

Leukocytes description of Mudskipper (*Periophthalmodon schlosseri*) of Barito River Estuary, Desa Tanipah, Kalimantan Selatan

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Abstract

Leukocytes have an important role in driving away infections from pathogen microorganism by phagocytosis together with macrophages. The aim of this research was to analyze the leukocytes' differentiation (eosinophil, basophil, neutrophil, lymphocytes, and monocytes) of mudskipper's blood (*Periophthalmodon schlosseri*). Samples were taken from desa Tanipah Kalimantan Selatan. The sampling locations were decided by purposive or taking the data intentionally according to the consideration of mudskipper's location which is not homogenous. The data collection were conducted using *Line Transect* which means the sample was taken according to how many encounters can be taken. There were 15 mudskippers taken from Desa Tanipah used in this research. The parameter observed was the differentiation of leukocytes which consists of the percentages of monocytes, lymphocytes, eosinophil, basophil, and heterophil. The result showed that the percentage of lymphocytes in mudskippers is $62 \pm 4,1\%$ and monocytes $24,7 \pm 0,8\%$, and the neutrophil is $0,5 \pm 0,1\%$ and eosinophil $0,6 \pm 0,1\%$ and no basophils were found. According to the result, it can be concluded that lymphocytes is the most dominant one amongst others.

Keywords: leukocytes' differentiation; Desa Tanipah; *Periathalmodon schlossery*; mudskipper

INTRODUCTION

The description of differentiation of leukocytes is one of several parameters to complete the blood's profile, because the leukocytes' differentiation is component which has important function as first self-defense when diseases or patogen struck Dellman & Brown, 1989). The leukocytes' differentiation consists of lymphocytes, monocytes, neutrophil, basophil, and eosinophil. Leukocytes have a role in driving away the infections from pathogen microorganism by phagocytosis together with macrophage. The number of leukocytes in fish is more than the number of leukocytes in human's blood (Fujaya, 2004). The number of leukocytes of human's blood is about 5.000-9.000 cells/ μL , whereas the number of leukocytes in fish's blood is 20.000-150.000 sel/ μL . If the infective materials come in, the number of leukocytes in blood will increase (Moyle & Cech, 1988).

Mudskipper/ikan timpakul/ikan gelodok (*Periophthalmodon schlosseri*) is found along the coastal beaches and the habitat is spread in Indonesia, Thailand, Peninsular of Malaysia, Singapore and some coastal beaches of Indonesia (Baker, 2011). This animal is included as threatened species because the condition of coastal mangroves increasingly damaged everyday (Bay Science Foundation, 2009; Fadli, 2010). For some provinces in Indonesia, the flesh of mudskipper is used as the medicine for asthma (Fadli, 2010). Moreover, *P.*

Schlosseri is known has potency as bio-indicator in coastal ecosystem (Shirani et al., 2010).

The estuary of Barito river is the mouth's river with mangrove forest which is more than 30 km along the west coastal and around 20 km along the river to the inland. The swamps of mangroves forest which are rich of fauna who can adapt to the mud which is changing from the inundated to opened (flux and reflux). One of the faunas that can be found in the coastal beaches at the Barito river estuary Kalimantan Selatan is mudskipper (*Periophthalmodon schlosseri*) (MacKinnon et al., 2000; Shirani et al., 2014).

According to Lavabetha et al. (2015) mudskipper's (*P. schlosseri*) blood profile from Muara Sungai Barito Kalimantan is the number of erythrocytes is $3,36 \pm 0,1 \times 10^6$ sel/ μL ; the HE level is $12,38 \pm 0,56$ gr%; the hematocrit is $41,53 \pm 0,60$ %; the number of MCV is $123,78 \pm 3,94$ μm^3 ; the number of MCH is $36,88 \pm 1,82$ pg/sel; the number of MCHC is $29,80 \pm 1,18$ g/dL. The average of leukocytes' total of mudskipper (*P. schlosseri*) is $22,62 \pm 5,09 \times 10^3$ cells/ μL , whereas the number of leukocytes in common fish is between 30.000 until 150.000 cells/ μL . It can be said that the amount of leukocytes of *P. schlosseri* is higher than the leukocytes' number in other animals (Hrubic & Smith, 2000).

The research was done to describe differentiation of leukocytes of mudskipper at Barito river estuary,

especially at Desa Tanipah, has never been reported before. Based on that consideration, this research was focused on the differentiation of leukocytes (eosinophil, basophil, neutrophil, lymphocytes and monocytes) of *P. Schlosseri* from Barito river estuary, Kalimantan Selatan.

The objective of this research was to analyze the differentiation of leukocytes (eosinophil, basophil, neutrophil, lymphocytes and monocytes) of mudskipper's blood (*Periophthalmodon schlosseri*) at Barito river estuary, Desa Tanipah, Kalimantan Selatan.

