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Growth and Molting Response of Mud Crabs (*Scylla tranquebarica*) Injected with Spinach Extract

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ABSTRACT

Mud crab is a superior aquaculture commodity with a wide market potential both domestically and internationally. One of the main problems in mud crab cultivation is the long maintenance period and unequal molting time. The purpose of this study was to determine the effect of spinach injection on the growth and molting response of mud crab (*Scylla tranquebarica*). This was conducted in June-August 2024 for 60 days at the Sea Cages Maju Mandiri Group, Madong Village, Kampung Bugis Subdistrict, Tanjungpinang Kota District, Tanjungpinang City, Riau Islands Province. The method used in this study was an experimental method using a Completely Randomized Design (CRD) of 4 treatments and 3 replications. The results showed that the vitomolt injection treatment gave a significant difference ($p>0.05$) to the percentage of crab molting tested. The injection method of spinach extract (vitomolt) with a dose of 90 µg/g gave the highest molting percentage results of 83.33%, molting time at d-46, absolute weight growth of 89.00 g. Molting stimulation with the spinach extract injection method (vitomolt) 90 µg/g can be applied to the cultivation of mud crab (*S. tranquebarica*) because it can accelerate the molting process and growth of mud crab.

INTRODUCTION

Mud Crab (*Scylla tranquebarica*) are a fishery resource that has the potential to be cultivated and have a high economic price. This commodity is one of the superior aquaculture products with broad market potential, in domestic and international. The countries where mud crab products are sold are America, Tiongkok and Korea.

The demand for mud crab exports has also increased. Muhlis *et al*. (2021) reported, the demand increased from 260.000 tons to 290.000 tons every year. The hight level of pressure is not followed by culture productions. The volume of productions from culture activities has only reached 15-25% (Andayani, 2022). Because people have not fully mastered the technology of mud crab culture.

However, mud crab culture still has big problems such as the availability of crablet from hatchery. Such as cannibalism, disease outbreaks and no commersial formulated feed that is spesifically designed for mud crab larvae (Liew *et al*., 2024).

Long periods to rering and unequal molting times can cause high mortality of mud crab during rearing. The periods of raering can take up 4-7 months if crab are raised naturally. The molting phase naturally occurs for approximately 49 days (Frastia, 2015). Therefore, the methods must be taken to shorthern the molting period. An innovation that allows to be developed natural ingredients as molting stimulants. One of the transformations that has been achieved to address this issue to produce a molting stimulant from spinach extract. This discovery promises technology for the production of softshell crab. Vitomolt can also be given with mixed in artificial feed or trash fish. Suyono, *et al*. (2021) reported injection method and artificial feed with spinach extract give the best growth in 50,40 grams Spinach extract contains hormones (ecdysteroids) that stimulated the molting process. This hormone also regulates physiological functions in crab such as growth, metamorphosis, reproductions.

Spinach extract to stimulate molting was introduced by Fujaya under the name vitomolt. This spinach extract contains phytoecdysteroids. Ecdysteroids are molting hormones for crab. Mud crab that received Vitomolt supplementation moulted faster than those without Vitomolt supplementation. However, the results of various studies (Aslamyah and Fujaya, 2010; Fujaya *et al*., 2010; Bangun, 2020), show that the molting rate of mud crab given vitomolt supplementation either through injection or feed only reached a peak after day of 30. Further research to optimize its use vitomolt in accelerating mud crab production is very necessary. The purpose of this study was to determine the effect of vitomolt injection with different doses on the molting rate and growth of mud crabs(*Scylla tranquebarica*).

METHOD

This reaserch was conducted in June-August 2024 at the Sea Cages Maju Mandiri Group, Madong Village, Kampung Bugis Subdistrict, Tanjungpinang Kota District, Tanjungpinang City, Riau Islands Province. The tools used are 12 baskets measuring 45,7x34x15cm, digital scales, vernier calipers, 5L bucket, wire, syringe with 1 ml, scissors, and multitester water quality check. A total of 24 mud crab with average weight 197±4 gram was collected from fisherman in Busung Distric, Bintan, Indonesia. Another material used are trace fish, vitomolt (spinach extract) and aquadest.

