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Original Article

Relationship between the carapace width and body weight increments and the confirmation of Stage 1 ovary after molting of the immature orange mud crab, *Scylla olivacea*, (Herbst, 1796) in captivity

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Abstract

This study describes the relationships between the carapace width (CW) and body weight (BW) increments and the confirmation of Stage 1 ovary after the molting of immature orange mud crabs, *Scylla olivacea*, in captivity. Morphological coloration and histological assessments were done on 165 immature female *S. olivacea*. Healthy crabs were sampled from the Setiu Wetlands in the coastal waters of Terengganu on the Malaysian Peninsula from July to September 2015. Thirty crabs were sacrificed for a preliminary study as a standard (control) in which the gonads (if available) were dissected for histological study. The remaining crabs (n=135) were selected for subsequent analysis (limb autotomy). Compared to the controls, the molted crabs generally did not produce any difference in the stage of the ovaries (remaining in Stage 1) but were observed to have larger oocytes. This demonstrated that the limb autotomy technique may activate hormone regulation, thus triggering vitellogenesis in the mud crab. There were also positive correlations between CW and BW ($P=0.001$, $P<0.01$) and significant differences through regression analysis ($P=0.002$, $P<0.01$) with the equation $y = 2.61x + 6.27$ ($R^2=0.069$). These results can be useful for developing baseline data for further crab management in Malaysia.

Keywords: molting, *S. olivacea*, carapace width, body weight, maturation

1. Introduction

Orange mud crabs (*Scylla olivacea*) have recently become the most important and valuable fishery species in Malaysia along with two other species, *S. tranquebarica* and *S. paramamosain* (Azra & Ikhwanuddin, 2016; Ikhwanuddin, Azmie, Juariah, Zakaria, & Ambak, 2011; Ikhwanuddin, Bachok, Hilmi, Azmie, & Zakaria, 2010a; Ikhwanuddin, Bachok, Mohd-Faizal, Azmie, & Abol-Munafi, 2010b). They are widely distributed around the South China Sea and extend into the Indian Ocean and the western Pacific (Ikhwanuddin *et al.*, 2011; Keenan, Davie, & Mann, 1998). The demand for this species has recently increased due to its quality, including high meat yield (Rattanachote & Dangwatanakul, 1992), large size (Ikhwanuddin, Nur-Atika, Abol-Munafi, & Muhd-Farouk, 2014), and rapid growth during culture (Millamena & Quinitio, 1999). Recently, the production of mud crabs has

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become an important import commodity (Ikhwanuddin *et al.*, 2014), leading to many great opportunities in crab farming (Begum, Shah, Mamun, & Alam, 2009), as they are in high demand for all size classes, including mature females for the premium market segment as well as soft-shell production for food materials (Hungria, Tavares, Pereira, Silva, & Ostrensky, 2017; Marichamy & Rajapackiam, 2001). The high demand in local markets for large-size crabs, together with the local fisheries practice of selling all sizes of mud crabs (Waiho, Fazhan, & Ikhwanuddin, 2016), prompted the present study of the size and carapace width increments of mud crabs.

A morphometric analysis based on Cadrin (2000) provides a powerful complement to genetic and environmental stock identification approaches and weight-width relationships in populations and is important for estimating the population size of a stock for exploitation purposes (Oluwatoyin, Akintade, Edwin, & Victor, 2013). According to Atar and Secer (2003), the weight increment and length-width ratio are widely used in a given geographic region to identify the growth and formation of a species. Many investigations based on Oluwatoyin *et al.* (2013) have studied the length-weight relationships of fin-fishes, but such information on the portunid species is still scarce (Sukumaran & Neelakantan, 1997). The length-width relationship is regarded as the most suitable measurement for evaluating fish (Dulcic & Kraljevic, 1996; Jones, Petrell, & Pauly, 1999; Petrakis & Stergiou, 1995; Stickney, 1972) and crustacean (Ikhwanuddin *et al.*, 2010a; Sukumaran & Neelakantan, 1997; Tabash, 2001; Villasmil, Mendoza, & Ferrer, 1997) populations. Moreover, there is little information on carapace width and body weight increments and estimation equations after the crabs have molted. There has also been disagreement concerning the ovarian maturation stage changes after each molt (International Workshop on Portunid Crabs Aquaculture and Sustainable Fisheries 2016, Universiti Malaysia Terengganu, Terengganu, Malaysia).

