Health Evaluation of Wader Fish (*Puntius* sp.) Caught from Brantas River, Malang City, Indonesia

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Abstract. Anthropogenic activities along watershed area are often causing river pollution. This will affect the sustainability of aquatic resources, such as fish. One of the fish species in Brantas River that can be employed as bioindicator of water pollution is Wader fish (*Puntius* sp.). The aim of this research was to analyze the health of Wader fish based on its hematological profile. This study utilized descriptive method and conducted in three sites of Brantas River that located in Malang city, Indonesia. We took water quality parameters data to obtain Pollution Index of the river and blood samples to evaluate the hematological profile of wader fish caught in research area. Further analysis to evaluate relationship between water quality factors and hematological profile of Wader fish was performed by using Canonical Correspondence Analysis (CCA) with the support of PAST software version 4.06. The results showed that pH, temperature, dissolved oxygen (DO), ammonia and total suspended solid (TSS) of the study area were within the normal range, while the biological oxygen demand (BOD) value was above the standard. In addition, Pollution Index of this research suggested that Brantas River condition was low polluted. Meanwhile, the analysis of hematological profile of wader fish in terms of erythrocytes and hemoglobin were below than the recommended value, leukocytes were normal, and micronuclei were above the standard.

1. Introduction

The Brantas River is the second longest river in the Province of East Java and it has an important role in the Province of East Java, especially in the area of Malang City [1]. Malang City is an area that depends on the DAS Brantas for its needs. The utilization of the DAS Brantas, Malang can be divided into two, namely commercial interests (needs of Regional Drinking Water Company, industry and State Electricity Company and non-commercial (household and agricultural needs). Water quality in the DAS Brantas, Malang City needs to be considered [2].

The population density of Malang City was increase in every year that can affect the water quality in the DAS Brantas of Malang City. It caused by any pollution such as waste from residents' activities was dumped directly into the river. The higher of industrial and domestic activities along the river will have an significant impact on changes in water quality [3,4]. The alteration of the water quality effect...
the health of fish because the waters are the habitat of fish in their survival [5]. To determine the condition of a waters, analysis of physical, chemical, and biological parameters can be carried out. The alteration of the water quality can be detected from organisms that live in the Brantas Watershed (DAS), Malang. Aquatic organisms that can be used to detect changes in water quality are fish [6]. Fish are one of the organisms that are easy to find and most live in river water environments [7].

Wader fish (Puntius sp) is an endemic fish species in the Brantas River. Wader fish (Puntius sp) are able to live with environmental conditions according to their habitat, but to spawn wader fish (Puntius sp) requires fairly cold temperatures, relatively clear water conditions, not too heavy currents and good oxygen [8]. The blood profile of wader fish (Puntius sp) is for adaptation and survival of the fish itself and does not affect its genetics. The blood profile can be used as an indicator of changes in water quality [9].

Therefore, it is important to evaluate the health of wader fish (Puntius sp) based on the blood profile in Brantas DAS river of the Malang City, East Java in relation to changes in water quality including temperature, pH, DO, BOD, ammonia and TSS. The aims of this study are 1) to analysis the water quality of the Brantas river in Malang City, 2) to analysis the blood profile of wader fish (Puntius sp) in the Brantas river of Malang City, 3) to analysis the relationship between levels of the water Quality and blood profile of wader fish (Puntius sp) from Brantas river in Malang city.

2. Material and Methods
This research was conducted from June 2021 to July 2021 at three stations, (Sukun, Pisang Candi River and Joyosuko River) which are the Brantas DAS river, Malang. The method used in this study is a descriptive method. The sampling method of this research used purposive sampling technique. The research sampling station in Brantas river are presented in the figure 1.

![Figure 1. Research station](image)

2.1 Temperature, Dissolved Oxygen (DO), pH, and Ammonia
Water quality parameters in this study (temperature, DO, pH, and ammonia) were measured [10]. Temperature was measured [11] using an Hg thermometer, DO was measured using a DO meter and pH was measured with a pH meter. Ammonia was measured using a spectrophotometer.
2.2 Total Suspended Solid (TSS)
TSS is the total suspended solids in the water. It can be calculated by the following formula [12]:

\[
\text{Mg TSS per litre} = \frac{(A-B) \times 1000}{\text{Sample volume (ml)}}
\]

**Description:**
- **A** = Filter paper weight and residue (mg)
- **B** = Filter paper weight (mg)
- **1000** = Liters Conversion (L) in milimeter (ml)

2.3 Biological Oxygen Demand (BOD)
BOD (Biological Oxygen Demand) is the amount of dissolved oxygen required by organisms for the decomposition of organic matter. It can be calculated by the following formula:

\[
\text{BOD} = (\text{DO}_1 - \text{DO}_5) \times \text{Dilution factor}
\]

