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EFFECTS OF ENVIRONMENTAL AND WATER QUALITY VARIATIONS ON HEMATOLOGICAL PARAMETERS OF NILE TILAPIA (*Oreochromis niloticus*) REARED IN POND AND RECIRCULATING AQUACULTURE SYSTEMS

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ABSTRACT

Aquaculture is crucial for global food security, yet environmental change threatens fish health, particularly in freshwater species like Nile tilapia (Oreochromis. niloticus). This study aimed to evaluate the effects of environmental and water quality variations on the hematological responses of Nile tilapia reared in outdoor pond systems and indoor Recirculating Aquaculture Systems (RAS). The study was conducted over a 12-month duration (June 2023 to May 2024) using three replicate outdoor earthen ponds and three indoor RAS tanks. Daily data regarding environmental variables were collected from the government Meteorological Department, while water quality parameters were monitored on-site daily and biological sampling was conducted four times monthly. Tilapia managed within the RAS system exhibited higher mean RBC counts (1.512 × 10v /mL), which may be associated with improved oxygen availability and stable environmental conditions, while outdoor pond fish showed higher mean WBC (389.188 × 10^3/mL) and elevated glucose (122.675 mg/dL) level, potentially reflecting physiological or immunological responses to fluctuating environmental conditions. All hematological parameters differed significantly between systems ($p < 0.05$). RAS maintained more stable water quality, with higher DO (8.53 mg/L vs. 7.03 mg/L in ponds, $p < 0.001$) and zero ammonia, though TDS was higher (242.301 ppm, $p = 0.034$). Pond environments experienced greater temperature fluctuations, with rainfall contributing to reduction in pH. Environmental and water quality variables strongly shaped hematological responses, highlighting their role in fish welfare. Environmental and water quality variables showed measurable associations with hematological responses, highlighting their potential influence on fish welfare. The findings suggest that RAS may support improved environmental stability and physiological condition through controlled water quality management, with potential implications for fish health, broodstock quality, and sustainable aquaculture development.

KEYWORDS: Climate change; Fish; *Oreochromis niloticus*, Hematology, Tilapia, Aquaculture, Growth, RAS

INTRODUCTION

Environmental change is seen as a significant threat to global food production, affecting both the quality and quantity of agricultural and aquaculture yields (Little *et al.,* 2007; Beach and Viator, 2008; Hamdan *et al.,* 2015; Haque *et al.,* 2021; Myers *et al.,* 2017). Over decades to millions of years, climate change alters weather patterns statistically. These alterations may be in the average weather or in the distribution of weather events around an average, and they may be regional or global (León-Ramírez *et al.,* 2022; IPCC, 2014). In this context, understanding how pond and Recirculating Aquaculture System (RAS) environments influence the hematological responses of Nile tilapia is important for evaluating physiological stability under variable culture conditions.

Bangladesh's favorable agro-environmental conditions, plentiful water resources, and vast network of rivers, ponds, and floodplains make it one of the most suited countries for freshwater aquaculture (DoF, 2022). Aquaculture in Bangladesh is essential for food and nutrition security, as well as for sustainable livelihoods, income generation, and export earnings (Rahman *et al.,* 2021). However, the sustainability of the sector is at stake by the predicted consequences of climate change, which are currently a reality, not

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just a future concern (Maulu *et al.,* 2021). Nile tilapia (*O. niloticus*) has become a significant species in the aquaculture sector of Bangladesh due to its rapid growth, high protein content, and adaptability to diverse environmental conditions and rendering it a favoured option for both small-scale and commercial aquaculture (Sayed *et al.,* 2015). But rising temperatures, variable rainfall, saline intrusion, and extreme weather events are making aquaculture more difficult. The negative effects of climate change, which present serious threats to water quality, fish physiology, and overall production, are posing a growing challenge to the sustainability of tilapia farming (Costa, 2024; Siddique *et al.,* 2022a; Siddique *et al.,* 2022b).