In this study, the relationships between carapace width and body weight were analyzed and presented. We aimed to determine the sizes of and relationships between the carapace width and body weight increments after immature female mud crabs, *S. olivacea*, molted. In addition, the ovarian maturation stage after the immature crabs molted was also determined to confirm a baseline as a starting point for future research.

2. Methodology

2.1 Sampling

A total of 165 immature female *S. olivacea* with carapace widths (CW) less than 9.06 cm (Ikhwanuddin *et al.*, 2010a, b) were sampled from Setiu Wetlands Mangrove Forest, Terengganu, Malaysia (5°31′23.1″N 102°55′56.1″E) from July to September 2015. The crabs were transported alive to the Marine Hatchery of Institute of Tropical Aquaculture and Fisheries Research, where their morphological characteristics were determined following the methods of Keenan *et al.* (1998). The CW and BW of each crab were measured using a six-inch liquid crystal display digital vernier caliper (accuracy, 0.01 cm; Kingsmart, Hong Kong) and a digital electronic balance (accuracy: 0.01 g; Shimadzu Corp., Japan) and were labeled with a cable tie tag (nylon, 3x150 mm). The CW distance was measured between the 9th antero-lateral spines of the mud crab carapace.

2.2 Induced molting in immature crabs

Thirty immature crabs were randomly chosen as a control, while the remaining 135 crabs were used for subsequent analyses. To induce molting, limb autotomy was applied (Amin-Safwan, Muhd-Farouk, Nadirah, & Ikhwanuddin, 20 16; Nadiah, Ikhwanuddin, & Abol-Munafi, 2012) to 135 immature crabs by cutting off the chelipeds and walking legs while leaving the pleopods (swimming legs) for the crab's movement. The autotomized crabs were then cultured in fiberglass tanks (320x138x60 cm) with an ambient salinity of 28-32 ppt and the temperature was maintained at 27-29 °C using a heater. Moderate aeration, ambient light intensity, and 100% water exchange every two days were given to the tanks. The crabs were fed with chopped yellowstripe scad, *Selaroides leptolepis*, at 10% of their body weight twice daily at 0900 h and 1700 h for observation of the molting event. The culture period ended after all the autotomized crabs had successfully molted.

2.3 Experimental design

Thirty immature crabs were randomly chosen and dissected for control data of the external morphology and histological assessment. A total of 135 immature crabs underwent limb autotomy to induce molting. Once the crabs molted and their carapace had fully hardened in an average of 7 days, their BW and CW were measured again to determine the increment size. Next, all the molted crabs with fully hardened carapaces were dissected to determine the ovarian maturation stage (based on coloration), and small portions of the ovarian lobes were fixed in Davidson's solution (24 h) for histological assessment and confirmation of the ovary stage. Davidson's solution was chosen as the fixative in the present study because it is the most suitable medium for crab tissues (Muhd-Farouk, Amin-Safwan, & Ikhwanuddin, 2016a).

2.4 Histological assessments

The histological study was based on the standard histological procedure following Muhd-Farouk, Jasmani, and Ikhwanuddin (2016b). Tissue processing of a small portion of the ovarian lobes, that had been fixed in Davidson's solution (24 h) and continued with 70% alcohol (overnight), was done in an automatic tissue processor for 18±1 h at 60 °C to infiltrate the fixed tissue samples. After processing and hydration of the tissue was complete, wax impregnation was performed by embedding in paraffin wax to form a solid block for easier handling. The solid block was then sectioned into 5 µm sections using a rotating microtome (Leica RM2135). The sections were placed on the paraffin section in a water bath maintained at 40-45 °C to allow for expansion. After the sections were fully expanded, a microscope slide was held at an angle and slid under one or two well-formed tissue sections (the fishing step). After the fishing step was complete, the microscope slides were dried on a hot plate at 40 °C overnight. The samples were then stained with modified hematoxylin-eosin (H&E). The diameters of 100 oocytes from each

crab were measured using an Advanced Research Microscope (Nikon Eclipse 80i, Japan) together with NIS-Elements D 2.30 software.