**Description:**
- **DO}_1 = \text{DO measurement results on day 1}
- **DO}_5 = \text{DO measurement results on day 5}
- **Dilution Factor** = 1

2.4 Index pollution
Index pollution based on PP No. 22 of 2021 [1] there are 4 categories, including 0 \(\leq\) IP \(\leq\) 1 means meeting the quality standard, 1 \(\leq\) IP \(\leq\) 5 means low polluted, 5 \(\leq\) IP \(\leq\) 10 means moderately polluted and IP \(\geq\) 10 means heavily polluted. To determine the pollution index, the formula is used:

\[
\text{IP} = \sqrt{\left(\frac{\text{Lij}}{\text{M}}\right)^2 + \left(\frac{\text{Lij}}{\text{R}}\right)^2}
\]

**Description:**
- **IP** = Index pollution
- **Ci** = concentration of water quality parameters
- **Lij** = concentration of water quality listed in the quality standard
- **M** = maximum
- **R** = average

2.5 Data Analysis
To analyze the pattern of the relationship between water quality including temperature, pH, DO, BOD, ammonia and TSS with blood profile variables consisting of erythrocytes, leukocytes, hemoglobin and micronuclei, analysis was used CCA (Canonical Correspondence Analysis) [13,14] using the Past 4.03 application.

2.6 Erythrocytes
Observation of the number of red blood cells in fish was carried out based on the method [15], which was taking blood using an erythrocyte pipette up to a limit of 0.5. Then, the blood is mixed with hayem solution as a dye solution on the observation of erythrocytes to a limit of 101 which is indicated on the pipette. Homogenize the contents of the pipette by making a number 8 motion to mix it, discard the
first drop, and put it into the haemocytometer counting chamber, and cover it with a cover glass and calculate under a microscope. Observe under a microscope at 1000X magnification using immersion oil. The formula for calculating the number of erythrocytes in below:

\[
E_{\text{rythrocytes}} = n \times \left( \frac{1}{5 \times 0.004} \right) \times 200 \text{ cell/mm}^3
\]

**Description:**

- \( n \) = the number of erythrocytes in the box taken
- \( 5 \) = field of view taken
- \( 200 \) = dilution factor
- \( 0.004 \) = konstanta

**2.7 Leukocytes**

White blood cells (leukocytes) were observed. Briefly, taking fish blood from the appendix using a leukocyte thoma pipette to a scale of 0.5 ml. Then, dilute the blood with turk's solution using an erythrocyte thoma pipette to a scale of 11. Homogenize the solution by shaking the thoma pipette in the shape of a figure eight. Discard the first 2 drops to remove air bubbles in the thoma pipette. Drops of blood into the haemocytometer and covered with a cover glass. White blood cells (leukocytes) are ready to be observed with a microscope [16].

\[
\text{Leukocyte (Sel/mm}^3) = n \left( \frac{1}{4 \times 0.1} \right) \times 20
\]

**Description:**

- \( n \) = leukocytes (cells)
- \( 4 \) = Number of fields of view observed
- \( 0.1 \) = Constant value
- \( 20 \) = Dilution

**2.8 Haemoglobin**

The equipment used to calculate the hemoglobin concentration in wader fish (*Puntius* sp) is a sahli tube to put of blood and HCL samples, a sahli pipette for taking blood samples by sucking, a dropper pipette for dripping HCL solution into a sahli tube and a stopwatch for calculating the time Hb calculation, while the material used to observe hemoglobin is fish blood samples that have been given anti-coagulant HCL 0.1 N for the formation of hemotin acid and distilled water for [16].

**2.9 Micronuklei**

Micronuclei was observed. Briefly, in fish blood cells are carried out by first taking blood from the lateral line near the gills using a syringe equipped with an anticoagulant so that the blood does not clot. The blood that has been taken is immediately made blood smear preparations on an object glass, and dried at room temperature. After that, the blood smear was fixed with methanol to maintain cell morphology for ± 10 minutes and continued with staining using Giemsa 10% to sharpen or clarify various tissue elements, especially the cells, so that they could be distinguished and examined under a microscope after 1 hour. Observations were made using a microscope with 1000X magnification using immersion oil [17].
3. Result and Discussion

Water quality parameters are used as supporting data in estimating pollution [18]. Water quality parameters will affect the blood profile of wader fish (Puntius sp). Thus, if the water conditions are bad, it will cause negative effect at the health of wader fish (Puntius sp) in the water.

