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# Hemocyte profile of *Sulcospira testudinaria* as a bioindicator for assessing water quality in the Ranu Grati Area, Pasuruan Regency, East Java, Indonesia

Asus Maizar Suryanto Hertika[1*], Nanik Retno Buwono[1], Muhammad Asnin Alfarisi[2], Marsa Fatin Halimah[3]

[1] Department of Aquatic Resource Management, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Veteran Str., Malang 65145, Indonesia
[2] Master Program of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Veteran Str., Malang 65145, Indonesia
[3] Faculty of Computer Sciences, University Brawijaya, Jl. Veteran, Malang, 65145, East Java, Indonesia
* Corresponding author's e-mail: asusmaizar@ub.ac.id

## ABSTRACT

*Sulcospira testudinaria*, a freshwater gastropod prevalent in Indonesian aquatic environments, serves as a bioindicator for assessing water quality. This study examined the hemocyte profile of *S. testudinaria* to evaluate water quality in Ranu Grati, Pasuruan Regency, East Java. Gastropod and water samples were obtained from four research stations with three repetitions of sampling at two-week intervals from August to October 2024. Water quality was evaluated using key parameters including temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), and ammonia levels. Physiological responses to environmental conditions were examined through hemocyte analysis, which evaluated both total hemocyte count (THC) and differential hemocyte count (DHC). The results indicated discrepancies in water quality, with Stations 3 and 4 exhibiting heightened amounts of TSS, BOD, and ammonia, signifying pollution. The circumstances markedly influenced the hemocyte profile, resulting in elevated THC and modified ratios of granulocytes, hyalinocytes, and semi-granulocytes. Canonical correspondence analysis (CCA) demonstrated significant connections between suboptimal water quality measures and stress responses in *S. testudinaria*. The results underscore the efficacy of *S. testudinaria* as a bioindicator, offering essential insights for the sustainable management of freshwater ecosystems.

**Keywords:** *Sulcospira testudinaria*, bioindicator, hemocyte profile, water quality, Ranu Grati.

## INTRODUCTION

Ranu Grati is a natural lake situated in Pasuruan Regency, East Java. The lake serves as a significant site for diverse community activities, including water supply, tourism, and freshwater aquaculture. Approximately 1.77% of the aquatic area of Ranu Grati is utilized for aquaculture [Utomo & Muzaki, 2022]. The outcomes of aquaculture are employed by three villages/sub-districts surrounding Ranu Grati: Sumber Dawesari Village, Ranu Klindungan Village, and Grati Tunon Sub-district. The growing population surrounding Ranu Grati has led to a surge in intensive human activities, including fisheries, agriculture, and industry, which have become unregulated and pose a risk of contaminating the lake with various pollutants, such as domestic waste, fisheries byproducts, industrial waste, heavy metals, and other hazardous chemicals [Safitri & Idajati, 2018]. The water quality at Ranu Grati has deteriorated, evidenced by physical alterations such as turbidity and an unpleasant stench. Inadequate environmental management has exacerbated the quality of the lake's waters, leading to significant adverse effects on its biota. Consequently, the assessment and evaluation of water quality are essential to furnish precise data regarding the pollution levels in Ranu Grati. An efficient way to evaluate pollution levels is by the examination of the hemocyte profile of the bioindicator species, gastropods. Gastropods are aquatic organisms that can indicate the state of an aquatic environment. Gastropods, a class of mollusca, constitute a component of the benthic community in aquatic environments and serve as biological markers of ecological changes [Budi et al., 2013]. Certain gastropods function as scavengers and deposit feeders, utilizing a proboscis to extract and agitate dirt in aquatic environments. Consequently, the presence of gastropods can signify water quality [Naldi et al., 2015]. Gastropods serve as pollution indicators due to their substantial populations, ease of location, straightforward collection and identification post-preservation, immobility, and varied responses to pollutant levels [Wulansari & Kuntjoro, 2018]. The water snail species *Sulcospira testudinaria* serves as a bioindicator of water pollution.

*Sulcospira testudinaria* is a gastropod species prevalent in freshwater habitats throughout Indonesia, especially on the island of Java. This species generally resides in rivers and lakes with either tranquil or rapid currents [Hertika et al., 2023]. *Sulcospira testudinaria* serves as a significant bioindicator of aquatic ecosystems, since it can indicate environmental conditions through alterations in its hemocyte profile [Hertika et al., 2021]. *Sulcospira testudinaria* is a freshwater snail classified under the gastropod class. Gastropods possess an open circulatory system. In gastropods, the circulatory fluid is termed hemolymph and comprises hemocytes [Paturakhman, 2017]. Hemocytes in the hemolymph serve a role in non-specific cellular defense mechanisms. Water pollution can impair the immune system and the response to environmental stressors, including pollutant exposure [Chifdiyah, 2012]. Exposure to pollutants, including heavy metals and other chemicals, can induce alterations in the hemocyte profile, serving as a biomarker of the immunological response to environmental stress [Wahyuni et al., 2015]. This haemocyte profile comprises the THC, which quantifies the overall quantity of blood cells, and the DHC, which classifies the various types of haemocytes present in the organism. Variations in THC and DHC may signify the degree of stress or cellular impairment encountered by the snail as a result of pollution exposure. This hemocyte profile analysis aims to anticipate the impact of pollution on the gastropod population and its ecology. Utilizing the gastropod hemocyte profile as a bioindicator is anticipated to yield precise data regarding the effects of pollution on the physiological well-being of the mollusk. The urgency of this research is significant due to the necessity for a more comprehensive water quality monitoring system that emphasizes biological consequences rather than solely physical and chemical factors. Methods employing bioindicators like *Sulcospira testudinaria* remain infrequently utilized; thus, our research holds substantial potential to address this deficiency and significantly advance the domain of aquatic environmental science. Furthermore, the findings of this study are anticipated to furnish a robust scientific foundation for the advancement of more efficient and cost-effective environmental monitoring technology. This project will enhance scientific knowledge and furnish practical tools for environmental managers to sustain water resources in Ranu Grati and other regions of Indonesia.

