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Effects of Autotomy on Haemolymph Profile and Mineral Composition in Mud Crab (*Scylla serrata*) at Pre and Post- Moulting Cycle

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Abstract. Soft-shell crab is a valuable product variant in aquaculture, with moulting being a critical phase for its production. Physiological indicators such as haemolymph profiles and mineral content in muscle tissue are essential for assessing crab health and moulting performance. This study aimed to evaluate the effect of autotomy treatment on the physiological condition of mud crab (*Scylla serrata*), focusing on haemolymph characteristics and mineral concentrations in muscle during the pre-moulting and post-moulting phases. The treatment involved inducing autotomy of walking legs and claws. Sampling was conducted at four distinct phases: pre-autotomy, 15 hours post-autotomy, at immediate post-moult stage, and two days post-moulting. The physiological parameters observed included Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC), comprising hyaline, semi-granular, and granular cell types. Muscle mineral content was assessed by measuring calcium, sodium, and phosphorus concentrations. Results indicated that autotomy reduced THC and the proportion of hyaline cells, while granular cells increased during the post-moulting phase. Calcium, sodium, and phosphorus concentrations decreased shortly after autotomy, peaked during moulting, and declined again after moulting. These findings provide insight into the physiological responses of mud crabs to autotomy and offer practical reference points for enhancing moulting success and supporting soft-shell crab production in aquaculture systems.

Keywords: soft-shell crab; autotomy-induced moulting; haemocyte profile; crab physiology; aquaculture management

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INTRODUCTION

Indonesia consists of vast mangrove areas and possesses high potential in natural resources (Kuseri et al., 2015). Mangrove forests serve as suitable habitats for various types of crustaceans (Lapolo and Baderan, 2018; Fujaya et al., 2020). Among them, the mangrove crab is a valuable species and an important commodity in the aquaculture industry. Soft shell crab products are a product variant in cultivation activities. Soft-shell crabs are those that have recently shed their exoskeleton and remain soft before mineralization or hardening occurs. Autotomy of legs and claws may induce moulting (Waiho et al., 2021). The autotomy method stimulates carapace turnover and is more efficient in the moulting cycle than conventional rearing methods (Fujaya et al., 2020).

It is hoped that understanding the physiological conditions of haemolymph and meat mineral content will contribute to the development of appropriate handling methods, particularly those that reduce stress levels in crabs. Such methods are expected to support successful autotomy, thereby accelerating moulting and enhancing soft-shell crab production. Observations of haemolymph and meat mineral content serve as physiological indicators to assess the health condition of crabs. The term soft-shell crab refers to crabs that have recently moulted and whose new shells are still soft, making the entire body edible (Waiho et al., 2021). However, the use of leg autotomy as a moulting trigger still faces several challenges in soft-shell crab production. These include asynchronous moulting, high mortality rates (Tavares et al., 2021), and incomplete moulting, often resulting in the failure of leg and claw regeneration.

The autotomy method can speed up the

moulting process in crustaceans and can be applied on a mass scale (Fujaya et al., 2020; Basyuni et al., 2020 ; Asmat-Ullah et al., 2025). This method makes it easier for crabs to change their capace more quickly (Mykles, 2021). In crabs, autotomy by breaking the walking legs and claws or eyestalk will stimulate the production of ovarian maturation, growth hormone, and reduce the activity of Molt Inhibiting Hormone (MIH) (Waiho et al., 2021). which is distributed throughout the body to the base of the walking legs via haemolymph. After MIH activity decreases, organ Y responds to produce moulting hormones called ecdysteroid hormones (Mykles, 2021). However, in other cases, autotomy will actually hinder the eating process, especially if the breaking is done on the claws. Autotomy can occur at the cellular level (e.g. coelomocytes), organisms (e.g. skeleton/muscle), population (e.g. intra/interspecific aggression), and within ecosystems (e.g. anthropogenic changes) (Jobson et al., 2024).

The distribution of aquatic organisms is restricted by salinity, which also affects physiological functions, including haemolymph osmolarity (Long et al., 2017; Zhang et al., 2022). Haemolymph is a fluid equivalent to blood in invertebrates that contains essential nutrients such as proteins, lipids, and other molecules, which are vital for the growth (Gianazza et al., 2021). It plays a role in initiating immune responses, carrying out immunological functions, performing phagocytosis, maintaining haemocyte balance, and supporting various protective mechanisms. Crustaceans have an open circulatory system, where nutrients, oxygen, hormones, and cells are channeled to the haemolymph, which contains haemocyanin, a copper-based protein, so it is blue. In crustaceans, the amount and variability of haemolymph are influenced by the moulting process (Djai et al., 2017; Nur et al., 2021). Haemolymph is used as a channel for ions in the crab body, which are distributed to all parts of the body, such as calcium, sodium, and phosphate (Truong et al., 2023). Organic and inorganic mineral reserves originating from haemolymph help in the process of ecdysis and shell hardening (Marzuki et al., 2025).

