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Ecdysis Patterns and Glucose Fluctuations in Mangrove Crabs Across Salinity Levels

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ABSTRACT
Mangrove crabs (*Scylla* sp.) have become one of the important fishery commodities in Indonesia. However, the cultivation of mangrove crabs still faces challenges due to the lack of understanding the biology of these crustaceans. Ecdysis, commonly known as molting, is a characteristic of mangrove crabs. This lifelong activity needs to be studied because it is closely related to the aquatic environment, such as salinity parameters that exhibit significant fluctuations, consequently impacting the physiological processes of aquatic organisms. For instance, the test crab species used (*Scylla olivacea*) weighing 70-90g each were kept solitarily in aquaculture boxes. They were fed at a rate of 5% of their body biomass per day. Four salinity treatments (21, 24, 27, and 30ppt) were employed, each with three replications. The research findings indicated that salinity significantly influenced the molting progress of mangrove crabs, and the tested salinity levels were deemed suitable for mangrove crab cultivation. The salinity level that resulted in the lowest glucose utilization and supported accelerated molting was 24ppt.

INTRODUCTION

The development of mangrove crab and related crustacean aquaculture in Indonesia holds promising prospects for the future due to the high demand for crab and other crustacean commodities, as well as the abundance of resources in various aquatic regions. The economically valuable crab species in Indonesia include *Scylla serrata*, *S. tranquebarica*, *S. paramamosain*, and *S. olivacea*. To effectively utilize crab and crustacean resources, various studies are needed **(Tahya *et al.*, 2016, 2017)**. Some studies have contributed to innovations in the cultivation of soft-shelled mangrove crabs **(Aslamyah & Fujaya, 2010; Fujaya, 2011)**.

The development of soft-shelled crab aquaculture still faces challenges such as the supply of natural seed and environmental fluctuations that can impact physiological processes. To support the development of soft-shelled crab aquaculture, understanding the biological requirements is crucial. Salinity is one of the key environmental factors that significantly influence the growth and molting of mangrove crabs. **Katiandagho (2014)** reported that fluctuating salinity levels have triggered molting responses in crabs.

Internal body responses to salinity changes, such as glucose fluctuations in response to aquatic environmental factors, have not been extensively studied. Glucose content exhibits highly sensitive fluctuations and serves as an indicator of energy utilization within the hemolymph. Hence, this research is necessary to establish reference points for determining suitable salinity levels to support successful molting of mangrove crabs. The objective of this study was to determine the appropriate salinity levels for maintaining mangrove crabs based on molting progress and hemolymph glucose fluctuations.

MATERIALS AND METHODS

Experimental animal and specimens

The test subject was the male mangrove crab *Scylla olivacea*, weighing between 70-80 grams, with a carapace length and width ranging from 70-75mm. The crabs were sourced from fishermen and were in the intermolt phase, indicating a healthy condition. The experimental crabs were acclimated for 5 days in containers filled with water adjusted to the specified salinity treatments of 21, 24, 27, and 30ppt. On the 6th day, the crabs were sorted according to the research requirements. Throughout the study, the crabs were maintained individually and fed with minced fish at a rate of 5% of their body weight every afternoon **(Tahya, 2016)**. The experimental period lasted for 30 days to evaluate the test crabs' responses to different salinity levels.

Observations and measurements

Several variables were observed during the study, including carapace retraction, body weight gain, and glucose measurement. The retraction of the exoskeleton was observed by noting the separation of the thin layer on the swimming leg, while the observation of the epipodite was conducted in the area around the mouth. These observations aided researchers in identifying the premolt stage by observing the visible retraction on the cuticle surface **(Tahya *et al.*, 2016b)**.

RESULTS AND DISCUSSION

Carapace retraction

Carapace retraction serves as an indication to determine the molting development in mangrove crabs maintained under different salinity conditions. Based on the observation results, retraction is observed in the cuticle and epipodite regions. As reported by **Tahya (2016)**, crabs have entered the ecdysis phase when they exhibit carapace retraction.

