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Effects of Eyestalk Ablation and Limb Autotomy on Moulting, Growth and Survival Rate of Orange Mud Crab, *Scylla olivacea* Juveniles for the Production of Soft-Shell Crabs

(Kesan Pembuangan Tangkai Mata dan Autotomi Anggota Badan terhadap Proses Penyalinan Kulit, Pertumbuhan dan Kadar Kemandirian Juvenil Ketam Bakau, *Scylla olivacea* untuk Pengeluaran Ketam Kulit Lembut)

MHD IKHWANUDDIN[1,2,*], HANAFIAH FAZHAN[1,2,3,4], YOUJI WANG[5], ALEXANDER CHONG SHU-CHIEN[3,6], MUYASSAR H. ABUALREESH[7], MOHAMAD JALILAH[1], AMBOK BOLONG ABOL-MUNAFI[1] & KHOR WAIHO[1,2,3,4]

[1]*Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia*
[2]*STU-UMT Joint Shellfish Research Laboratory, Shantou University, Shantou, Guangdong, China*
[3]*Centre for Chemical Biology, Universiti Sains Malaysia, 11900 USM, Pulau Pinang, Malaysia*
[4]*Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand*
[5]*International Research Center for Marine Biosciences, Ministry of Science and Technology, Shanghai Ocean University, Shanghai, China*
[6]*School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia*
[7]*Department of Marine Biology, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia*

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ABSTRACT

Soft-shell crabs are highly sought after due to their ease of storage and can be eaten whole, including the outer carapace. The high demand for soft-shell crabs leads to the exploration of different production techniques and methods. This study explores the effect of limb autotomy, eyestalk ablation and their combinations on the duration and success rate till the next moulting, and the size increment and survival rate of male juveniles of *Scylla olivacea* after moult. Additionally, female juveniles were also subjected to limb autotomy to investigate the potential impact of sex on their response to the procedure. Full limb autotomy (T2), either alone or with eyestalk ablation (T4), induced the fastest moulting (average 33 d and 39 d, respectively), followed by eyestalk ablation (average 57 d) and control (average 82 d). Limb autotomy, either alone or with eyestalk ablation, resulted in 100% and 90% moulting success, respectively, whereas that of eyestalk ablation and control was 50% and 56.83%, respectively. Crabs subjected to both moult-inducing techniques had the lowest size and weight gain after moulting. Females moulted faster than males in natural conditions, but this was negligible when limb autotomy was introduced. Limb autotomy had no impact on the CW increment and survival rate of both sexes, but greatly reduced their BW increment. Thus, limb autotomy can be incorporated in the production of soft-shell crabs to reduce turnover time.

Keywords: Eyestalk ablation; limb autotomy; mud crab; *Scylla olivacea*; soft-shell crab

ABSTRAK

Ketam kulit lembut sangat digemari kerana kemudahan penyimpanannya dan boleh dimakan keseluruhan, termasuklah cangkerang luarnya. Permintaan yang tinggi untuk ketam kulit lembut membawa kepada penerokaan teknik dan kaedah pengeluarannya yang berbeza. Kajian ini meneroka kesan autotomi anggota badan, pembuangan tangkai mata dan gabungannya terhadap tempoh dan kadar kejayaan sehingga proses bersalin cangkerang seterusnya, serta peningkatan saiz dan kadar kemandirian juvenil jantan *Scylla olivacea* selepas bersalin cangkerang. Tambahan pula, ketam betina juvenil juga dikenakan autotomi anggota badan untuk mengkaji kemungkinan kesan jantina terhadap tindak balas mereka bagi prosedur tersebut. Autotomi anggota badan sepenuhnya (T2), sama ada secara bersendirian atau gabungan bersama dengan pembuangan tangkai mata (T4) mempercepatkan proses penyalinan cangkerang (purata masing-masing 33 hari dan 39 hari) diikuti oleh ablasi tangkai mata (purata 57 hari) dan kawalan (purata 82 hari). Autotomi anggota badan, sama ada secara bersendirian atau bersama ablasi tangkai mata, masing-masing menghasilkan kejayaan bersalin cangkerang 100% dan 90%, manakala ablasi tangkai mata dan kawalan masing-masing hanya mencapai 50% dan 56.83%. Ketam yang dikenakan kedua-dua teknik induksi penyalinan cangkerang mencatatkan peningkatan saiz dan berat badan yang paling rendah selepas bersalin cangkerang. Ketam betina bersalin cangkerang dengan lebih pantas berbanding jantan dalam keadaan semula jadi, tetapi perbezaan ini menjadi tidak ketara apabila autotomi anggota diperkenalkan. Autotomi anggota badan tidak memberikan kesan terhadap peningkatan lebar cangkerang (CW) dan kadar kemandirian hidup kedua-dua jantina, tetapi mengurangkan peningkatan berat badan (BW) secara ketara. Oleh itu, autotomi anggota badan boleh diamalkan dalam penghasilan ketam kulit lembut untuk memendekkan masa kitaran.

