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Hematological Analysis of Red Bader Fish (*Barbonymus altus*) as a Biomarker in Assessing Pollution Status in Brantas River, Surabaya, East Java

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Abstract: The Brantas River has many functions for both humans and the aquatic organisms in it. The type of fish that is often found in the Brantas River in Surabaya is the red bader fish (Barbonymus altus). Hematology is a sensitive parameter and can be used as a marker in assessing water conditions. The aim of this research is to analyze the hematological profile of red bader fish (Barbonymus altus) to assess the condition of the Brantas River, Surabaya. The research was carried out at 3 locations on the Brantas River in Surabaya with 3 sampling times with an interval of 1 month starting in January – March 2022. The parameters observed were water quality (temperature, TSS, TDS, pH, DO, BOD, ammonia and heavy metals Hg) and hematological parameters (erythrocytes, hemoglobin, hematocrit, leukocytes and micronuclei). Analysis of the relationship between water quality parameters and hematology using the Correspondence Correlation Analysis (CCA) method. The results show that there are water quality parameters that do not meet quality standards and there are abnormal hematological parameters. CCA analysis shows that there is a correlation between water quality and hematological parameters with moderate concentrations (erythrocytes, hemoglobin and hematocrit) and strong concentrations (leukocytes and micronuclei).

Keywords: *Barbonymus altus*; Brantas River Surabaya; Hematology; Water pollution

Introduction

The Brantas River in Surabaya has a large function for human activities, namely as a source of consumption for as many as 2.7 million people for their drinking water needs and industrial production activities. However, due to the many uses of the river, the river becomes polluted by domestic and industrial waste (Asrori, 2021). As much as 29% of the total land area of the Brantas River in Surabaya is dominated by industrial areas so that the input of industrial waste is very large (Budiono et al., 2017).

Monitoring the condition of river waters is very important considering the many benefits of the Brantas River. One way that can be done in monitoring environmental conditions is to use biomarkers. Biomarkers are one way to monitor the quality of river waters by looking at biological markers on organisms. The working principle of this biomarker is by looking at the characteristics of organisms and populations in response to changes in environmental conditions (Hedayati, 2018).

Fish is one of the aquatic organisms that can be used as a biomarker. Fish can be good organisms as biomarkers because fish are very sensitive to environmental changes (Dewi et al., 2014). One of the fish species found in the Brantas River in Surabaya is the red bader fish (*Barbonymus altus*). These fish include cyprinidae fish which are generally often used for toxicity tests so that they can be used as biomarkers in viewing river pollution, especially the Brantas River in Surabaya. These fish also live freely in water bodies so that they can reflect river waters better than fish that

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only live on the bottom or surface of the waters (Dewi et al., 2014).

Hematology is an important parameter in assessing the condition of the aquatic environment. Blood plays a role in circulating food substances resulting from digestion, carrying oxygen, and carrying enzymes and hormones to all parts of the body (Susilowati & Farfar, 2019). Hematology is an important biomarker parameter in evaluating environmental conditions both in the field and in the laboratory (Puttipong et al., 2020). Hematological changes can be used as an indicator of fish health. This is because fish can respond to variations in environmental conditions with changes in hematological conditions (Dhanalakshmi et al., 2012).

The purpose of this study was to analyze the condition of the waters to the presence of pollution and to analyze the relationship of water quality to the expression of biomarkers in the Brantas River, Surabaya.

Method

The research was carried out starting with a survey and determining the appropriate research location. Then samples were taken, both water samples and blood and gill samples from the fish, which were carried out 3 times with an interval of one month. After the data was collected, statistical analysis was carried out. This research was conducted in the Brantas River, Surabaya, East Java (Figure 1). Determination of sampling points was determined using the (Ani et al., 2021). The research location is divided into three locations from west (Upstream) to east (Downstream) on the Brantas River in the Surabaya area. The research location was determined from the distribution of the Surabaya area with different waste pollution. The first location is an industrial area, the second location is a densely populated area because it is in the city center and the third area is a less dense residential area which is the final stream before heading to the estuary. The research was carried out three times every month from January - March 2022.

