

GLUCOSE AND CORTISOL VARIATIONS: PRELIMINARY STUDY ON PHYSIOLOGICAL RESPONSE OF MUD CRAB AFTER TRANSPORTATION ACTIVITY

VARIASI GLUKOSA DAN KORTISOL: STUDI AWAL RESPON FISILOGIS KEPITING BAKAU SETELAH KEGIATAN TRANSPORTASI

Sulistiono^{1*}, Marisa¹, Dudi Muhammad Wildan¹, Wildan Nurussalam²

¹Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, IPB University,
Agatis Street, Campus of IPB Dramaga, Bogor 16680, Indonesia

²Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University,
Agatis Street, Campus of IPB Dramaga, Bogor 16680, Indonesia

*Corresponding author: onosulistiono@gmail.com

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ABSTRACT

The system of transporting mud crabs (*Scylla* spp.), carried out by the community by placing them in styrofoam boxes, was suspected of triggering stress in the biota. This study aims to determine the glucose and cortisol variation values of mud crabs as physiological response of the biota after transportation activity. Analysis was carried out on 2 groups of mud crab populations (I, II). Each population was observed 3 times, the crab had just arrived from the field (H_0), had been cultivated for 5 days (H_5), and had been cultivated for 10 days (H_{10}) in a plastic box (30x25x20 cm³) in cement pond (180x180x75 cm³) filled with seawater (25 PSU). Crabs were fed with fish pieces twice per day. In population I, the average glucose and cortisol values of H_0 ranged 78.3±30.5 mg/100 ml and 0.5±0.1 ng/dL, H_5 ranged 42.7±4.9 mg/100 ml and 1.0±0.3 ng/dL, and H_{10} ranged 47.0±8.5 mg/100 ml and 0.6±0.2 ng/dL, respectively. In population II, H_0 ranged 60.4±37.4 mg/100 ml and 0.5±0.2 ng/dL, H_5 ranged 44.2±3.6 mg/100 ml and 0.9±0.3 ng/dL, and H_{10} ranged 75.2±19.7 mg/100 ml (glucose). Based on these observations, the glucose values in the two mud crab populations varied (3.6-37.4 mg/100 ml), and according to the ANOVA test, they are insignificantly different ($p>0.05$). However, there is a downward trend from H_0 to H_5 .

Keywords: cortisol, glucose, mud crab, stress

ABSTRAK

Sistem pengangkutan kepiting bakau (*Scylla* spp.) yang selama ini dilakukan oleh masyarakat dengan meletakkan pada kotak styrofoam diperkirakan dapat menjadi pemicu tingkat stress pada biota tersebut. Penelitian ini bertujuan untuk mengetahui nilai variasi glukosa dan kortisol kepiting bakau sebagai respons fisiologis setelah kegiatan transportasi. Analisis dilakukan terhadap 2 kelompok populasi kepiting bakau (I-Februari dan II-Maret 2024). Masing-masing populasi diamati sebanyak 3 kali, yaitu populasi kepiting yang baru datang dari pengambilan di lapang (H_0), populasi kepiting yang sudah dipelihara 5 hari (H_5), dan populasi kepiting yang sudah dipelihara selama 10 hari (H_{10}) di karanjang plastik (30x25x20 cm³) dalam bak semen (180x180x75 cm³) yang berisi air laut (25 PSU). Kepiting diberi pakan berupa potongan ikan sebanyak dua kali. Pada populasi I, nilai glukosa dan kortisol rata-rata dari perlakuan H_0 adalah 78,3±30,5 mg/100ml dan 0,5±0,1 ng/dL, pada H_5 berkisar 42,7±4,9 mg/100ml dan 1,0±0,3 ng/dL, dan pada H_{10} berkisar 47,0±8,5 mg/100ml dan 0,6±0,2 ng/dL. Pada populasi II, pada H_0 berkisar 60,4±37,4 mg/100ml dan 0,5±0,2 ng/dL, pada H_5 berkisar 44,2±3,6 mg/100ml dan 0,9±0,3 ng/dL, dan pada H_{10} berkisar 75,2±19,7 mg/100ml (glukosa). Berdasarkan pengamatan tersebut, nilai glukosa pada kedua populasi kepiting bakau bervariasi (3,6-37,4 mg/100ml), dan melalui ANOVA nilai tersebut tidak berbeda nyata ($p>0,05$). Namun demikian terdapat tren penurunan pada H_0 sampai H_5 .