Kata kunci: Autotomi anggota badan; ketam bakau; ketam kulit lembut; pembuangan tangkai mata; *Scylla olivacea*

INTRODUCTION

Mud crabs, *Scylla* spp. are common euryhaline species distributed throughout tropical to warm temperate regions within the Pacific and Indian oceans. As a mangrove crustacean species, they are frequently present in inter-tidal and sub-tidal zones of mangrove swamps (Ikhwanuddin et al. 2011; Keenan, Davie & Mann 1998; Waiho, Fazhan & Ikhwanuddin 2016) with salinity ranging from 20 to 30 ppt (Nurdiani & Zeng 2007). Mud crabs are highly sought after for their substantial size, exquisite flavor, and plentiful meat content (Fazhan et al. 2017b; Paul et al. 2016; Rattanachote & Dangwatanakul 1992). On top of that, they are not only known as a good protein source but also contain antioxidant properties which is excellent in protecting human cells from free radicals as well as comprising various natural antimicrobial peptides (AMPs) capable of acting as antibiotics to defend the human body from pathogenic microorganisms (Fredrick & Ravichandran 2012; Salaenoi et al. 2006; Yang et al. 2020; Yusof, Ahmad & Swamy 2017; Zheng et al. 2016). Traditionally, Malaysian folks consume mud crab soup as a remedy for treating dengue fever. Furthermore, van Oosterom et al. (2010) states that mud crabs have been used as biomarkers to detect the effects of pollutants on the quality of the environment in aquatic ecology. Based on their numerous benefits, mud crabs are considered an important income generator for many coastal fishing societies all over the Asia-Pacific countries as they can simply caught by nets or traps, remain alive for a significant duration after caught and are of high value (Gillespie & Burke 1992). The increasing price of mud crabs in both local and international markets has encouraged many culturists to initiate culture trials for commercial invention of mud crabs (Ikhwanuddin et al. 2011; Waiho, Fazhan & Ikhwanuddin 2016). Nowadays, mud crabs are being actively cultured in several countries in Asia including Malaysia, Thailand, Indonesia, Taiwan, Myanmar, Vietnam, Sri Lanka, China, and India (Azra & Ikhwanuddin 2016; Bir et al. 2020; Bonine et al. 2008; Zannatul, Zhang & Hasan 2010).

Soft-shell crabs are known to fetch higher market prices than their hard-shell counterparts due to their ease of consumption and high nutritional content. Compared to hard-shell crabs that are often sold in live conditions (thereby increasing culture costs during temporary storage), soft-shell crabs are normally sold in frozen form, enabling easier handling, storage, and transportation. In addition, the culture of soft-shell crab can be carried out all year round, in various types of systems (dos Santos Tavares et al. 2018), thus providing a steady income for culturists with its higher market price and demand compared to normal hard-shell mud crabs. FAO (2020) reported that the total profit of USD1,469,819.00 was obtained in 2015 from the crab industry, of which 75% was derived from *Scylla* spp. production. Mud crabs are often used in soft-shell production (Shelley & Lovatelli 2011; Tobias-Quinitio et al. 2015) and their price is highly dependent on body size. The retail price of soft-shell crab in Malaysian Market is about RM30 to RM60 per kilogram, while the price of ready-to-cook is between RM70 and RM130 per kilogram (Sidom 2017). Even though the crab industry has grown rapidly in the last 100 years ago in China (Yalin & Qingsheng 1994), other Asian countries including Malaysia, Thailand and Vietnam have just started this crab farming business in the last 30 years. In contrast, the biggest worldwide producer and exporter of soft-shell crabs are pioneered by Myanmar with a total production of 40 tonnes/month (FAO 2020).