3.1 Water quality

The results of water quality measurements at the three research stations are; The temperature at station 1 ranged from 21.9 °C - 24.2 °C, station 2 obtained results ranging between 22.0 °C and 23.3 °C and station 3 obtained results ranging between 23.1 °C and 24.1 °C. The pH value obtained at station 1 ranged from 6.7 to 7.0. The pH obtained at station 2 ranged from 6.3 - 7.1 and at station 3 the results ranged from 6.9 - 7.2. Measurement of pH at the three research stations based on PP No. 22 of 2021, class 2 quality standards are classified as normal, which is between 6.3 – 7.2. The pH pH is too acidic and above the threshold causes death in aquatic organisms, while pH is too alkaline inhibits the growth of aquatic organisms [19].

The Dissolved oxygen (DO) value at station 1 obtained results ranging from 6.6 mg/l - 6.8 mg/l, station 2 obtained results ranging from 6.1 mg/l and 6.9 mg/l and at station 3 the results ranged from 6.1 mg/l – 6.8 mg/l. DO measurements at the three research stations were classified as normal for fish life, namely 6.1 – 6.9 mg/l. Dissolved oxygen (DO) is good for the life of aquatic organisms is more than 4 mg/l while less than 2 mg/l causes death in several types of fish [3]. BOD values obtained at station 1 ranged from 4.07 mg/l - 4.88 mg/l, station 2 obtained results ranging from 4.07 mg/l - 4.88 mg/l and station 3 obtained results ranging from 4.07 mg/l - 4.88 mg/l. BOD measurements at the three research stations were classified as exceeding the threshold for fish life, which is between 4.07 – 4.88 mg/l based on PP No. 22 of 2021, class 2 quality standard.

Ammonia results at station 1 obtained results ranging from 0.011 mg/l - 0.081 mg/l, station 2 obtained results ranging from 0.058 mg/l - 0.070 mg/l and station 3 obtained results ranging from 0.051 mg/l - 0.066 mg/l . Ammonia measurements at the three research stations were classified as normal, namely 0.011 mg/l - 0.081 mg/l based on Government Regulation (PP No. 22 of 2021), class 2 quality standard. The results of TSS at station 1 obtained results ranging from 31 mg/l - 41 mg/l, station 2 the results ranged from 31 mg/l - 35 mg/l and at station 3 the results ranged from 31 mg/l - 39 mg/l. TSS measurements at the three research stations were classified as normal, which is between 31-41 mg/l based on Government Regulation No. 22 of 2021, class 2 quality standards. The results of water quality measurements are presented in table 1.

Table 1. Results of water quality measurements at three observation stations (each station is taken at three sub-stations)

| Parameter | Sampling | Station 1 | Station 2 | Station 3 | Class (PP No 22 Th. 2021) |
|-----------|----------|-----------|-----------|-----------|--------------------------|
|           | Sub St 1 | Sub St 2  | Sub St 3  | Sub St 1  | Sub St 2  | Sub St 3  | Sub St 1  | Sub St 2  | Sub St 3  |                |
| Temparatur (°C) | 21.9 | 22.3 | 22.1 | 22.2 | 22.2 | 20.0 | 21.3 | 23.2 | 23.2 | Deviasi 3 |
| ure (mg/L) | 2 | 23.8 | 24.2 | 24.0 | 23.1 | 23.0 | 23.3 | 23.9 | 24.1 | 24.2 |
| (mg/L) | 2 | 31 | 32 | 33 | 31 | 33 | 36 | 36 | 39 | 50 |
| pH | 1 | 6.9 | 7.0 | 6.9 | 7.1 | 7.1 | 7.0 | 7.0 | 6.9 | 6-9 |
| DO (mg/L) | 2 | 6.8 | 6.7 | 7.0 | 6.5 | 6.3 | 6.5 | 7.0 | 7.2 | 7.1 |
| (mg/L) | 2 | 6.7 | 6.8 | 6.8 | 6.9 | 6.8 | 6.7 | 6.8 | 6.6 | 6.5 | 4 |
| (mg/L) | 2 | 4.88 | 4.07 | 4.07 | 4.07 | 4.88 | 4.07 | 4.07 | 4.07 | 4.07 |
| BOD | 1 | 4.07 | 4.88 | 4.07 | 4.88 | 4.88 | 4.07 | 4.88 | 4.88 | 4.88 |
| (mg/L) | 2 | 0.013 | 0.011 | 0.017 | 0.07 | 0.059 | 0.067 | 0.055 | 0.060 | 0.051 | 0.2 |
| (mg/L) | 2 | 0.078 | 0.081 | 0.078 | 0.062 | 0.058 | 0.070 | 0.066 | 0.061 | 0.061 | 0.61 |