This study used a Completely Randomized Design (CRD) with 4 treatments and 3 replicates. The treatments given is as follows:

Treatment A: injection vitomolt 60 µg/g
Treatment B: injection vitomolt 90 µg/g
Treatment C: injection vitomolt 120 µg/g

Treatment D: without injection vitomolt (control)

Research Parameters

The molting percentage

The molting percentage is the ratio between the number of crab that experienced molting and the number of mud crab. The molting percentage is calculated using the formula (Djunaedi, 2016):

$$Molting\ Percentage = \frac{The\ Number\ of\ Crab\ molting}{The\ Total\ of\ Crab} x\ 100\%$$

The Molting Time

Data is displayed in a table and calculated how many days it takes for the crab to moult. Next, the total time needed will be calculated and the average time for the mud crab to moult is found.

Absolute Weight Growth

Absolute Weight Growth can be calculated using the following formula:

$$Wm = Wt - Wo$$

Description:
Wm = Absolute growth
Wo = Average weight of mud crab at the beginning of the study (grams)
Wt = Average weight of mud crab at the end of the study (grams)

Survival Rate (SR)

By using information on the number of mud crab at the beginning and end of the study, the survival rate of mud crab (SR) was calculated using the following formula:

$$SR = \frac{Nt}{No} x100\%$$

Description:
SR = Survival rate (%)
No = Number of mud crab (*S. tranquebarica*) at the beginning of the study (ind)
Nt = Number of mud crab (*S. tranquebarica*) at the end of the study (ind)

The water quality parameters measured in this study were pH, temperature, salinity and DO. Salinity was measured using a refractometer, temperature, pH, DO were measured using a multitester. Water quality was measured once a week during the study in addition to daily temperature. The results of the research material can be presented in the form of tables and diagrams, after which the material was analyzed descriptively

Data Analysis

The data obtained were then analyzed using analysis of variance (Anova) if the results were significantly different, further testing would be carried out using the

Duncan test using SPSS 16. Water quality data were analyzed and presented descriptively.

RESULT AND DISCUSSION

The molting response of mud crab (*Scylla tranquebarica*) injected with spinach extract (vitomolt) to the percentage molting and time of molting show in figure 1 and Tabel 1. the highest percentage of mud crab molting was obtained in treatment B with an average value of 83.33±28.87% followed by treatment C with an average value of 50.00±0.00%, treatment A with an average value of 33.33±28.87% while in treatment D the average value was 0. Based on the results of the analysis of variance, the percentage of molting was significantly different (p>0.05).

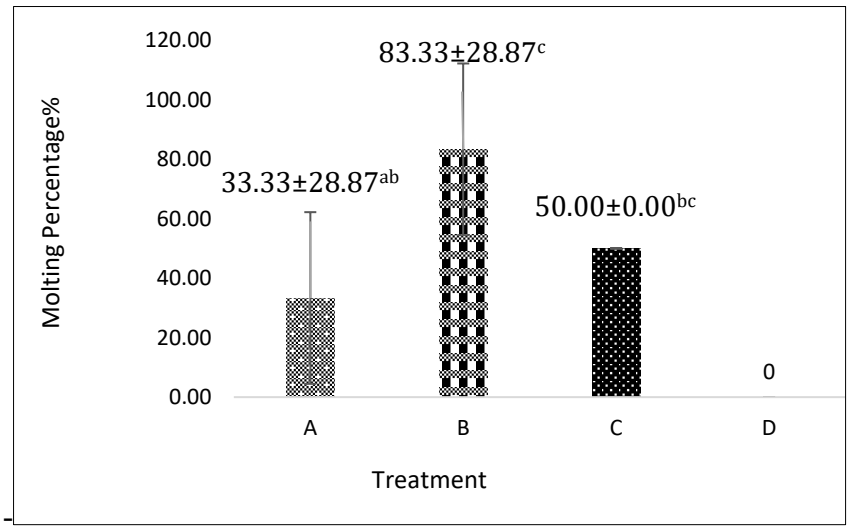

Figure 1. Percentage of Mud Crab Molting

The percentage of molting and the duration of molting of mud crab after injection of various doses of vitomolt on mud crab are showed in Figure 1 and Table 1. The fastest percentage and molting time values were found in treatment B (injection of spinach extract (vitomolt) 90 µg/g) with a molting percentage of 83.33±28.87% and average molting time at d-47, followed by treatment A with a molting percentage of 33.33±28.87% and an average molting time at d-49. While the molting time in treatment C with percentage 50.00±0.00% with a molting time at d-58. While in treatment D (control) no molting occurred and the average molting percentage was 0. The absence of molting in treatment D was due to several environmental factors that inhibited the molting process in mud crab.