The relationship between environmental factors and water quality parameters is intricate and significant. Air temperature, humidity, rainfall, and sunlight intensity affects pond water quality, which is essential for ensuring the balance of these aquatic environments. For instance, increasing air temperatures can result in higher water temperatures, which may stress or hinder aquatic organisms (Siddique *et al.,* 2023; Siddique *et al.,* 2024a; Siddique *et al.,* 2024b; Siddique *et al.,* 2024c). Water temperature is a critical abiotic factor influencing fish growth and health throughout all developmental stages; thus, fluctuations resulting from global climate change may hamper metabolic and physiological processes (Islam *et al.,* 2022; Elgendy *et al.,* 2024). Variations in water temperature also influence reproductive behaviour, feed intake, and overall growth, thereby affecting productivity (Elarabany *et al.,* 2017; Siddique *et al.,* 2024b). By altering vital water quality indicators including temperature, dissolved oxygen (DO), pH, and ammonia levels, climate change has an impact on fish health and productivity. Fish physiological functions, such as their haematological and biochemical profiles, which are crucial markers of health and stress reactions, may be affected by these alterations (Fazio, 2019). Additionally, a major concern to tilapia farming is the rise of disease outbreaks brought on by variations in water quality and temperature (Combe *et al.,* 2023). Extreme pond temperatures can affect fish growth and cause changes in hemato-physiological, metabolic, immune, and molecular responses (Islam *et al.,* 2022; Hamed *et al.,* 2024; Siddique *et al.,* 2025a; Siddique *et al.,* 2025b; Siddique *et al.,* 2025c). Fish hematological profiles are affected by various factors, including age, sex, nutritional status, and environmental conditions like temperature, salinity, and dissolved oxygen levels (Fazio, 2019). Seasonal fluctuations in temperature and dissolved oxygen levels can notably influence haematological parameters, with higher values recorded during warmer periods attributed to increased metabolic activity (Ezzat *et al.,* 1974; Brix *et al.,* 2004). Elevated levels of RBC and Hct in tilapia correlate with increased water temperatures, reflecting physiological adaptations to thermal stress. Haematological parameters, including red blood cell (RBC) count, haemoglobin concentration, haematocrit (Hct) values, and white blood cell (WBC) count, offer important insights into the adaptive capacity of fish in response to environmental stressors (Zuhrawati, 2014; Jeong *et al.,* 2012). Furthermore, stress, whether resulting from environmental changes or handling procedures, can modify haematological profiles, with acute stressors causing decreases in RBC count, haemoglobin levels, and Hct values (Akinrotimi *et al.,* 2009). Environmental stability plays an important role in fish physiological responses. Outdoor pond systems are more exposed to seasonal environmental fluctuations, whereas RAS maintain comparatively stable water conditions. Therefore, hematological parameters such as RBC, WBC, and glucose can provide useful indicators of physiological responses under different culture environments.

To counteract these challenges, RAS have become a popular sustainable alternative to pond farming. RAS allows fish production in controlled environments with precisely maintained temperature, DO, pH, and ammonia levels. This system requires less space while maintaining high productivity, allows production close to the consumer market, reduces water consumption, and increases stocking densities, making it ideal for regions with water scarcity or extreme environmental variability. RAS also reduces aquaculture's nutrient pollution and habitat deterioration, boosting environmental sustainability (DeLong *et al.,* 2009; Ebeling and Timmons 2012; Mugwanya *et al.,* 2022; Mahalder *et al.,* 2023; Haque *et al.,* 2025; Siddique *et al.,* 2025d; Nova *et al.,* 2026a; Siddique *et al.,* 2026a). While previous studies have examined the effects of individual stressors on fish hematology, limited information exists on the long-term comparative physiological responses of Nile tilapia cultured in pond and RAS environments across seasonal variations. In Bangladesh, longitudinal comparative studies under monsoon-driven climatic conditions remain particularly scarce. The novelty of this study lies in its year-round comparative assessment of hematological parameters and environmental variables in Nile tilapia reared under pond and RAS conditions. By integrating seasonal climate variation, water quality dynamics, and blood physiological responses over a 12-month period, this study provides important insights into fish physiological adaptation and sustainable aquaculture management under tropical farming conditions.

Therefore, this study comparatively evaluated the effects of environmental and water quality variations on the hematological responses of Nile tilapia cultured in traditional pond systems and RAS. The study specifically investigated the influence of climatic factors (temperature, humidity, and rainfall) and water quality parameters (DO, pH, ammonia, and TDS) on seasonal hematological responses between the two culture systems over a 12-month period. It was hypothesized that the controlled indoor RAS environment would maintain more stable water quality conditions and comparatively stable hematological profiles than the open pond system exposed to seasonal environmental fluctuations. Evaluating adaptive aquaculture frameworks, such as RAS, offers insights into potential management options for tilapia farming under shifting environmental regimes in regions like Bangladesh. Furthermore, evaluating controlled aquaculture infrastructure such as RAS may provide an empirical basis for understanding how reduced exposure to direct atmospheric fluctuations can contribute to improved physiological stability in cultured fish. To enhance the resilience and long-term sustainability of tilapia farming in Bangladesh and similar climate-vulnerable regions, adaptive aquaculture approaches such as RAS may represent a promising management strategy under increasing environmental and climatic variability.

MATERIALS AND METHODS

Ethical approval and animal welfare

All experimental procedures involving Nile tilapia were conducted following standard animal handling and welfare protocols. Prior approval was obtained from the Animal Welfare and Ethics Committee of Bangladesh Agricultural University for the use of fish in this study and for adherence to scientific animal procedures (Ref. no. BAURES/ESRC/FISH-11/2022). Fish handling, blood collection, and sampling were performed carefully to minimize stress and discomfort.

Study Area and Research Design

The study was conducted in two distinct aquaculture environments: earthen pond and Recirculating Aquaculture Systems (RAS) in the Laboratory of Climate Research for Fishes (LCRF) at Bangladesh Agricultural University (BAU) in Mymensingh, Bangladesh (Figure 1).