2.5 Data analysis

The CW and BW increment sizes were measured and recorded. The external morphology of the ovaries was photographically recorded, and the oocyte structures and diameter sizes were histologically measured and recorded. The collected data were analyzed using statistical correlation to determine the strength of a relationship with the available statistical data and regression analysis to estimate the relationships among the variables using Statistical Package for the Social Sciences (SPSS) software (version 22.0 for Windows; SPSS Inc., Armonk, NY: IBM Corp.). The data are shown as mean±standard deviation (SD).

3. Results

3.1 External morphology and histological assessment

Morphologically, all 30 immature female crabs dissected for control data were in Stage 1 ovary (Figure 1a). The ovary was very small, thin, and very hard to differentiate from the digestive gland. The ovaries were seen as a strand- and ribbon-like tissue structure. However, the results for the 135 crabs that underwent induced molting by limb autotomy showed development after the crab had molted, but remained in Stage 1 ovary with increased ovary volume (Figure 1b).

Table 1 shows the mean oocyte diameter for the control and treatment (autotomized crab) of *S. olivacea* in this study. Regarding the histological assessment, the mean oocyte diameter for the control crabs was 25.67±4.38 µm, which was referred to as Stage 1 ovary (Figure 1c). The mean oocyte diameter after the crabs molted showed an increment in oocyte sizes, but still maintained a Stage 1 ovary at 34.76±12.13 µm. The oocyte, follicle cells, nucleus, and oogonia were still present (Figure 1d).

3.2 Size of carapace width and body weight increments

Table 2 shows the mean, standard deviation, and highest and lowest recorded CW and BW increments of *S. olivacea* in this study. Figure 2 and Figure 3 show the frequencies of the CW and BW increments in *S. olivacea*. On average, the CW and BW increments were 0.816±0.27 cm and 8.395±2.72 g, respectively.

3.3 Correlation and regression analyses

Table 3 shows the correlation analysis in this study. There was a positive correlation between CW and BW ($P=0.001$, $P<0.01$). As CW increased, BW also significantly increased in *S. olivacea*. The regression analysis (Table 4) showed a significant difference ($P=0.002$, $P<0.01$), with $y= 2.61x + 6.27$ ($R^2=0.069$) (Figure 4) between CW and BW in *S. olivacea*.

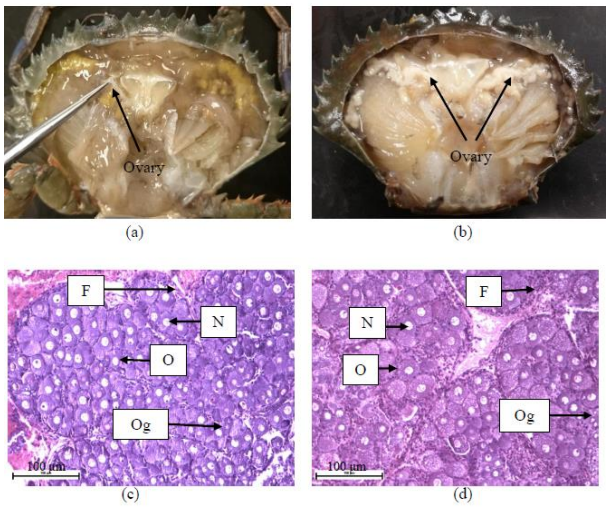

Figure 1. (a) External morphology of Stage 1 ovary before limb autotomy, (b) External morphology of Stage 1 ovary after molting, (c) Histological assessment of Stage 1 ovary before molting, (d) Histological assessment of Stage 1 ovary after molting. O: oocyte, F: follicle cell, N: nucleus, Og: oogonia.

Table 1. Mean oocyte diameter of *S. olivacea* before (control) and after the molt induced by limb autotomy.

	Mean oocyte diameter (µm)	Standard deviation	n
Before (Control)	25.67	4.38	30
After limb autotomy	34.76	12.13	135

Table 2. Mean, standard deviation, highest and lowest carapace width and body weight increments recorded after the crabs molted (N=135).

