Haematology Profile in Silver Barb (*Barbonymus Gonionotus*) Caught From

**Jagir River, Surabaya City, East Java, Indonesia**

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**Abstract**

The impact of human activities around the river can lead to the degradation of river water quality and affect the fish blood condition. The aim of this research was to observe the haematology profile of silver barb (*Barbonymus gonionotus*) caught in the Jagir River based on the water quality in each sampling station. The research was conducted by employing a survey method in Jagir River Surabaya, East Java, Indonesia. The samples were taken from three stations (10 fish each): station 1 (upstream side); station 2 (residential area) and station 3 (around paddy fields). The water quality parameters were temperature, pH, dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), and mercury level. Meanwhile, the haematology profile was conducted from erythrocyte, leukocytes, haemoglobin concentration, haematocrit, and micronuclei assay. The highest of erythrocyte (1,384,000 ± 145,235 cells/mm³), haemoglobin (7.14 ± 0.6 g%), and haematocrit (29.5 ± 6.2%) was on the fish captured at station 1 and the highest of leukocyte (643,288 ± 57,818 cells/mm³), and micronuclei (12 ± 2.0 cell/1000) was on the
fish at station 2, then the station 3 showed improvement the haematology profile compared to station 2. The water quality affected fish health based on their haematology profile. It is necessary to supervise and enforce the law in order to comply with the river utilization rules.

**Keywords:** *Barbonymus gonionotus*, haematology, Jagir river, silver barb, water quality

1. Introduction

Fish haematology is the science of studying the anatomical, physiological, and pathological aspects of fish blood. Haematological parameters have been known as valuable tools for monitoring fish health (Satheeshkumar *et al.*, 2011). Haematological parameters can be used as the indicators of the health status of fish in response to changes related to water quality, nutrition, and disease (Fazio, 2018). Karimi *et al.* (2013), using red blood cells (RBC); white blood cells (WBC); hematocrit (Hct); mean corpuscular haemoglobin (MCH); mean corpuscular volume (MCV); mean corpuscularhaemoglobin concentration (MCHC) and leukocyte differential as haematology parameters on their research.

*Barbonymus gonionotus* also called silver barb classified as Cyprinidae family which originally freshwater fish (U.S. Fish & Wildlife Service, 2018). *Barbonymus gonionotus* found a lot in Jagir river. Jagir River is one of the rivers which flows over the lowland part of Surabaya City. As time goes by, this river has been polluted by several pollutants such as domestic waste, agricultural waste and industrial waste which had given an adverse effect on fish health as like silver barb. This study was aimed to observe the haematology profile of silver barb (*Barbonymus gonionotus*) caught in the
Jagir River, Surabaya City, East Java, Indonesia with supporting analysis in the form of water quality parameters.

2. Materials and Methods

2.1 Sampling Preparation

The main material in this study was the blood of silver barb (*Barbonymus gonionotus*) were caught in every station by fisherman using the fishing rod at Jagir river, Surabaya City, East Java, Indonesia from 5 a.m to 12 p.m in one day. This research was held from March to April 2018. In this research the site study determined by three locations, those were upstream side (close to ceramic industry); residential area; and nearby paddy fields as station 1, 2, and 3 respectively (Fig. 1). The blood was taken directly by using 1 mL spuit on the lateral line of fish which filled anti-coagulant (sodium citrate 3,8% solution) about 10 µL before, to avoid blood clots of fish. The fish blood was taken around 90 µL or more. Then, it was moved to the 1.5 mL Eppendorf according to the label and was kept to the coolbox with dry ice and were carried out immediately to the laboratory on the Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Indonesia for further assay. Every sampling location was taken 10 fish for analyzing the haematology profile. The haematology profile was erythrocytes (Blaxhall and Daisley, 1973); leukocytes (Anderson and Siwicki, 1993); haemoglobin concentration (Wedemeyer and Yasutake, 1977); hematocrit (Wennecke, 2004) and micronuclei (Palacio Betancur *et al.*, 2009).
In addition, the water quality parameters also measured \textit{in-situ} including temperature (Thermometer Hg), pH (Universal pH paper MERCK 109535), dissolved oxygen (DO) (Lutron PDO-519), and \textit{ex-situ} including chemical oxygen demand (COD) (UV-Vis.Spechtrophotometer 1601), biological oxygen demand (BOD), total suspended solids (TSS) (Gravimetric method) and levels of mercury heavy metal (AAS Shimadzu type AA-6800).