## MATERIALS AND METHODS

### Research site

This research was performed in Ranu Grati, Pasuruan Regency, which comprises three villages: Ranuklindungan, Gratitunon, and Sumberdawesari (Figure 1). Sampling was conducted at four strategically located stations that mirrored the environmental conditions. The four research locations were identified through purposeful sampling, intentionally chosen based on Ranu Grati land use data. This investigation was conducted with three repetitions biweekly from August to October 2024 to ensure measurement accuracy. Table 1 presents the specific geographic coordinates for the Ranu Grati sampling location.

### Methods

#### *Water sampling and analysis*

Water samples were obtained utilizing sanitized polyethylene bottles with a 200 ml capacity [Olasoji et al., 2019]. Upon filling, the bottles were sent to the laboratory in a cooler with ice cubes to preserve sample integrity. Subsequent analyses were performed at the Freshwater Fisheries Unit Sumberpasir, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia. The concentrations of TSS

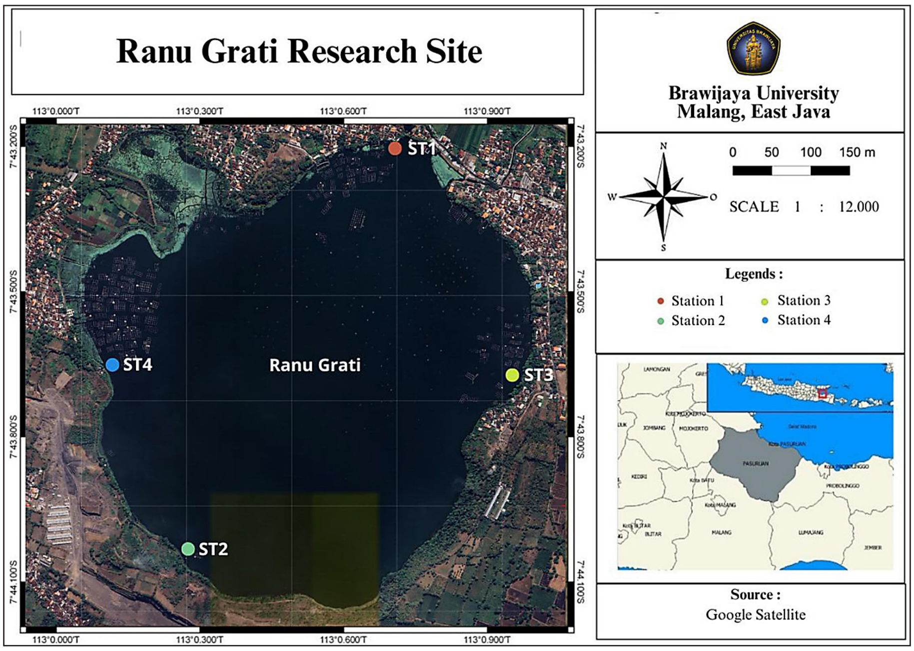



**Figure 1.** The sampling points at Ranu Grati

**Table 1.** Sampling station and their coordinates

| Sampling locations | GPS Coordinates |
|---|---|
| Station 1 | 7°43'37.8"S 113°00'51.2"E |
| Station 2 | 7°43'59.1"S 113°00'12.8"E |
| Station 3 | 7°43'36.2"S 113°00'56.5"E |
| Station 4 | 7°43'38.1"S 113°00'05.7"E |

and unionized ammonia ($NH_3$) were measured in accordance with the specifications outlined in SNI 06-6989.3-2004 and SNI 19-7119.1-2005. The biochemical oxygen demand (BOD) testing method is specified in SNI 06-6989.14-2004. Optimal water quality thresholds were derived from Government Regulation Number 82 of 2001 about Water Quality Management and Pollution Control. Water quality measurements including pH and total dissolved solids were conducted in the field with an EZ-9901 Automatic Calibration Digital Water Quality Tester. At the same time, we measured dissolved oxygenconcentration and temperature using a DO9100 Dissolved Oxygen Meter Analyzer. The comprehensive procedure for assessing water quality parameters is presented in Table 2.

### *Sampling of gastropods*

The gastropod samples included in this investigation were the *Sulcospira testudinaria* species, a freshwater snail prevalent in tropical aquatic habitats. Susuh kura samples were contained in plastic bottles filled with water to preserve their physiological state during transit. Sampling was conducted at four designated stations, considering environmental variables encompassing physical, chemical, and biological aspects of the study site. All collected samples underwent additional examination at the Fish Disease and Health Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, for hemocyte profile analysis.