The role of haemolymph as a transport medium for various nutrients in the crustacean body in terms of calcium, sodium and phosphate levels shows changes during the moulting process(Da Silva et al., 2019). Moulting is the process of changing old shells to produce new shells, requiring the help of meat minerals because they support the formation of body organs (Demarchi et al., 2011; Truong et al., 2023; Nurdin et al., 2024) it goes on to say that moulting and reproduction are often intertwined, with moulting occurring before or after the reproductive period, depending on the species and environmental conditions. The moulting process likely plays a role in maintaining population balance, allowing for effective growth and reproduction. This process is important for a comprehensive interpretation of the reproductive and growth dynamics of crustaceans in general.

The research purposes are to assess the levels of essential minerals such as calcium, sodium, and phosphorus in the crabs meat before and after moulting. These minerals are vital for exoskeleton hardening. In addition to examining crab health, it is characterized by its haemolymph profile. Through advanced understanding of crustacean physiology, especially on the biological responses to autotomy, can support eco-friendly aquaculture innovations by using natural physiological triggers (like autotomy) instead of chemical or hormonal methods to induce moulting.

METHODS

Experimental Crabs: The test organisms used in this study were wild-caught male and female mangrove crab (*Scylla serrata*) with body lengths ranging from 7 to 8 cm. Crabs were collected using traditional traps operated by local fishermen in the Morosi coastal waters, Konawe Regency, Southeast Sulawesi, Indonesia. Prior to the experiment, all crabs underwent a seven-day acclimatization period to ensure they exhibited normal behavior and physiological responses under laboratory conditions.

Experimental Design: The study used an experimental method with a randomized block design, consisting of one treatment with two groups, classified by sex. Haemolymph and meat samples were collected before autotomy, 15 hours after autotomy, during moulting, and 2 days after moulting.

Autotomy Method: Autotomy was performed only on healthy, active crabs with complete appendages. The procedure involved manually inducing autotomy of one pair of walking legs by applying gentle pressure with pliers until the limbs detached naturally. In addition, both claws (chelae) were removed by cutting, while the swimming legs were left intact. This method was adapted from previously established protocols (Waiho et al., 2021; Fazhan et al., 2022; Jobson et

al., 2024).

Rearing Condition: Following autotomy, the crabs were reared individually in plastic net baskets (15×18.5×20 cm) placed within larger fiber tanks (210×110×45 cm) containing seawater at a depth of 30 cm. Crabs were fed daily with trash fish at 5% of their body weight. The rearing period continued until each crab had completed the moulting process and its new exoskeleton had hardened. Maintenance procedures included daily siphoning to remove uneaten feed and fecal matter, provision of continuous aeration, and partial water changes (20–30%) every seven days.

Water Quality Maintenance: Water quality parameters were regularly monitored and maintained within optimal ranges to support the crabs physiological processes: salinity (22–24 ppt), temperature (24–26°C), pH (6.9–7.2), dissolved oxygen (5–6 ppm), and alkalinity (76.2 ppm). These environmental conditions were managed to minimize stress and promote successful moulting and recovery post-autotomy.

Sampling and Observations

Sampling of Haemolymph: Haemolymph samples (0.9 mL) were collected from the base of the walking legs using a 1 mL syringe preloaded with 0.1 mL of 3.8% sodium citrate solution as an anticoagulant. Sampling was carried out at four distinct phases: (1) Pre-autotomy, (2) 15 hours post-autotomy, (3) Immediately after moulting (soft-shell stage), and (4) Two days post-moulting (hardened shell stage). All samples were stored on ice (at temperatures below 5°C) prior to analysis.

Observation of Differential Haemocyte Count (DHC): For DHC analysis, a drop of haemolymph was placed on a clean glass slide, air-dried, and fixed with 70% methanol for 10 minutes. The slide was then stained with Giemsa solution for 10 minutes, rinsed with distilled water, and observed under a light microscope at 100× magnification.

Observation of Total Haemocyte Count (THC): For THC analysis, haemolymph was diluted and loaded into a Neubauer haemocytometer, and observed under a light microscope at 10× magnification. The total number of haemocytes was counted to quantify the immune cell concentration in each sample.

Observed Parameters: The main physiological parameters recorded during the study included:

(1)Total Haemocyte Count (THC), (2) Differential Haemocyte Count (DHC), (3) Muscle mineral content (calcium, sodium, and phosphorus), (4) Moulting percentage, and (5) Survival rate.