Water sampling was carried out in situ and ex situ. In situ measurements were carried out on temperature parameters using a thermometer (Mainassy, 2017), pH measurements using a pH meter (Purwaningsih et al., 2016) and DO measurements using a DO meter (Yustiani et al., 2019). Ex situ measurements are carried out by taking water using sample bottles made of polyethylene and then stored in a coolbox container with additional ice gel to then be analyzed at the Laboratory of the Faculty of Fisheries and Marine Sciences, Brawijaya University. Total suspended solid (TSS) and total dissolved solid (TDS) were carried out using the gravimetric method,, Biological Oxygen Demand (BOD) was carried out using the Winkler method with incubation for 5 days (Santoso, 2018), Ammonia (NH_3) using a spectrophotometer (Nurdin et al., 2023), heavy metal Hg using spectrophotometer.

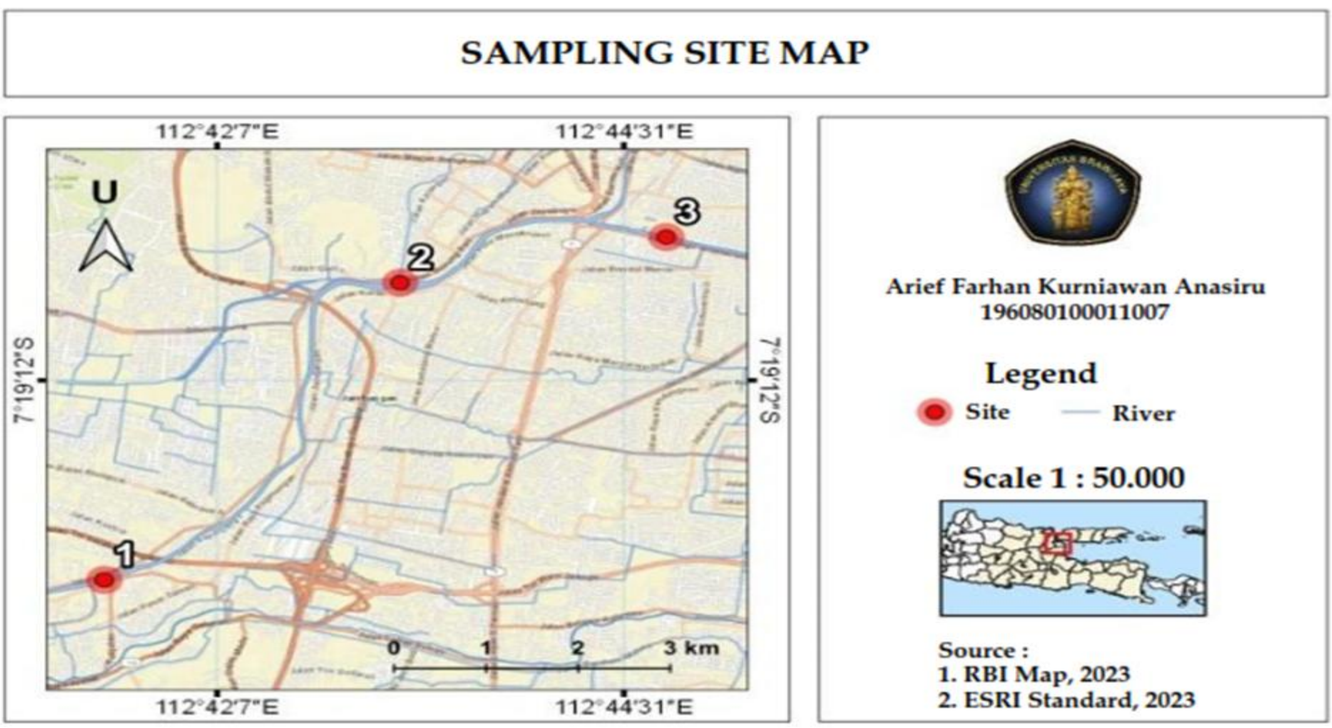

Figure 1. Map of sampling locations

Fish sampling was carried out using a fishing net. The number of fish caught was 3 at each sampling location. The fish used in this research was the red bader fish (*Barbonymus altus*) (Figure 4). Blood samples were taken from the lineal latiralis section of the caudal fin as much as 9 µl using a 1 ml syringe containing 10 µl of EDTA (Etylene Diamine Tetraacetic Acid). After that, the blood was put into Ependorf tube, then labeled and put into a cool box containing ice gel. The blood samples were then taken and analyzed at the Laboratory of the Faculty of Fisheries and Marine Sciences, Brawijaya University. The hematological parameters analyzed were erythrocytes, leukocytes, hemoglobin, hematocrit and micronuclei. Observation of erythrocytes and leukocytes using a haemocytometer (Putranto et al., 2019), hemoglobin was observed using the Sahli method, hematocrit was observed using microhematocrit and micronuclei using the smear method and calculations using a microscope at 1000x magnification (Naqvi et al., 2016).