Mangrove crabs maintained in media with salinity levels of 21 and 24ppt exhibited carapace retraction on the 5th day, which was the fastest progression among all treatments. The increase in salt concentration in the media, as observed in the 27 and 30ppt salinity treatments, led to a delay in molting progress, resulting in carapace retraction being observed on the 15th day. The observation of retraction in the swimming leg serves as an indicator of accelerated molting occurring from within the test crab's body through retraction in the endocuticle layer **(Tahya, 2016)**.

The premolt phase exhibited by crabs in media with salinity levels of 21 and 24ppt indicates that the body responds differently when the salinity is increased to 27 and 30ppt, suggesting that the body requires suitable conditions for the molting process. Crabs maintained at higher salinity levels displayed delayed molting progress, as indicated by the observation of the intermolt phase on the 5th day, contrasting with the lower salinity levels. Further maintenance until the 15th day indicated a change in progress towards premolt, confirming a slower molting response. Another observable indication in each group that aids researchers in inferring molting progress is the observation of behavioral responses to food. As **Tahya *et al.* (2016a)** stated, signs of crabs entering the premolt phase include reduced feeding activity and a decrease in aggressive behavior.

Ecdysis, or the phenomenon of shedding the exoskeleton, presents an opportunity for mangrove crabs to significantly increase their body weight. Individual body mass increase is caused by cell multiplication, resulting in linear weight gain. The development of cell numbers, along with the growth of flesh and muscles, exerts pressure on the carapace, leading to physiological accommodation within the crab's body through the formation of a new cuticle layer (endocuticle). This mechanism results in the formation of a layer inside the old cuticle, which is visually evident and can be observed through retraction in the walking leg.

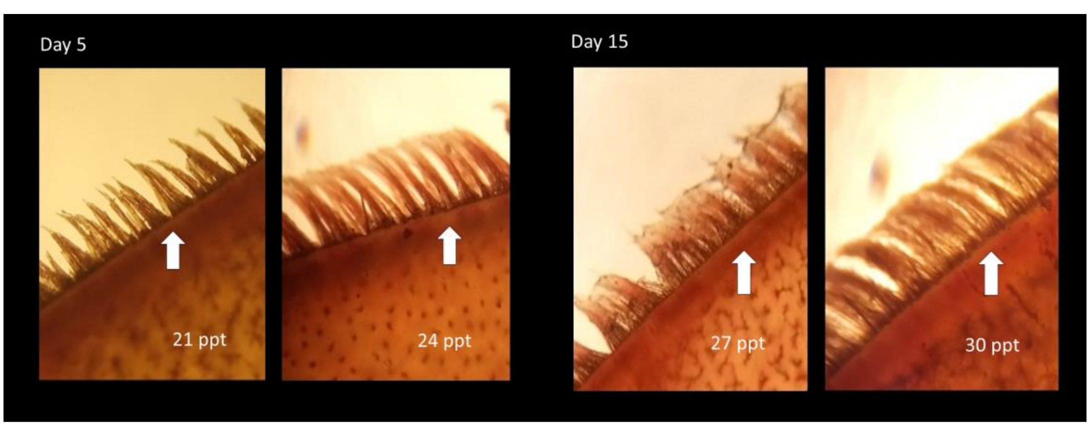

Fig. 1. Carapace retraction (→) in swimming leg of crab

Carapace retraction corresponds with molting development, which can occur naturally or due to triggers. Natural development arises from biological needs, such as during stages that require multiple metamorphoses or during adulthood when crabs grow larger and adapt to reproductive phases. On the other hand, stimulation processes can be triggered by the environment or human intervention, regarding the influence of media salinity on the development of mangrove crab stages **(Misbah *et al.*, 2017)**.

