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Hematological Profile of Tilapia (*Oreochromis niloticus*) as a Bioindicator to Evaluate Water Quality of Floating Net Cage Cultivation System in Ranu Grati, Indonesia

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Abstract

Ranu Grati is a volcanic lake situated in Grati District, Pasuruan Regency, East Java, Indonesia. The rapid expansion of floating net cage aquaculture in Ranu Grati has raised concerns about water quality deterioration and its impact on fish health. This study aimed to evaluate the hematological profile of tilapia (*Oreochromis niloticus*) as a bioindicator for assessing water quality in floating net cage cultivation systems. The purpose of this research was to examine the hematological profile of tilapia fish alongside a series of water quality parameter measures as an environmental factor influencing fish life. Sampling was conducted with three repetitions every three weeks from September to November 2024 at five distinct locations representative of each floating net cage group distributed around the region. The Canonical Correspondence Analysis (CCA) approach was utilized to ascertain the relationship between the hematological profile of tilapia and water quality. The water quality at Stations 2 and 3 in the research area was somewhat polluted, but Stations 1 and 5 were heavily polluted. The elevated pollution levels at Stations 1 and 5 are attributed to the significant quantity of floating net cages relative to other stations. The investigation indicated that low dissolved oxygen levels and elevated ammonia concentrations impacted the hematological parameters of Tilapia fish. All water quality characteristics influenced the status of leukocytes, erythrocytes, and hemoglobin at moderate quantities. Moreover, micronuclei and hematocrit levels were significantly affected by elevated concentrations of ammonia, temperature, BOD, and pH, whereas DO, TDS, and TSS influenced lower values.

INTRODUCTION

Ranu Grati is a volcanic lake located in Grati District, Pasuruan Regency, East Java, Indonesia. The area of this lake reaches around 198 hectares and is surrounded by three villages in Pasuruan Regency, namely Sumber Dawesari Village, Ranu Klindungan,

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and Gratitunon. (Utomo and Muzaki, 2023). As a strategic natural resource, Ranu Grati has a vital role as a water source, tourist attraction, agricultural land, industry, and fisheries, both capture and cultivation (Safitri and Idajati, 2017). The aquaculture industry in Ranu Grati has grown rapidly, with a floating net cage system covering an area of 35.5 hectares or 17.9% of the total lake area, the largest in Pasuruan Regency (Nugroho, 2015). The main commodities cultivated include tilapia, freshwater milkfish, tombro, gourami, patin, and pomfret. Among the various types of fish, tilapia (*Oreochromis niloticus*) is the most popular superior commodity and dominates the cultivation activities of the community around Ranu Grati (Azhar and Wirasisya, 2019).

Although fish cultivation in floating net cages provides significant economic benefits for fishermen, this practice also harms the balance of the aquatic environment. The accumulation of organic waste from leftover feed and fish metabolism in the form of nitrogen and phosphorus from feces can reduce water quality and water fertility (Yildiz *et al*., 2017). High market demand has driven the proliferation of uncontrolled floating net cages, thus increasing the burden of organic waste in the waters. This condition can cause eutrophication, decreased dissolved oxygen levels, and increased pathogenic microorganisms, especially in intensive cultivation systems with high densities and the use of artificial feed (Akinnawo, 2023). Water quality problems in Ranu Grati are increasingly complex due to the entry of domestic waste, agricultural and industrial runoff, and tourism activities that produce organic and inorganic pollutants (Akhtar *et al*., 2021). These anthropogenic inputs can disrupt the balance of aquatic ecosystems and endanger farmed fish and native aquatic biota.

Given the complexity of the relationship between cultivation practices and environmental quality, it is necessary to develop efficient monitoring methods to provide early warning of ecosystem stress before irreversible damage occurs to this vital water resource. One effective approach is the use of bioindicators to assess water quality through the biological response of organisms to changes in environmental conditions (Akinnawo, 2023). Tilapia is an ideal bioindicator for environmental monitoring in tropical freshwater ecosystems due to its wide distribution, high economic value, and well-documented physiological responses to environmental disturbances. This species exhibits remarkable adaptability to a wide range of water quality conditions, yet still provides measurable physiological responses when exposed to environmental stressors (Omar *et al*., 2015). The relatively sedentary nature of tilapia in fish cage culture systems ensures that the observed physiological responses reflect local environmental conditions, rather than transient exposure during migration (Dewi *et al*., 2014). For example, a study revealed that tilapia showed a significant physiological response to heavy metal pollution in the Nile River waters, with the accumulation of heavy metals such as cadmium, lead, and zinc in fish tissues impacting blood biochemical parameters such as total protein, albumin, glucose, cholesterol, and liver enzymes (Said *et al*., 2021). While the effects of profenofos exposure on Nile tilapia, particularly focusing on the alterations in hematological parameters and the structure of peripheral erythrocytes. Specifically, glucose and white blood cell (WBC) levels increased, while hemoglobin, red blood cell (RBC) count, and packed cell volume (PCV) decreased (Al-Emran *et al*., 2022).