The experimental trials were structured across a 12-month study duration spanning from June 2023 to May 2024, utilizing three independent outdoor traditional earthen ponds in parallel with three indoor climate-controlled recirculating aquaculture system (RAS) tanks. To maintain experimental rigor, physical and chemical water quality configurations across all six

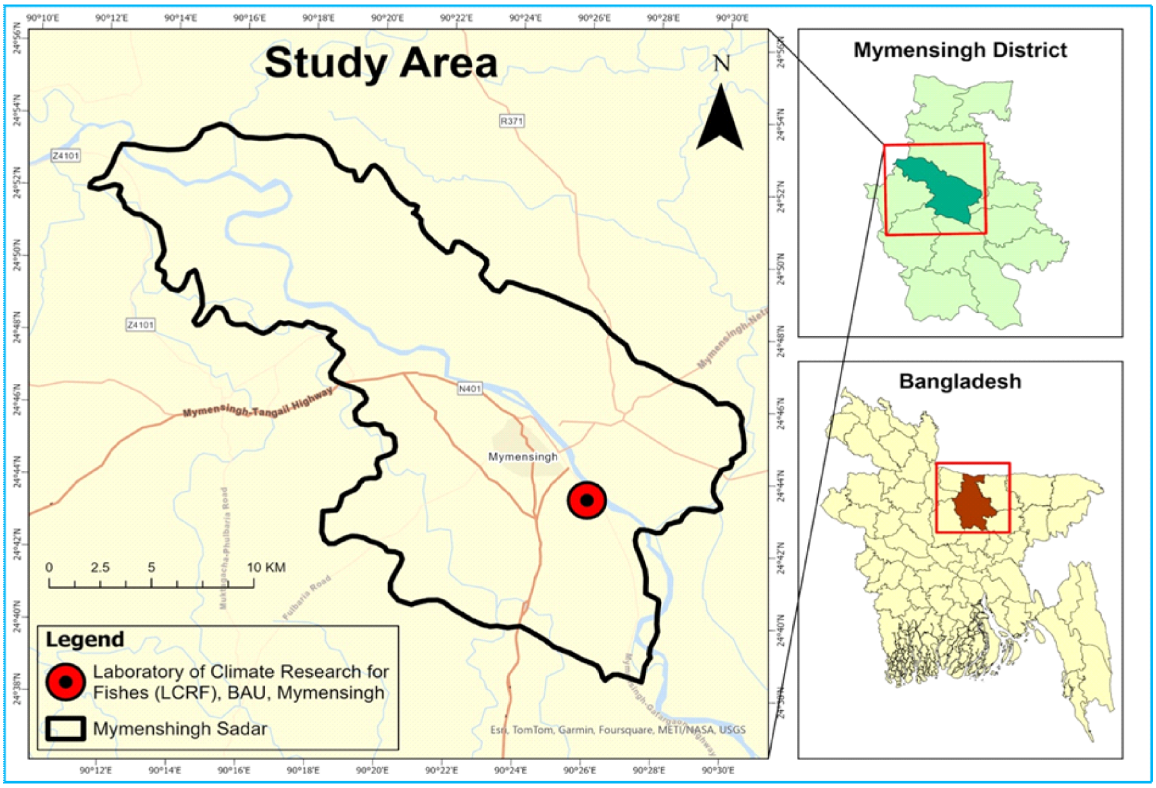


Figure 1. Study area indicated in Bangladesh country map.

units were documented through continuous daily monitoring, whereas fish growth performance and hematological sampling profiles were evaluated at a standardized monthly frequency.

Three earthen ponds (26 ft × 17 ft × 2.6 ft; area 1149.2 ft^3 or 32.54 m^3 of each) were dried out completely. The undesired small fish, aquatic weeds etc. were removed from the ponds. Excess bottom mud was removed and used to repair the broken and uneven dykes. Lime was applied at 1 kg/decimal in the bottom and dyke during pond preparation.

The RAS system consists of three rearing concrete tanks (10,000 L of each), a clarifier (settling tank) to remove solid wastes (feces and uneaten feed), and another two upper plastic tanks used as biofilters to remove toxic waste products (ammonia and nitrites) that are produced by the fish. Chlorine-free tap water is used in operating the system. Smooth surfaces were set on the inside of the tanks to prevent skin abrasions and infections to the fish and to permit cleaning and sterilization. All the tanks were aerated by a complete system of air pumps during the whole period of the experiment. Besides air pumps, blowers were used to supply oxygen (Figure 2).

Mature *O. niloticus* were collected from Asia Scientific Hatchery and Nursery in Dhala, Trishal Upazila, Mymensingh District, Bangladesh, in May 2023. Prior to stocking, the fish were acclimatized for 24 hours in fiber tanks. The average age, weight, and length of the fish were 6 months, 60.47 g, and 15.5 cm, respectively. The broodfish were stocked at a density of 300 individuals in each RAS tank and pond, with a male-to-female sex ratio of 40:60, into previously prepared RAS tanks and earthen ponds.

The broodfish were fed a commercial floating feed containing 35% crude protein, administered twice daily at a feeding rate of 2–4% of their body weight in both systems. Both environmental factors (air temperature, humidity, rainfall, and sunlight intensity) and water quality parameters (water temperature, dissolved oxygen, pH, ammonia, and total dissolved solids) were studied from June 2023 to May 2024 to evaluate their effects on the culture systems. This data was subsequently employed for longitudinal analysis in conjunction with tilapia hematology. This experimental design enabled a comprehensive evaluation of the influence of environmental factors and water quality on the growth and hematological profiles of Nile tilapia in both systems.