Kata kunci: glukosa, kepiting bakau, kortisol, stress

INTRODUCTION

Indonesia has abundant natural resources, one of which is the mud crab (*Scylla* spp). This animal is one of the important fishery commodities and has high economic value in Indonesia (Koniyo 2020). The increasing demand and increasing prices, both in the domestic market and in the international market, have encouraged the community and government to increase production to increase income and foreign exchange earnings. In the period January-June 2021, crabs (and swimming crabs), which generally come from catches, were in the top 3 commodities sent abroad after shrimp, tuna/skipjack/mackerel and squid/cuttlefish/octopus (Kompas 2021).

This type of crab is popular with the community because it has high nutritional value and contains various important nutrients (Kanna 2002). Kordi (2012) stated that every 100 g of mud crab meat (fresh) contains 13.6 g of protein, 3.8 g of fat, 14.1 g of carbohydrates, and 68.1 g of water, while Motoh in Oktamalia *et al.* (2018) stated that mud crab meat and eggs (in dry weight) contain quite high protein (67.5%) and relatively low-fat content (0.9%). According to Kanna (2002), in addition to having high economic value. Mud crab is one of the mainstay commodities for export. Export destination countries include the United States, Japan, Australia, Benelux, Hong Kong, Taiwan, Singapore, North Korea, and South Korea.

The mud crabs, as one of the trade commodities, are widely traded in live form. If the biota dies, the price becomes low or even unsaleable. Such conditions are important for fishermen and traders to pay attention to so that the crabs can remain alive in the media, either through transportation or in showcases at stalls or trade stores. The activities of catching, handling, and moving aquatic biota to other places through transportation activities can cause the biota to experience stress. This condition can even cause fatality. Causes of stress include changes both externally and internally. External changes that can cause stress responses include temperature fluctuations and lack of oxygen during transportation. According to Masjudi *et al.* (2016), the transfer of living biota is forcing them to be placed in a new environment that is different from their original environment, accompanied by very sudden changes in environmental properties. Furthermore,

sudden temperature changes can cause stress to the biota and even death (CNN Indonesia 2016). As a result of significant temperature changes, fish experience difficulties in the acclimatization process, which will affect fish activity and can cause death due to failure in the acclimatization process.

In poikilothermic animals, including mud crabs, changes in environmental temperature will directly affect the metabolic process (Sugiharto 2021). Therefore, temperature changes will affect the high need for blood glucose supply for thermogenesis. The energy needed from glucose to deal with stress can be met if glucose in the blood can immediately enter the target cells. The success of glucose supply to cells is determined by insulin performance. Meanwhile, during stress, insulin inactivation occurs, thus closing the use of glucose by cells (Wendelaar 1995). One way to determine the stress level of the biota is through observation of glucose and cortisol. For this reason, it is important to treat the biota so that it remains alive and fresh and that it has a good selling value. So far, people have touched the biota to see its movement. However, this technique is sometimes less valid because crabs reflexively move when touched. One method to see a more precise level of freshness is to observe the concentration of glucose and cortisol.

This study aims to analyze the variation values of glucose and cortisol in mud crabs as physiological response of the biota after transportation activity. The results of this observation are expected to be the basis of information in the study of the influence of transportation on the health of mud crabs.

METHODS

Time and location

This experiment was conducted from February to March 2024. In this study, two population groups (I and II) were populations that were taken randomly from populations that were not differentiated based on size. This experiment was conducted in a basket container (plastic) placed in a concrete pond in the Aquatic Animal Physiology Laboratory, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, IPB University.