\subsection*{2.2 Statistical Analysis}

Haematology profile data were analysed statistically followed an average formula from 10 fish in each station with the standard of deviation (SD) as the error bars with $\alpha < 0.05$.

\section*{3. Results and Discussion}

The total length of fish were caught between 10-12 cm and the weight ranged of fish from 10.8 to 12.32 grams. Morphologically, the fish should be alive and well (intact). The haematology profile was observed based on the following components: erythrocytes, leukocytes, haemoglobin concentrations, haematocrit and micronuclei. In each station showed different results of haematology profile, the average number of
erythrocytes was 1,384,000± 145,235 cells/mm$^3$ from 1,160,000-1,640,000 cells/mm$^3$; 438,000± 31,552 cells/mm$^3$ from 410,000-490,000 cells/mm$^3$; 624,000± 24,404 cells/mm$^3$ from 610,000-680,000 cells/mm$^3$, the average number of leukocytes was 198,978± 35,916 cells/mm$^3$ from 133,500-252,125 cells/mm$^3$; 643,288± 57,818 cells/mm$^3$ from 548,500-706,750 cells/mm$^3$; 556,508 ± 29,718 cells/mm$^3$ from 512,650-595,875 cells/mm$^3$, the average level of haemoglobin concentrations was 7.14± 0.6 g% from 6-8 g%; 2.5 ± 0.2 g% from 2.1-2.7 g%; 4.8± 0.5 g% from 4.1-5.4 g%, the average value of haematocrit was 29.5± 6.2% from 24-35%; 14± 2.4% from 10-17%; 20± 2.0% from 16-24%, and the average value of micronuclei was 6 ± 1.1 cells/1000 from 4-7 cells/1000; 12 ± 2.0 cells/1000 from 9-15 cells/1000; 10 ± 2.0 cells/1000 from 7-13 cells/1000, sequentially on station 1, station 2 and station 3.

In this study, the highest erythrocyte, haemoglobin and hematocrit value was on station 1. Meanwhile, the highest leukocyte and micronuclei number were on station 2. While at station 3 the total leukocyte and micronuclei values showed lower than station 2 but higher than station 1 and vice versa on erythrocyte, haematological and haemoglobin values (Fig.2-6). This happened because there has related to different environmental conditions in each sampling station. Fish blood parameters can be considered as biomarkers for environmental monitoring (Seriani et al., 2013). At station 1 (upstream side) was the least amount of pollution waters, while at station 2 (residential area) was the waters with the most household contamination such as garbage, human and animal feces, then at the station 3 (nearby paddy fields) the amount of contamination decreased when compared to the station 2. This explanation was supported by the analysis of water quality in all stations (Table 1.). The water quality parameters followed Indonesian Government Regulation Number 82 of 2001 class II.
displayed that on the all stations DO and pH was appropriate to the standard, and for BOD; COD; TSS; and mercury level showed exceed the standard. Mercury is one of the heavy metals classified as pollutants. Its presence in the environment in excess, especially in the waters can give bad impact on aquatic organisms until to humans. Mercury has been known as the most toxic heavy metal in the environment which is produced by anthropogenic activities including municipal wastewater discharge, industrial wastewater discharge, mining, and agriculture activities (Chen et al., 2012 and Jan et al., 2009). Although the water conditions of all stations were not too good, there were appeared that at station 1 has a better water quality than the other two stations, and station 2 has the worst water quality. While at station 3 was no better than station 1 but better than station 2 which mean at station 3 showed an improved water quality compared to station 2.

![Figure 2](image_url)

**Figure 2.** Erythrocytes level of *Barbonymus gonionotus*on all sampling station
Figure 3. Leukocytes level of *Barbonymus gonionotus* on all sampling station

Figure 4. Haemoglobin level of *Barbonymus gonionotus* on all sampling station
Figure 5. Haematocrit level of *Barbonymus gonionotus*on all sampling station