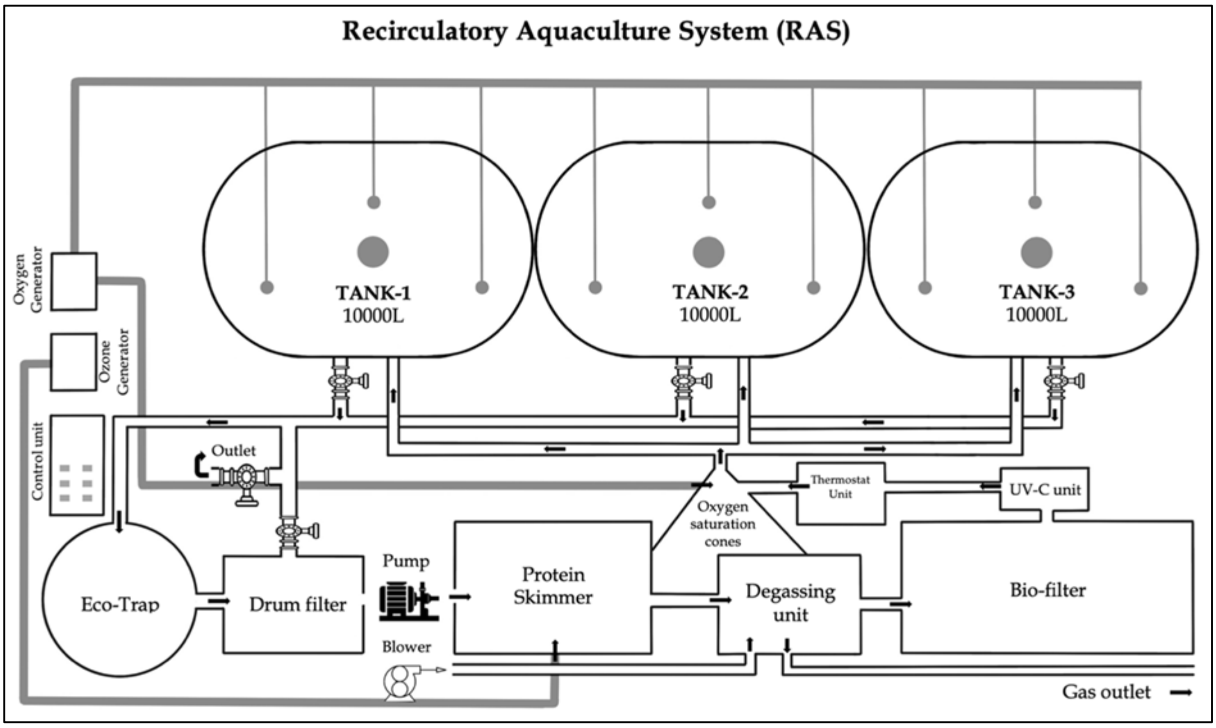


Figure 2. Operational Flow Diagram of the Recirculatory Aquaculture System (RAS) at Laboratory of Climate Research for Fishes.

Determination of environmental factors and water quality parameters

Daily data on environmental variable, including air temperature, rainfall, humidity, and sunlight intensity, were collected from the government Meteorological Department located near the study area from June 2023 to May 2024. Water quality parameters were monitored daily as described in Table 1.

Table 1. Daily measurement of water quality parameters

Parameters	Measuring frequency	Time of measure	Equipment
DO (mg/L)	Four times daily (8 am, 12 pm, 4 pm, 8 pm)	At the beginning, middle, and end of the experiment (to capture monthly fluctuations)	Lutron DO-5509 DO meter
Ammonia (ppm)			API testing kit
pH			HANNA pH-107 pH meter
Temperature			SMART Sensor AR 867
TDS			TDS- 3 TDS meter

Growth Monitoring

Randomly selected tilapia from both the pond and RAS systems were evaluated for growth monitoring. Sampling was conducted four times per month during the morning hours (between 10:00 and 11:00 AM), with 10 fish collected from each replicate unit during every sampling event. After collection, fish specimens' total length (TL) in cm was measured using a measuring scale to the nearest of 0.1 cm, and total body weight (TBW) in grams was measured for each individual specimen using a digital balance (model TANITA China, KD-160, Max 2kg). To evaluate growth performance, the average length (cm) and weight (g) of sample fish from pond and RAS were separately documented over the stocking period and on each sampling day. A variety of metrics were employed to assess growth performance, including:

$$Length\ Gain\ (cm) = Mean\ Final\ Length - Mean\ Initial\ Length$$

$$Weight\ Gain\ (cm) = Mean\ Final\ Weight - Mean\ Initial\ Weight$$

$$\mathrm{SGR}\ (\%/\mathrm{day}) = \left(\frac{ln\ (Final\ Weight) - ln\ (Initial\ Weight)}{Culture\ Period\ (days)}\right) \times 100$$

$$\mathrm{FCR} = \left(\frac{Feed\ Intake\ (kg)}{Weight\ Gain(kg)}\right)$$

$$\mathrm{PER}\ (\%) = \left(\frac{Weight\ Gain\ (kg)}{Protein\ Intake\ (kg)}\right) \times 100$$