The hight molting percentage in treatment B (with dose 90 µg/g) is thought to be due to the ecdystreroid content is accaptable for stimulating the mud crab to molting. The lower result in treatmen A (with dose 60 µg/g) and treatmen B (with dose 120 µg/g) on molting percentage because ecdystreroid hormone in their dose is not in accordance with needs for mud crab to molting procces. The dose that too

high than required can result in negative effect. Sustained high concentrations of ecdysteroid hormone trigger a process called downregulation or obstration production of hormone receptors to be inhibited and the ability of receptor cells to bind hormones to decrease (Fujaya, 2012).

Hasil penelitian yang sama juga telah dilaporkan oleh Herlina et al (2015) bahwa kepiting bakau (*Scylla olivacea*) yang diinjeksi ektrak daun murbei (*Morus* spp) dengan dosis 100 ppm menghasilkan persentase molting sebesar 50% dibandingkan dengan dosisi 125 ppm dan 150 ppm yang hanya menghasilkan persentase molting sebesar 33,3%.

The same research results have also been reported by Herlina *et al* (2015) that mud crab (*Scylla olivacea*) injected with mulberry leaf extract (*Morus* spp) at dose 100 ppm produced a molting percentage of 50% while doses of 125 ppm and 150 ppm which only produced molting percentage of 33.3%. Vitomolt injected into the base of the mud crab's swimming legs contains ecdysteroids. The right dose of ecdysteroids will optimally stimulate the molting process, whereas if the dose is low, it cannot stimulate molting properly and a higher dose will ultimately not show a better response because it will result in inhibition (Almaliki, 2021).

Table 1. Molting time of mud crab in each treatment.

Treatment	Molting Time (day)			amount	Average
	Repetition 1	Repetition 2	Repetition 3		
A	0	49	49	98	49.00
B	46	47	48	141	47.00
C	57	58	59	174	58.00
D	0	0	0	0	0

Table 1 shows the fastest molting process was obtained in treatment B with an average value of d-47, then followed by treatment A with an average value of d-49, and treatment C with an average value of d-58. The molting process of mud crab during the study tended to occur at night and in the afternoon. The time for crab to moult in the afternoon was around 17.00 WIB. Then the time needed for mud crab to release the old shell in one crab molting period was around 30-60 minutes. The hardening time for the new shell took 5-7 days to harden the crab shell. Based on the results of the analysis of variance, the molting time was not significantly different ($p>0.05$).

The molting process is a critical stage in the life of crustaceans. Because 30% of crab die at this stage, ranging from failed molting, pathogen infection, and cannibalism of the crab itself. The process of changing the old crab shell to a new shell causes the crab's body to become soft, this condition is very vulnerable for crab undergoing the molting process, because they do not have any protection if attacked by enemies. Therefore, the crab maintenance system should use a single room or individual maintenance system. This system has been proven to overcome

cannibalism in mud crab and can be applied to mud crab rearing activities (Muhlis *et al*., 2021).

According to Fujaya *et al*. (2012), molting is influenced by a number of factors, including internal and external factors. External factors are influenced by temperature, light, salinity, pH, DO, and food availability. Then the internal factor is the scope or broad maintenance media. Both of these elements will affect the brain and cause organ Y to release hormones that cause molting. Molting hormones come from organ Y. The crab molting process occurs in the afternoon and evening, then the molting process is accompanied by an increase in weight, width and length of the carapace in the crab. This can be seen from the nature of crustaceans that experience growth by molting. The increase in weight and body size will only be seen after the molting process is complete. The results of the mud crab that moulted with treatment B: injection of vitomolt 90 µg/g are presented in Figure 2 below.

Figure 2. Shell Replacement of Mud Crab Treated with Injection Vitomolt 90 µg/g

The crab molting process takes about 30-60 minutes for the crab to shed its old shell with a new shell. The process of enlarging the new exoskeleton to its maximum size takes about 15 minutes, water absorption continues until the exoskeleton hardens perfectly in about 5-7 days. Crab will experience 17-20 skin changes during their lifetime. There are several factors that cause crab not to moult at all, namely because of the presence of moss attached to the carapace, this causes the crab to become stressed and inhibits the molting process. Jalpano *et al* (2023) also showed similar results that exiting moss in container and shell can cause the crab to become stressed and its immune system will be disrupted.

Figure 3. Moss on The Shell of a Mud Crab

This will disrupt molting and even the crab can die. Documentation of moss on the shell of a mud crab is presented in Figure 3.

The absolute weight growth of the test crab at the end of the study is presented in Figure 4.