Observation of Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC) Procedure for THC observation, the haemolymph suspension was observed in a Neubauer haemocytometer under a light microscope (10× magnification).

$$\text{THC} = \text{average number of cells} \times 1/\text{volume of chamber}) \times \text{dilution factor}$$

Procedure for DHC observation: a drop of haemolymph was spread on a glass slide, air-dried, fixed with 70% methanol (10 minutes), stained with Giemsa solution (10 minutes), rinsed, and observed under 100× magnification.

$$\text{DHC (\%)} = (\text{Number of haemocyte type/Total haemocytes}) \times 100\%$$

Measurement of Minerals: Muscle tissues were collected from crabs at each experimental phase. The samples were dried and ground into a fine powder. One gram of the powdered tissue was digested with 10 mL of 65% nitric acid (HNO_3) and heated until nearly dry. After cooling, 1 mL of chloride solution was added, and the volume was adjusted to 100 mL using distilled water. The concentrations of calcium and sodium were determined using Atomic Absorption Spectrophotometry (AAS), while phosphorus was analyzed using UV-Visible spectrophotometry following the standard method outlined in SNI 01-2332.3-2006.

Crab Growth and Survival: Crab growth was assessed during the rearing period by observing moulting success and qualitative changes in body size. Although specific growth parameters were not quantified, moulting as an indirect indicator of growth performance. Survival rate was calculated at the end of the rearing period using the following formula:

$$\text{SR (\%)} = (\text{Number of surviving crabs / Initial number of crabs}) \times 100\%$$

Moulting Percentage: Autotomy was induced by applying gentle pressure along the walking legs using pliers, allowing the limbs to detach naturally. In contrast, the claws were removed by cutting, while the swimming legs were left intact. The number of crabs that successfully underwent moulting during the rearing period was recorded to determine the moulting percentage.

Moulting (%) = (Number of moulting crab/Total test crabs) × 100%

Water Quality: Environmental parameters, including salinity, pH, temperature, dissolved oxygen (DO), and alkalinity, were monitored regularly to ensure optimal rearing conditions. Consistent measurement of these factors was essential to support the physiological processes of the crabs, particularly during the moulting cycle.

Data Analysis: The data obtained from this study, consisting of THC, DHC, calcium, sodium, and phosphorus, water quality data and moulting are presented in the described descriptively.

RESULTS AND DISCUSSION

Total Haemocyte Count (THC)

Crabs that were reared after being treated, the highest THC content was found in moulting crabs with a THC value of 4.85×10^6 cells mL^{-1} followed by the phase two days after moulting crabs, namely 2.71×10^6 cells mL^{-1}, then in pre-autotomy crabs, it was 1.87×10^6 cells mL^{-1} and the lowest 15 hours after autotomy was 1.09×10^6 cells mL^{-1}. The phase of the crab's condition affects the THC content, a drastic increase in THC immediately after moulting, is presented in Figure 1.

The amount of THC in mud crabs at the start of pre-autotomy was 1.87×10^6 cells mL^{-1}, then experienced a change in the amount of THC 15 hours after autotomy, namely 1.09×10^6 cells mL^{-1}, in this case autotomy of the walking legs and claws of mud crabs reduced the amount of THC. When moulting, the amount of THC in mud crabs is 4.85×10^6 cells mL^{-1}. Then two days after moulting, the amount of THC changes, namely 2.71×10^6 cells mL^{-1}, in this case the amount of THC increases during moulting and decreases again after moulting.

This difference in the amount of THC is caused by stress caused by the removal of the walking legs and claws so that the crab cannot freely walk or take food and requires some recovery time due to the trauma resulting from the detachment of the movement organs. This was also stated by (Darnell et al., 2020) that after the walking legs and claws are cut, the mud crab is vulnerable to predators or experiences stress due to the injuries experienced, thereby reducing its physiological condition has the effect of reducing the amount of THC. The low amount of THC is caused by defense activity, in this case the haemocyte cells will carry out a degranulation process and initiate a phagocytosis process (Liu et al., 2020).

The amount of THC increases during moulting and then decreases again 2 days after moulting. The high amount of THC is a result of the process of releasing the old exoskeleton to form a new exoskeleton. At this stage the crab is in a critical state so the male crab's stress level increases which is indicated by moulting failure due to the crabs inability to release the old framework. This is in accordance with the opinion that during moulting the stress level increases thereby reducing the survival rate, supported by the opinion of (Chang et al., 2005) stated that during ecdysis there was a large increase in the amount of THC in coagulated haemolymph and in absolute amounts, however the amount of THC decreased 24 hours after ecdysis but there was no change in the number of haemocytes until it reached per cubic milliliter because the volume of haemolymph returned to normal. The increase in the amount of haemolymph after ecdysis is caused by an increase in the cytotic number. The amount of THC is said to be normal, indicated by a high level of immune system activation of phagocytic cells. The amount of THC that is too high or too low is said to be an indicator of a stress response (Perez and Fontanetti, 2011).