MATERIALS AND METHODS

Tools and Materials

The tools used in this study were a set of fishing equipment, bucket, drain, analytic balance, tray, tissue paper, object's glass, syringe, haemocytometer, camera, and microscope. The materials were blood (*Periophthalmodon schlosseri*), EDTA solution (*Ethylene Diamine Tetra Acid*), Giemsa solution 10%, alcohol 70%, methanol and clean water.

The Procedure of Research

The Decision of Location in Taking Samples

The determination of sampling location in taking samples was conducted in purposive or taking the samples incidentally according to the consideration of heterogeneity of the sample location. The samples collected in a region flux and reflux of Bahagia River, Desa Tanipah Kecamatan Tabungane Kabupaten Barito Kuala, South Borneo. The samples was taken by *Line Transect* method namely the samples were taken according to its encounter.

Preparation of Blood Samples

The blood's samples of mudskipper were taken from caudal vein between the squama near the tail using syringe which was dampened with anticoagulant EDTA (*Ethylene Diamine Tetra Acid*). Syringe's needle was inserted from the anal into vertebra until the needle touched the bone. The blood was suctioned for 1 ml then the needle will be put off, and the blood's samples was moved to the tube (Erika, 2008).

Preparation of Blood Cell Slides

Blood cell preparation was conducted by placing a drop of blood in object glass. The second object's glass was put with angle 45° above the first one, then moved it to the back and touched the blood so the blood was spread. The second object glass was moved to the opposite direction so it made a thin layer of blood. The blood cell slide preparation was then air dried. After that, fixation would be conducted by saturated the preparation in methanol for 5 minutes, then it would be dried. The slide was then immersed to Giemsa solution for 30 minutes. After that, it was washed with clean water and let it dry. Then, the preparation was observed under light microscope with strong magnification, and it was counted for each type of leukocytes (Roberts, 1992).

The Observation of Leukocytes' Differentiation

The observation of leukocytes' differentiation was conducted to decide the percentage of each leukocyte's type in blood, by observed the blood cell slides under microscope. The micro setting in microscope was used, then it was counted from the side to the underside, then it moved to the right and to the top and so on. The calculation of leukocytes was conducted with strong magnification. The method used was *Shilling* method which the calculation was conducted with different viewing field as 10 viewing fields or until the leukocytes' number was reached 100 (Mitruka & Rawnsley, 1977).

The Technique of Data Collection

The data collection was quantitative. Quantitative data consisted of the number of leukocytes' blood of mudskipper (eosinophil, basophil, neutrophil, lymphocytes and monocytes). The quantitative data taken in this research was processed statistically. The data was served in mean and standard deviation.

RESULTS AND DISCUSSION

Results

The result of laboratory's analysis of the measurement of leukocytes' differentiation of mudskipper in Barito river estuary was served in table 1 and figure 1 as follows:

Table 1. Description of Percentages (%) Leukocytes' Differentiation of mudskipper.

No	Fish (n)	Length (cm)	Weight (g)	Lymphocytes	Monocytes	Neutrophils	Basophil	Eosinophil
P1	5	23,8	157,4	58,6	25,4	0,6	0	0
P2	5	24,12	162,6	60,8	25	0,2	0	0,8
P3	5	23,32	153,64	66,6	23,8	0,8	0	1,0
Average	5	16,30 ± 13,27	157,88± 4.5	62±4,13	24,73±0,83	0,53±0,1	0	0,6±0,1