The occurrence of soft-shell crabs in the wild is rare (Waiho et al. 2015), thus, the only viable way to procure these unique delicacies is through hatchery-induction (dos Santos Tavares et al. 2018; Waiho et al. 2021). The moulting process is the key factor in the innovation of soft-shell crabs. For a short period after moulting, their new exoskeleton will be soft, pliable and easy to eat. Crustaceans, including mud crabs, must moult away their old exoskeleton and change to a new and larger one in order to increase in size (Abdullah-Zawawi et al. 2021). Later, the body volume of the crab will increase by around 30% to 40% after the moulting process (Romano & Zeng 2006). According to Freeman et al. (1987), generally, the average soft-shell crab production in a culture system is around 30 to 45 days per cycle. Such long waiting periods incur high costs of production in terms of monitoring costs, maintenance costs and labor costs (Rahman et al. 2020). Significantly, this leads to the finding of alternative and effective ways to shorten the moulting period to improve soft-shell crab productivity.

It is commonly known that the eyestalk ablation technique reduces moulting cycle in crustaceans (Browdy & Samocha 1985; Chu & Leong 2009; Molyneaux & Shirley 1988). Through this technique, the eyestalks of crustaceans, either unilateral (one) or bilateral (both) were removed to trigger the moulting process. Stella, López Greco and Rodríguez (2000) stated that the eyestalk ablation technique will suppress or stop the production of moult inhibiting hormone (MIH) from the X-organ and vice versa will trigger the Y-organ to produce ecdysteroids, which act to stimulate the molting process. The eyestalk ablation technique can be performed in several methods which is; 1) Direct cutting of the eyestalk; 2) enucleating, that is a small cutting was apply at the distal part of the eye and all the content of eyes were removed via the cut opening; 3) burn/cauterizing the eyestalk to completely destroy the eye tissue using a hot-burning forceps or electrocautery tools; and 4) ligation of the eyestalk by using a medical thread (Chu & Chow 1992; Diarte-Plata et al. 2012; Taylor et al. 2004; Waiho et al. 2021). However, among these four methods, the cauterizing and ligation procedures are the best choice due to the speedy recovery of wound closure. On the other hand, the loss of limbs is also known to be able to induce moulting as well (Chu & Leong 2009; Hopkins 1982; Skinner & Graham 1970). Normally, self-amputation or limb autotomy of limbs happens in crustaceans as well as crabs, in their effort to get away from danger or in reaction to stress trauma including injury (Emberts et al. 2017). Thus, the full regeneration of new limbs will occur through the moulting process (Cooney, Korey & Hughes 2017; Mykles 2001; Rahman et al. 2020). Limb autotomy can be done by gripping or putting pressure on the segment of the limb, thus forcing the crab to release its limbs (Emberts et al. 2017).

Therefore, both techniques can be practiced in the production of soft-shell crabs (Waiho et al. 2021). In addition to eyestalk ablation and limb autotomy, other factors such as the use of ecdysteroid (Raghavan & Ayanath 2019), phytoecdysteroid (Sorach et al. 2013), changes in environmental parameters (Gong et al. 2015), the use of biogenic amines (Sainath & Reddy 2020), application of methyl farnesoate (Reddy, Nagaraju & Sreenivasula 2004), and the inhibition of moult-inhibiting hormone (Qiao et al. 2018) could induce moulting in crustaceans as well. However, due to their complexity in handling and administration, eyestalk ablation and limb autotomy are the two most cost-effective and simple methods for crab culturists. Limb autotomy is frequently applied to stimulate moulting during soft-shell crab invention, especially in Asia (Quinitio & Estepa 2011) whereas eyestalk ablation is more often used in the induction of gonadal maturation of crustacean broodstock (Meng et al. 2020; Sittikankaew et al. 2020). The utilization of eyestalk ablation, specifically as a moult induction method during soft-shell crab production has yet to be explored. Furthermore, it has been suggested that the limb autotomy method is quite easy to conduct as opposed to the eyestalk ablation method and the renewal of limbs is commonly quicker (Desai & Achuthankutty 2000). Nonetheless, de la Cruz-Huervana, Quinitio and Corre Jr. (2019) and Quinitio and Estapa (2011) stated that the growth of crab juveniles was stunted after applying the limb autotomy method.

Albeit considered extreme procedures, both limb autotomy and eyestalk ablation are commonly used in the fisheries and aquaculture sectors, particularly in Southeast Asia (Quinitio & Estepa 2011). Autotomy is a naturally occurring defense mechanism in *Scylla* species, allowing them to shed limbs to evade predators (Fujaya et al. 2020; Waiho et al. 2021), a process that is essential for their survival in the wild (Lawton 1989). These techniques have become standard practice in the management and culture of *Scylla* species, helping to optimize their reproductive and growth performance under captive conditions.