Differential Haemocyte Count (DHC)

The Differential Haemocyte Count (DHC) in crab haemolymph varies across different physiological phases. In the pre-autotomy (normal) phase, the proportion of hyaline cells is highest at 61.73%, followed by semi-granular cells at 22.95%, and granular cells at 15.32%. Fifteen hours after autotomy, the percentage of hyaline cells decreases to 48.14%, while granular and semi-granular cells increase to 27.62% and 24.25%, respectively. Immediately after moulting, a significant shift is observed, with granular cells dominating at 69.85%, while hyaline and semi-granular cells drop sharply to 14.11% and 16.03%, respectively. Two days after moulting, when the crabs shell has hardened, the proportions are more balanced: hyaline cells at 24.40%, semi-granular at 24.85%, and granular at 50.75%. These data indicate a trend in which hyaline cells predominate prior to moulting, semi-granular cells remain relatively stable throughout, and granular cells increase markedly following moulting. This pattern is illustrated in Figure 2.

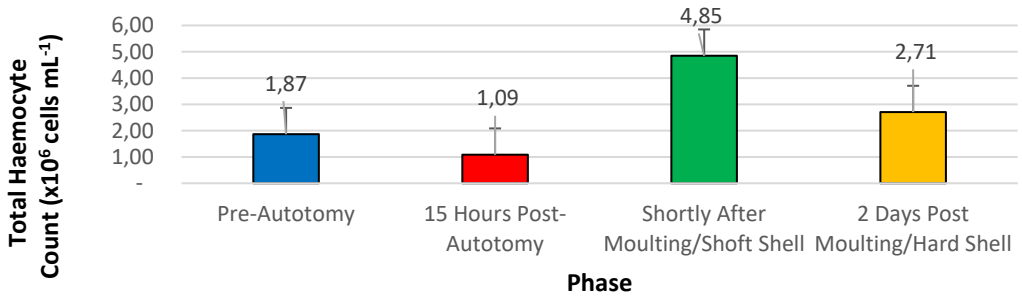

Figure 1. Total Haemocyte Count (THC) in the haemolymph of mud crab (*Scylla serrata*) in the phase before and after autotomy of walking legs and claws, n=6.

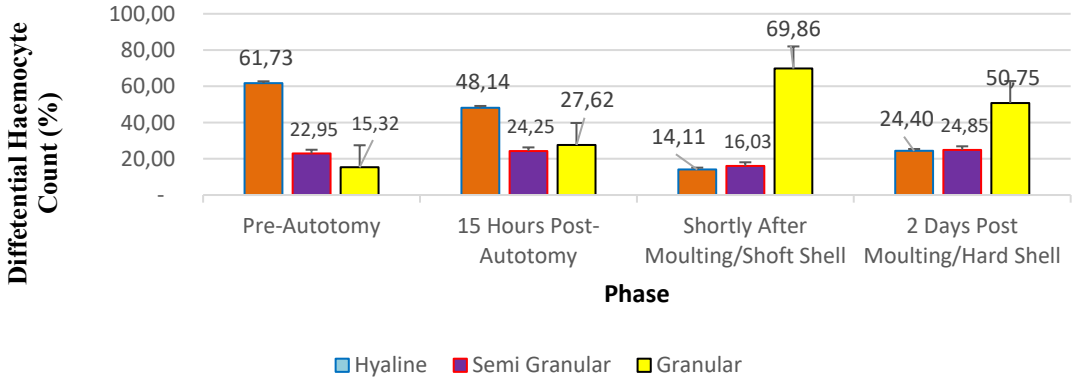

Figure 2. Percentage of Differential Haemocyte Count (DHC) in the haemolymph of mud crab (*Scylla serrata*) in the phase before and after autotomy of the walking legs and claws. DHC consists of hyaline cells (H), semi-granular (SG) and granular (G) cells, n=6

The Differential Haemocyte Count (DHC) in mangrove crabs is classified into three main types based on their morphology: hyaline cells, semi-granular cells, and granular cells. Hyaline cells are the smallest and smoothest in appearance, while semi-granular and granular cells are larger and contain granules. Granular cells are the largest and contain more granules than semi-granular cells (Li et al., 2019). At the beginning of the trial (pre-autotomy phase), the proportion of hyaline cells was 61.73%. This value declined to 48.14% fifteen hours after autotomy, further decreased to 14.11% during moulting, and slightly increased to 24.40% two days after moulting. These fluctuations suggest that autotomy reduces the proportion of hyaline cells, which then decreases further during moulting before partially recovering post-moulting.