Materials and equipment

Materials

The materials used in this study include test animals, feed, and seawater. The test animals used in this study were mud crabs measuring small size crabs or 20-30 per kg (30-90 g per head). The feed used in this study was trash fish, which was given 2 times (morning and afternoon).

Container for rearing

To rear the mud crabs, 2 concrete ponds were needed, which are used in the acclimatization stage (crab stress test) for 2 months. The container in the form of a plastic basket measuring 30x25x20 cm³ is placed in a concrete tub measuring 180x180x75 cm³, the water depth is about 60 cm, which is equipped with an aerator and water filter (circulation) (Figure 1).

Measurement equipment

Several pieces of equipment are needed for the observation of weight, length, and water environment, including scales, calipers, water pumps, and crab traps. A bench scale with an accuracy of 0.1 g (capacity of 5 kg) is used to weigh individual crabs. A caliper (accuracy of 0.1 cm) is used to measure the carapace width of the crab.

Glucose and cortisol analysis

Small-size crabs (populations I and II) originating from the wild were kept in 2 large concrete ponds/tanks, and plastic baskets for the acclimatization stage. At this stage, glucose and cortisol were studied through observations of blood (hemocyanin) as much as 1 cc, which was taken from the legs of the mud crab (hard carapace) (Romadhon et al. 2021). Observations were carried out at the initial stage before being placed in the aquarium (H₀), after 5 days (H₅) and the 10th day (H₁₀).

Data analysis

Data on the distribution of carapace-width of mud crabs are presented descriptively, while the observation results as glucose and cortisol values are compared between observations (H₀, H₅, H₁₀) using the ANOVA test (Walpole 1993).

RESULTS AND DISCUSSION

Results

Crab sample transportation and size distribution

Crab transportation was carried out using a car equipped with air conditioning (AC) to avoid excessive heat. The trip from Muara Gembong to the IPB campus took about 4-5 hours. During the trip, no crab samples died. This condition shows that the transportation technique using styrofoam and transported by a car equipped with air conditioning (AC) is very suitable for mud crabs. Before being put into the tub, the crabs were given hymolymph for glucose and cortisol observation. The samples were immediately put into the storage tub after arriving at the Physiology Laboratory. Transportation using styrofoam is quite popular among crab traders. The author's experience: the transportation system using styrofoam has been carried out several times over a fairly long distance (Bintuni-Jakarta), taking more than 1 day. The crabs in the container were 100% alive until Jakarta. Sometimes to create a natural atmosphere in the styrofoam, some mangrove leaves are given and brackish water is sprinkled (10-20 per mil).

The length of small-size mud crabs is spread from around 5.0-8.9 cm, both in the first population (n=130, February 2024) and the second population (n=135, March 2024). The size that is quite often found is the carapace width of 6.0-7.9 cm, while the size that is rarely found is in the range of 5.0-5.9 cm and 8.0-8.9. Small-sized mud crabs (1 kg consists of 30-40 crabs) are a common ingredient used for making soft shell crabs. This crab size is also used by fishermen in Subang and Bekasi Regencies to make soft-shell crab crabs. Soft-shell crabs that are quite large (500 g-1 kg) are less suitable for making soft-shell crabs. Large sizes also require a longer time compared to small soft-shell crabs (1 kg consists of 30-40 crabs) (Figure 2).

Glucose analysis

This study is a preliminary study conducted to prepare for the manufacture of softshell crabs. In this initial observation, the stress level of the crabs that are the target biota for the manufacture of softshell

crabs was observed as a result of stress from transportation. For this reason, it is necessary to observe the glucose and cortisol content. Stress is a condition that causes physical or psychological discomfort that results in the release of hormones related to stress or can cause certain physiological responses. Stress can occur in all living organisms and can cause changes and adjustments both physically, psychologically, and physiologically. Stress is also considered the sum of all physiological responses that animals use to maintain or rebuild normal metabolism. Stress in aquatic biota is generally a state of disruption of the homeostasis of the fish body, which produces an adaptive response to compensate for the disturbance/stressor that can cause physiological disorders, disease, and death in fish (Lestari and Syukriah 2020).