The relationship between water quality parameters and hematological parameters was analyzed using the Correspondence Correlation Analysis (CCA) method using PAST application version 3.0. According to Kusumawati (2017), Correspondence Correlation Analysis is a multivariate statistical model that can identify and quantify the relationship between two sets of variables. This analysis focuses on the correlation between the linear combination of one set of variables and the linear combination of another set of variables.

Result and Discussion

The temperature at the research location ranges from 30 – 32.5 °C. At each site it ranges between 30 - 31 °C (Site 1), 31 - 32.5 °C (Site 2), 31-32°C (Site 3). The highest temperature is at site 2, this is due to the influence of hot weather. According to Patty et al. (2020), water temperature is influenced by the intensity of the sun and wind. However, the temperature is still at optimal levels for fish growth, namely around 25 – 32 °C. Indonesia is a tropical region, where in the summer there is a high increase in water temperatures and it is predicted that this will increase in the next few years due to climate change. This causes seasonal changes in the fish hematological index and will also have an impact on fish eating habits, fish growth and also fish reproduction and fish survival (Ashaf-Ud-Doulah et al., 2019). Stress due to environmental changes can be seen from changes in red blood cells, Hemoglobin dan Hematocrit.

Total Suspended Solid (TSS) measurement results ranged from 0.087 – 0.206 mg/L. TSS at each site is 0.138 – 0.199 mg/L (Site 1), 0.116 – 0.206 mg/L (Site 2), 0.087 – 0.189 mg/L (Site 3). Total Suspended Solid is sediment that floats in waters without touching the bottom of the waters which consists of various materials including soil, mud, organic particles, microorganisms, waste and other particles (Khairunna et al., 2021). TSS levels in the three samples tended to be low. Based on Government Regulation No. 21 of 2021 according to class II quality standards, the TSS range in river waters does not exceed 50 mg/L. This means that TSS levels in the waters of the Brantas River in Surabaya are still relatively low and safe for the environment.

Total Dissolved Solid (TDS) measurement results ranged from 0.0089 – 0.0153 mg/L. TDS levels at each site are 0.0121 – 0.0153 mg/L (Site 1), 0.0101 – 0.0117 mg/L (Site 2), 0.0089 – 0.0103 mg/L. Based on Government Regulation No. 21 of 2021 according to class II quality standards, the TDS range in river waters does not exceed 1,000 mg/L. This means that TDS levels in the waters of the Brantas River in Surabaya are still relatively low and safe for the environment. If the TDS value is excessive, it will increase the turbidity value which will inhibit the penetration of sunlight into the water column and ultimately affect the photosynthesis process in the waters (Rinawati et al., 2018). An increase in TDS can cause blockages or obstructions in the fish's gills, causing the fish to experience oxygen exchange disorders, osmoregulation disorders and cause inflammation and gill damage (Masita & Afdal, 2023).

The results of pH measurements at the research location ranged from 6.4 – 7.3. At each site it ranges from 6.5 – 7.2 (Site 1), 7.1 – 7.2 (Site 2), 7.2 – 7.3 (Site 3). The pH value states the intensity of the acidic or alkaline properties of water. It is defined as the negative logarithm of the hydrogen ion concentration. pH is expressed on a scale of 0 to 14. A good pH range for fish survival is between 6.5-7.5 (Aliyas et al., 2016). So it can be concluded that the pH levels at the three research sites are still in a good range.

Changes in pH, either up or down, can inhibit the physiological or metabolic functions of fish such as growth, reproduction and ecological distribution (Swain et al., 2020). The pH at site 1 is lower than at other sites due to the organic waste content resulting from industrial activities. The decrease in pH is caused by the organic acid content produced through the process of aerobic decomposition of organic materials. The degree of acidity (pH) is one of the important factors and acts as a limiting factor in waters. This is because most air biota are very sensitive to changes in pH values (Puspitasari et al., 2016).