Determination of Hematological Parameters

For hematological analysis, fish were randomly collected from both the pond and RAS culture systems during each sampling event, with at least one fish sampled from each replicate pond and each replicate RAS tank. Sampling was conducted four times monthly, and the obtained values were averaged for subsequent analysis. To ensure statistical independence and avoid handling stress effects on hematological parameters, sampled fish were not returned to the experimental units after blood collection. Therefore, no individual fish was repeatedly sampled during the study period. To minimize handling stress during blood sampling, fish were anesthetized in a separate bath containing clove oil at a concentration of 40–50 mg/L prior to blood collection, following the effective and low-toxicity anesthetic protocol reported for Oreochromis niloticus (Bona *et al.,* 2024). All fish handling and sampling procedures were conducted following standard ethical guidelines for animal care and use. Blood samples were collected from the caudal peduncle using a sterile syringe and pipette for RBC count (5 µL), WBC count (5 µL), and glucose analysis. To ensure accuracy, three blood samples were collected from each fish, and the mean blood count value was used for analysis. To prevent clotting, samples were immediately transferred into the Eppendorf tubes with RBC and WBC diluting fluid. Immediately after blood collection from the fish, a small drop of blood was placed on the test strip, which was inserted into the glucose meter, level of blood glucose (mmol/L) was determined through a digital EasyTouch® GHB meter (Model ET 232, Bioptic Technology Inc. Taiwan). The entire blood collection procedure took approximately 1 minute per fish, minimizing stress-related effects and reducing potential errors in normal blood values. The blood sample was prepared with precision in a 200-times dilution for RBC and 20-times dilution for WBC. For RBC and WBC count, five ìl blood was diluted in 995 µl RBC solution, and another five µl blood was diluted in 95 µl WBC solution. The diluted sample was then placed on a hemocytometer, covered with a cover slip, and examined under a compound microscope (Olympus CX 43) equipped with a camera (Olympus EP 50) at 40x magnification. A standard hemocytometer was used in the counting of the white blood cells and red blood cells according to the method of Blaxhall and Daisley (1973) and observed under microscope (Figure 3). RBC and WBC were counted using the following formula:

$$\mathrm{RBC}\ (10^6/\mathrm{ml}) = \left(\frac{C\ X\ D\ x\ 100\ x\ 4000}{(S\ x\ 80)}\right)$$

where, C = Number of cells counted

D = Diluting factors (200 for RBC)

S = Number of 1 square mm counted

$$\mathrm{WBC}\ (10^3/\mathrm{ml}) = \left(\frac{C\ X\ D\ x\ 100\ x\ 4000}{(S\ x\ 80)}\right)$$

where, C = Number of cells counted

D = Diluting factors (20 for WBC)

S = Number of 1 square mm counted

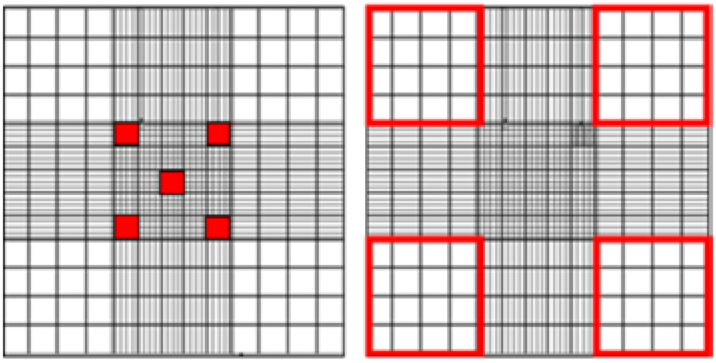
Statistical Analysis

Descriptive statistics were calculated to summarize key data. Blood cell counts were obtained by manual counting and verified using ImageJ software through digital image analysis of microscopic blood smear images. In ImageJ, cells were identified and counted from calibrated microscope images to validate the accuracy and consistency of the manual counts.. Before conducting parametric tests, normality was assessed using Shapiro-Wilk and Lilliefors (Kolmogorov-Smirnov) tests ($p > 0.05$) and supported by Q-Q plots and histograms. A series of Pearson correlations were performed in SPSS (version 2023) to explore the relationship between environmental factors and water quality with hematological parameters. Independent Samples t-test was conducted to evaluate significant differences in water quality parameters, RBC, WBC, and glucose levels between the two systems. Principal Component Analysis (PCA) was also performed to analyze multivariate relationships among environmental, water quality, and blood parameters in the pond. Visual graphs are presented by using MS Excel, SPSS, OriginLab 2024 and RStudio (Version 2023.12.0+369). The significance level for all statistical tests was set at $p < 0.05$.

RESULTS AND DISCUSSIONS

Determination of Climatic Factors

During the 12-month study period, notable seasonal variations in environmental conditions were observed in the pond system. Average air temperature ranged from a low of 21.21 ± 0.29°C in January to a peak of 30.48 ± 0.19°C in July, reflecting the typical tropical climate of the region. Relative humidity fluctuated between 52.33 ± 1.72% and 85.77 ±1.19%, reaching its maximum in August. Rainfall patterns followed a monsoonal trend, with the highest average monthly precipitation recorded in August



3(a) 3(b)

Figure 3. Microscopic view of hemocytometer (rectangular shape detecting the area counted for blood cell estimation); a) counting of RBC, b) counting of WBC.