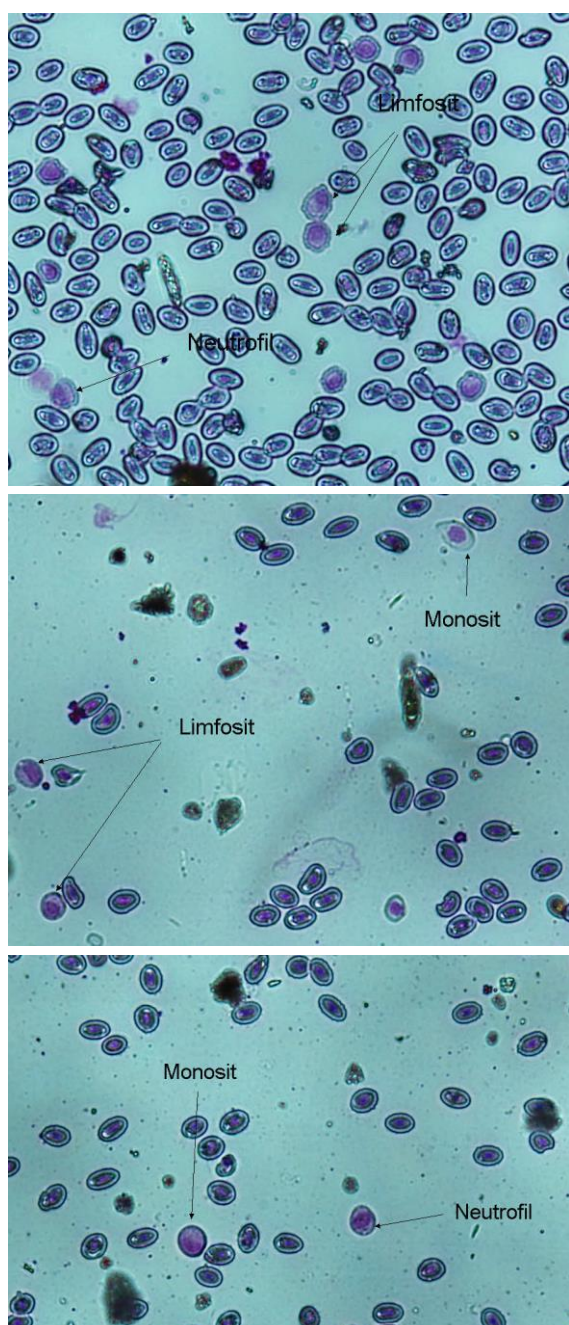


Figure 1. Description of differentiation of mudskipper leukocytes. Magnification 40 x.

Discussion

Lymphocytes Percentage

From the observation of lymphocytes percentage, it was found around $62 \pm 4,13\%$. According to the observation's result, the taken average percentage was under normal range. The normal percentage of lymphocytes in fish was between 71,12 - 82,88% (Robert, 1978). This condition showed the reduction of lymphocytes' number which called as lymphocytosis.

Lymphocytes found in mudskipper were more than human lymphocytes around 20% - 40%. The more lymphocytes in body the more body can attack the

disease. Lymphocytes had an important role in immune's respond and it produced the antibody. According to Jain (1986) & Jain (1993), lymphocytes had important roles in producing the hormonal and cellular immunity to attack and destroy the disease agent.

The increase of leukocytes number in blood circulation was called lymphocytosis; however, the decrease was called leucopenia. The decrease of lymphocytes' number in peripheral blood occurred because most lymphocytes were pulled from the circulation and concentrated into the tissue where there was inflammation (Jain, 1993).

Moyle & Cech (1988) reported that lymphocytes functioned as antibody's production to face foreign materials from outside if there were a decreasing percentage of lymphocytes in circulation when an infection occurred, it predicted as lymphocytes activity in antibody production was disturbed.

The response of leukocytes' number and leukocytes' differentiation will be affected by several stressor, such as, temperature, season, activities, hunger's condition, self-defense, maintenance, and density, diseases, infections, parasites, poisoned and metals. The leukocytes' number and leukocytes' differentiation will be increased or decreased (Schalms, 2000). Mudskipper had higher activities if we compared to other fish because it is *air-breathing* and it can adapt in land and water. The difference of haematological parameter of fish reflected the ecological condition in their habitat and it was their physiological adaptation in their life's way.

Monocytes' Percentages

From observation, the monocytes found was 24,73% which the monocytes percentage in fish commonly was 0,1% (Robert, 1978). According to Moyle & Cech (1988), there would be less monocyte in leukocyte unless there were infection in blood circulation. Roberts (1978) reported that the monocytes percentage in fish was 0,1% from the total population of circulated leukocytes. Monocytes had a role as macrophage and it was found in an infection's area (Dellman & Brown, 1989). Monocytes and macrophage of the tissues will do the phagocytosis to the tissues' cells and the disease agent (Nabib & Pasaribu, 1989).

Neutrophil's Percentage

From observation, the neutrophil found was 0,53% which the monocytes percentage was below the normal average. The percentage of normal average of neutrophil of fish was around 6-8% (Roberts, 1978). The neutrophil's cell functioned in the blood's vein in phagocytosis the bacteria faster because *P. Schlosseri* was spending more time outside the water. In this case, it can be said that neutrophil cell functioned in blood's veins in phagocytosis the bacteria faster because *P. Schlosseri* was spending more time outside the water.

Eosinophil and Basophil Percentage

The observation result from mudskipper blood samples were there was no basophil cell (0) and the eosinophil was 0,6%. The average percentage of basophil cell of mudskipper was under the normal average, which the normal average was between 0.5–1%. Nabib & Pasaribu (1989) reported that eosinophil and basophil was rarely participated in fish blood circulation. The eosinophil and basophil play a role in parasitic infections and allergic response which connected to the critical disease.

CONCLUSION

According to the result of this research, the lymphocytes percentage was found as the most dominant amongst other leukocyt types, which was 62%. The monocy percentage was 24.73% and the eosinophil percentage was 0.6%. The neutrophil percentage was found as the lowest one amongst all the population of leukocyt type which was 0.5% whereas basophil was not found.

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