The results of Dissolved Oxygen (DO) measurements at the research location ranged from4.88 – 7.47 mg/L. DO at each site ranges from 4.88 – 6.07 (Site 1), 5.19 - 5.73 (Site 2), 5.34-7.47 (Site 3). Based on Government Regulation No. 22 of 2021, according to class II quality standards, the DO range in river waters is a minimum of 4 mg/L. The results show that the third site has a DO value above the quality standard so it is still good for the life of aquatic organisms. The lowest DO range is at site 1, this is threatening because of the high levels of pollutants contained in the waters, especially pollutants from industrial activities. Low DO values are influenced by high organic material contamination. With high levels of organic matter in the waters, the oxygen in the waters will decrease due to its use for the decomposition of organic matter (Azizah, 2017). If DO conditions in the water decrease, it will cause fish to experience hypoxia. Hypoxia is a condition where the body experiences a lack of oxygen due to the difference between oxygen supply and oxygen consumption (Sinansari et al., 2021). Hypoxia can cause effects on fish hematology such as reducing the hormones cortisol and Hb (Zulfahmi, 2018). Stress due to environmental changes can be seen from changes in red blood cells, hemoglobin and hematocrit. Hypoxic conditions will make fish adapt by increasing levels of red blood cells, hemoglobin and hematocrit (Jia et al., 2021).