Figure 6. Micronuclei level of *Barbonymus gonionotus*on all sampling station
Table 1 Water Quality Measurement Results of Jagir River

| No. | Parameter   | *                | Station 1 | Station 2 | Station 3 |
|-----|-------------|------------------|-----------|-----------|-----------|
| 1.  | Temperature | Based on its environment | 28.6      | 29.2      | 31.7      |
| 2.  | pH          | 6-9              | 7         | 7         | 7         |
| 3.  | DO (mg/L)   | >4               | 10.1      | 9.6       | 10.2      |
| 4.  | COD (mg/L)  | 25               | 23.65     | 63.95     | 53.8      |
| 5.  | BOD (mg/L)  | 3                | 7.1       | 12.2      | 9.1       |
| 6.  | TSS (mg/L)  | 50               | 100.75    | 222.76    | 200.73    |
| 7.  | Hg (mg/L)   | 0.002            | 0.008     | 0.012     | 0.0097    |

* Indonesian Government Regulation Number 82 of 2001 class II
Erythrocytes, haemoglobin concentration, and hematocrit value indicate an inversely proportional to leukocytes and micronucleinumber. This happened because of the different roles of each haematological component. In nature, erythrocytes (red blood cells) has the main physiological role to transport of gases (O₂, CO₂) from the lung to the tissues and to maintain systematic acid/base equilibria, in addition, the damage of erythrocytes define the hemolysis and contribute to severe pathologies (Kuhn et al., 2017).

Moreover, various physical and functional characteristics of erythrocyte show a strong correlation on oxygenation of haemoglobin, whose molecular mechanism involves the interaction with another abundant protein at the membrane, viz. the band 3 protein. While the haemoglobin (Hb) is the protein contained in red blood cell with function is to carries out the oxygen from lungs or gills to the tissues (Billett et al., 1990; Russo et al., 2008). In other words, the red blood cells or erythrocytes is a carrier of haemoglobin as a carrier of oxygen from the lungs in the form of bicarbonate ions (HCO⁻). When erythrocytes are less, the binding of oxygen will be hampered so that the metabolism of the fish is disturbed. At the same time, hematocrit shows the percentage of the volume occupied by red blood cells compared to the total blood volume (Brunken et al., 2016).

Hematocrit is used to measure the comparison between erythrocytes and plasma so that the hematocrit gives the total ratio of erythrocytes to the total volume of blood in the body. Hematocrit values are influenced by the size and number of erythrocytes. A high hematocrit number may reflect an absolute increase in a number of erythrocytes or decrease in plasma volume (Wennecke, 2004). Haemoglobin and hematocrit are very
strongly correlated with erythrocytes. When the amount of erythrocyte decreases, the value of haemoglobin and hematocrit will also decrease. The standard of Teleostei erythrocyte is $1.05 \times 10^6$ cell/mm$^3$ and $3 \times 10^6$ cell/mm$^3$ (Roberts, 2001); Haemoglobin standard of Tilapia is 5.05-8.33 g/100 mL (Salasia et al., 2001) and Teleostei standard of Haematocrit is 20-30% and some saltwater fish is 42% (Bond, 1979). Whereas higher concentrations of pollutants can inhibit normal cell division which could give rise to micronuclei (Fig. 7). Luzhna et al. (2013), explained that micronuclei are an extra-nuclear body that contains damaged chromosome fragments and/or whole chromosomes which not incorporated into the nucleus after cell division.

![Figure 7](image)

**Figure 7. Micronuclei on the blood of *Barbonymus gonionotus***

Leukocytes or white blood cells have a role in cellular and humoral defence organisms against foreign substances that enter the body. An increase in the number of leukocytes exceeds the maximum amount defined as leukocytosis, usually as a physiological response to protect the body from attack by microorganisms. An increase in total leukocyte occurs due to the response of the fish body to poor environmental conditions of maintenance, stress factors and disease infections. White blood cell
behaviour is adaptive in nature and depends on their environment (King et al., 2018). The standard of fish leukocyte is 20,000-150,000 cell/mm$^3$ (Moyle and Cech, 2004).

Even though the water quality classified lack of appropriate eligibility, but based on the fish haematology standards showed that fish health status on the station 1 was good enough than fish which were captured on the station 2 and 3. The fish health on station 2 could be classified sick which were observed from their haematology profile and at station 3 the health status of fish was still relatively poor but starts to improve when compared to the fish on station 2.

4. Conclusions

Water quality influences the haematological profile of silver barb (*Barbonymus gonionotus*). The haematological profile of silver barb is directly proportional to the quality of the waters in the Jagir River, Surabaya. At station 1, the haematology profile showed properly, and worsen at station 2 which indicated that the silver barb experienced environmental stress and sick, then get improved at station 3. Supervision and law enforcement are needed to maintain the water quality of Jagir River and begin to concern the aquatic organism who live in their waters especially fish.

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