	Mean	Standard deviation	Highest increment	Lowest increment
CW (cm)	0.816	0.27	1.54	0.30
BW (g)	8.395	2.72	19.00	3.20

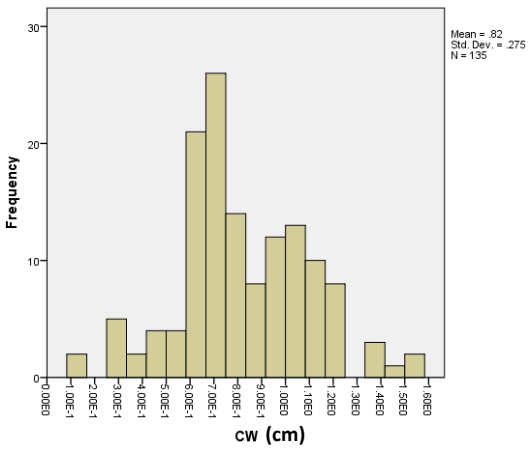

Figure 2. Frequency of carapace width increments of *S. olivacea* (N=135).

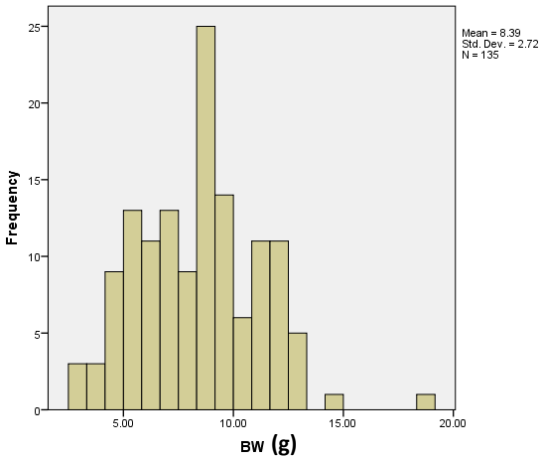

Figure 3. Frequency of body weight increments of *S. olivacea* (N=135).

Table 3. Correlation analysis for carapace width and body weight increments of *S. olivacea* (N=135).

		CW	BW
CW	Pearson Correlation	1	0.263**
	Sig. (1-tailed)		0.001
	N	135	135
BW	Pearson Correlation	0.263**	1
	Sig. (1-tailed)	0.001	
	N	135	135

Table 4. Regression analysis for carapace width and body weight increments of *S. olivacea* (N=135).

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	68.665	1	68.665	9.897	0.002[b]
	Residual	922.702	133	6.938		
	Total	991.366	134			

4. Discussion

Crustaceans undergo molting (ecdysis) numerous times throughout their lives (Ryer, Montfrans, & Moody, 19 97). The molting event, a complex, energy-demanding process, is a difficult and stressful time in the life of crustaceans and is the most vulnerable time for cannibalization. A crab molts 20 to 25 times during its life (Gaude & Anderson, 2011) normally for growth, ovarian maturation, mating, and stressful conditions, and molting is also influenced by several environmental factors. On the basis of previous studies of spiny crab species by Atar and Secer (2003) and Oluwatoyin *et al.* (2013) the length measurement of crabs is said to be difficult. During attempts to measure them either the extremities of the crab can be broken or the investigator can be injured by the crab. In addition, determinations of the increments after crabs have molted in the wild are still scarce. Only by rearing the crabs in captivity can the increments be confirmed. It is therefore convenient to estimate and convert into width (length) when only the weight is known or vice versa (Atar & Secer, 2003; Czerniejewski & Wawrzyniak, 2006; Josileen, 2011; Oluwatoyin