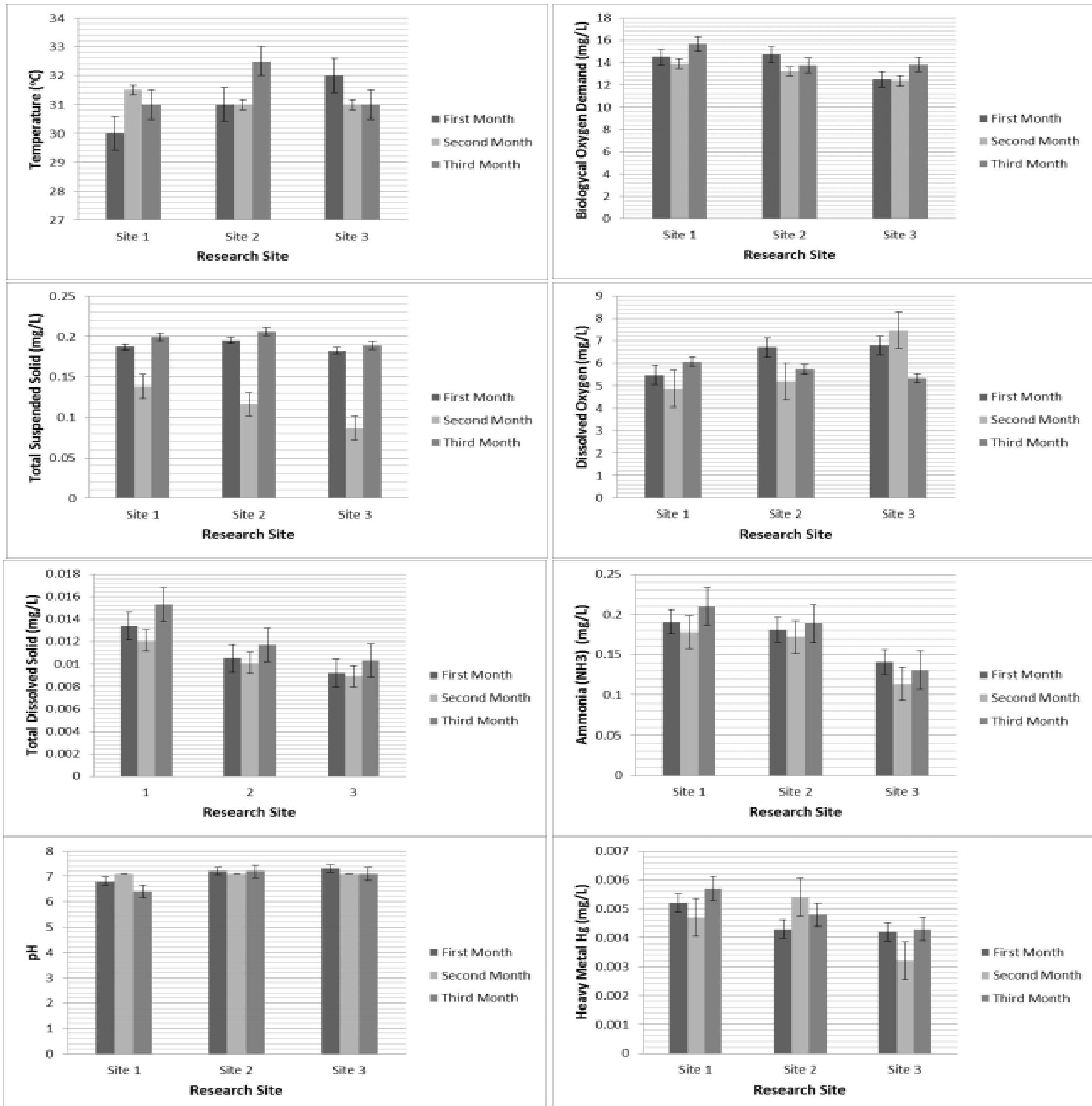

Figure 2. Water quality measurement results

The results of Biological Oxygen Demand (BOD) measurements at the research location ranged from 12.34–15.67 mg/L. BOD levels at each site were 13.88–15.67 mg/L (Site 1), 13.19–14.7 mg/L (Site 2), 12.34–13.8 mg/L (Site 3). Based on Government Regulation No. 21 of 2021, according to class II quality standards, the BOD range in river waters does not exceed 3 mg/L. Based on these results, the BOD range in the Brantas River in Surabaya is in a bad range because it has exceeded the quality standards. The highest BOD levels are at site 1 which is an industrial area. High BOD levels at the research location are due to the effects of high industrial activities such as the paper industry and food processing in the area. High BOD levels come from various wastes, including domestic waste (household waste, detergent, food waste), industrial waste (textiles and food processing industry) where the waste products ultimately flow into rivers (Aktar & Sabrina Moonajilin, 2017). The high BOD value is caused by several factors such as domestic waste which is thrown directly into the water and also from agricultural waste. A high BOD value indicates a high concentration of organic matter (Supardiono et al., 2023). High BOD levels cause oxygen consumption in waters to increase, which causes a decrease in DO. Low DO values can cause problems in the life of aquatic organisms (disturbing the respiration process, causing damage to fish function, causing oxidative stress) (Chakraborti, 2021).