Hematological profiles are sensitive bioindicators for identifying physiological responses to environmental stressors, such as water pollution, in fish populations experiencing suboptimal conditions (Puttipong *et al*., 2020). A 2024 study on *Barbonymus altus* found correlations between water pollution parameters (ammonia, heavy metals, BOD) and abnormal hematological values such as reduced erythrocyte counts and elevated leukocytes and micronuclei, indicating

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physiological stress and genotoxic damage (Anasiru *et al.*, 2024). This is possible because fish can respond to changes in environmental conditions through alterations in hematological parameters, considering that blood plays a vital role in the circulation of nutrients from digestion, oxygen transport, and the distribution of enzymes and hormones throughout the body (Dhanalakshmi *et al.*, 2012; Susilowati and Farfar, 2019). This study aims to examine the hematological profile of tilapia as a bioindicator in evaluating water quality in the fish net cage cultivation system in Ranu Grati. Through hematological profile analysis, this study attempts to explain the impact of adverse aquatic environmental variables on fish health. The results of the study are expected to be a scientific basis for the development of pollution mitigation techniques and improving fish net cage management in Ranu Grati. These findings have the potential to be a reference for the development of cultivation methods that are both environmentally sustainable and biologically sound, emphasizing the importance of early detection of water quality degradation through hematological monitoring.

METHODOLOGY

Ethical Approval

No animals were harmed or subjected to inappropriate treatment throughout this investigation. All treatments involving live fish were executed in compliance with established animal care protocols. The test animals were meticulously managed, guaranteeing ideal environmental circumstances, encompassing suitable water quality metrics and temperature control. Appropriate and fitting feed was continuously supplied, and all handling and sampling techniques were devised to reduce stress and prevent harm. All collected fish were immediately returned to their natural habitat after blood sampling and length-weighting procedures to ensure their well-being and survival. This study was conducted in strict adherence to aquaculture ethical standards, with animal welfare paramount throughout the entire study period.

Place and Time

This research was conducted from September to December 2024. The location of this research is in the floating net cages of Ranu Grati, Pasuruan Regency, East Java, Indonesia. Sampling was carried out at five different stations, which were considered to be able to represent the existence of each floating net cage group spread across the Ranu Grati area. This research was also carried out with three repetitions every three weeks to obtain measurement accuracy. Fish blood sample analysis was carried out at the Fish Parasite and Disease Laboratory, while water quality analysis was carried out at the Freshwater Laboratory, Sumber Pasir. Both laboratories are under the auspices of the Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia. Figure 1 displays the map of the research station.

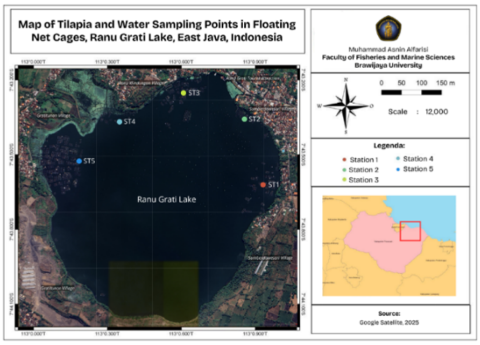

Figure 1. Map of water and tilapia sampling stations in floating net cages located in Ranu Grati, Pasuruan Regency, East Java, Indonesia.

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Research Materials

The apparatus utilized in this investigation included trays, 1 cc syringes, Eppendorf tubes, glassware, cover slips, micropipettes, test tubes, beakers, microscopes (Olympus CX-23, Japan), cuvettes, 1.5 ml microtubes, haemocytometers (Neubauer Set, Germany), cool boxes and ice gels for fish protection, DO meter analyzer (model DO9100, Indonesia), water quality tester model EZ-9901, spectrophotometers (Thermo Scientific GENESYS 10S UV Vis, Japan) microhematocrit tubes, Winkler bottles, filter paper, hot plates, rulers, aluminum foil, and tissues for equipment sanitation. The materials utilized in this experiment comprised tilapia fish (O. Niloticus) measuring between 17-24 cm, water samples collected from each station, aquades for rinsing research equipment, labeling paper, 3.8% sodium citrate as a coagulant, Turk's solution for leukocyte dilution and erythrocyte lysis, Hayem's solution for erythrocyte dilution and leukocyte lysis, and Giemsa solution (Merck, Germany) for staining erythrocytes. SnCl imparts a blue hue to the orthophosphate solution, ammonium molybdate serves as a reagent for orthophosphate quantification, phenol disulfonic acid is utilized to solubilize fat on nitrate deposits, and NH4OH is employed to color the nitrate solution, while Nessler's solution is used to detect ammonia concentrations in a water sample.

Research Design

This study used a purposive sampling method to determine the location of the sample points. The purposive sampling method, according to Budiarti *et al.* (2023), is a non-random sampling method where researchers take samples by applying certain assessments to obtain more representative information and in accordance with the objectives of the research being carried out. The location for taking bioecological samples and fish samples is at 5 station points on floating net cages located in Ranu Grati, Pasuruan Regency, East Java. The determination of these station points was carried out based on considering data on the presence of dominant floating net cages and was divided into 5 points. Each station has different characteristics as described in Table 1.