Based on observations, the test crabs maintained at various salinity levels exhibited the premolt phase. This phase indicates the initiation of molting development, gradually leading to the shedding of the old cuticle. Environmental factors, such as salinity, contribute to molting development through a mechanism controlled by neuropeptides in the brain and organ-X. The responses generated by the crabs showed differences based on salinity treatments. A similar mechanism was achieved through the administration of phytoecdysteroids as exogenous hormones, with varying doses leading to varying crab responses **(Fujaya, 2011)**.

Hemolymph glucose level

Observations of hemolymph glucose levels in mangrove crabs maintained in controlled containers with various salinity levels have been successfully documented in Fig. (2). The glucose levels of the test crabs in Fig. (2) show that the observation before the treatment was 30mg/ dl and fluctuated during the maintenance period, reaching 57mg/ dl. Mangrove crabs placed in various salinity conditions exhibited different response values, but generally, these fluctuations can be interpreted as a quick response of the body to environmental changes.

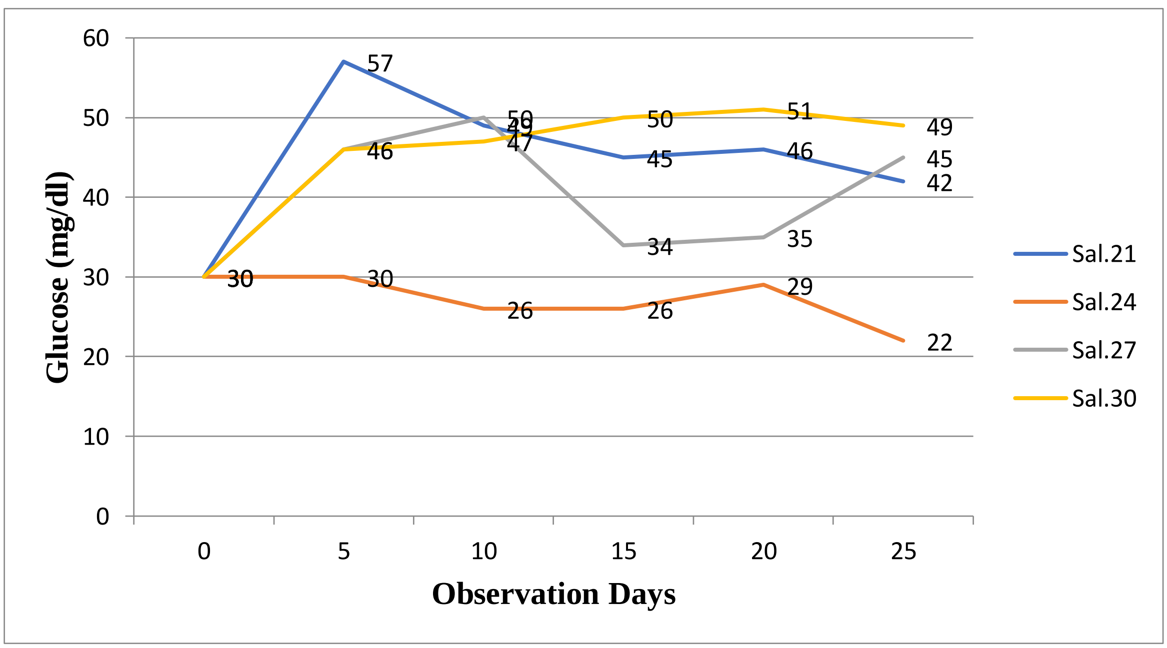

Fig. 2. Hemolymph glucose in various salinity

While the process of glucose availability in the body of mangrove crabs has not received much attention from crustacean researchers, it has been observed in other aquatic animals in general, such as those of **Tilden *et al.* (2001)**, **Verri *et al.* (2001)**, **Matsumasa and Murai (2005)**, **Wang *et al.* (2016)** and **Zhang *et al.* (2022)**. The common mechanism of glucose availability involves the digestion of carbohydrates into simple sugars, including fructose, galactose, and glucose.