In the case of semi-granular cells, their proportion was 22.95% prior to autotomy, increased slightly to 24.25% fifteen hours after autotomy, decreased to 16.30% during moulting, and rose again to 24.85% two days after moulting. This indicates a relatively stable pattern, with mild fluctuations across the phases. Granular cells constituted 15.32% of total haemocytes before autotomy, increasing to 27.62% fifteen hours after autotomy. A substantial rise was observed during moulting, reaching 69.86%, followed by a decline to 50.75% two days after moulting. The marked increase during moulting is likely associated with physiological stress and immunological activation in response to exoskeleton shedding.

Among the three haemocyte types, granular cells demonstrated the highest proportions overall, particularly during moulting. The observed reduction in hyaline cells is likely due to stress from physical injury post-autotomy, which simultaneously stimulates the production of semi-granular and granular cells. This is consistent with the findings of Mousavi (2010), who reported species-specific variations in total and differential haemocyte counts in crustaceans.

These shifts in DHC are associated with the immune response and neurogenesis processes (Benton et al., 2014). According to Liu et al. (2021), the presence of hyaline cells is linked to phagocytic activity, enabling crustaceans to defend against pathogens. A decline in hyaline cells typically corresponds with an increase in

granulocytes as part of the immune defense.Semi-granular cells consistently showed the lowest proportions among the three types. Their numbers tend to recover post-moulting, as they are involved in immune functions such as exocytosis and release of antimicrobial compounds (Sheshachalam et al., 2014). Haemocyte classification and immune functionality in crabs have also been described by Chang et al. (1987), who noted higher esterase activity in granular cells.

The surge in granular cell numbers during moulting is likely due to the need for exoskeleton regeneration, involving the activation of the prophenoloxidase system. Othman et al. (2022) reported that a high proportion of granular cells correlates with elevated phenoloxidase activity and respiratory bursts occurring 24–48 hours post-moulting, after which the number of granular cells begins to decline. Similarly, Vargas et al. (1998) highlighted that a higher total haemocyte count can stimulate prophenoloxidase activation, which enhances the crabs immune defense mechanisms.

Calcium

Calcium concentration in mangrove crab meat demonstrates moderate variation across different physiological phases. In the pre-autotomy phase, calcium content is 1.12 ppm, which decreases to 0.97 ppm fifteen hours after autotomy. The concentration then increases during moulting to 1.24 ppm and slightly decreases again to 1.16 ppm two days post-moulting (**Figure 3**). These changes suggest that both autotomy and moulting influence calcium dynamics within the crab's body.

At the beginning of the pre-autotomy phase, the calcium concentration in mangrove crabs was recorded at 1.12 ppm. Fifteen hours after autotomy, this value decreased to 0.97 ppm. During the moulting phase, the calcium level increased significantly to 1.24 ppm, before slightly declining to 1.16 ppm two days after moulting. These fluctuations indicate that autotomy treatment leads to a reduction in calcium levels, whereas the moulting process is associated with a temporary increase in calcium concentration, followed by a post-moulting decline.

The observed decrease in calcium levels after autotomy is likely caused by the physiological stress and metabolic demands triggered by the loss of walking legs and claws. This is supported by previous studies indicating that calcium deficiency can disrupt normal physiological functions such as bone formation, soft tissue maintenance, homeostasis, and acid-base balance (French et al., 2002; Zhu et al., 2024).

Conversely, the increase in calcium during moulting plays a crucial role in the processes of gastrolith formation and exoskeleton hardening. The success of moulting is highly dependent on the availability of calcium in the crab's body, and calcium deficiency is one of the contributing factors to moulting failure. During this phase, calcium is stored in the form of gastroliths, which are reabsorbed and utilized in the calcification of the new exoskeleton. The primary sources of calcium for mangrove crabs include dietary intake, absorption from water, and recycling from old exoskeletons or through cannibalism. This is consistent with the findings of Zhu et al. (2024), who reported that dietary supplementation with 25-hydroxyvitamin D_3 can enhance growth performance, calcium-phosphorus metabolism, lipid metabolism, antioxidant capacity, and immune function in *Litopenaeus vannamei* reared in low salinity conditions.