Therefore, this study investigated the outcome of eyestalk ablation as well as limb autotomy techniques on the moulting duration, developmental phases (carapace width and body weight increments) and survival rate until subsequent moulting in juvenile *S. olivacea*. The effect of crab gender coupled with limb autotomy was studied as well in order to select the best candidate and method for soft-shell crab production. Although any crab species can be used to produce soft-shell crabs, the orange mud crab, *S. olivacea* was chosen as the suitable candidate in this study due to its abundance and availability in Malaysian waters (Fazhan et al. 2017a; Ikhwanuddin et al. 2011). The findings from this research will provide farmers with practical, science-based protocols for molt induction, potentially reducing production cycles and improving harvest predictability. By optimizing these techniques, the industry can enhance production efficiency through better molting synchronization, improved growth rates, and increased survival - factors directly impacting profitability. Additionally, this research contributes valuable data for developing standardized operating procedures in soft-shell crab farming, ultimately supporting the sustainable growth of this lucrative aquaculture sector that serves high-end seafood markets worldwide.

MATERIALS AND METHODS

ETHICAL NOTES

No license was needed for the acquisition of *S. olivacea* as they are commercial crustacean species harvested by local fishermen. Manual removal of limbs (limb autotomy) and eyestalk ablation were necessary to conduct the following experiments.

PRELIMINARY INDUCED MOULTING

The experiment was carried out at the hatchery of Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, Terengganu, Malaysia. All crab samples were obtained from Setiu Wetland, Terengganu, Malaysia (5°40.583'N, 102°43.089'E). *S. olivacea* juveniles each of 60-80 g body weight were used in this study. Species identification relied upon the taxonomic work by Fazhan et al. (2020) and Keenan, Davie and Mann (1998). The crab was sexed based on their abdomen morphology (Figure 1) (Ikhwanuddin et al. 2011) - adult female crab has a broader and spherical abdomen while the young female crab has invariably triangular abdomen; the young and adult male crab has a straight and slender abdomen. Crabs were selected based on *Scylla serrata*'s moulting stage

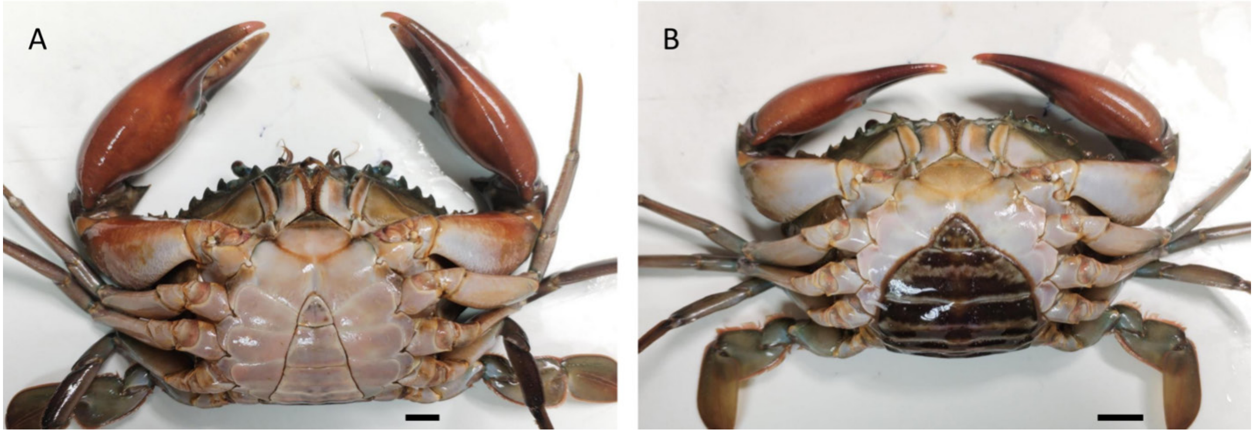

FIGURE 1. The abdomen morphology of orange mud crabs *Scylla olivacea* is useful to differentiate between sexes. (A) Male *S. olivacea* has pointed triangular abdomen; (B) female *S. olivacea* has broader and spherical abdomen

classification, specifically aiming those in the intermoult phase (Quinitio & Estepa 2011). To synchronize the experiment, all juveniles were cultured and only subjected to either one of the treatments (as described herewith) randomly right after moulting. Autotomised crabs were individually cultured in a triangle culture tank (80 cm L × 60 cm W × 40 cm D). Feeding was carried out daily at 0800 h and 2000 h with one chopped fresh fish of 2 cm cube size per crab during the culture period. Uneaten food and wastes were siphoned out 2 h after feeding, prior to water exchange of 50% daily. Water quality parameters were checked daily using a ProQuatro Multiparameter Meter (model PRO1030, manufacturer YSI) and were maintained at a temperature of 26-30 °C, pH 6.5-8.5, salinity of 28-32 ppt, and dissolved oxygen levels > 6 mg/L.

