ($P < 0.05$). For partial and full limb autotomy treatments, BW increment represents the difference between initial BW (before limb autotomy) and final BW (after molting).

4. Discussion

Limb autotomy promotes molting and the molt cycle duration is inversely correlated with the number of lost limbs, as observed in *S. olivacea* in this study and in other *Scylla* species, i.e., in *S. serrata* juveniles with an internal CW of 20–23 mm (De la Cruz-Huervana et al., 2019). In addition to the number of lost limbs, the type of limbs removed also affects the molt interval. The chelipeds of *Scylla* spp. are essential in combat, mating, and feeding (Yap et al., 2013; Waiho et al., 2015; Fazhan et al., 2017b). Therefore, the loss of all limbs, including the chelipeds, in the full autotomy group hastened the molting process and resulted in higher molting percentages compared to the intact or the partially autotomized crabs. However, the molt cycle duration increases in limb autotomized *S. paramamosain* crablets of C1 stage (Gong et al., 2015). Compare with the large juveniles used in this study, C1 crablets are rapidly growing and have a very short molting interval (4–7 days). Thus, additional time is needed for nutrient accumulation to promote limb regeneration (Gong et al., 2015).

Limb autotomy did not have a negative effect on the survival of *S. olivacea*, as shown by the comparable survival rates among the treatments. A similar trend has even been observed in *Scylla* juveniles of much smaller sizes (Gong et al., 2015; De la Cruz-Huervana et al., 2019) as well as in juveniles of other crab species (He et al., 2016). Crabs' ability to shed their limbs voluntarily is a useful adaptive mechanism to escape predators and minimize injuries (Zhang et al., 2018). If performed correctly, autotomy, which is the self-induced limb loss by crabs, should have minimal adverse effects on their survival.

As shown by the low molting percentage of the control group, unsynchronized molting is common in brachyuran species and is considered as one of the factors leading to cannibalism, limb autotomy, and mortality (Zhang et al., 2018). Partially synchronous molting was observed after limb autotomy was performed (Fig. 3a). The effect of limb autotomy on the synchronization of the molt cycle has also been reported in other brachyuran species (O'Brien, 1999) and is attributed to the secretion of limb autotomy factor anecdysis (LAF_{an}) (Skinner, 1985; O'Brien, 1999; Mykles, 2001). The relationship between LAF_{an} and



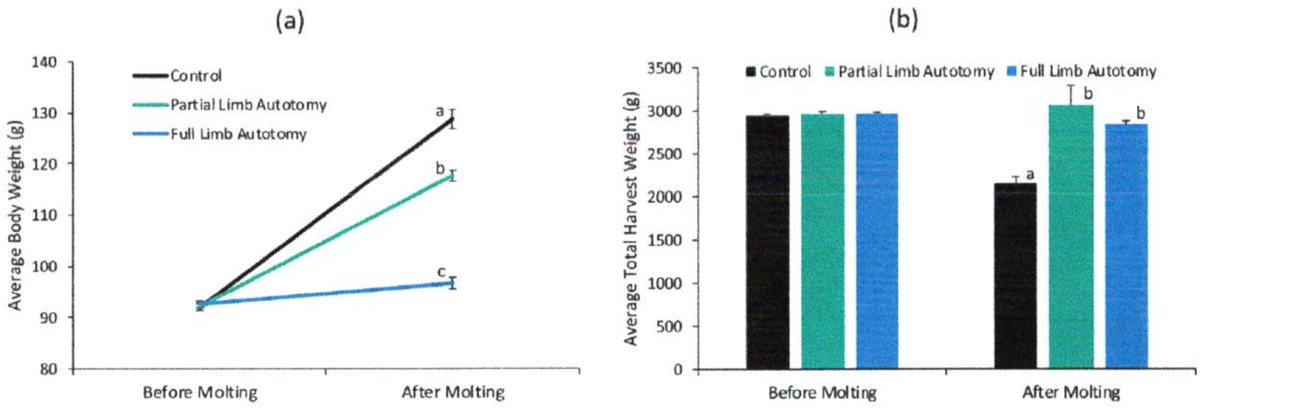


Fig. 3. The (a) average body weight (BW) and (b) average total harvest weight of *Scylla olivacea* before and after molting under different treatments. Different lowercase letters indicate significant difference among treatments ($P < 0.05$). No significant difference in average BW and average total harvest weight before molting were found among treatments. For partial and full limb autotomy treatments, BW increment represents the difference between initial BW (before limb autotomy) and final BW (after molting). Error bar represents standard error.