Observations of glucose content were observed in 3 samples (each population) with the assumption that the stress on the crabs was similar in each individual. The samples were taken from populations kept in plastic baskets placed in concrete tubs. For population I, on observation on February 17 (H_0), crab samples ranged from 7.6-8.2 cm (carapace width) or 77-109 grams (body weight). On observation on February 22 (H_5), crab samples measured 7.1-7.4 cm (carapace width) or 67-85 grams (weight). While on observation on February 27 (H_{10}), crab samples measured 7.1-7.4 cm (carapace width) or 66-80 grams (body weight).

For population II, an observation on March 11 (H_0) showed that crab samples

ranged from 7.3-7.6 cm (carapace width) or 86-89 grams (body weight). On observation on March 16 (H_5), crab samples measured 6.7-7.6 cm (carapace width) or 67-72 grams (weight), while on observation on March 21 (H_{10}), crab samples measured 7.1-7.6 cm (carapace width) or 63-80 grams (body weight). In the observation, 3 crabs were taken to take blood (hemolymph) as much as 1 cc each, which was taken from the fifth leg. The results of glucose observations of mud crabs kept in maintenance tanks are presented in Figure 3. Based on the figure, there appears to be a decreasing trend in glucose levels from H_1 to H_5 .

From the results of these observations, it is known that there is quite a large variation between individual mud crabs. In population I, the variation in glucose content ranges from 37.7 to 106.3 mg/100 l. While in population II, the variation in glucose ranges from 36.6 to 95.7 mg/100 l. This condition depends on the condition and also the size of each individual. The condition of stressed individuals is generally indicated by higher or increased glucose content (population II). However, this is not shown in population I.

Cortisol analysis

The parameters observed in order to determine the stress level of crabs that arrived from transportation and then kept in plastic baskets placed in concrete tubs were cortisol content. Cortisol observations were carried out simultaneously with glucose content observations. The results of the cortisol content observations are presented in Figure 4.

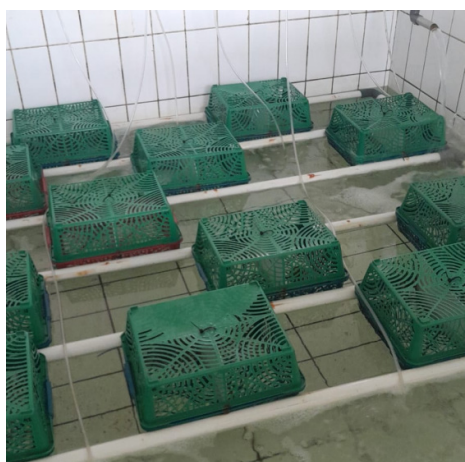


Figure 1. Container for maintaining small-size crabs, as a material for making soft shell crabs in the Physiology Laboratory, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, IPB University.

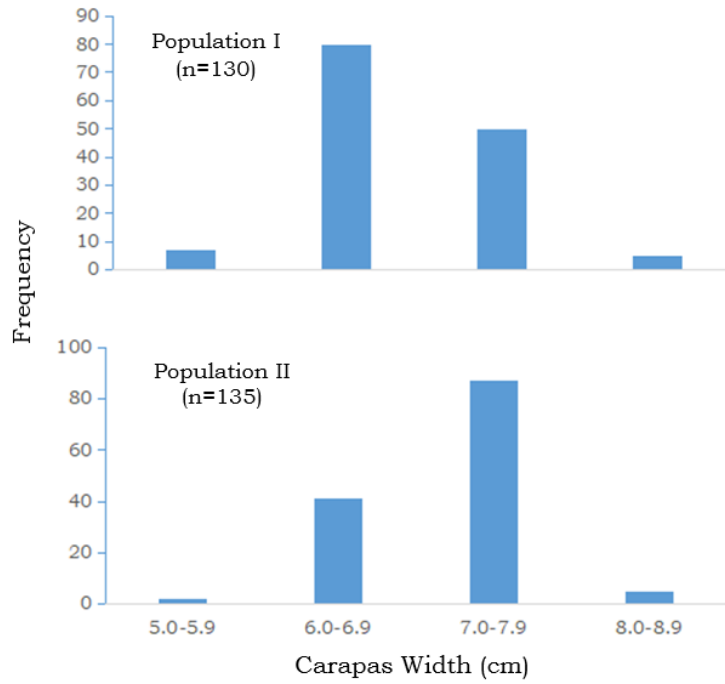


Figure 2. Population structure of mud crabs (material for making softshell crabs).