EFFECTS OF EYESTALK ABLATION AND LIMB AUTOTOMY

Newly moulted crabs (20 males) were used for each treatment to identify the outcome of eyestalk ablation and limb autotomy method on the moulting period, growth increment and survival rate until the next moulting. In this experiment, four different treatments were used, i.e., Treatment 1 (T1) – control, Treatment 2 (T2) – limb autotomy group, Treatment 3 (T3) – bilateral eyestalk ablation group, and Treatment 4 (T4) – limb autotomy and bilateral eyestalk ablation group. Limb autotomy was conducted by cutting the joint between the carpus and merus of right and left chelipeds, and the mid-section of the merus of the three pairs of walking legs using scissors. The last pair of swimming legs of crabs was not autotomised to allow minimal mobility for feeding purposes. The eyestalk ablation technique was via the application of the cautery pinch method, involving clamping the base of the eyestalk with a hot pair of pliers or forceps, the same method used by Mann, Asakawa and Blackshaw (1999).

To ascertain the impact of crab gender on the moulting duration, growth increment and survival rate till the next moulting, an additional 10 females of newly moulted crabs were also used in T1 and T2. Crabs in all treatments were cultured individually and a collective of 60 crabs was utilized for this experiment. Water quality parameters and feeding regime were maintained as mentioned earlier in the first phase of the study.

Crabs were observed daily for the formation of new appendage buds, moulting and mortality during water exchange. Emergence of new appendage buds signifies that moulting is near. Newly moulted crabs were maintained for two days until their crab carapace hardened. External carapace width (CW) – the distance between the tips of the 9th antero – lateral spines of the crab carapace and body weight (BW) were measured before treatments (limb autotomy and eyestalk ablation) and after the carapace of newly moulted crabs hardened. The CW was measured with an RS PRO 300 mm, 12-inch Vernier Caliper (0.001-inch resolution), rounded to the nearest 0.1 cm, and the BW was determined using an analytical balance (model ADJ 100-4, manufacturer Kern) with a sensitivity of 0.1 g, rounded to the nearest gram.

The experiment was conducted over a 90-day period. It should be noted that T3 and T4 only involved male crabs, as the experimental design for these treatments was limited to males. Treatment 1 (Control): No eyestalk ablation + No limb autotomy (10 males + 10 females), Treatment 2: No eyestalk ablation + Limb autotomy (10 males + 10 females), Treatment 3: Unilateral eyestalk ablation + No limb autotomy (10 males), and Treatment 4: Unilateral eyestalk ablation + Limb autotomy (10 males).

DATA COLLECTION AND ANALYSIS

Moulting period is measured as the number of days needed for the newly moulted crabs to moult again after being subjected to different treatments. The growth increment is the increment in CW or BW between preliminary moult and subsequent moult. The percentage of survival rate was measured after moulting from each treatment during the second phase of the study.

Statistical analysis was carried out using IBM SPSS Statistics version 28, with a significance level set at 95%. The Shapiro-Wilk test was applied to observe normality, while Bartlett's test was used to measure variance homogeneity. One-Way Analysis of Variance (ANOVA) was performed to establish significant differences ($P < 0.05$) between the treatments, followed by post-hoc Tukey's test (Sokal & Röhlf 1981; Zar 1984).