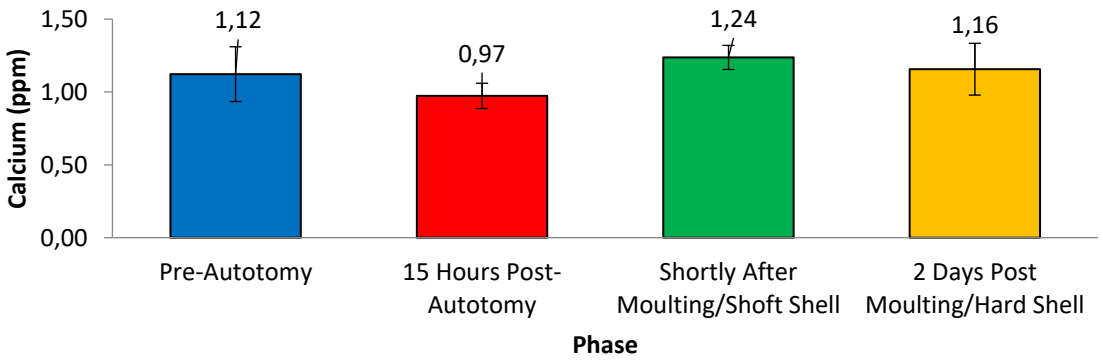

Figure 3. Calcium content in mud crab meat (*Scylla serrata*) in the phase before and after walking leg and claw autotomy, n=4.

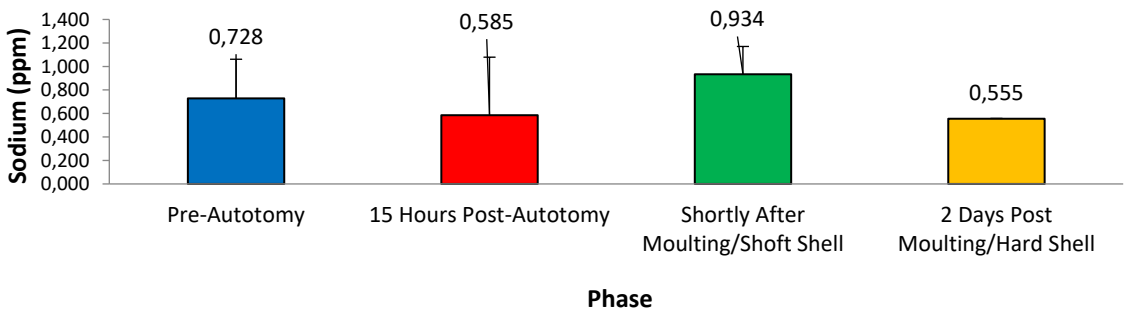

Figure 4. Sodium content in mangrove crab (*Scylla serrata*) meat in the phase before and after autotomy of walking legs and claws, n=4

Sodium

Sodium concentration in mangrove crab meat varies across different physiological phases. The highest sodium level is recorded immediately after moulting, reaching 0.934 ppm. In comparison, the pre-autotomy phase contains 0.728 ppm, which decreases to 0.585 ppm fifteen hours after autotomy and slightly drops again to 0.555 ppm two days post-moulting (Figure 4). These results indicate that moulting significantly increases sodium concentration, whereas autotomy leads to a marked reduction.

Autotomy causes a decline in sodium levels, likely due to physiological stress and injury, which interfere with normal mineral metabolism. Guo and Dixon (2021) emphasized that stress can disrupt mineral homeostasis, including sodium, which plays a critical role in maintaining the organism's fluid balance. The observed reduction in sodium post-autotomy aligns with this understanding of stress-induced mineral imbalance. Conversely, sodium levels increase during moulting due to active absorption processes. Campli et al. (2024) reported that peak sodium absorption occurs during moulting and is closely associated with water uptake. After moulting, sodium concentration declines rapidly, even within 30 minutes post-ecdysis. This decline is attributed to the redistribution of sodium through body fluids during the moulting cycle. Furthermore, during this process, sodium concentration in extracellular fluids is reduced due to water absorption, thereby enhancing sodium uptake by two- to fivefold to maintain ionic equilibrium.

These findings highlight the dynamic role of sodium in relation to stress, moulting physiology, and osmoregulatory mechanisms in mangrove crabs. Both internal and external factors, including injury and water salinity, contribute significantly to sodium fluctuations, which can ultimately affect the success of moulting and post-moult recovery.

Phosphorus

Phosphorus concentration in mangrove crab meat shows a relatively consistent pattern across different physiological phases. In the pre-autotomy phase, the phosphorus content is 0.072 ppm. This concentration decreases to 0.0715 ppm fifteen hours after autotomy, then increases to 0.075 ppm immediately after moulting, and slightly declines again to 0.073 ppm two days post-moulting (Figure 5). These fluctuations suggest that autotomy and moulting might influence phosphorus levels in crab tissues.

Autotomy, particularly the loss of walking legs and claws, leads to a marked reduction in phosphorus concentration. This decline is attributed to injury-induced stress, which disrupts metabolic balance and temporarily lowers phosphate availability. According to Amini et al. (2019), phosphate plays an essential role in energy storage and release; thus, the stress-induced energy demand following autotomy likely contributes to decreased phosphate levels, followed by gradual recovery during the healing process.