Table 1. Sampling area coordinates and description.

Station	Geographical Position	Location Characteristics
1	7°43'39.6"S 113°00'50.9"E	Sumberdawesari Village. Close to residential areas
2	7°43'24.4"S 113°00'48.0"E	Sumberdawesari Village. Close to residential areas and lake outlets
3	7°43'17.9"S 113°00'36.1"E	Ranuklindungan Village. Close to residential areas, Ranu Grati tourist attraction area, and lake outlets
4	7°43'25.8"S 113°00'21.2"E	Ranuklindungan Village. Close to residential areas and lake outlets
5	7°43'33.9"S 113°00'12.6"E	Gratitunon Village. Close to residential areas, agricultural areas, and lake inlets

Work Procedure

Fish blood sample

Before blood sampling on tilapia, fish samples were first taken using a fishing net. The number of fish caught was 5 at each sampling station. The fish used in this study were tilapia (Figure 2). Fish blood sampling was carried out using the technique of piercing the tail vessel or caudal blood vessel (Yuniastutik, 2021). Blood was taken by puncturing a 1 mL syringe needle containing ±10 µL of natrium citrate anticoagulant on the lateral line of the fish behind the caudal fin, inserting the needle into the muscle until it reaches the spine (columna spinalis). Then pull slowly until approximately 90 µL of

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blood enters the syringe. After that, the blood was put into an Ependorf tube, then labeled and put into a cool box containing ice gel. Blood samples were then taken and analyzed at the Fish Parasites and Diseases Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia.

The investigated hematological parameters included erythrocytes, leukocytes, hemoglobin, hematocrit, leukocyte differential, phagocytic activity, and micronuclei. Examination of erythrocytes and leukocytes via a hemocytometer (Fazio, 2019), hemoglobin was observed using the Sahli method, hematocrit was observed using the microhematocrit method, phagocytosis activity was observed using baker's yeast homogenized with blood, stained using Giemsa, and the amount of phagocytosis activity was calculated (Yanuar and Manoppo, 2017). Micronuclei and leukocyte differential using the smear method and calculations using a microscope with 1000x magnification (Naqvi *et al.*, 2016).

Figure 2. Specimens of tilapia (O. Niloticus) collected from floating net cages situated in Ranu Grati.

Water sampling and measurement methods

Water sampling was conducted both in situ and ex situ. In situ measurements of temperature and dissolved oxygen parameters were conducted with a DO meter analyzer model DO9100 (Mainassy, 2017), while pH and TDS were assessed with with pH meter and TDS meter model EZ-9901 (Purwaningsih *et al.*, 2016). Ex situ measurements involve collecting water samples in polyethylene bottles, which are subsequently stored in a cooler with ice gel for analysis at the Freshwater Fisheries Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University (Olasoji *et al.*, 2019). BOD was determined using the Winkler method with a 5-day incubation period specified in SNI 06-6989.14-2004 (Santoso, 2018). Total suspended solids (TSS) were analysed using the gravimetry method as per SNI 06-6989.3-2004 (Yustiani *et al.*, 2019). Ammonia (NH_3), nitrate, and orthophosphate were measured using a spectrophotometer according to SNI 19-7119.1-2005, SNI 06-69897.9-2011, and SNI 06-6989.31:2021 (Nurdin *et al.*, 2023).

Data Analysis

The haematological profile and water quality data were analysed and shown in graphs utilising Microsoft Excel. The results were subsequently analysed descriptively by correlating them with field circumstances and pertinent literature. The correlation between haematology profiles and water quality was assessed by canonical correspondence analysis (CCA) with the PAST application version 4.03. Correlation Analysis is a multivariate statistical model capable of identifying and quantifying the relationship between two variable sets (Zhuang *et al.*, 2020). This study examines the relationship between a linear combination of one set of variables and a linear combination of another set of variables. The two variables are the independent and dependent variables. The independent variable is described as one

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that is not affected by external causes, whereas the dependent variable is influenced by the independent variable. This method seeks to analyze the influence of independent variables (water quality parameters) on dependent variables (erythrocytes, leukocytes, hemoglobin, differential leukocyte counts, hematocrit, phagocytic activity, and micronuclei) and to identify the environmental factors that most significantly impact the dependent variables (Jin *et al.*, 2016).

RESULTS AND DISCUSSIONS

Hematological Profile of Tilapia Fish

Hematological parameters are highly sensitive to water quality indicators and environmental stress. The results of the hematological profile analysis of tilapia, including erythrocytes, leukocytes, hemoglobin, hematocrit, micronuclei, and phagocytic activity, are presented graphically in Figure 3. These variations in the hematological profile reflect the overall impact of local environmental conditions such as changes in water temperature, dissolved oxygen levels, and pollutant concentrations. The wide range of hematological responses observed in tilapia underscores the fish's ability to adapt to fluctuating environmental stressors and highlights the potential of hematological profiles as reliable indicators of water quality and ecosystem health. Similarly, hematological analyses are widely used to evaluate fish health and welfare in aquaculture and environmental monitoring, reflecting the physiological state and immune competence under different water conditions (Witeska *et al.*, 2022).