RESULTS AND DISCUSSION

MOULTING DURATION

Moulting is a crucial process in the growth, development, reproduction, and regeneration of crustaceans (Chang & Mykles 2011; Loeb 1993; Skinner & Graham 1970; Waiho et al. 2017). Moulting duration of *S. olivacea* juveniles recorded in this study ranged from 24 (T2) to 90 (T1) days. The mean duration to the next moulting (days) of *S. olivacea* was significantly different ($P = 0.014$) among treatment as shown in Figure 2(A). Overall, the mean duration to the next moulting for T1 was the longest compared to the other treatment groups. Limb autotomy technique (T2) induced *S. olivacea* to moult within the shortest period in comparison to those subjected to eyestalk ablation (T3) and approximately three times faster than the control group (T1) ($P < 0.05$). This is similar to our previously reported results, where mud crabs subjected to whole limb autotomy (the removal of all appendages except the walking legs) moulted approximately three times faster (average 19.8 days) compared to the control (average 59.3 days) and half limb autotomy (the removal of only the chelipeds, 28.1 days) (Fujaya et al. 2020). This is consistent with findings in other crab species, such as the fiddler crab *Uca pugilator*, as well (Hopkins 1982). In addition, when limb autotomy and eyestalk ablation were used in combination (T4), *S. olivacea* moulted significantly faster compared to T1 ($P < 0.05$) and no significant difference in duration to the next moulting was found when compared to those subjected to only either one of the moult-inducing techniques (i.e., limb autotomy or eyestalk ablation) ($P > 0.05$). Meanwhile, the moulting success rate (%) of *S. olivacea* was significantly different ($P = 0.014$) among treatments as shown in Figure 2(A). The moulting success rate in T1 was the lowest

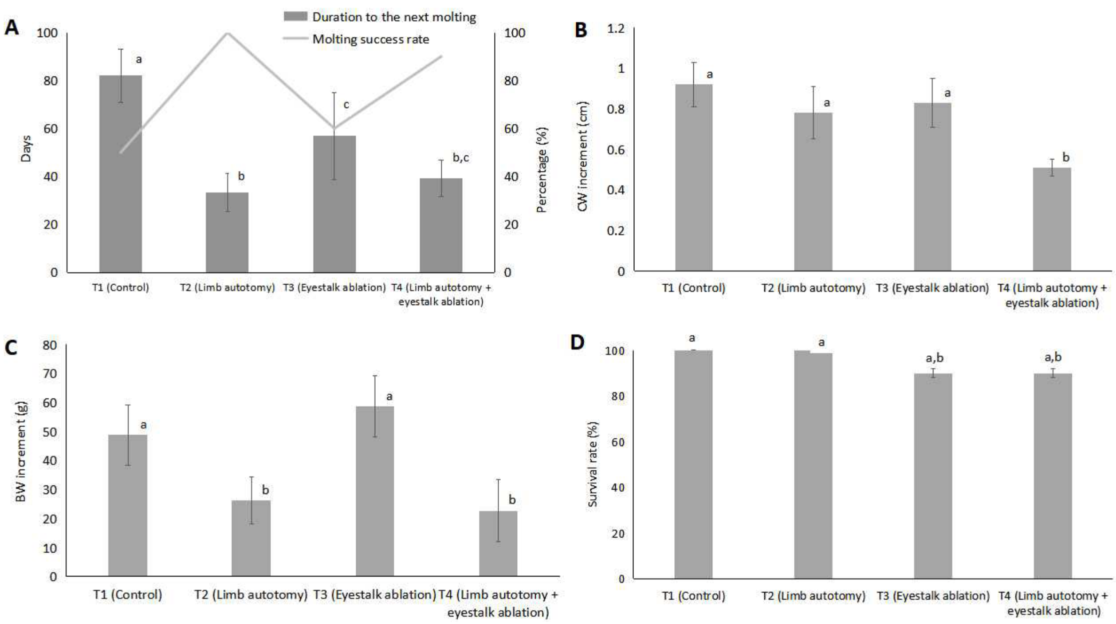

FIGURE 2. Growth increment and survival rate of *Scylla olivacea* juveniles subjected to four treatments over a 90-day study period. (A) Mean duration to the next molt (days) and molting success rate (%); (B) Mean carapace width (CW) increment; (C) Mean body weight (BW) increment; and (D) Survival rate (%) of *Scylla olivacea* juveniles. Values are presented as average ± standard deviation (n=10). Different superscripts of the alphabet letters indicate statistically significant differences between groups ($P < 0.05$) according to Tukey's test

which is 50%, while in T2 was at the highest peak, which is 100%. A combination of both moult-inducing techniques (T4) was more superior (higher moulting success rate) than purely relying on eyestalk ablation technique (T3) to induce moulting, although still slightly less successful as using only limb autotomy technique. This phenomenon occurs because eyestalk ablation inhibits the X-organ (sinus gland) complex to produce moult-inhibiting hormone, resulting in immediate rise of ecdysteroids in crab's hemolymph (Skinner 1985). Limb autotomy, on the other hand, induces crabs to produce Limb-Autotomy Factor-Anecdysis, stimulating them to moult faster (Mykles 2001).