During moulting, phosphorus concentration increases, likely due to its role in the synthesis of new cuticles. However, phosphorus levels decrease again after the moulting process is complete. This pattern may reflect phosphorus absorption and utilization during the exoskeleton formation phase. A low phosphorus level, particularly in crabs, has been associated with unsuccessful moulting events. In this context, haemolymph and gastroliths serve as internal phosphate reservoirs for shell development. Phosphate, together with calcium, is essential for forming the carapace or exuvia, a structure that plays a critical role in the moulting process, especially in hardening the new shell (Lafont et al., 2021).

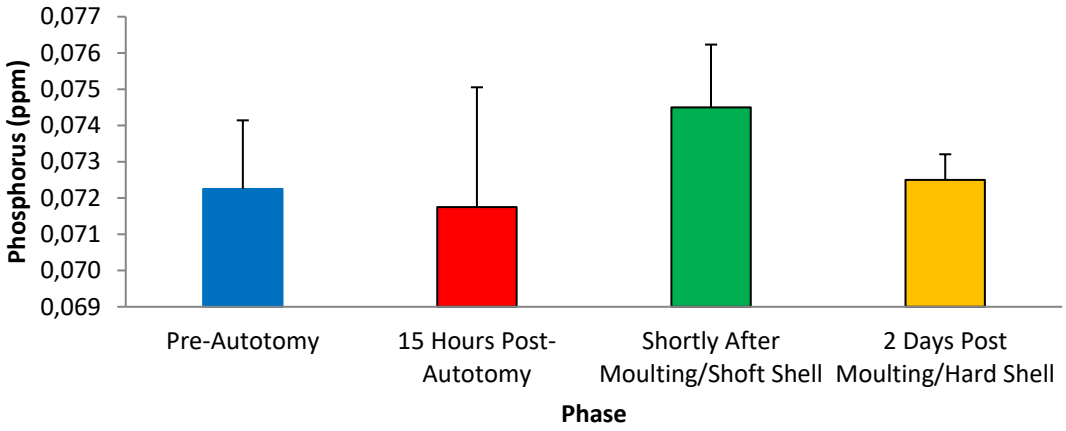

Figure 5. Phosphorus content in mangrove crab (*Scylla serrata*) meat in the phase before and after autotomy of walking legs and claws, n=4

Environmental conditions, particularly salinity, also influence post-moult shell hardening. The duration required for the carapace to fully harden tends to be shorter-approximately 9±0 days-when crabs are maintained in water with a salinity range of 23–28 ppt (Kurkute et al., 2019). These findings underscore the importance of both physiological and environmental factors in the regulation of phosphorus metabolism and successful moulting in mangrove crabs.

Moulting Percentage

The highest percentage of moulting after autotomy of walking legs and claws was found in female crabs, 100%, and the lowest in male crabs, namely 50%. The low percentage of male crabs moulting is a result of the inability of male crabs to shed their old shells.

The high moulting percentage is related to the abundance of THC and increased granular cells (DHC) as well as high absorption of meat minerals such as calcium, sodium and phosphate. This is supported by (Da Silva et al., 2019; Campli et al., 2024); Astutik et al., 2025) that the high amount of THC and the abundance of granular cells help facilitate the moulting process because the presence of haemolymph in the body increases the concentration of calcium, sodium and phosphate also increases.

Autotomy of the walking legs and claws can speed up moulting due to the functioning of the ecdysteroid hormone at the base of the walking legs and claws so that the crab is stimulated to molt. Then increases the amount of THC because when the crab molts there is a respiratory explosion which stimulates an increase in granular cells to stimulate prophenoloxidase activity to produce phenoloxidase activity as defense activity but the amount of THC and DHC in hyaline cells was higher in crabs. According to (Nur et al., 2021), a high amount of THC can increase the number of granular cells, whereas if we look at gender differences. Meanwhile, if we look at the levels of calcium, sodium and phosphate when the crab molts, there is an increase, during moulting crabs absorb more meat minerals than male crabs, thereby increasing the percentage of moulting. Calcium, sodium and phosphate are minerals that play a role in the formation of bones and teeth, exoskeleton, regulating fluid volume, fluid balance and storing energy. Calcium and phosphate are minerals that play a role in the formation of bones and teeth which act as exuvia during moulting, high absorption occurs to form a new exoskeleton (Mmanda, 2025), sodium increases when crabs molt as a result of high sodium absorption. It causes body fluids to increase thereby increasing blood volume.