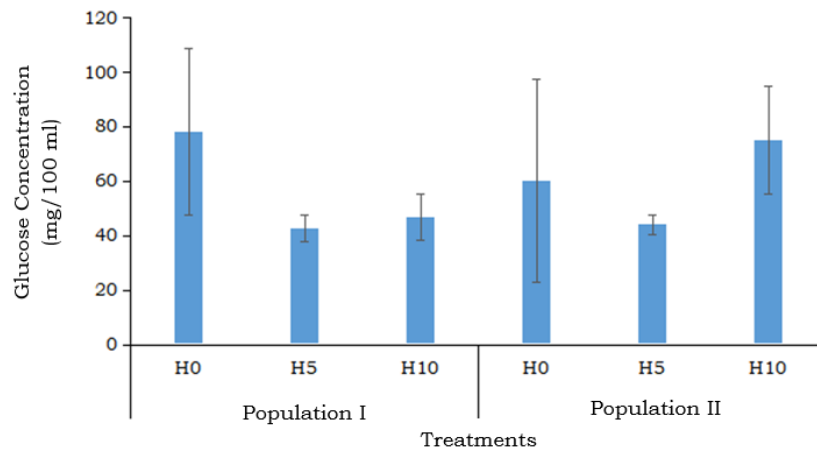


Figure 3. Results of observations of glucose concentration of mud crabs kept in maintenance tanks.

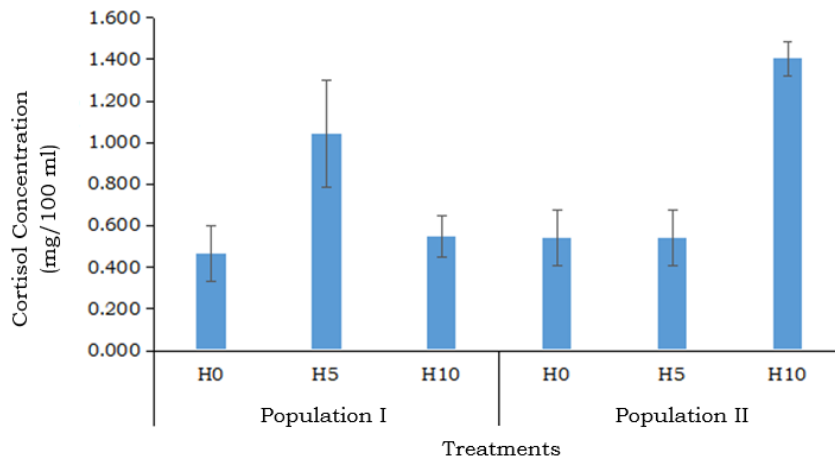


Figure 4. Results of observations of cortisol concentrations of mud crabs kept in maintenance tanks.

The results of observations of mud crab cortisol content were less varied (0.3-1.2 ng/dL) in Population I, and 0.3-1.3 ng/dL in Population II. However, it appears that in Population I, the content fluctuates from low to high and back to low again. In population II, the cortisol condition becomes higher from H₀, H₅ to H₁₀. It is not yet known for certain the cause of the increase in cortisol concentration in crabs; however, it is thought to be influenced by several environmental factors, including the fairly warm temperature around the maintenance pond, so that the biota will become stressed. Further research related to these factors is important to do.

Discussion

Mud crab (*Scylla* spp.) is one of the fishery commodities that has the potential to support the lives of people, especially for small-scale fisheries. Mud crab is a coastal fishery resource that has important economic value and has a high price.