The simple sugars produced are absorbed through the intestine and they enter the hemolymph circulation. During the process of absorbing simple sugars, including glucose, transporters that are membrane proteins facilitate their transportation. This transportation starts from the intestine until the hemolymph circulation is reached. Subsequently, the simple sugars enter the hemolymph circulation and flow throughout the body, serving as an energy source for various biological activities.

In this study, the adaptation of the body to salinity conditions requires a certain amount of energy. The crab's body undergoing salinity adaptation breaks down glucose through cellular respiration, producing ATP as an energy source for the body's cells. Energy reserves stored in the form of glycogen in the liver and muscles will be used when the crab has excess unused glucose during adaptation.

The crab boxes managed using a recirculation system can lead to continuous evaporation, thus impacting the increase in salinity in the maintenance media. This process cannot be denied due to various factors, such as room temperature, water movement within the recirculation system, and aeration processes.

Fluctuations in salinity will affect crab adaptation, thereby indicating a positive correlation with energy, as evident in the glucose dynamics of the test crabs. An increase in glucose in the hemolymph of the test crabs serves as an indicator of the amount of glucose flowing within the hemolymph, while a decrease indicates glucose flowing into the cells. Stress experienced by aquatic organisms in response to environmental changes can be chemical, physical, or biological in nature **(Li *et al.*, 2009)**.

Increase in body weight

The increase in body weight of the test crabs serves as an expressive indication of cell number augmentation. However, significant body weight increase was not observed in this study due to the absence of molting among the test crabs throughout the observation period. Nonetheless, the slight weight increase found in all test crabs across different salinity levels ranged between 5.4 to 5.9g.

Environmental factors significantly contribute to the growth process **(Islamy, 2019; Islamy *et al.*, 2024; Serdiati *et al.*, 2024)**. Salinity is a crucial environmental factor for mangrove crabs, as this crustacean species requires transitional environments during its developmental stages up to molting. Foraging activities and growth are influenced by the environment, and they correlate with energy utilization within the crab's body. Physiologically, osmotic processes occur to adapt to changes in salinity.

Energy stored in the crab's body is redirected for adaptation purposes, including osmotic work. This physiological process is known as osmoregulation, involving the regulation of osmotic pressure between the body and the environment. The energy expended on adaptation is quite fluctuating and depends on the habitat where mangrove crabs live, in accordance with the conditions of brackish and marine waters. Significant environmental changes lead to increased energy utilization. This phenomenon should be supported by adequate nutrient supply, as shown by **Karim *et al.* (2017)**, who found differing physiological responses and growth in mangrove crabs provided with different diets.

The process of adaptation occurs naturally and becomes a habit for crabs living in their natural habitat. This adaptation process differs when mangrove crabs are raised under controlled conditions in cultivation media. The research findings from observations, with growth rates of 5.4 to 5.9g, indicate no significant differences among mangrove crabs raised under controlled conditions with salinities ranging from 21 to 30ppt. Similarly, there was no increase in carapace width compared to the initial measurements.

The insignificant growth observed in the study can be interpreted as a mechanism of preparation for mangrove crabs to enter the premolt phase. During this phase, mangrove crabs accumulate energy to prepare for the molting process. Significant weight growth and carapace width increase occur during the postmolt phase.

In addition to interpreting growth data, researchers can also ensure that the salinity range of 21-30ppt is suitable for controlled cultivation of mangrove crabs using a closed recirculation water system. Within this range, body adaptation doesn't require substantial energy expenditure, thus delaying the molting activity.

The research did not find an increase in carapace length and width among the test crabs. According to **Tahya (2016)**, carapace size supports crab weight growth, but no increase in length or width was observed due to the hardness of the crab's shell, which doesn't grow before the new carapace forms.

CONCLUSION

The study successfully identified molting progress in mangrove crabs maintained under controlled conditions with varying salinity levels. The observable indicator was carapace retraction, which signifies endocuticle formation and is a stage leading to molting in mangrove crabs. The salinity level of 24ppt resulted in the lowest glucose utilization response and supported accelerated molting.

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