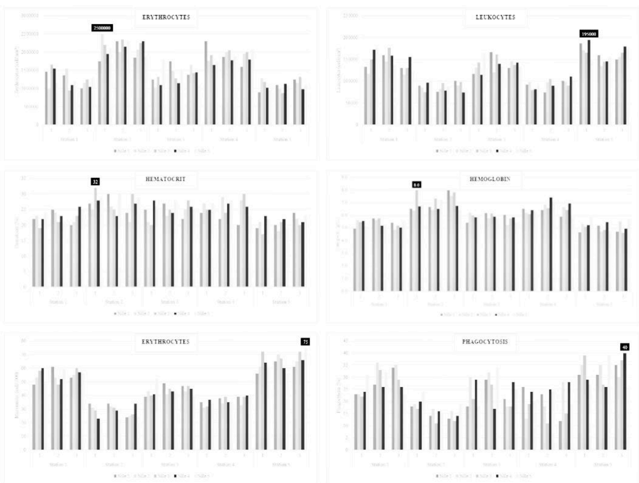

Figure 3. Result of tilapia fish (*O. niloticus*) hematological profile.

The significant spatial variations of erythrocytes are linked to localized environmental stressors. Station 2, located near residential areas and lake outlets, exhibited the highest erythrocyte range (1,150,000–2,500,000 cells/mm³), likely reflecting compensatory erythropoiesis due to hypoxia caused by organic pollutants or reduced oxygen solubility, as observed in aquaculture systems impacted by nutrient runoff (Iyiola *et al.*, 2024). Conversely, Station 5, located near agricultural areas and lake inlets, exhibited the lowest erythrocyte values (900,000–1,180,000 cells/mm³), suggesting chronic stress or anemia potentially linked to agrochemical exposure, which is consistent with findings on pesticide-induced hematotoxicity in fish (Kanu *et al.*, 2023). The standard number of red blood cells in *O. niloticus* generally ranges from 1.05 to 3.0 × 10^6 cells/mm³, widely accepted as the normal physiological interval for healthy tilapia, with values influenced by factors such as age, sex, environmental conditions, nutrition, and oxygen availability (Fazio *et al.*, 2024). At

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station 5, erythrocyte variation was below standard, possibly due to exposure to toxins and waste from residential and agricultural areas. From the erythrocyte value, we can determine the level of stress of the fish in the oxygen transport system, which is affected by toxins (Ariweriokuma *et al*., 2016).

Inversely proportional to erythrocyte, the leukocyte count, indicative of differing environmental stressors and immune responses, has the highest range in Station 5 (135,000–195,000 cells/mm^3), located near residential, agricultural area, and lake inlets, suggesting an elevated immune response possibly triggered by exposure to agricultural runoff, pathogens, or pollutants, consistent with findings by Fazio (2019), who reported increased leukocyte counts in fish exposed to environmental contaminants. The lowest range in Station 2 (74,500–96,000 cells/mm^3), potentially indicating immunosuppression or poor health status, aligns with studies reporting decreased leukocyte levels under chronic pollutant exposure (Kanu *et al*., 2023). Stations 1 and 3 exhibited moderate leukocyte counts, reflecting intermediate environmental pressures, while Station 4 also showed relatively low leukocyte levels, possibly due to similar stressors as Station 2. These leukocyte variations underscore their utility as sensitive hematological biomarkers for assessing fish health and water quality in aquaculture systems impacted by anthropogenic activities. Increased leukocytes mean that antibody production also increases to fight fish from toxins. Monitoring leukocyte profiles can thus aid in early detection of environmental stress and guide management practices to improve fish welfare and productivity (Sinha *et al*., 2022).

The concentration of hemoglobin reflects the rate of transport process between oxygen and carbon dioxide in erythrocytes (Susandi *et al*., 2017). The standard concentration of hemoglobin (Hb) in *O. niloticus* reported by Adebayo (2023) generally ranges around 5.8-9.5 g/dL when expressed as a percentage by volume in blood. This range reflects normal physiological values for healthy Nile tilapia under typical aquaculture or natural conditions. Station 2, located near residential areas and lake outlets, showed the highest hemoglobin levels (6.4–8.0 g/dL), indicating a possible physiological adaptation to environmental conditions such as lower dissolved oxygen or increased pollutant load, which can stimulate erythropoiesis and hemoglobin synthesis to enhance oxygen transport (Wang *et al*., 2023).

Similarly, Station 4 also demonstrated relatively high hemoglobin concentrations (5.9–7.4 g/dL), suggesting moderate environmental stress or better water quality supporting fish health. In contrast, Station 5, near residential, agricultural areas, and lake inlets, showed the lowest hemoglobin range (4.6–5.7 g/dL), which may indicate chronic stress, anemia, or impaired oxygen-carrying capacity possibly due to agrochemical contamination or eutrophication effects (Kanu *et al*., 2023). Stations 1 and 3 presented moderate hemoglobin values, reflecting intermediate environmental conditions. These findings align with recent studies highlighting hemoglobin concentration as a sensitive hematological biomarker for assessing fish health and environmental quality, indicating the oxygen availability within organisms and their ability to regulate it to maintain stability. A decline in hemoglobin levels caused by industrial pollution can lead to reduced oxygen delivery to fish tissues, resulting in diminished physical activity in the fish (Witeska *et al*., 2022). Monitoring hemoglobin levels in tilapia can thus provide valuable insights into the impacts of anthropogenic activities on aquatic ecosystems and inform sustainable management practices.