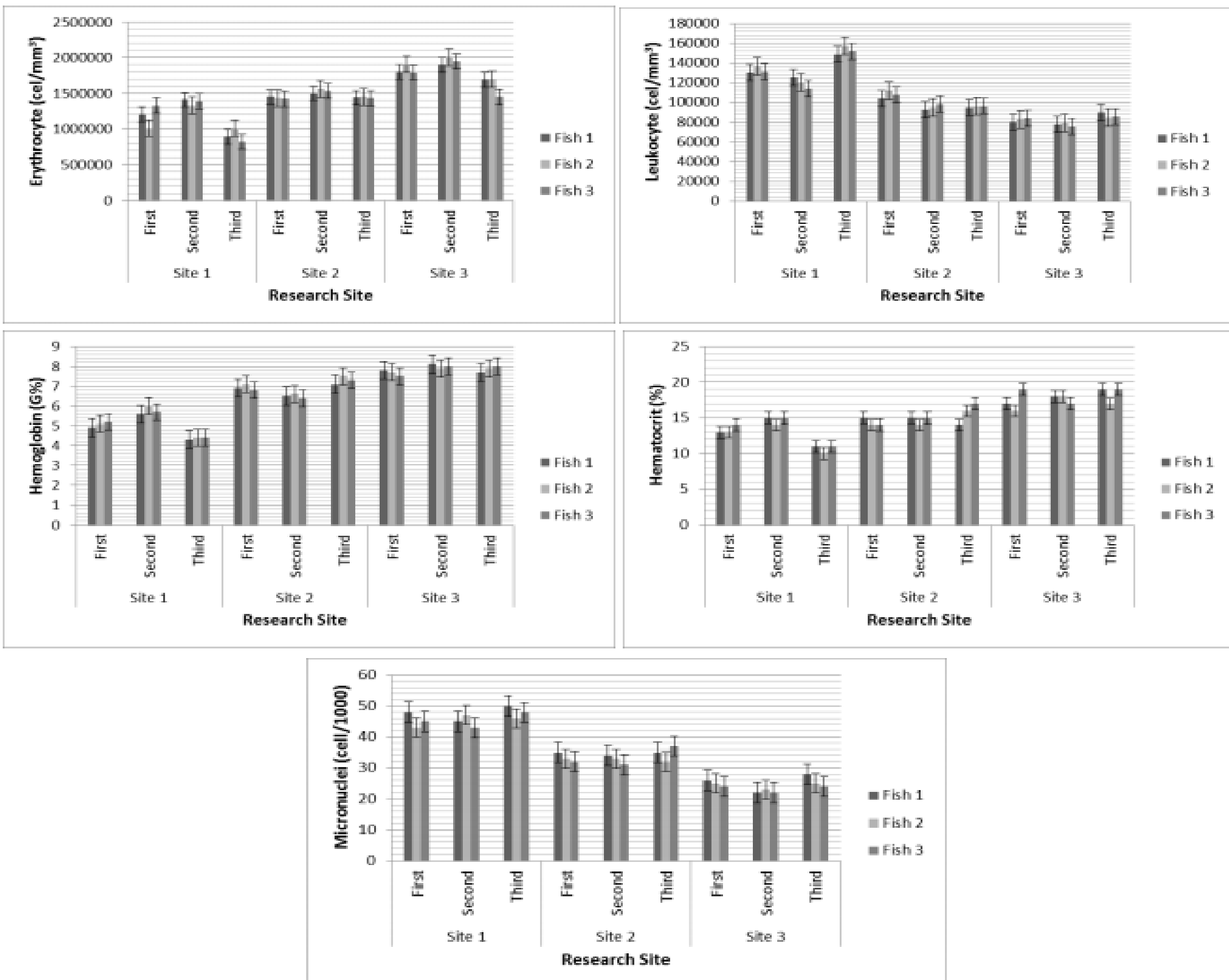

Figure 3. Results of measurements of the hematological profile of red bader fish

The results of ammonia (NH_3) measurements at the research location ranged from 0.114 – 0.210 mg/L. Ammonia at each site ranges from 0.178 – 0.210 mg/L (Site 1), 0.172 – 0.189 mg/L (Site 2), 0.114 – 0.141 mg/L (Site 3). Based on Government Regulation No. 21 of 2021 according to class II quality standards, the range of ammonia in river waters does not exceed 0.2 mg/L. The results show that site 1 tends to be higher than the other sites and at the third sampling time it has passed the quality standard. This is because there are effects from industrial waste and domestic waste. Ammonia in waters comes from the breakdown of organic nitrogen such as protein and urine and also from inorganic nitrogen such as the decomposition of organic material by decomposers (ammonification process) found in the soil of the water column (Supriyantini et al., 2017). High concentrations of ammonia in river bodies indicate pollution, one of which is caused by the discharge of domestic wastewater, both fresh (untreated) and treated (Setianto & Fahritsani, 2019).

The results of the heavy metal Hg at the research location ranged from 0.0032 – 0.0057 mg/L. at each site it ranges from 0.0047 – 0.0057 mg/L (Site 1), 0.0043 – 0.0054 mg/L (Site 2), 0.0032 – 0.0043 mg/L (Site 3). Based on Government Regulation No. 21 of 2021 according to class II quality standards, the range of heavy metal Hg in river waters does not exceed 0.002 mg/L. The heavy metal levels of the Brantas River in Surabaya are already dangerous because they have exceeded the quality standard limits. The heavy metal levels of the Brantas River in Surabaya are already dangerous because they have exceeded the quality standard limits. The results from the three locations have exceeded the quality standard threshold and the highest is at site 1. This is because waste, both domestic and industrial waste, is discharged into the waters especially at site 1 which is the center of various industries. According to Ibrahim and Aris (2021), mercury in waters can come from industrial emulsions, coal burning and gold mining mineral extraction waste as well as from nickel ore mining. Heavy metals are metal chemical elements that have a high atomic weight that are toxic in small amounts. Heavy metals have been found in the atmosphere, soil, water, sediments, and biota including aquatic species. Heavy metals are influenced by environmental factors such as season, water pH, temperature and dissolved oxygen (Elfiza et al., 2023).