The condition of mud crabs that have just arrived from transportation generally experiences stress. This condition is possible because, during transportation, the biota is tied and placed in a container (styrofoam) with limited conditions. However, after transportation, the crabs are placed in a plastic basket container and placed in a concrete pool container with water equipped with an aerator so that the oxygen is good enough to meet the needs of the biota.

Based on observations, the glucose conditions in populations I and II vary. In population I, the glucose content fluctuates. However, in general, there is a downward trend. This condition is thought to be closely related to the environmental conditions in which the biota are maintained. The environment (low DO or high temperature) can trigger an increase in glucose content in individual biota. In the case of Population I, the biota observed in H₅ and H₁₀ were in better environmental conditions compared to conditions in H₀. In H₅ and H₁₀, the water had begun to stabilize, and there was a treatment of aerators, so the environment was better compared to conditions in H₀ (which had just arrived from transportation).

This condition is in accordance with the observations of Widiastuti *et al.* (2022), which state that high blood glucose (hyperglycemia) occurs in fish experiencing stress due to an environment that is not in accordance with their living requirements

(high temperature). Environmental conditions (high temperature) cause the fish's body to secrete stress hormones that function to inhibit insulin secretion (namely at a temperature treatment of 31°C), producing 161.8-192.0 mg/dL. In conditions of non-stressed biota, it shows a fluctuating blood glucose content (at a temperature of 29°C producing 115.2-117.8 mg/dL). This is by Masjudi *et al.* (2016), who stated that lower temperatures (29°C) can lower blood glucose. Temperature also affects blood glucose levels, urea, uric acid, and protein levels, but the pattern is inconsistent. In this observation, there is a difference in the results between the expressions indicated by glucose and cortisol concentrations. However, these values are not statistically significantly different.

Observations made on cortisol were less varied than observations on glucose. The average cortisol content in Population I ranged from 0.3-1.2 ng/dL, while observations in Population II ranged from 0.3-1.3 ng/dL. However, from these results, it can be said that in Population I there was a decrease in cortisol values, which is estimated that the population is quite comfortable (observation H₅). From these observations, it can be stated that the condition of crabs experiencing stress due to transportation is not shown in higher cortisol values in the initial observation (H₁). Cortisol levels increased in observation H₅, which is expected to decrease cortisol content because the crabs have been placed in better media in the maintenance tank. A similar condition was conveyed by Widiastuti *et al.* (2022), who stated that the results of the analysis test showed that the influence of the environment (temperature interaction) on the cortisol hormone had no effect. This may be part of other nongenomic actions of cortisol, and this action may be partly independent of the general cortisol receptor. Cortisol can ultimately be metabolized and inactivated, primarily via the hepato-biliary-fecal route. In the liver and bile, cortisol is inactivated by reduction and conjugation to glucuronide or sulfate.

Stress conditions due to fishing and transportation activities as indicated by the value of glucose and cortisol occur in most biota after those activities. Effect of fishing and transportation activities occurs in fish and other biota (Sabu and Sasidharan 2020). Fishing activity on lobster (*Nephrops norvegicus*) was reported in Norway (Neil 2012), and effect of air transportation of red

king crab (*Paralithodes camtschaticus*) was reported in Northern Pacific Ocean, Sea of Japan and Bering Sea regions (Mota *et al.* 2021). To reduce the effect of the fishing and transportation activities, it is necessary to think about the methods of capture and transportation, so that the biota until the market is in good condition.

CONCLUSION

Based on the results of observations on the population of mud crabs that had just arrived from transportation using styrofoam (H_1), maintained for 5 days (H_5), and maintained for 10 days (H_{10}) in plastic baskets ($30 \times 25 \times 20 \text{ cm}^3$) in cement tubs ($180 \times 180 \times 75 \text{ cm}^3$) provided with seawater (salinity 25 per mil). Based on these observations, the glucose values in both mud crab populations varied quite a bit from 3.6-37.4 mg/100m, but the cortisol values varied less (0.1-0.3 ng/dL). Through ANOVA the values were not significantly different ($p > 0.05$). However, there was a downward trend from H_0 to H_{10} .

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