Hematocrit is the percentage of red blood cells in the fish's blood volume. Observing hematocrit in fish can be used as an indicator of the fish's health. The highest hematocrit value occurred during sampling 1 at station 2, while the lowest hematocrit value occurred during sampling 1 at station 5. The hematocrit value at station 2 was higher than at the other stations. The normal

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hematocrit value in tilapia ranges from 22%–60% (Rimalia and Kisworo, 2021). Based on this, it can be said that some tilapia caught in the Ranu Grati floating net cage are classified as abnormal. A hematocrit value of less than 22% indicates that the fish are anemic. A low hematocrit value is a stress response from fish due to changes in environmental conditions or environmental pollution (Yuniar, 2017). Changes in hematocrit values in the fish's body indicate the fish's health condition. High hematocrit values in fish indicate that the fish are exposed to contaminants or other problems that cause stress. Meanwhile, a low hematocrit value indicates that the fish is experiencing an infection, vitamin deficiency, protein deficiency, or anemia. Anemia in fish can inhibit growth (Dahlifa *et al*., 2016). Differences in hematocrit levels in fish are influenced by the size and number of red blood cells in the fish's body. A decrease in hematocrit levels can also be caused by pathogenic bacteria (Putranto *et al*., 2019).

Micronuclei (MN) are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division (Luzhna *et al*., 2013). Micronuclei counts, indicative of chromosomal damage and genotoxic stress (D'Agostini and La Maestra, 2021), were highest at Station 5, which is affected by household waste, agricultural pesticides, and lake inlets. This suggests elevated exposure to genotoxic agents such as agrochemicals or pollutants commonly associated with agricultural runoff (Kanu *et al*., 2023). Conversely, Station 2 exhibited the lowest micronuclei frequency, indicating relatively better water quality or lower genotoxic stress levels. Stations 1, 3, and 4 showed moderate micronuclei counts, reflecting intermediate environmental conditions (mid pollution). These findings align with recent research demonstrating the micronuclei assay as a sensitive biomarker for environmental genotoxicity in fish. Micronuclei were found in fish inhabiting polluted aquatic environments. An increase in micronuclei numbers indicates abnormalities in chromosome number and structure caused by various cellular agents. This situation may lead to cytotoxic effects from metals and genotoxic substances affecting the organism's body (Arslan and Parlak, 2017).

Phagocytic activity, a measure of innate immune competence, was highest at Station 5 (26–40%), suggesting an activated immune response possibly due to increased pathogen or pollutant exposure. In contrast, Station 2 showed the lowest phagocytic activity (11–24%), which may indicate immunosuppression or compromised health status due to environmental stressors (Fazio, 2019). Moderate phagocytic levels at Stations 1, 3, and 4 further support a gradient of immune activation corresponding to environmental quality. Together, elevated micronuclei counts and heightened phagocytic activity at Station 5 highlight the dual impact of genotoxic stress and immune system activation in tilapia exposed to agricultural and residential pollutants (Usman *et al*., 2020). These hematological parameters serve as robust bioindicators for monitoring water quality and fish health in aquaculture systems subjected to diverse anthropogenic pressures (Ahmed *et al*., 2020).

Water Quality Parameters

The measurements of water quality parameters at five distinct stations conducted in the Ranu Grati floating net cages can be seen in Figure 2. These water quality parameters serve as crucial indicators of the overall health of the aquatic environment. Alterations in water quality, including fluctuations in dissolved oxygen, pH, temperature, nitrat, ammonia, biological oxygen demand, orthophosphate, total dissolved solid, total suspended solid and the presence of pollutants (heavy metals, pesticides, organic contaminants, etc.), directly influence fish blood profiles, including erythrocytes, leukocytes, hemoglobin, and genotoxic indicators such as micronuclei. By monitoring these parameters, we gain valuable insight into the environmental conditions that the fish are exposed to, which in turn reflect their health status.