The results of observations of red bader fish erythrocytes ranged from 810,000 – 2,000,000 cells/mm3. The results at each site obtained values ranging from 820,000–1,4100,000 cells/mm3 (Site 1), 1,420,000– 1,560,000 cells/mm^3 (Site 2), and 1,450,000 – 2,000,000 cells/mm3 (Site 3). In normal fish, erythrocyte levels range from 1,010,000 – 2,430,000 cells/mm3 (Harianto et al., 2021). Erythrocyte levels at site 1 showed low values from the normal fish range. This is because site 1 has a large amount of pollution from both organic and inorganic contamination. Location 1 is a location dominated by industrial activity. A decrease in erythrocyte values can show symptoms of anemia, an increase in free radicals such as ammonia can cause damage to red blood cells. In addition, ammonia toxicity can inhibit the hematopoietic ability of red blood cells (Gao et al., 2021). The decrease in red blood cells can be caused by the destructive action of pollutants in the water which then causes damage to erythrocytes and hemolysis. Pollutants cause changes in the properties of the erythrocyte membrane to become more fragile and permeable. This allows cell swelling, deformation (elongated erythrocytes, roundness) and cell damage (Ugokwe & Awobode, 2015). apart from that, handling when collecting blood samples (injection) or because pathogenic infections may cause stress in fish can increase erythrocytes (Fauzana et al., 2023).

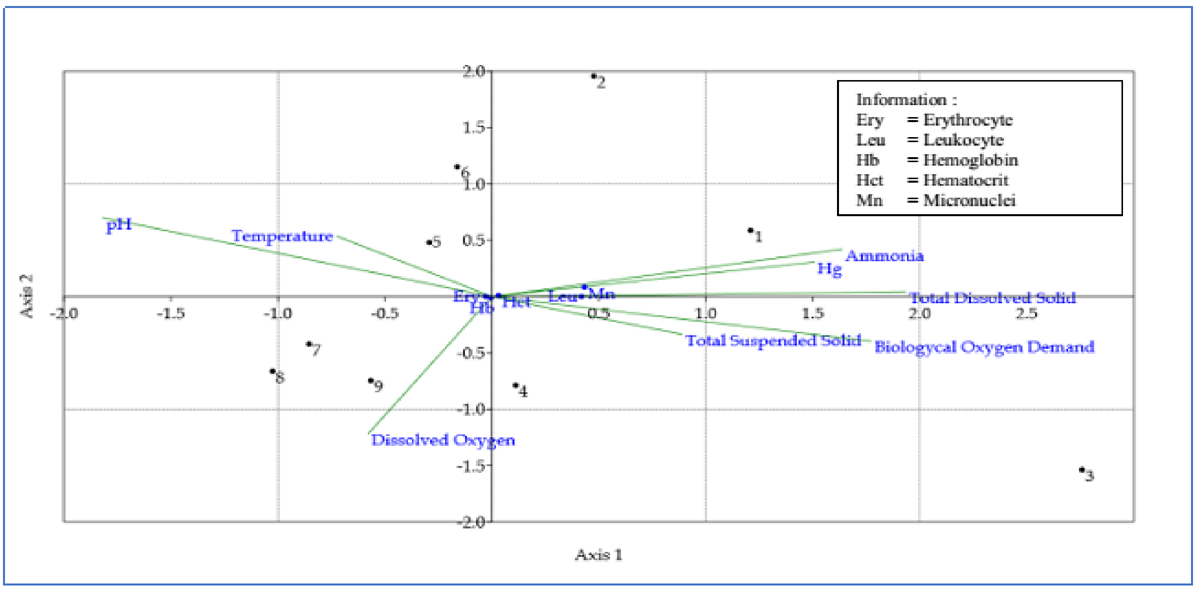

Figure 4. CCA (Correspondence Correlation Analysis) results between water quality and hematology profile

The results of observations of red bader fish leukocytes ranged from 75,500–1,575,000 cells/mm$^{3.}$ Each site obtained values ranging from 114,000–1,575,000 cells/mm^3 (Site 1), 92,500 – 112,000 cells/mm3 (Site 2), and 75,500 – 89,500 cells/mm^3 (Site 3). The leukocyte value in normal fish ranges from 20.000–150.000 cells/mm^3 (Herliani & Herlina, 2023). The results showed that the leukocyte value at site 1 exceeded the normal range. This is because of the high level of contamination at site 1 due to high industrial activity at that location. Leukocytes play a role in cleaning the body from foreign objects, including pathogen invasion through the immune response system and other responses (Simorangkir et al., 2020). The leukocyte value is influenced by several factors such as environmental parameters, infection and also the age and eating habits of the fish (Parrino et al., 2018). High leukocyte values indicate that the water conditions are polluted by pollutants (Sunardi et al., 2021).