**Table 2.** Technique for assessing water quality parameters

| Parameter | Unit | Sampling method |
|---|---|---|
| Temperature | °C | Dissolved oxygen analyzer tipe DO9100 |
| pH | | Water quality tester tipe EZ-9901 |
| Dissolved oxygen | mg/L | Dissolved oxygen analyzer tipe DO9100 |
| Biological oxygen demand | mg/L | SNI 06-6989.14-2004 |
| Ammonia | mg/L | SNI 19-7119.1-2005 |
| Total dissolved solid | mg/L | Water quality tester tipe EZ-990 |
| Total suspended solid | mg/L | SNI 06-6989.3-2004 |

*Hemocyte study*

Hemocyte parameter assessment encompasses the total hemocyte count and the differential hemocyte count, which comprises hyalinocytes, semigranulocytes, and granulocytes. THC and DHC are evaluated utilizing a hemocytometer [Priya et al., 2015; Nwani et al., 2016]. THC is analyzed by the methodology established by Blaxhall and Daisley (1973) as referenced in [Accorsi et al., 2013]. Additionally, DHC is evaluated by the Mix & Sparks approach, as referenced in [Prastiti et al., 2023].

### Data analysis

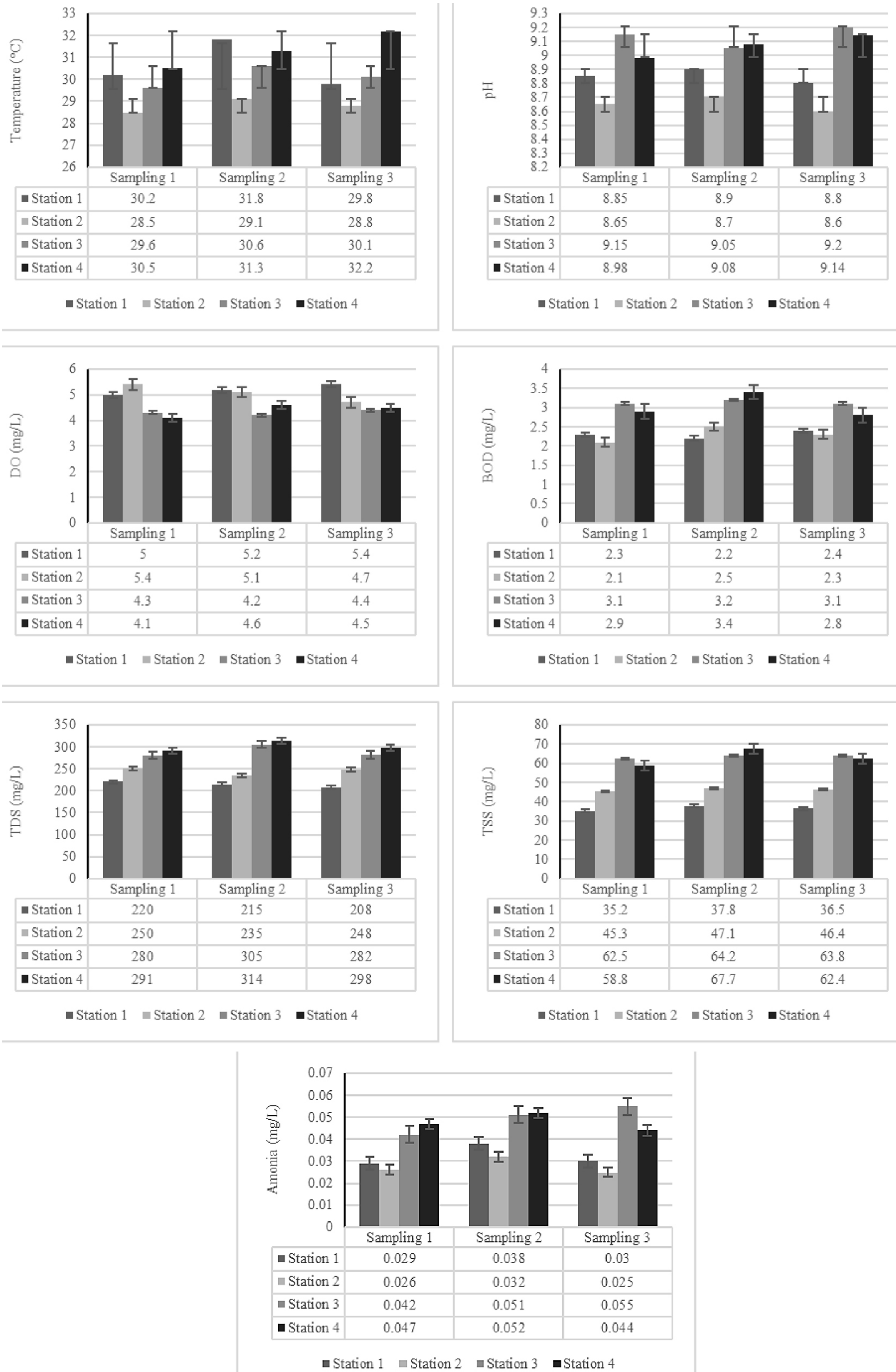
Water quality data is analyzed and presented in the form of tables or graphs using Microsoft Excel. The data is then interpreted descriptively by relating it to field conditions and relevant literature. Furthermore, the relationship between water quality parameters and the hemocyte profile is evaluated using canonical correspondence analysis (CCA). This multivariate analytical technique is frequently utilized to clarify the relationships between biological communities and environmental variables via ordination [Ghiffari et al., 2021]. This study employed PAST software version 4.03 for the CCA analysis. This method aims to evaluate the impact of independent variables on dependent variables and to determine the environmental factors that most significantly affect the dependent variables [JiaXin et al., 2016]. Independent variables are characterized as those not influenced by external factors, while dependent variables are affected by the measured independent variables. In this context, water quality measures function as the independent variables, whereas the dependent variable is the hemocyte profile of *Sulcospira testudinaria*, encompassing THC, hyalinocytes, semi-granulocytes, and granulocytes.