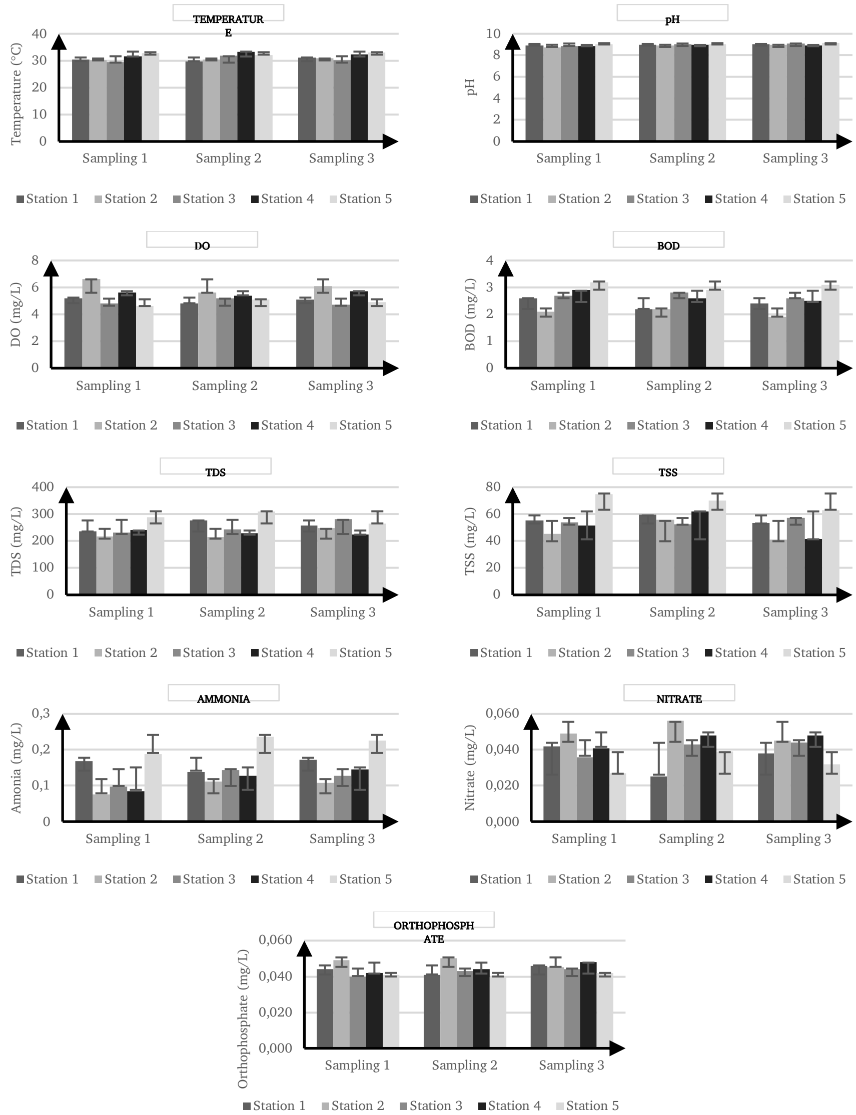

Figure 4. The outcomes of water quality parameter assessments conducted in the Ranu Grati floating net cages.

The temperature varied from 29.5 to 33.4°C across all stations and sampling periods. The temperature patterns varied between stations, with some showing increases in Sampling 2 (particularly Station 4) while others remained relatively stable or showed slight fluctuations across the sampling periods. But the number of temperatures still can be tolerated for the fish growth, with the optimum range between 27–33.1°C for freshwater fish (Tang *et al.*, 2017). The observed temperature increase during Sampling 2 at Station 4 may be attributed to localized environmental conditions, such as reduced cloud cover or water flow changes during the rainy season. Temperature fluctuations within this range are typical in tropical freshwater systems but can influence fish metabolism, growth, and oxygen consumption (Ongh *et al.*, 2025). Elevated temperatures can exacerbate stress in tilapia, potentially affecting hematological parameters and immune function (Witeska *et al.*, 2022).

The pH levels at the research site fluctuated from 8.76-9.14 across all stations and sampling periods. The pH variations between stations were relatively small, indicating fairly stable alkaline conditions across all monitoring locations. This stable alkaline condition is favorable for tilapia culture, as tilapia generally tolerate pH between 6.5 and 9.0, with optimal growth near neutral to slightly alkaline conditions (Arfiati *et al*., 2020). The slight pH elevation may be influenced by photosynthetic activity during daylight or the buffering capacity of the lake system. The increase in pH can be influenced by ammonia, which cannot be ionized and becomes toxic, so that pH has a strong correlation with ammonia concentration. Stable pH reduces physiological stress and supports efficient metabolic processes in fish (Hertika *et al*., 2021a).

The dissolved oxygen (DO) concentrations varied from 4.6 to 6.7 mg/L throughout all stations and sampling intervals. The DO variations between stations were moderate, with Station 2 showing significantly higher oxygen levels than the other four stations. DO is essential for aquatic life as it supports the system of respiration, as well as osmoregulation. Variations in DO levels among different stations are influenced by factors such as temperature, varying concentrations of organic matter, and the characteristics of land cover surrounding the water bodies (Patty *et al*., 2015). Higher DO at Station 2 could be due to better water circulation near lake outlets or lower organic pollution loads. DO values above 5 mg/L are generally considered adequate for tilapia health, although values near the lower limit (4.6 mg/L at Station 5) may impose suboptimal conditions, potentially leading to hypoxic stress (Wang *et al*., 2023). The rainy season and gloomy weather likely contributed to DO fluctuations by reducing photosynthesis and increasing organic matter input, which enhances microbial oxygen consumption (Sahiti *et al*., 2018).