GROWTH INCREMENT AND SURVIVAL RATE

Induced moulting by either limb autotomy, eyestalk ablation or the combination of both techniques appears to have an adverse effect on growth in terms of size. T4 showed significantly lower CW increment compared to the other three treatments (T1, T2 and T3) ($P = 0.022$) (Figure 1(B)). However, the pattern of body weight (BW) increment differed slightly from that of carapace width (CW) increment. Although there was no significant difference between them ($P = 0.058$), treatments T1 and T3 exhibited a substantially higher BW increment compared to T2 and T4 ($P = 0.043$) (Figure 2(C)). The mean BW increment in T2 was comparable to that of T4 ($P > 0.05$). The combination of both limb autotomy and eyestalk ablation techniques should be avoided in the production of soft-shell crabs as this method appears to be too stressful on crabs and showed the least growth in terms of CW and BW increment. Waiho et al. (2021) emphasized that the eyestalk ablation method can be considered inhuman because it causes serious stress to the crab due to the loss of eyesight and increase the probability of diseases infection. Moreover, even though limb autotomy naturally occurs during stressful events or to escape predator, this technique should be avoided because it generates uneven newly generated limbs and induce significant weight loss, although it shortens the moult intervals (Hopkins 1982). Quinitio and Estepa (2011) showed that autotomised *S. serrata* juveniles experienced lower specific growth rate after first and second moult in comparison to intact juveniles. The combination of both limb autotomy and eyestalk ablation (T4) resulted in the lowest CW and BW increments, potentially owing to the high stress induced from the execution of both moult-inducing techniques. Eyestalk ablation is proven stressful in other crustacean species. For example, swimming behaviour and the onset of feeding were disrupted following eyestalk ablation of the Whiteleg shrimp *Litopenaeus vannamei* broodstock (Taylor et al. 2004). Thus, in the future, research into different strategies to minimize the effects of stress during eyestalk ablation of mud crabs, such as the use of various topical anesthetic and the potential application of coagulating agents can be conducted.

The survival rate was considerably high in all four treatments, with control (T1) and autotomised crabs (T2) showing a 100% survival rate while eyestalk ablated (T3) and crabs subjected to both autotomy and eyestalk ablation (T4) had a 90% survival rate ($P = 0.054$) (Figure 2(D)). Similar high survival rate (83.3%) of *S. serrata* after eyestalks ablation was reported by Allayie, Ravichandran and Bhat (2011). Although able to accelerate growth, eyestalk ablation appears to impose greater stress to juvenile crabs in comparison to limb autotomy. The mortality observed in eyestalk ablated crabs in both treatments (T2 & T4) right after moulting may be due to severe physiological stress resulting from visual impairment, direct injury to the nervous system, and impairment of hormone-mediated functions, particularly those related to moulting and growth regulation (Chang 1989; Sainz-Hernández et al. 2008).

EFFECT OF CRAB GENDER

Limb autotomy resulted in a significantly shorter mean duration to the next molting, regardless of gender, compared to the control group (T1) ($P = 0.064$) and was significantly shorter ($P < 0.05$) than T2 (Figure 3(A)). Females moulted faster than males in T1 ($P = 0.022$, $P < 0.05$) but showed no significant difference in the duration to the next moulting in T2 ($P = 0.066$, $P > 0.05$), and this may be attributed to the requirement for the female crabs to moult and hasten maturity in order to copulate and spawn (Mykles 2001). No significant difference in CW increment ($P = 0.071$, $P > 0.05$) was detected in the males and females of T1 and T2 (Figure 3(B)). However, the BW increment in control group (T1) was almost two times larger compared to the limb autotomized group (T2) in both sexes ($P = 0.029$, $P < 0.05$) (Figure 3(C)). A 100% survival rate was achieved by females in T1 and T2, and males in T1 ($P = 0.054$, $P > 0.05$) (Figure 3(D)). Limb autotomy proves to be a useful tool to reduce moulting intervals significantly, regardless of gender. Limb autotomy appears to have a stronger influence on inducing moulting than other natural factors, such as crab gender, making those factors insignificant. However, crabs of both sexes manifested significantly better growth in terms of CW and BW increments when they are fully intact. Limb autotomy reduced moulting intervals but at the cost of smaller carapace size and lighter body weight as the duration for growth is much lesser compared to controls. In addition, limb autotomy also increases the risk of crabs not being able to fully regenerate new limbs, resulting in crabs with uneven limb sizes, thereby reducing their market value due to lower aesthetic factor (Fujaya et al. 2020).