## RESULTS AND DISCUSSION

### Water quality analysis result

Temperature measurements taken at four different stations within the Ranu Grati area are depicted in Figure 2. The temperature ranged from 28.5 to 32.2 °C, specifically 29.8 to 31.8 °C at Station 1, 29.8 to 29.1 °C at Station 2, 29.6 to 30.6 °C at Station 3, and 30.5 to 32.2 °C at Station 4. The temperature rose during the second week at all sites; nevertheless, there was minimal variation across sites 1 to 4. When the water temperature is high, *Sulcospira testudinaria* may have to use energy to adapt to its surroundings, which can hurt its metabolic system [Gunawan et al., 2019]. According to Hartinah et al., [2014], the hemocyte concentration in *Sulcospira testudinaria* increases as temperature rises, while lower temperatures lead to a decline in hemocyte levels. Factors such as seasonal variations, climate conditions, and surrounding vegetation can affect lake water temperature. The optimal temperature range for the growth and survival of gastropods is between 25 and 32 °C. Environmental temperatures exceeding 35 °C will interfere with the metabolic processes of organisms, especially gastropods [Dinata et al., 2022]. Gastropods will react to hemocytes to rejuvenate the immune system [Hertika and Putra, 2023]. The measurements indicate that the water conditions in Ranu Grati, Pasuruan Regency, provide a normal temperature range conducive to the survival of *Sulcospira testudinaria*.

The pH results at the research site varied from 8.6 to 9.15. Each station yielded slightly varying pH measurements. Station 1 maintained pH readings between 8.8 and 8.9, while Station 2 recorded slightly lower values of 8.6 to 8.7. Higher alkalinity was observed at Station 3 (9.05–9.20) and Station 4 (8.98–9.14). Throughout the monitoring period, we noted that pH levels generally increased from the initial week to the third week of sampling, with significant fluctuations observed throughout the study duration. Reducing the uptake of carbon dioxide and bicarbonate during photosynthesis can elevate pH. Reduced carbon dioxide concentrations may correlate with elevated pH levels, and physicochemical alterations in the water affect the equilibrium between carbonate and bicarbonate [Rahmawati & Retnaningdyah, 2015]. If a body of water has a pH level above or below the threshold, it might interfere with the metabolic processes of organisms and induce stress in gastropods [Mathius et al., 2018]. Elevated pH levels can influence the ammonia concentration in gastropod physiology, hence affecting their overall state. Photosynthetic activity, which requires CO2 ions, and the influx of trash surrounding the lake are responsible for the rise in pH levels in aquatic environments [Putra et al., 2014]. Water with a low pH has a sour taste, whereas water with a high pH is alkaline and has a bitter taste [Wahyuni et al., 2023]. Furthermore, due to water's properties as an effective




| Temperature (°C) | Sampling 1 | Sampling 2 | Sampling 3 |
|---|---|---|---|
| Station 1 | 30.2 | 31.8 | 29.8 |
| Station 2 | 28.5 | 29.1 | 28.8 |
| Station 3 | 29.6 | 30.6 | 30.1 |
| Station 4 | 30.5 | 31.3 | 32.2 |

| pH | Sampling 1 | Sampling 2 | Sampling 3 |
|---|---|---|---|
| Station 1 | 8.85 | 8.9 | 8.8 |
| Station 2 | 8.65 | 8.7 | 8.6 |
| Station 3 | 9.15 | 9.05 | 9.2 |
| Station 4 | 8.98 | 9.08 | 9.14 |

| DO (mg/L) | Sampling 1 | Sampling 2 | Sampling 3 |
|---|---|---|---|
| Station 1 | 5 | 5.2 | 5.4 |
| Station 2 | 5.4 | 5.1 | 4.7 |
| Station 3 | 4.3 | 4.2 | 4.4 |
| Station 4 | 4.1 | 4.6 | 4.5 |

| BOD (mg/L) | Sampling 1 | Sampling 2 | Sampling 3 |
|---|---|---|---|
| Station 1 | 2.3 | 2.2 | 2.4 |
| Station 2 | 2.1 | 2.5 | 2.3 |
| Station 3 | 3.1 | 3.2 | 3.1 |
| Station 4 | 2.9 | 3.4 | 2.8 |

| TDS (mg/L) | Sampling 1 | Sampling 2 | Sampling 3 |
|---|---|---|---|
| Station 1 | 220 | 215 | 208 |
| Station 2 | 250 | 235 | 248 |
| Station 3 | 280 | 305 | 282 |
| Station 4 | 291 | 314 | 298 |

| TSS (mg/L) | Sampling 1 | Sampling 2 | Sampling 3 |
|---|---|---|---|
| Station 1 | 35.2 | 37.8 | 36.5 |
| Station 2 | 45.3 | 47.1 | 46.4 |
| Station 3 | 62.5 | 64.2 | 63.8 |
| Station 4 | 58.8 | 67.7 | 62.4 |

| Amonia (mg/L) | Sampling 1 | Sampling 2 | Sampling 3 |
|---|---|---|---|
| Station 1 | 0.029 | 0.038 | 0.03 |
| Station 2 | 0.026 | 0.032 | 0.025 |
| Station 3 | 0.042 | 0.051 | 0.055 |
| Station 4 | 0.047 | 0.052 | 0.044 |