(15.97 ± 3.98 mm), while negligible rainfall was observed in January and April. Mean daily sunlight intensity varied from 3.40 ± 0.65 hours during overcast months to 7.19 ± 0.52 hours in sunnier periods. These fluctuations in environmental parameters likely contributed to variations in pond water quality, influencing the physiological responses of cultured tilapia (Figure 4). These environmental trends align with the local monsoonal climate, characterized by high temporal variability and a strong influence on open water systems. Such fluctuations have been shown to directly affect pond water quality and, subsequently, fish health (Sen *et al.,* 2020; Kikuchi *et al.,* 2023). For instance, air temperature variations were closely mirrored by changes in pond water temperature, confirming the susceptibility of open aquaculture systems to ambient weather conditions (Abdel-Tawwab & Ahmad, 2009; Yu *et al.,* 2021; Mahalder *et al.,* 2025a). The observed peaks in humidity and rainfall during the monsoon season (July–August) likely reduced light penetration and increased organic runoff, thereby influencing dissolved oxygen dynamics and pH variability in pond environments. Similar patterns have been observed in other tropical aquaculture studies, in which seasonal environmental shifts modulate physicochemical stability and fish performance (Martins *et al.,* 2010; Ayaz *et al.,* 2015). Overall, these environmental fluctuations underscore the environmental sensitivity of earthen pond aquaculture, where unregulated exposure to temperature, rainfall, and sunlight intensity can induce stress responses in fish populations. In contrast, recirculating aquaculture systems (RAS) insulate cultured fish from these external ambient variations by maintaining controlled indoor conditions, which results in greater consistency of water quality matrices and a corresponding stabilization of host physiological parameters (Kubitza *et al.,* 2000; Martins *et al.,* 2010; Mahalder *et al.,* 2025b).

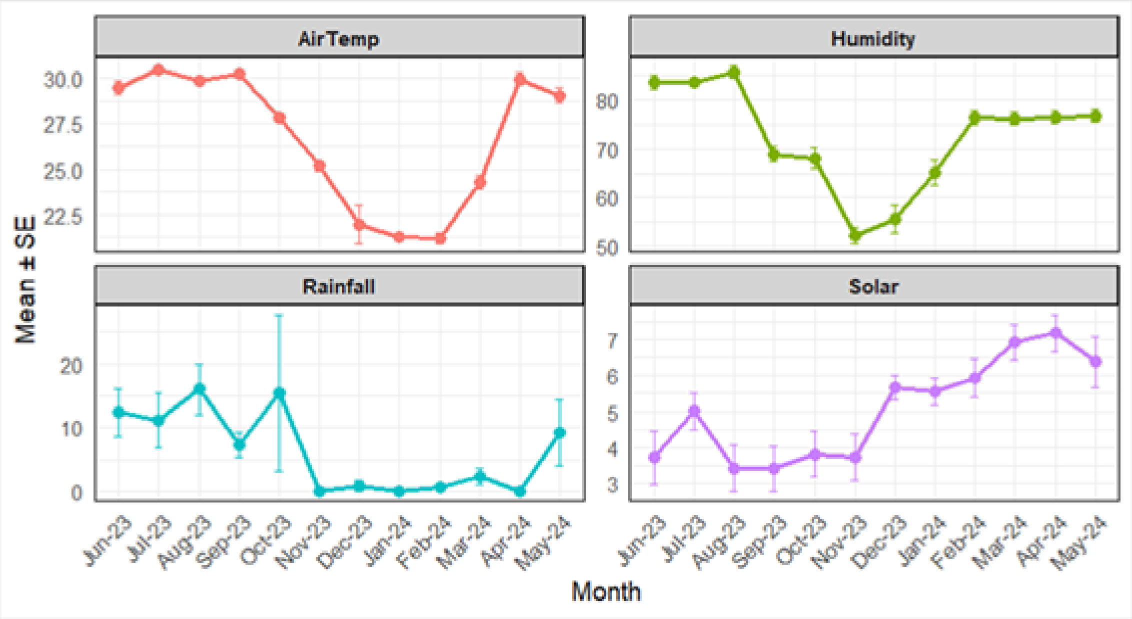


Figure 4. Monthly changes of environmental factors over the year in the pond system (June 2023 – May 2024), including air temperature (°C), relative humidity (%), rainfall (mm), and sunlight intensity (hours). Values are presented as mean ± standard error (SE). Each subplot represents the monthly trend of a specific environmental parameter, with error bars indicating variability.