The biochemical oxygen demand (BOD) values ranged from 1.9-3.2 mg/L across all stations and sampling periods. The BOD variations between stations were moderate, indicating different levels of organic pollution, with Station 5 showing the highest organic load. BOD values of Station 5 show the highest BOD (2.9–3.2 mg/L), indicating elevated organic pollution likely due to agricultural runoff and domestic waste inputs. According to the water quality guidelines and various aquaculture standards, BOD values below 3 mg/L are generally considered acceptable for freshwater fish culture, though values approaching 3 mg/L suggest a moderate organic load that could affect fish health if sustained (Hertika *et al*., 2022). Other stations showed moderate BOD levels (1.9–2.9 mg/L), indicating relatively better water quality but still impacted by organic matter. The BOD is an important indicator of organic pollution, as it reflects the amount of oxygen required by microorganisms to break down organic matter in the water (Bader *et al*., 2022). The higher BOD values suggest a higher level of organic material, which can lead to oxygen depletion and negatively affect aquatic life (Vigiak *et al*., 2019).

The total dissolved solids (TDS) values ranged from 215-310 mg/L across all stations and sampling periods. Station 5 demonstrated consistently higher TDS levels (265–310 mg/L) compared to other stations. These values fall within acceptable ranges for freshwater aquaculture (generally <500 mg/L), but elevated TDS can affect osmoregulation and fish stress (Ongh *et al*., 2025). The higher TDS at Station 5 suggests greater inputs of dissolved minerals and possibly pollutants from agricultural and residential runoff. TDS represents the amount (mg/L) of substances that cause turbidity in the water. However, high TDS values do not always correlate with high turbidity, as observed in some water bodies (Ngantu and Boma, 2023). The amount of dissolved solids in the water affects light penetration. The higher the dissolved solids, the more it will hinder light from penetrating the water. This directly impacts the rate of photosynthesis by chlorophyll-containing organisms in the water, such as hydrophytes and phytoplankton (Boyd, 2020).

The total suspended solids (TSS)

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values ranged from 41.2-74.8 mg/L across all stations and sampling periods. Station 5 demonstrated consistently higher TSS levels compared to other stations. TSS refers to the combined quantity of organic and inorganic particles suspended in water, often associated with pollution from micropollutants and heavy metals, as well as influencing primary productivity. The highest concentrations of total suspended solids may reduce water clarity and increase turbidity, which negatively impacts fish gill function and oxygen exchange (Hertika *et al*., 2021b). Government Regulation Number 82 of 2001 has approved the maximum level of TSS to be 50 mg/L for class II water quality. The high TSS levels can decrease light penetration into the water, thereby affecting the coverage and health of aquatic ecosystems (Guimarães *et al*., 2019). The TSS levels in this lake surpass the threshold, and the elevated TSS may also signify soil erosion and runoff during the rainy season, consistent with the prevailing meteorological conditions at the time of the test.

The ammonia concentrations varied from 0.076 to 0.236 mg/L across all stations and sampling intervals. The ammonia values showed variable patterns across sampling periods, with Station 5 consistently having higher ammonia levels compared to other stations. Ammonia is toxic to fish even at low concentrations; the safe limit for ammonia in aquaculture is generally below 0.05 mg/L unionized ammonia (Zhou and Boyd, 2015). Although the measured total ammonia includes both ionized and unionized forms, the elevated ammonia at Station 5 suggests potential toxicity risks, likely exacerbated by agricultural waste, domestic waste, organic matter decomposition, and nutrient runoff during the rainy season. Elevated concentrations of unionized ammonia in lake water can lead to the mortality of aquatic organisms. Unionized ammonia becomes hazardous at high concentrations as it can decrease DO levels due to the dissociation process of ammonia. Additionally, high levels of unionized ammonia can interfere with the function of hemoglobin (Hertika *et al*., 2021a).

The nitrate concentrations varied from 0.027 to 0.056 mg/L throughout all stations and sampling intervals. The nitrate variations between stations were moderate, indicating different levels of nitrogen cycling processes, with Station 2 (0.045–0.056 mg/L) showing the most active nitrification activity and Station 5 the least. The highest value of nitrate concentrations suggests enhanced nitrification processes, perhaps attributable to improved oxygenation near lake outflows (Windusari and Sari, 2015). The nitrate concentrations are low and typically regarded as safe for fish (<50 mg/L), indicating efficient nitrogen cycling at most stations. As the final, least toxic product of the nitrogen cycle, nitrate poses minimal impact on fish health at these concentrations (Banerjee *et al*., 2023). Unlike ammonia and nitrite, which can affect fish physiology even at low levels, nitrate becomes problematic only at concentrations much higher than those measured in this study. While nitrate monitoring is essential for understanding nitrogen cycling and assessing potential risks, particularly with increased organic loading, its current levels do not pose significant stress to cultured tilapia.

The orthophosphate concentration varied between 0.040 and 0.050 mg/L at all sampling locations. The orthophosphate concentration showed relatively stable values across all stations and sampling periods, indicating moderate nutrient loads. These values are below thresholds that typically trigger eutrophication (>0.1 mg/L), suggesting limited risk of algal blooms during the sampling period despite the rainy season (Sahiti *et al*., 2018). Orthophosphate is a key nutrient that plays a crucial role in the growth of aquatic plants and phytoplankton, but when present in excessive amounts, it can contribute to nutrient imbalances and lead to eutrophication (Naeem *et al*., 2014). The observed orthophosphate concentrations in this study are relatively low, which suggests that nutrient levels are being efficiently managed in the water body, minimizing the

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risk of harmful algal blooms and the negative ecological impacts typically associated with high orthophosphate concentrations.