**Figure 2.** The assessment of water quality parameters, including temperature, pH, dissolved oxygen, biological oxygen demand, total dissolved solids, total suspended solids, and $NH_3$, was conducted in Ranu Grati

solvent, a non-neutral pH promotes the breakdown of diverse contaminants, including heavy metals. The pH of normal water can maintain the health of aquatic life.

The dissolved oxygen levels at the sampling locations varied from 4.1 to 5.4 mg/L. The highest concentrations were found at Station 1 (5.0–5.4 mg/L) and Station 2 (4.7–5.4 mg/L). In contrast, Station 3 showed lower values ranging from 4.2 to 4.4 mg/L, while Station 4 had the lowest readings, between 4.1 and 4.6 mg/L. The diminished dissolved oxygen levels at stations 3 and 4 may result from nearby activities that elevate organic loads, including the presence of floating net cages and domestic trash. According to Government Regulation Number 22 of 2021 on Environmental Protection and Management, the minimum dissolved oxygen level required for class 2 water quality is 4 mg/L. This suggests that the dissolved oxygen levels at the three observation stations remain within the acceptable range, ensuring no harm to aquatic organisms. Dissolved oxygen levels in water are primarily regulated by the activities of microbes that decompose organic materials into inorganic substances. In addition, dissolved oxygen levels can be influenced by several factors, including temperature, respiratory activity of organisms, salinity, water turbidity, turbulence, the presence of easily oxidized chemicals, and atmospheric pressure [Patty et al., 2015]. The obtained DO values are categorized as normal to low for freshwater. Gastropods may inhabit and endure in aquatic environments with dissolved oxygen levels of 2–3 mg/L; other determinants include organism resilience, temperature variations, activity levels, and pollution presence [Mainassy, 2017]. Nevertheless, excessively low DO levels might impede physiological functions like as respiration and development, while diminishing reproductive rates. This state may also elevate oxidative stress, hence impacting the gastropod's resilience to environmental fluctuations or pollution exposure [Patty, 2013].

The biological oxygen demand measurements across the study site ranged between 2.1 and 3.4 mg/L. Station 1 showed BOD values from 2.2 to 2.4 mg/L, while Station 2 had similar levels between 2.1 and 2.5 mg/L. Higher concentrations were recorded at Station 3 (3.1 to 3.2 mg/L) and Station 4 (2.8 to 3.4 mg/L). Government Regulation Number 22 of 2021 concerning environmental protection and management establishes a BOD limit of 3 mg/L for Class II waters. The BOD value at station 3 surpasses the specified quality standard, suggesting the presence of dirty waters. BOD is a measure used to assess water quality. Elevated BOD levels may signify insufficient dissolved oxygen in aquatic environments. The study of BOD in water is a method to reduce toxin levels after the values are determined, facilitating biological treatment processes. The elevated organic matter concentration in water contributes to an increase in BOD levels [Daroini & Arisandi, 2020]. The BOD number does not reflect the precise quantity of organic matter; rather, it serves as a relative measure of the oxygen required by microbes to oxidize that organic matter. Increased oxygen demand correlates with diminished dissolved oxygen levels, indicating a higher concentration of organic waste that need oxygen [Putra & Yulis, 2019].

The TDS findings at the research site ranged from 208 to 314 mg/L. The values varied by station: Station 1 showed concentrations between 208 and 220 mg/L, while Station 2 had readings from 235 to 250 mg/L. Higher levels were observed at Station 3 (280–305 mg/L) and Station 4 (291–314 mg/L). Based on Government Regulation Number 22 of 2021 on Environmental Protection and Management, class 2 water quality standards permit TDS levels up to 1000 mg/L. All monitoring stations maintained TDS levels below this threshold, indicating suitable conditions for aquatic organisms. According to Astuti [2014], TDS in water bodies originates primarily from inorganic substances, particularly ions that naturally occur in aquatic environments. Furthermore, elevated TDS concentrations can diminish water transparency, restricting light penetration, which subsequently reduces photosynthetic activity and overall water productivity. Elevated TDS can influence the coloration of the water surface, attributable to the multitude of particles present that refract light [Rahadi et al., 2020]. Dissolved particles in natural waters are non-toxic; nonetheless, excessive quantities can elevate turbidity levels and contaminate aquatic environments. Moreover, it may adversely affect aquatic ecosystems and pose detrimental health risks to humans upon consumption [Kustiyaningsih and Irawanto, 2020].