Water Quality Parameters

Over the study period, the Recirculating Aquaculture System (RAS) maintained stable water quality parameters, in contrast to the pond environment. The water temperature in the RAS ranged from 27.11 ± 0.17°C to 29.33 ± 0.22°C, with minimal fluctuation, while the pond temperature ranged from 19.13 ± 0.46°C to 33.70 ± 0.54°C. Dissolved oxygen (DO) levels were higher and more consistent in the RAS (8.05 ± 0.10 mg/L to 8.93 ± 0.20 mg/L) compared to the pond (6.43 ± 0.09 mg/L to 7.57 ± 0.17 mg/L). The pH in the RAS was slightly alkaline and stable (7.30 ± 0.04 to 8.21 ± 0.13), whereas the pond showed greater variability (7.15 ± 0.06 to 8.37 ± 0.12). Ammonia remained undetectable in the RAS throughout the study, while the pond recorded low, non-toxic levels from 0.04 ± 0.02 mg/L to 0.18 ± 0.04 mg/L. Total dissolved solids (TDS) were elevated in the RAS (230.07 ± 0.21 ppm to 255.70 ± 1.07 ppm) compared with the pond (227.87 ± 5.71 ppm to 244.67 ± 2.28 ppm), indicating a more stable ionic milieu in the controlled system. These findings demonstrate the capacity of the evaluated RAS configuration to maintain more uniform water quality parameters relative to the open pond system under the observed seasonal conditions (Figure 5). These results highlight the greater environmental stability of the RAS, where insulation and controlled heating/cooling systems kept temperatures within the optimal thermal window for tilapia (27–30°C), thereby minimizing thermal stress and enhancing physiological performance (Kubitza *et al.,* 2000). In contrast, the pond environment closely tracked ambient air temperature, confirming that open systems are highly susceptible to environmental fluctuations (Abdel-Tawwab & Ahmad, 2009; Yu *et al.,* 2021; Refaey *et al.,* 2025; Mahalder *et al.,* 2025c). Seasonal increases in pond temperature during the summer months corresponded with reduced DO, illustrating the inverse relationship between temperature and oxygen solubility and the potential for stratification-induced hypoxia. Conversely, the RAS maintained significantly higher and steadier DO levels, owing to mechanical aeration and continuous water recirculation, which enhance aerobic metabolism and prevent oxygen depletion, findings consistent with Martins *et al.* (2010). The pH in both systems remained within tolerable limits for *O. niloticus* (6.5–9.0). Yet, diel fluctuations in the pond, likely due to photosynthetic activity, CO, accumulation, and acid rainfall, caused wider variation (Ayaz *et al.,* 2015). In contrast, the buffering capacity of the RAS ensured tighter pH control, which is crucial for maintaining enzyme activity and overall homeostasis. Ammonia dynamics further highlighted system differences: the pond showed measurable ammonia levels, particularly during warmer months, suggesting limited nitrification and possible organic matter accumulation. Elevated ammonia concentrations, even at sub-toxic levels, can stress fish and impair gill function, whereas the RAS's biofiltration efficiently converted ammonia to nitrate, maintaining undetectable levels (Ebeling *et al.,* 2006; Khangembam *et al.,* 2018). Although TDS values were slightly higher in the RAS, this was likely due to limited water replacement and mineral buildup from feed and metabolite recycling. These concentrations remained within the acceptable range for tilapia and posed minimal osmotic stress, though long-term accumulation could require management through partial water exchange (Siddique *et al.,* 2026b). Overall, these findings indicate that the RAS setup provided greater stabilization of monitored environmental parameters, maintaining water quality conditions within narrow ranges, whereas parameters in the pond system varied in alignment with external meteorological shifts.

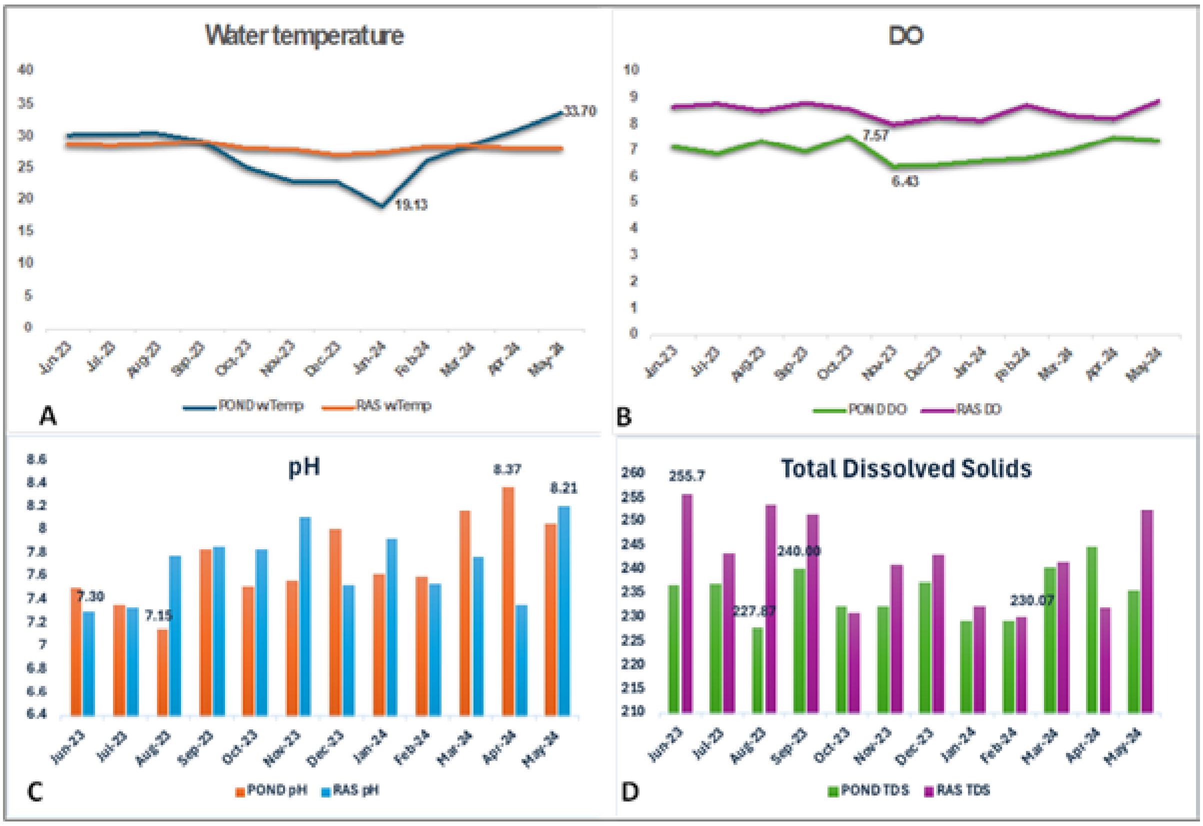


Figure 5. Monthly changes of water quality in Pond and RAS. A) Water temperature, B) DO (Dissolved Oxygen), C) pH, D) Total Dissolved Solids.