3.2 Pollution index

The calculation of the pollution index value for water quality follows the class II water quality standards. Class II water quality standards according to PP No. 22 of 2021. The results of the
measurement of the pollution index at the three stations, namely at station 1 in sampling 1 and 2 are classified as low polluted with IP values of 1.28 and 1.54. Values that meet the quality standards in sampling 1 and 2 at station 1 are pH, ammonia and TSS, and DO, while those that do not meet the quality standards are BOD. Station 2 in sampling 1 and 2 is classified as low polluted water with IP values of 1.24 and 1.54. Values that meet the quality standards at sampling 1 and 2 at station 2 are pH, ammonia and TSS and DO, while those that do not meet the BOD quality standards. Station 3 in sampling 1 and sampling 2 are classified as low polluted with IP values of 1.26 and 1.05. Values that meet the quality standards in sampling 1 and 2 at station 3 are pH, ammonia and TSS and DO, while those that do not meet the quality standards are BOD.

3.3 Profile of Blood Wader Fish (Puntius sp)

Figure 2. The average number of erythrocytes of wader fish (Puntius sp) in sampling 1 and 2 at the three stations were 740,000 cells/mm3 and 780,000 cells/mm3. Station 1 ranges from 450,000 – 1,060,000 cells/mm3. Then the number of erythrocytes at station 2 ranged from 460,000 – 1,050,000 cells/mm3 and the number of erythrocytes at station 3 ranged from 430,000 – 1,200,000 cells/mm3. The number of erythrocytes of teleostey ranged from 1.05x10^6 – 3x10^6 cells/mm3. The number of erythrocytes in the three stations is dominated by low erythrocyte counts [19].

The average leukocytes of wader fish (Puntius sp) in sampling 1 and 2 was obtained 60,422 cells/mm3 - 34,622 cells/mm³. Station 1 ranges from 43,400 – 127,600 cells/mm³. Then the number of leukocytes at station 2 ranged from 25,400 – 103,200 cells/mm³ and the number of leukocytes at station 3 ranged from 34,600 – 67,400 cells/mm³. The number of fish leukocytes ranges from 20,000 – 150,000 cells/mm³. Wader fish (Puntius sp) in the three stations are still classified as blood quality standards because the number of white blood cells (leukocytes) is still in the normal range of teleost fish [20].

The average hemoglobin concentration of wader fish (Puntius sp) in the three stations in sampling 1 and 2 obtained the same result, namely 4.9 gram%. The hemoglobin of wader fish (Puntius sp) at station 1 ranged from 4 – 6 gram%. Then the results of the hemoglobin concentration at station 2 ranged from 4-6 grams%/ and the results of the hemoglobin concentration at station 3 ranged from 4 - 6 grams%. The normal levels of teleost fish hemoglobin are between 12-14 grams%. The concentration of hemoglobin in the three stations can be interpreted as low or under the blood quality standard [21].

The average number of micronuclei of wader fish (Puntius sp) at the three stations in sampling 1 and 2 obtained the results of 16 cells/1000 and 19 cells/1000. station 1 ranges from 11 – 24 cells/1000. Then the results of the calculation of the number of micronuclei at station 2 ranged from 14 - 24 cells/1000. and the results of the calculation of the number of micronuclei at station 3 ranged from 12 to 27 cells/1000. The polluted river waters show a high number of micronuclei. The highest number of micronuclei is located at station 3 and the lowest number of micronuclei is at station 1. Station 3 is a station that is often used even every day for residents' activities, one of which is washing clothes, carpets, or motorbikes, where waste from various residents' activities enters water bodies [19, 22].
3.4 Relationship between levels of water quality and blood profile

The results of Canonical Correspondence Analysis (CCA) in Figure 3 can be interpreted that the number of erythrocytes tends to be influenced by high overall water quality. Because the erythrocyte point can be projected to all water quality parameters with a close distance, it can be interpreted that the number of erythrocytes tends to be influenced by high water quality values. The number of leukocytes tends to be influenced by high DO values, moderate to low ammonia, moderate to low pH, moderate to low TSS, moderate to low temperature and medium to low BOD. Hemoglobin concentration values tend to be influenced by high BOD values, high DO, moderate to low ammonia, moderate to low pH, moderate to low temperature and moderate to low TSS. Micronuclei values tend to be influenced by high BOD values, high DO, moderate to low pH, moderate to low ammonia and moderate to low temperatures.

4. Conclusion

The result of blood profile revealed the erythrocyte and hemoglobin were in low value, but micronuclei have high value. For leukocytes were observed in normal value in this study. Based on that result in this study can be considered the fish in unhealthy condition. The results of the CCA interpretation obtained are that the fish blood profile is predominantly influenced by BOD and DO parameters, while the parameters pH, temperature, TSS and ammonia tend to influence moderate to low.

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