The results of TSS at the research location across the study area varied between 35.2 and 64.2 mg/L. At Station 1, TSS values were recorded in the range of 35.2 to 37.8 mg/L, while Station 2 exhibited slightly higher concentrations ranging from 45.3 to 46.4 mg/L. The TSS findings at Stations 3 and 4 fluctuated between 62.5–64.2 mg/L and 58.8–67.7 mg/L, respectively. According to Rahmawati & Retnaningdyah [2015], the TSS value fluctuates between 0.75 and 1.25 mg/l. This number corresponds with the TSS quality standard categories I-IV, which prescribe a maximum allowable concentration of 50 mg/l. The concentration of total suspended particles significantly affects turbidity measures, hence reducing light penetration in aquatic environments. This may obstruct the photosynthetic activities of aquatic organisms. The absorption of solar heat can raise temperatures, resulting in reduced oxygen levels, which then affects the growth and respiratory functions of aquatic species. As the respiratory processes of aquatic species deteriorate, there will be a concomitant reduction in the oxygen levels within their bodies. Increased TSS levels can cause physiological stress in gastropods due to suspended particles [Putri et al., 2017]. This stimulates immune system involvement, as seen by changes in the hemocyte profile, including an increase in the THC and the ratio of hyalinocytes to granulocytes.

Unionized ammonia ($NH_3$) levels ranged between 0.043 and 0.246 mg/L across all sampling locations. Specifically, Station 1 showed $NH_3$ concentrations between 0.081 and 0.169 mg/L, while Station 2 recorded lower values ranging from 0.043 to 0.115 mg/L. At Station 3, $NH_3$ levels fluctuated between 0.057 and 0.214 mg/L. The highest concentrations were observed at Station 4, where values ranged from 0.186 to 0.246 mg/L. Ammonia constitutes the predominant waste in the agricultural process. Ammonia in aquatic environments originates from the aerobic degradation of organic waste by degrading microorganisms [Chrisnawati et al., 2018]. According to Government Regulation Number 22 of 2021, the recommended ammonia concentration limits are established at 0.1 mg/L for class one waters, 0.2 mg/L for class two waters, and 0.3 mg/L for class three waters. Aquatic organisms or species that are particularly sensitive to water contamination, the ammonia threshold is set at a more stringent level of 0.02 mg/L [Tatangindatu et al., 2013]. The introduction of household garbage (derived from community activities near the research site) and waste from local fish farming are determinants that influence ammonia levels. The breakdown of nutrients by bacteria leads to elevated oxygen levels, consequently increasing ammonia concentrations [Azizah, 2017]. Moreover, elevated ammonia concentrations can influence the quantity of blood cells, including the hemocyte count in gastropods.

### *Hemocyte profile of Sulcospira testudinaria*

The THC of *Sulcospira testudinaria* from the four locations averaged 48.08 x$10^4$ cells/ml. The range of THC was $29 \times 10^4 – 54 \times 10^4$ cells/ml at Station 1 and $24 \times 10^4 – 51 \times 10^4$ cells/ml at Station 2. Simultaneously, the THC concentrations at Stations 3 and 4 varied from $45 \times 10^4 – 72 \times 10^4$ cells/ml and $47 \times 10^4 – 69 \times 10^4$ cells/ml. THC is the total hemocytes found in the body of gastropods, which can indicate the level of pollution (Figure 3) [Hertika et al., 2021]. Therefore, THC levels increase in polluted environments. The number of freshwater gastropod cells with THC is around $58 \times 10^4$ cells/ml. This value serves as a measure and indicates that if the value is higher, gastropods will produce hemocytes to prevent exposure to pathogens indicating that the waters are polluted. The total hemocytes contained in the body of gastropods are called THC which consists of hyaline cells, semi-granulocyte cells, granulocyte cells to dead cells [Kilawati et al., 2021]. The characteristics of hyaline cells are relatively few granules, forming small round granules with an irregular structure. Semi-granulocyte cells have an irregular structure, a small number of granules, and there are small granules in their cytoplasm. Meanwhile, granulocytes have a round oval shape with a large size and cytoplasm filled with many granules [Prastowo et al., 2020]. THC activates various immune cells, including granulocyte cells, hyalinocytes, and semi-granulocyte cells as well as dead cells [Ermantianingrum et al., 2012]. Each cell has unique features. Hyalinocyte cells are oval and do not have granules or small spheres; granulocyte cells, on the other hand, are larger and have many granules in their cytoplasm, while semi-granulocytes have few granules and small granules.

The average hyalinocyte cell count from the four locations was 46%. The proportion of hyalinocytes varied from 23% to 52% at Station 1 and from 25% to 52% at Station 2. Simultaneously, the hyalinocyte counts of *Sulcospira testudinaria* at Stations 3 and 4 varied between 44–74% and 42–72%, respectively. Hyalinocyte cells identify foreign particles that infiltrate the organism's body. Elevated levels of gastropod hyalinocyte cells suggest alterations resulting from contaminants in the aquatic environment, leading