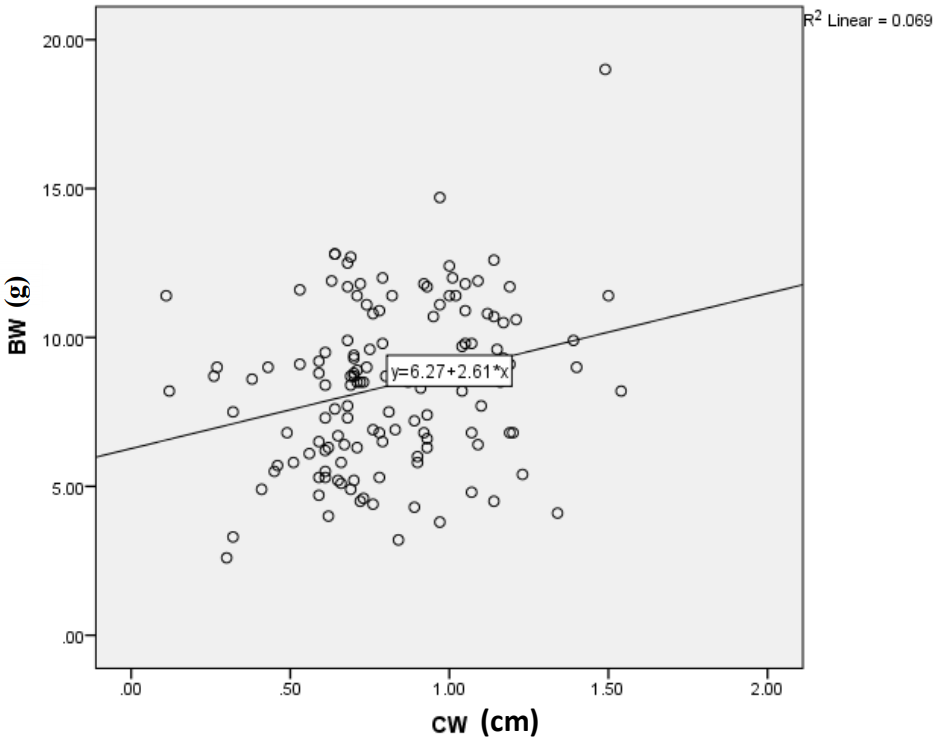

Figure 4. Regression analysis for carapace width and body weight increments of *S. olivacea* using the equation y = 2.61x + 6.27 (R^2 =0.069) (N=135).

et al., 2013). These relationships are often used to calculate the standing stock biomass, condition indices, ontogenetic changes, and several aspects of fish or crustacean population dynamics (Atar & Secer, 2003; Oluwatoyin *et al.,* 2013). Moreover, according to Sukumaran and Neelakantan (1997), body weight, total length, carapace width, and carapace length are the most frequently used dimensions, especially in the field of crustacean studies.

A previous study by Smith (1990) on the blue crab, *Callinectes sapidus*, found that this species can regenerate almost 90% of its normal limb length in the first molt and nearly 100% in the second molt after the loss of a single cheliped. Josileen and Menon (2005) stated that blue swimming crabs, *Portunus pelagicus*, were able to regenerate 90% of their normal size in the next molt, supporting a previous study by Smith (1990), and this does not affect the molt increment or the molt interval. Our study recorded a similar result with 85% regeneration of their normal size; however, the size of the cheliped became smaller. This may due to the multiple limb loss from the limb autotomy technique used in our study, which affected the rate of cheliped growth. Bennett (1973), and Kuris and Mager (1975) noted that the molt increment decreased proportionally with increasing numbers of limbs lost which supports our assessment of the cause of the decreased size of the mud crab chelipeds.

Our study showed that as the crabs molted, both the carapace width (CW) and body weight (BW) increased. These results were supported by a strong positive correlation ($P= 0.001$, $P<0.01$) between CW and BW. The regression analysis showed a significant result ($P=0.002$, $P<0.01$) with $y = 2.61x + 6.27$. However, our value of $R^2=0.069$ showed a very weak coefficient of determination compared to previous studies (Atar & Secer, 2003; Oluwatoyin *et al.,* 2013; Sudha Devi & Smija, 2015) on crabs. On average, the CW increment was 0.816±0.27 cm, with the highest and the lowest recorded increments of 1.54 cm and 0.30 cm, respectively. Meanwhile, the average BW increment was 8.395±2.72 g, with the highest and lowest recorded increments at 19.0 g and 3.2 g, respectively. Our findings proved that as female crabs molted, their carapace size, body weight, and ovary increased in size, similar to the results recorded by Josileen and Menon (2005) for *P. pelagicus*. However, their study did not include ovarian development. By focusing on the CW-BW relationship, the equation obtained can be used to determine the size of the *in situ* population in an area and thus can predict the population.