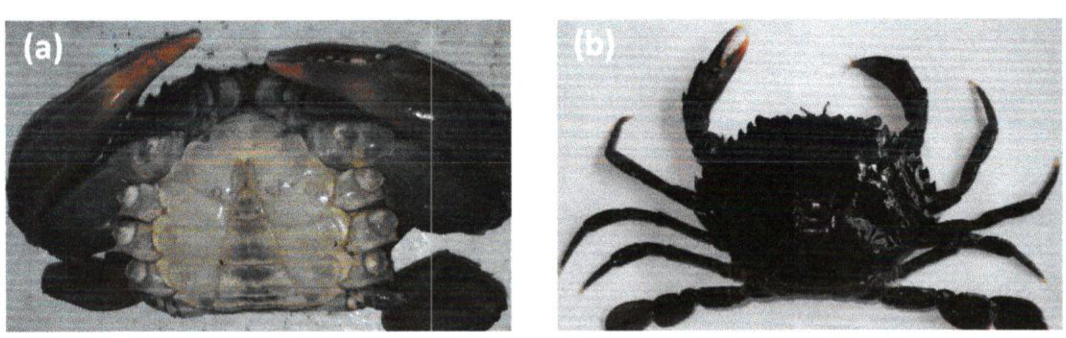
other known molt-regulating factors such as ecdysteroids, molt-inhibiting hormone (MIH), and crustacean hyperglycemic hormone (CHH) (Techa and Chung, 2015) are still unclear. A recent transcriptomic analysis conducted on *Portunus trituberculatus* following limb autotomy revealed the involvement of the WNT signaling pathway in limb regeneration and of WNT4 as the essential mediating factor (Liu et al., 2018).

Although limb autotomy is commonly used to induce molting in many soft-shell crab production farms, this technique may not be as efficient as rearing crabs until they molt naturally. In terms of body size (CL and CW), intact crabs grew more compared to partially and/or fully autotomized crabs. This was so because after limb autotomy, most of a crab's energy is diverted to the promotion of limb regeneration instead of growth (Hartnoll, 1982; Mariappan et al., 2000). A similar reduced growth trend after limb autotomy has been reported in *S. serrata* juveniles (De la Cruz-Huervana et al., 2019) and in other crab species (Bennet, 1973; Mclain and Pratt, 2011).

The crabs' wet weight determines the farmers' final profit. Crabs lost up to approximately 33 % of their initial BW when subjected to partial or full limb removal. This resulted in a staggering difference in the mean growth of the full limb autotomy group compared to the control group (3–5 g and 36–38 g, respectively). A similar BW increment reduction after limb loss has been observed in the blue crab *Callinectes sapidus* (Smith, 1990) and in *S. serrata* (De la Cruz-Huervana et al., 2019). *Scylla* juveniles (CW = 17–23 mm) are only able to fully regenerate their limbs after two molts (Quinitio and Estepa, 2011) and the regeneration process is age-dependent (Mariappan et al., 2000). Thus, the full limb regeneration of the *S. olivacea* used in this study could only occur after the completion of more than two molts, owing to their larger size (average CW = 76.4 ± 2.2 mm).

The use of pre-molt individuals will no doubt shorten the production period of soft-shell crabs and prevent the need for mutilation (leg removal) as is the case in the *Callinectes* soft-shell crab production industry (Ostrensky et al., 2015). However, this method is not preferred among *Scylla* farmers owing to a lack of in-depth knowledge regarding the molt stage identification of *Scylla* spp. and the fattening process that intermolt individuals before molt induction could be subjected to. Additionally, as the *Scylla* soft-shell crab industry still relies on wild-caught juveniles, crabs in the intermolt stage (the longest stage within the molt cycle) are the most abundant (Freeman et al., 1987). Nonetheless, the increasing potential of the soft-shell crab industry highlights the urgency to optimize mud crab hatchery technologies and to incorporate them fully into the aquaculture sector, in order to mitigate the negative impact of mud crab capture fisheries on their wild populations.

In conclusion, our results showed that, from the perspective of soft-shell crab production, limb autotomy induced faster molting and resulted in a higher molting percentage. At the same time, however, it resulted in significantly smaller body size (CL and CW), lighter BW, and crabs of lower aesthetic value, thereby decreasing their market price and the economic profits per rearing cycle. Further critical economic analyses, including comparisons of the net financial gain of these two methods (limb autotomy and traditional rearing), as well as research on the effects of the soft-shell crab industry on natural resources, should be undertaken. Currently, other molt induction methods that do not involve crab mutilation, such as the manipulation of physical parameters like temperature (Gong et al., 2015; De la Cruz-Huervana et al., 2019) and salinity (Gong et al., 2015), the use of natural plant extracts such as spinach extract (Aslamyah and Fujaya, 2011), vitomolt™ (Fujaya, 2011), and *Vitex glabrata* extract (Sorach et al., 2013), and the administration of chemicals including phytoecdysteroids (Nikhlani and Sukarti, 2017) are gaining attention.



CRediT authorship contribution statement

Yushinta Fujaya: Conceptualization, Methodology, Writing - original draft. **Nita Rukminasari:** Investigation, Data curation. **Nur Alam:** Investigation, Formal analysis. **Muhammad Rusdi:** Investigation, Validation. **Hanafiah Fazhan:** Conceptualization, Methodology, Writing - review & editing. **Khor Waiho:** Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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