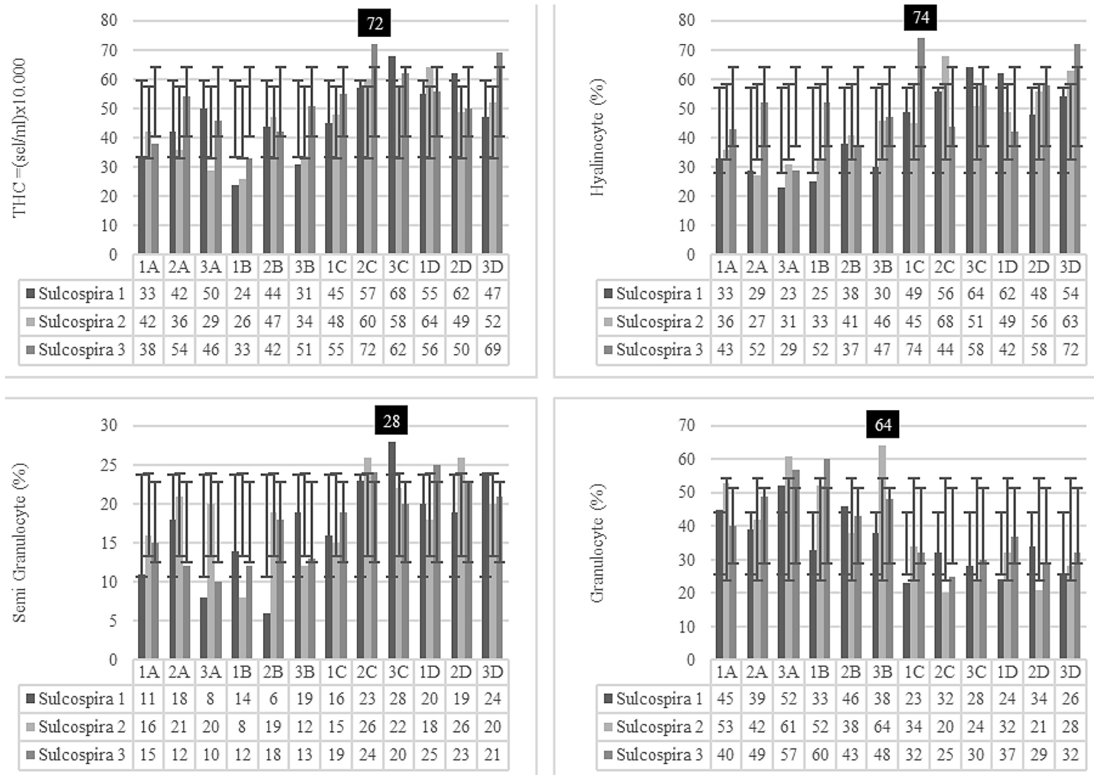



**Figure 3.** Result of Sulcospira testudinaria hemocyte profile

to enhanced phagocytic activity [Arfiati et al., 2018]. The snail possesses a pollution tolerance threshold within its environment. Intense water pollution can jeopardize the existence of *Sulcospira testudinaria*. Hyalinocyte cells serve to identify foreign particles or diseases that infiltrate the body [Arfiati et al., 2018]. An increase in the number of hyalinocytes correlates with elevated phagocytic activity [Kurniaji et al., 2015]. This is due to exposure to pollutants, which enhances the body's immunity. Excessive pollution, when intolerable, might adversely affect gastropods. Freshwater snail hyalinocytes are considered contaminated if the hyalinocyte value exceeds 62.2% [Accorsi et al., 2013].

The average semi granulocyte cell count from the four locations was 17.81%. The proportion of semi granulocyte cells varied from 8–21% at Station 1 and 6–19% at Station 2. Simultaneously, the proportions of semi granulocyte cells of *Sulcospira testudinaria* at Stations 3 and 4 varied between 15–28% and 18–26%, respectively. Semi-granulocyte cells originate from the maturation of hyalinocyte cells. Semi-granulocyte cells participate in encapsulation [Ekawati et al., 2012]. Encapsulation is the mechanism by which semi-granulocyte cells envelop foreign particles or pathogens [Jamilah & Suryanto, 2015). The elevated quantity of semi-granulocyte cells at stations 3 and 4 is attributed to their superior phagocytic and encapsulating capabilities relative to stations 1 and 2 [Ermantianingrum et al., 2013]. Semi-granulocyte cells originate from the maturation of hyalinocyte cells and can perform large-scale encapsulation when phagocytosis is insufficient to manage foreign particles or pathogens entering the body [Wangi et al., 2019].

The average granulocyte cell count from the four locations was 38.08%. The granulocyte cell values ranged from 39% to 61% at Station 1 and from 33% to 64% at Station 2. At Stations 3 and 4, the granulocyte cell levels varied between 20–34% and 21–37%, respectively. Granulocyte cells exhibit a spherical morphology, although irregular forms are often present. Furthermore, the cytoplasm contains granules that regulate the Prophenoloxidase (proPO) system, facilitating phagocytic activities [Arfiati et al., 2018]. Granulocyte cells primarily regulate phagocytosis activities within the body. The results indicate that the lowest values were recorded at stations 3 and 4, reflecting an increase in hyalinocyte and semi-granulocyte cells at these locations. Consequently, this diminishes the quantity of granulocyte cells. Granulocyte cells in freshwater snails under unpolluted environments exceed 18.5% [Accorsi et al., 2013]. Consequently, it may be inferred that pollution at stations 3 and 4 arises from KJA cultivation waste and residential waste, leading to alterations in the hemocyte composition of *Sulcospira testudinaria*. Regions with elevated pollution levels can induce physiological problems in aquatic organisms. These illnesses encompass alterations in the composition of THC and DHC.