Four ovarian stages of maturation were recorded in the *S. olivacea* on the basis of recent studies (Amin-Safwan *et al.,* 2016; Azmie, Azra, Noordiyana, Abol-Munafi, & Ikhwanuddin, 2017; Ikhwanuddin *et al.,* 2014; Muhd-Farouk *et al.,* 2016b). However, no study has been done on the ovarian maturation stage after immature crabs molted, and biological information on mud crabs is scarce. There is also confusion among crab breeders and researchers concerning ovarian maturation after molting of immature crabs (International Workshop on Portunid Crabs Aquaculture and Sustainable Fisheries 2016, Universiti Malaysia Terengganu, Terengganu, Malaysia). Some suggested that the crabs have advanced to the next stage, while others suggested that the cycle of ovarian maturation started again (Personal communications: Amirul, 2016; Aziz, 2015). Therefore, this present study focused on the ovarian stage after molting of immature crabs. We believe this is the first report on immature crab ovarian development after a molting event occurs.

Our study recorded ovarian development as the crabs molted. However, they remained in Stage 1 ovary. The external morphology showed that the ovaries of the molted crabs were much more developed compared to the control ovaries, which were very small, thin, and very difficult to differentiate from the digestive gland. Moreover, the ovaries were seen as strand- and ribbon-like tissue both before and after the molting event. To confirm the stage of ovarian maturation, a histological assessment was done to obtain more accurate results (Madlen, Khadiga, & Montaser, 2012). From a histological perspective, the ovarian maturation of *S. olivacea* after the molting event was confirmed as Stage 1 ovary with a mean oocyte diameter of 34.76±12.13 µm. The oocyte, follicle cells, nucleus, and oogonia were present. Compared to the control crabs with a mean oocyte diameter of 25.67±4.38 µm, the oocyte diameter after the molting event showed an increase in size, thus demonstrating the occurrence of ovarian development after crab molting. The ovarian development could have resulted from the limb autotomy technique, which leads to the activation of hormone regulation and vitellogenesis. However, a further in-depth study is needed as our understanding of vitellogenesis, particularly in the mud crab, is still limited. Moreover, a better understanding of hormone regulation after limb autotomy is performed is crucial as this technique is currently one of the favorite practices in soft-shell production. Nevertheless, our results show that the ovary remained in Stage 1, and this information can be used as a new finding about mud crab biology. In addition, this information can become a new guideline and an indicator for future research perspectives.

Temperature and the quality and quantity of food are the most important environmental factors that can affect molting and growth in crustaceans (Josileen & Menon, 2005). In this study, these two parameters were maintained as well as possible. The temperature was maintained in the range of 27-29 °C throughout the study period, which was similar to the temperature range of a previous study done by Josileen and Nemon (2005) on *P. pelagicus*, while feeding 10% of the body weight twice daily (0900 h and 1700 h). Compared to these parameters, salinity and light intensity have little effect on the molting event and inter-molt period of *S. olivacea* in captivity. These findings were supported by Hartnoll (1982) and Josileen and Menon (2005) who concluded that no significant change was found in the inter-molt period with regard to salinity in several species of crustaceans.

It is recommended that further studies are needed on CW-BW as the equation in this study showed a weak coefficient of determination. It is also suggested that male crabs and a higher number of samples be included in future studies. In addition, we also suggest that further studies focus more on limb autotomy, as we believe that this technique could trigger some hormones that activate vitellogenesis and growth in crustaceans. Further study is thus crucial to fully understand hormone regulation and the vitellogenesis mechanism after limb autotomy is introduced. With a complete understanding of the CW-BW relationship and the stage of ovarian maturation, the data obtained can be used as an important baseline for future mud crab resource management in that particular area.

5. Conclusions

This study showed a positive and strong correlation between CW and BW (P=0.001, P<0.01) and that the mean CW and BW increments of female *S. olivacea* were 0.816± 0.27 cm and 8.395±2.72 g. The regression analysis showed a significant difference (P=0.002, P<0.01) with an estimation equation of y = 2.61x + 6.27 for BW and CW. However, the coefficient of determination (R^2=0.069) showed a very weak estimation value in this study. We recommend further studies to obtain a strong coefficient of determination, as the R^2 value is important to ascertain how strong a linear relationship is between the variables determining the population in a particular area. Meanwhile, both the external morphological assessment and the histology have shown that after immature crabs molted, the ovaries remained in Stage 1. However, this study may have demonstrated that vitellogenesis was activated by hormone regulation during the performance of limb autotomy and thus can be used as the baseline for future studies.

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