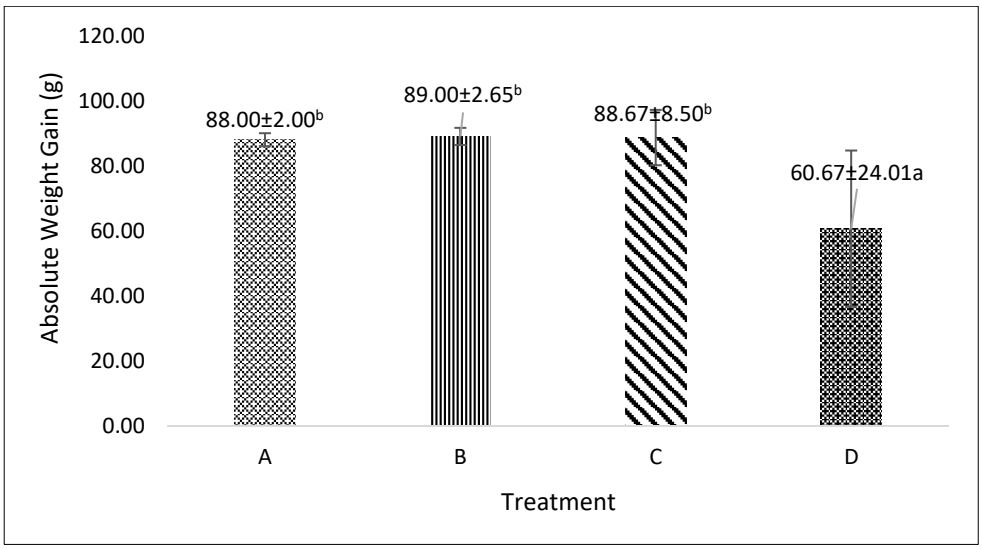

Figure 4. Absolute Weight Growth of Mud Crab

The molting process is an important activity in influencing crab growth, because without the molting process, weight gain, length and carapace width will not occur. Based on the results of measurements of the highest absolute weight gain of crab during the study, the highest was obtained in treatment B with an average value of 89.00 ± 2.65 grams, and the lowest weight growth was obtained in treatment D with an average value of 60.67 ± 24.01 grams.

The high weight growth in treatment B compared to other treatments is thought to be due to the stimulation of vitomolt given. The ecdysteriod hormone in vitomolt not only stimulates molting, but also stimulates growth first. The presence of ecdysteroids also affects protein synthesis which causes increased growth and accelerates molting (Mahdaliana, *et al*., 2022).

The growth of mud crab is influenced by the molting process, because it causes an increase in weight, length, and width of the carapace. Throughout its growth phase until it reaches adulthood, crab molt more often during its life.

In treatment D (control treatment) absolute growth only occurred at 60.67 ± 24.01 grams. In this treatment, crab utilize more energy from feed to adapt to the environment. The large amount of moss attached to the crab's carapace makes it difficult for the crab to grow optimally. The crab also has difficulty moving and breathing, as a result the crab becomes stressed. This result is better than the research by Fujaya (2011), which only produced a growth of 26.97 ± 2.54 grams.

The water conditions at the research location have relatively strong currents, causing moss plants to stick very quickly to the maintenance container and the crab's body. Based on observations during the study, moss plants grew very quickly after cleaning the moss on the crab's body with an interval of 2-3 days. According to Sagala *et al* (2013), moss that grows on the crab's body can inhibit molting and can even cause death.

The survival rate of mud crab at the end of the study is presented in Figure 5.