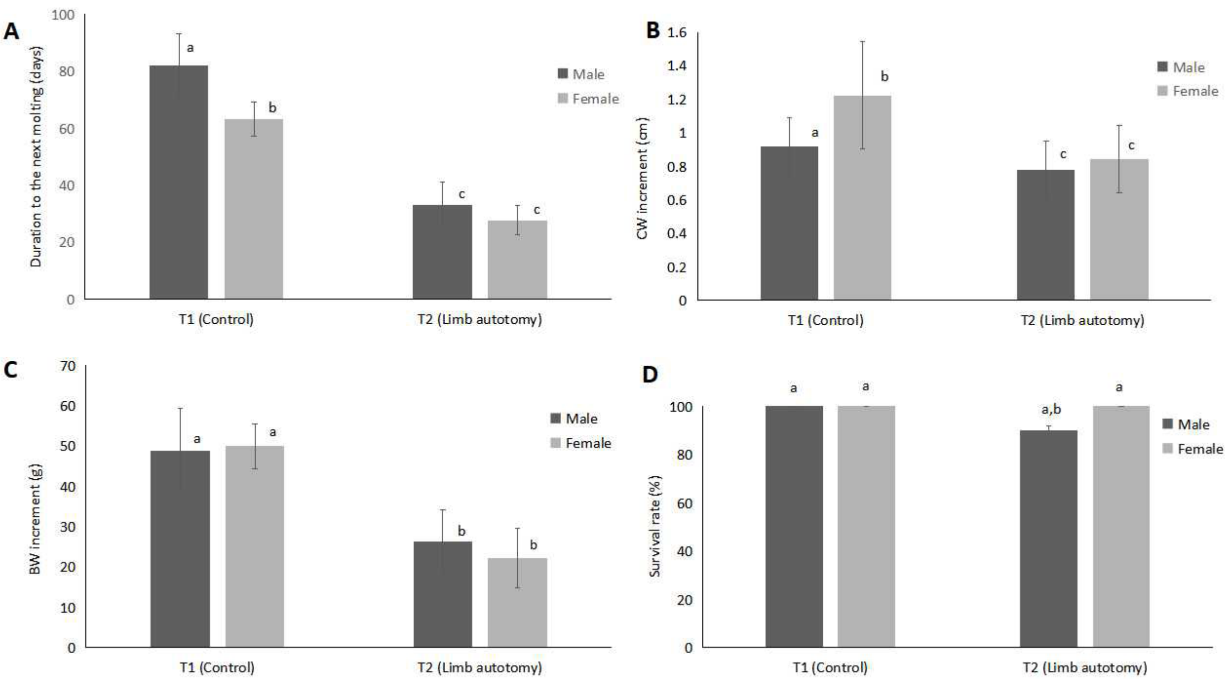

FIGURE 3. The (A) Mean duration to the next moulting (days) and moulting success rate (%), (B) Mean carapace width (CW) increment, (C) Mean body weight (BW) increment, and (D) Survival rate (%) of *Scylla olivacea* juveniles subjected to limb autotomy (T2) and control (T1) within 90-d study period. Values are presented as average ± standard deviation (n=20). Different superscripts of the alphabet letters indicate statistically significant differences between groups ($P < 0.05$) according to Tukey's test

CONCLUSION

Eyestalk ablation and limb autotomy techniques affected moulting duration, growth increment and survival rate until the next moulting. Combined limb autotomy and eyestalk ablation technique should not be used in soft-shell crab production due to its minimal growth increment. Although intact crabs showed the best results in CW and BW increment, limb autotomy technique will be more suitable to be used in the invention of soft-shell crabs as it decreases moulting duration significantly. Eyestalk ablation technique can be used as well when crab's body weight is far more of a concern than moulting duration because even though it is not as efficient as limb autotomy in reducing moult duration, eyestalk ablation is able to induce significant increase in body weight. The effect of gender is insignificant. Both males and females have equal potential to be used as candidates for soft-shell crab production.

Therefore, limb autotomy technique is suitable to be used to shorten the moult cycle during the production of soft-shell crabs, allowing farmers to achieve higher harvest volume compared to traditional culture methods. However, it is important to note that the use of limb autotomy technique requires skill and experience, and the environmental complexity of large-scale commercial production settings might increase the risk of infection to crabs compared to the well-controlled laboratory environment.

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*Corresponding author; email: ikhwanuddin@umt.edu.my