Hb observation results for red bader fish range 4.1 –8.1 G%. Meanwhile, the values at each site ranged from 4.3–6 G% (Site 1), 6.4–7.5 G% (Site 2), and 7.5–8.1 G% (Site 3). Normal hemoglobin values in fish range from 6–11 G%. (Azhari & Hidayaturrahmah, 2020). Hemoglobin has a function in oxygen distribution so that it is in harmony with the value of red blood cells. The hemoglobin value at site 1 was low and was within the normal range compared to sites 2 and 3, this was thought to be due to the high level of pollutants at that site. Low hemoglobin values in fish indicate that the fish is anemic. This is due to the effects of pollutants in the waters. This condition occurs due to disruption of erythrocyte production, hemodelusion and destruction of cells involved in the production of vitamin B12 which is used in the formation of erythrocytes and hemoglobin (Ugokwe & Awobode, 2015).

The hematocrit results for red bader fish range from 10–19%. Meanwhile, the value at each site ranges from 10–15% (site 1), 14–17% (site 2), 16–19% (site 3). Hematocrit is the volume percentage of red blood cells in fish blood. Normal hematocrit values in teleost fish range from 20–30%. Fish that are anemic have a hematocrit value of at least 10% (Yanuhar et al., 2021). Hematocrit is directly proportional to erythrocytes and hemoglobin. Hematocrit levels at site 1 are also lower than sites 2 and 3. This is because of the high level of industry at that location. A decrease in hematocrit can be an indication that the fish is experiencing an uncomfortable condition and can cause the fish to experience anemia (Sari et al., 2017).

The results of observations of red blood cell micronuclei in red bader fish showed results ranging from 22–50 cells/1000. Meanwhile, at each site it is around 43-50 cells/1000 (site 1), 31-37 cells/1000 (site 2) and 22–28 cell/1000 (site 3). One indicator of DNA damage is the formation of micronuclei. Induction of micronuclei in aquatic organisms and fish indicates mutagenic damage. This damage is a mutagenic effect of heavy metals in contaminated water (Ali et al., 2020). The frequency of MN depends on several factors such as season, stress, type of pollutant and heavy metals (Anifowoshe et al., 2022). Mercury has a strong toxic effect on cell membranes which has the ability to cross cell membranes and disrupt the cell's metabolic system. Mercury can also cause aneugenicity and clastogenicity which affects mitotic division which causes the formation of micronuclei (Cruz-Esquivel et al., 2023).

Correspondence Correlation Analysis (CCA) is a nonlinear multivariate direct gradient analysis method that combines correspondence analysis with multiple regression analysis. This method is traditionally used to evaluate environmental gradients in ecological studies, and it can easily identify causal relationships between species distributions and environmental variables (Zárate-Santana et al., 2021). This method relates dependent parameters, namely hematological profiles (erythrocytes, hemoglobin, hematocrit, leukocytes and micronuclei) with dependent parameters, namely water quality (temperature, total suspended solid, total dissolved solid, pH, DO, BOD, ammonia and heavy metal Hg). Based on the results of the CCA analysis as in Figure 3, there is two interpretation results were obtained. Firstly, because erythrocytes, hemoglobin and hematocrit are in the middle of all variables, it can be said that erythrocytes, hemoglobin and hematocrit are associated with temperature, TSS, TDS, pH, DO, BOD, Ammonia with moderate concentrations. Secondly, leukocytes, micronuclei are associated with strong concentrations with Ammonia, TSS, TDS, BOD and also associated with low levels with temperature, pH, DO.

Figure 5. Red Bader Fish (*Barbonymus altus*) from the Brantas River, Surabaya

Conclusion

The results of water quality analysis and hematological analysis show that location 1 is classified as polluted compared to locations 2 and 3. CCA analysis shows a relationship between hematological profiles and water quality, especially Leukocyte and Micronuclei parameters which correlate with high concentrations of TSS, TDS, BOD, Ammonia and Heavy Metals Hg shows that this parameter can be a biological marker for assessing water quality.

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Conflicts of interest
Authors declare that there are no conflicts of interest related to the publication of this paper.

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