Relationship between hematological profiles and water quality

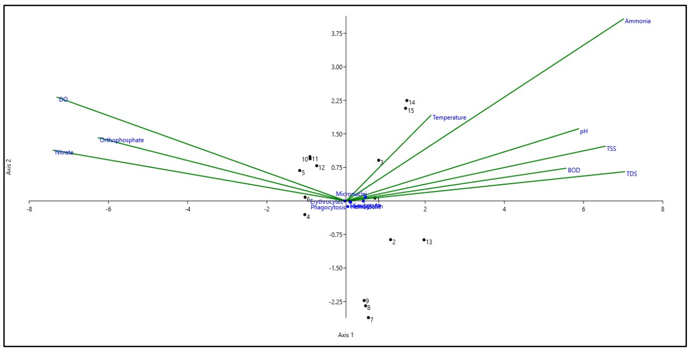

Figure 5. The outcome of Canonical Correspondence Analysis (CCA) concerning the hematological profile of O. Niloticus and water quality.

Water quality evaluation based on blood characteristics was conducted using the Canonical Correspondence Analysis (CCA) approach. CCA is a multivariate technique applied to explain the relationship between biotic communities and environmental factors through the ordination method. In this research, the CCA approach was used to analyze the impact of independent variables, namely variables with constant values that are not affected by other variables, on dependent variables, namely variables whose values are influenced by observed external factors (Hertika *et al.*, 2021a). The independent variables in this study were water quality (temperature, pH, DO, BOD, ammonia, nitrate, orthophosphate, TSS, and TDS) as environmental parameters. The dependent variables were hematological parameters (erythrocytes, leukocytes, leukocyte differentiation, hematocrit, hemoglobin, micronuclei, and phagocytosis activity).

The CCA results, as presented in Figure 5, can be interpreted as follows: (i) Because the value of erythrocytes, hemoglobin, and hematocrit tended to fall at the center of all water quality variables, they could be said to associate with temperature, pH, DO, BOD, ammonia, nitrate, orthophosphate, TDS, and TSS at moderate concentrations; (ii) Micronuclei levels, leukocytes and phagocytosis activity are also related to temperature, ammonia, BOD, TDS, and TSS at moderate to high concentrations. Based on CCA, micronuclei and leukocytes, and phagocytosis parameters were associated with temperature, ammonia, TDS, TSS, and BOD at high concentrations, while DO, orthophosphate, and nitrate at low concentrations. The CCA indicated that temperature, ammonia, TDS, TSS, and BOD at high concentrations, while DO, orthophosphate, and nitrate significantly affect the micronuclei, leukocytes, and phagocytosis conditions. Meanwhile, DO, orthophosphate, and nitrate affected the micronuclei and hematocrit conditions when the two were in low concentration, considering that both are closely related to oxygen supplies. The aquatic environment exerts both physical and chemical effects on the blood components of fish. When exposed to specific chemical agents, the hematological parameters of fish can either increase or decrease (Lestari *et al.*, 2019).

CONCLUSION

The results of the research analysis indicate that air quality in the Floating Net Cage Area located in Ranu Grati is lightly polluted at Stations 2 and 3, and moderately polluted at Stations 1 and 5. This is evidenced by several water quality indicators that exceed the quality standards based on Government Regulation No. 22 of 2021 for Class II water classification. Various hematological indicators including erythrocyte population, leukocyte, and

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hemoglobin concentration indicate the occurrence of physiological disorders, which are associated with poor water quality. Phagocytic activity and micronuclei levels in fish also indicate physiological stress caused by suboptimal environmental conditions. The high levels of pollution at Stations 1 & 5 are suspected to be caused by the large number of floating net cages in the area compared to other stations. In addition, agricultural runoff and dense household waste around the stations are also causes. There is a strong correlation between the bioecological conditions of the Ranu Grati waters and the health level of farmed tilapia. Dissolved oxygen is the bioecological factor that most influences the number of erythrocytes and hemoglobin. Meanwhile, ammonia levels had a dominant influence on leukocyte counts and micronuclei levels. Nitrate parameters showed the greatest influence on hematocrit values and phagocytic activity in fish.

CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest pertaining to the composition and dissemination of this paper. All authors have seen and sanctioned the work for publication, and it is not presently under review by any other journal.

AUTHOR CONTRIBUTION

The contribution of each author is as follows: Muhammad Asnin Alfarisi was responsible for the conceptualization, methodology, data collection, data analysis, and drafting of the manuscript. Asus Maizar Suryanto Hertika provided supervision, contributed to the methodology, managed the project, and conducted a critical review of the manuscript. Uun Yanuhar assisted in supervision, contributed to the methodology, interpreted the data, and conducted a critical review of the manuscript. All authors have examined and sanctioned the final version of the manuscript.

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