The fastest moulting occurs in crabs after autotomy of walking legs and claws, namely 80 days (Fujaya et al., 2020). The fast time required for crabs to molt is related to the lack of the MIH hormone in accordance with the opinion (Hosamani et al., 2017). The autotomy of some of the movement organs was achieved by moulting at 43, 46 and 50 days. This treatment achieved long moulting. Supported by the statement (Mykles and Chang, 2020) that autotomy treatment inhibits the production of the MIH hormone found in the crab's locomotion organs. After the amount of MIH in the haemolymph has decreased, the Y organ is stimulated to produce ecdysteroid hormones so that the crabs molts. Meanwhile, the factor causing the long moulting time for crabs that have autotomy of walking legs and claws is also related to the presence of the MIH hormone

in the walking legs so that organ x can still work to inhibit moulting. Supported by the statement that the skin moulting process in crustaceans does not take place at the same time and in the same amount due to differences in the body's physiological mechanisms of each organism, especially osmotic capacity (Tian and Mu, 2012)

Survival Rate

Based on the results of observations during the research, it was found that the highest survival rate was found in male and female crabs without autotomy, namely 100%, followed by female crabs after autotomy, namely 100%, while the lowest was in male crabs after autotomy, namely 67%. At the stage after autotomy, the walking legs and claws of the crab will force themselves to molt, some crabs will molt after their movement organs have fully grown and there are also mangrove crabs whose movement organs have not yet grown and have already carried out the moulting process, this is one of the causes of moulting failure.

The low survival rate of male crabs is due to failure to molt as a result of the crabs inability to shed its old shell and cannibalism. This is in accordance with the statement (Techa and Chung, 2013; Fujaya et al., 2020) that cutting walking legs and claws functions to increase the presence of moulting hormones; however this method can cause imbalance or stress so that the crab will respond to regenerate body parts by moulting. The high level of stress after a crabs autotomy will force its body to molt, even though sometimes its limbs are not yet fully grown so that the crab fails to molt. Besides, this stage requires a lot of energy. Supported by the statement (Fu et al., 2022) during moulting there is a high increase in stress levels because at this stage the physical condition is still very weak, and the flesh emits a fishy smell due to its exposed body frame. The fishy smell from the body is what attracts predators to eat it.

Water Quality Parameters

Water quality measurements taken during the study showed that temperature ranged from 26 to 35°C, salinity from 15 to 25 ppt, pH between 6.5 and 8.0, dissolved oxygen (DO) levels ranged from 4.0 to 8.0 ppm, and alkalinity was recorded at 76.2 ppm. These environmental parameters are closely associated with the moulting success of autotomized mud crabs (*Scylla* spp.) (Suyono, 2021). Temperature plays a critical role in accelerating metabolic processes during moulting, while salinity supports the exoskeleton shedding process through its influence on osmoregulation. Additionally, pH affects overall metabolic activity, which is essential for energy production during the moulting phase (Shimada et al., 2000; Long et al., 2017; Ren et al., 2021; Marzuki et al., 2025).

Haemocyte counts may decline following moulting when environmental conditions deteriorate, such as during periods of low dissolved oxygen, suboptimal temperature, or reduced salinity. Under hypoxic conditions, elevated levels of crustacean hyperglycemic hormone (CHH) have been detected in the haemolymph(Chang et al., 2005). Furthermore, in certain crustacean species, new haemocytes are consistently generated and released from hematopoietic tissues to sustain a balanced and functional haemolymph composition. This ongoing process plays a crucial role in maintaining an effective immune defense, enabling the organism to cope with diverse challenges and preserve its overall health. Although the rate of haemocyte production may fluctuate, the process remains continuous throughout the crustacean's lifespan (Liu et al., 2021).

The novelty of this study is combining a behavioral trigger (autotomy) with physiological and biochemical measurements specifically haemolymph and mineral concentrations in crab meat at different moulting stages. Some findings before have not linked them directly with changes in internal mineral dynamics and meat quality. The results of this study can provide new information by integrating immunological (THC and DHC) and physiological (muscle mineral content) analysis of mangrove crab responses to autotomy during the moulting phase. This approach to analyzing crustacean physiological adaptation during the moulting phase can provide a scientific basis for managing health and moulting success in soft-shell crab aquaculture

CONCLUSION

Autotomy reduced total haemocyte and hyaline cell counts; however, post-moult crabs exhibited increased THC and a higher proportion of granular cells. Calcium, sodium and phosphate concentrations decreased 15 hours after autotomy but increased when the crab was moulting and decreased again after moulting. Although haemolymph and mineral levels provide valuable biochemical indicators, the hormonal or genetic pathways involved in moulting and mineral regulation remain unclear. Future research should

study the expression of moulting-related genes (e.g., ecdysone receptor, MIH) and hormone fluctuations during pre-moult and post-moult in autotomized crabs.

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AUTHOR CONTRIBUTION STATEMENT

YY and EY designed and conducted the experiments, validated the data, and performed data analysis. IN and KS contributed to parameter measurements, while LF assisted in the crab rearing process. The manuscript was written by YY and LF, in consultation with IN.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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