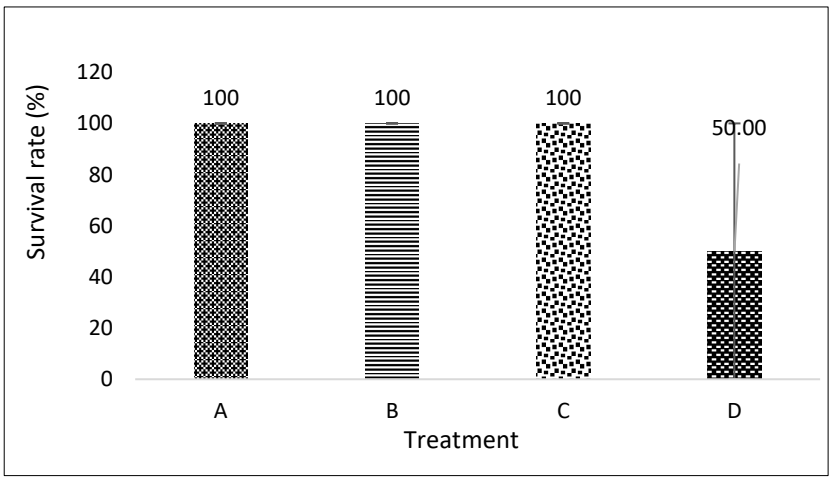

Figure 5. Survival Rate of Mud Crab

The weight growth process occurs due to the development of the integument part of the crab that undergoes the molting process first. Absorption of water content, an important element as a result of differences in osmotic pressure. This is thought to be so that the crab utilizes the feed well to maintain the body condition of the mud crab, so that the feed given can be used well for growth. The growth process in crab is influenced by the quality of feed, hormones and optimal environmental conditions. Growth can occur due to the input of energy from feed. This energy will be used by the body to repair damaged cells and add tissue due to cell division by mitosis (Prajayati *et al*., 2020).

Good water conditions and in accordance with the requirements for crab life will affect the survival rate, because the better the water conditions, the higher the survival rate. The water conditions at the research location are classified as good, resulting in a high survival rate in the test crab. However, in treatment D, the test crab died. This is because cannibalism between fellow crab and moss attached to the crab's body inhibits the molting process, resulting in death in the crab. Documentation for treatment D is presented in Figure 6.

Water quality is a major factor in the success of aquatic organism cultivation. In this study, there are several water quality factors that have been measured, including temperature, salinity, pH and dissolved oxygen (DO). The growth rate of crab weight and molting can be influenced by the water quality of the waters in the

research location. Water temperature is an important abiotic factor that affects the survival, growth and molting of crustaceans.

Figure 11. Mortality in treatment D

Tabel 2. Water Quality

No	Parameter	Range Value	Optimal Water Quality	Sources
1	Salinity	20-25 ppt	15-25 ppt	(Herlinah *et al*,. 2017)
2	Temperature	30,9-31,5 ℃	28-33 ℃	Katiandagho (2014)
3	pH	7,63-7,93	>5	Sagala *et al*,. (2013)
4	Dissolved Oxygen	>6.9-7,0	>5,0	(Tahmid *et al*,. 2015)

The temperature range measured during the study ranged from 30.9-31.5℃. This range is categorized as good for mud crab cultivation. According to Katiandagho (2014), the optimum temperature for mud crab growth is 25-35℃. Water temperature can affect the growth, activity and appetite of mud crab. The next water quality parameter, namely salinity, is the main parameter that is very important for aquatic organisms that live in brackish waters such as mud crab.

The salinity range measured during the study ranged from 20-25 ppt. This range is quite good from the normal limit for mud crab cultivation. According to Syafaat *et al.* (2021) salinity range for growth and nurshery 10-35ppt. The high and low salinity is influenced by several factors, including evaporation water circulation, rainfall, and river flow. The appropriate salinity for mud crab maintenance is 15-25 ppt. Temperature and salinity isi are two key parameters greatly to affect the physiological processes, having an impact on the growth of portunid crab.

The results of pH measurements during the study ranged from 7.63-7.93 and this condition is included in the normal limits in mud crab cultivation. Low pH values can reduce growth rates. This is in accordance with the statement of Sagala *et al* (2013) which explains that low pH can cause acute pH toxicity in crustaceans. Maximum mud crab growth should be cultivated in a pH medium between 7.5-8-5.

Measurement of DO or dissolved oxygen determines the growth and survival of mud crab. The level of oxygen solubility in water depends on the increase in temperature and salinity, because dissolved oxygen is closely related to these 2 water quality parameters. Dissolved oxygen measured during the study ranged from

6.9-7.0 ppm. This range is still within the tolerance limit for mud crab growth. Suryani *et al* (2018) stated that the dissolved oxygen content in waters> 4 ppm is still in the good category for the life of mud crab. Dissolved oxygen consumption is a physiological parameter that can be used to estimate the metabolic rate indirectly, namely by measuring the oxygen used in the oxidation process. The range of dissolved oxygen suitable for mud crab life is 3-15 ppm, Suryani *et al* (2018).

CONCLUSION

Based on the results of this research, spinach extract injection has effecting on the growth and molting response of mud crab (*S. tranquebarica*). Injection of spinach extract (vitomolt) with dose of 90 µg/g gave the highest molting percentage and absolute weight growth were 83.33±28.87% and 89.00±2.65 grams. Injection of spinach extract (vitomolt) at 90 µg/g can be applied in mud crab culture to accelerate molting and increase growth.

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