### *Relation of water quality and hemocyte profile*

The analysis of the CCA test results, as illustrated in Figure 4, reveals that the dependent variable THC in *Sulcospira testudinaria* (Figure 5) is significantly affected by essential water quality parameters. These parameters include temperature, total suspended solids, pH, total dissolved solids, dissolved oxygen, ammonia, and biological oxygen demand levels. The dependent variable granulocytes tends to be influenced by high pH values, medium to low DO, medium to low temperature, medium to low BOD, medium to low ammonia, medium to low TDS and medium to low TSS. The dependent variable Hyalinocytes tends to be influenced by high TDS, TSS, ammonia, BOD, temperature, and medium to low DO and pH values. The number of dependent variables semi granulocytes tends to be influenced by high TDS, TSS, ammonia, and BOD

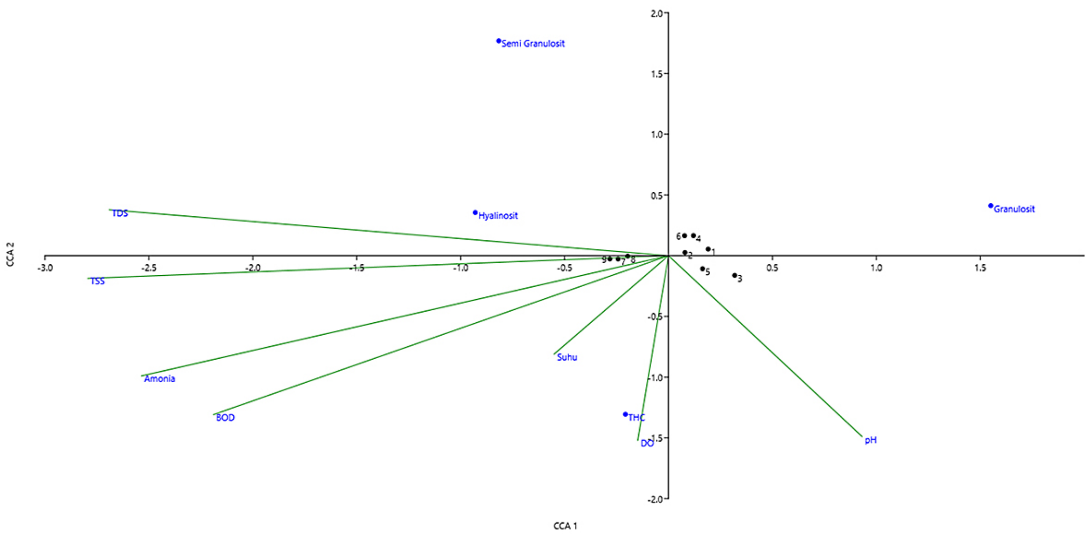



**Figure 4.** The result of CCA between Sulcospira testudinaria hemocyte profile and water quality

**Figure 5.** Sulcospira testudinaria collected from Ranu Grati, Pasuruan Regency, East Java, Indonesia

values, while the temperature, DO, pH values tend to be medium to low. It can be concluded that the distribution of hemocyte profiles (Granulocytes, Semi Granulocytes, and Hyalinocytes) in waters is greatly influenced by water quality conditions. Parameters such as pH and DO contribute positively to the presence of granulocytes, while poor water quality factors (such as BOD, TDS, and TSS) tend to be associated with hyalinocytes. Environmental stress such as changes in water quality can affect the hemocyte profile in aquatic organisms, including the number and proportion of hemocyte types such as granulocytes, semi-granulocytes, and hyalinocytes [Hertika et al., 2021]. Granulocytes, which have defensive immune functions such as phagocytosis, are more active in conditions with stable pH and sufficient dissolved oxygen, indicating that organisms tend to be healthier in good quality water conditions. Conversely, increased TDS, TSS, ammonia, and BOD indicate pollution that can increase stress levels in organisms, thereby increasing the presence of hyalinocytes which are often indicated as indicators of stress responses. In addition, high TDS and TSS can inhibit oxygenation, reduce the efficiency of aquatic organism respiration, which affects changes in hemocyte distribution [Maulinawati & Lembang, 2022]. Factors such as high BOD and high ammonia concentrations indicate the activity of organic matter decomposition and nitrogen pollution, which are known to affect the metabolism and immune system of aquatic organisms [Supono, 2015]. Overall, the distribution of hemocytes in *Sulcospira testudinaria* reflects the direct influence of water quality conditions on the physiological and immunological responses of the species.

## CONCLUSIONS

The investigation indicates that the water quality of Ranu Grati varies among different stations, with Stations 3 and 4 exhibiting symptoms of potential pollution. The pollution categorization was determined by the water quality indicators and hemocyte profiles of *Sulcospira testudinaria* that diverged from ideal conditions. Elevated levels of total dissolved solids, total suspended solids, and biological oxygen demand at Stations 3 and 4 specifically signified environmental stress. Reduced dissolved oxygen and elevated unionized ammonia concentrations resulted in alterations to the hemocyte profile, characterized by a significant rise in hyalinocyte cells as an immunological reaction. Canonical correspondence analysis demonstrated that water quality characteristics greatly affected the hemocyte composition of *Sulcospira testudinaria*. The total hemocyte count elevated in reaction to environmental stressors, with granulocytes exhibiting best functionality in stable aquatic conditions marked by balanced pH and sufficient dissolved oxygen. Stations 3 and 4 exhibited elevated pollution levels, presumably attributable to waste from floating net cage aquaculture and domestic refuse, resulting in physiological problems and alterations in hemocyte composition. The research illustrates the sensitivity of *Sulcospira testudinaria* as a bioindicator, able to indicate water quality conditions via its immunological responses and alterations in hemocyte profiles.

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