Relationship Between environmental Factors and Water Quality Parameters

The associations between environmental variables and water quality measures in the pond culture system were examined using Pearson correlation analysis (Table 2). Air and water temperatures were strongly and positively correlated (r = 0.793), indicating that higher air temperatures directly increased water temperature. This finding reinforces the close thermal coupling of open aquaculture systems with ambient environmental conditions, as previously reported by Abdel-Tawwab & Ahmad (2009) and Yu *et al.* (2021). Water temperature also showed a positive correlation with dissolved oxygen (DO) (r = 0.684). Although oxygen solubility typically declines with rising temperature, this relationship likely reflects enhanced algal photosynthetic activity during warmer, sunnier months, which can temporarily elevate DO levels (Martins *et al*., 2010). However, such diel fluctuations often lead to subsequent oxygen depletion during night hours, underscoring the dynamic nature of oxygen balance in open ponds. Ammonia concentrations were negatively correlated with humidity (r = –0.808) and water temperature (r = –0.758), suggesting that higher humidity and water temperature enhance microbial nitrification or dilution processes that lower ammonia accumulation. This trend supports the view that temperature-driven microbial activity and rainfall dilution can improve nitrogen turnover, though prolonged high temperatures may still promote ammonia toxicity under eutrophic conditions (Ebeling *et al.,* 2006; Khangembam *et al.,* 2018). Rainfall exhibited negative correlations with both pH (r = –0.579) and sunlight intensity (r = –0.583), reflecting the impact of acidic runoff and reduced sunlight exposure during monsoonal periods. These findings align with Ayaz *et al.* (2015), who noted transient pH declines following storm events due to organic acid influx and CO, accumulation. The observed positive correlation between DO and humidity (r = 0.628), as well as with rainfall (r = 0.625), further highlights the moderating influence of wet conditions on pond aeration and cooling, whereas DO's negative correlation with ammonia (r = –0.615) suggests that nitrogenous waste buildup compromises oxygen availability through increased biological oxygen demand. The positive correlation between pH and TDS (r = 0.753) indicates that higher ionic concentrations influence water buffering capacity and chemical equilibria, subtly affecting acidity. Although the TDS levels observed remained within tolerable limits for *O. niloticus*, sustained ion accumulation could pose long-term challenges if unmanaged (Siddique *et al.,* 2026b). Overall, these correlation patterns emphasize the complex interdependence between environmental factors and water chemistry in pond aquaculture. The results demonstrate that temperature, humidity, and rainfall collectively shape nutrient dynamics, oxygen balance, and pH regulation, underscoring the need for climate-responsive management strategies. In contrast, the structural configuration of Recirculating Aquaculture Systems (RAS) demonstrated a strong association with environmental stabilization, where its controlled indoor conditions decoupled water parameters from external ambient fluctuations, offering a resilient alternative under increasing climate variability (Martins *et al.,* 2010; Sen *et al.,* 2020).

Table 2. Correlations between environmental factors and water quality parameters in the pond

Correlations between environmental factors and water quality parameters in pond									
	Air temp (°C)	Humidity (%)	Rainfall (mm)	S. intensity (W/m^2)	W. temp (°C)	DO mg/L	pH	NH_3 mg/L	TDS (ppm)
Air temp	1								
Humidity	.550	1							
Rainfall	.681*	.561	1						
S. intensity	-.325	.075	-.583*	1					
W. temp	.793**	.755**	.488	.101	1				
DO	.705*	.628*	.625*	.028	.684*	1			
pH	-.111	-.211	-.579*	.758**	.149	.106	1		
NH_3	-.478	-.808**	-.327	-.337	-.758**	-.615*	-.144	1	
TDS	.371	.063	-.215	.422	.435	.235	.753**	-.261	1

*. Correlation is significant at the 0.05 level (2 -tailed).

**. Correlation is significant at the 0.01 level (2 -tailed).

Air temp = Air temperature, W. temp = Water temperature, S. intensity = sunlight intensity

Growth performance of *O. niloticus* in Pond and RAS

During the study, tilapia reared in the RAS exhibited higher observed metrics for growth and feed efficiency parameters than those managed in the traditional pond system. Although initial weights were identical (60.47 ± 6.14) in both culture systems, fish in RAS reached a significantly higher final weight (658.40 ± 20.15g) and length (28.63 ± 2.69 cm) than pond fish (540.57 ± 10.62 g, 26.96 ± 4.57 cm). The growth trend was exponential during the study period (Figure 6a-d), and there was a linear correlation between length and weight (Figure 7). Mortality rates were consistently higher in the pond system (5%) compared to the RAS (2%) over the entire study period. In contrast, pond fish experienced greater fluctuations in water temperature, DO, and pH due to ambient environmental conditions, which likely contributed to increased metabolic stress and reduced energy allocation to growth (Kubitza et al., 2000; Yu *et al*., 2021). The enhanced growth rate and body condition of RAS-reared tilapia also reflect improved feed conversion efficiency. Although the FCR data were reported separately, the observed higher biomass gain under RAS conditions indicates a lower FCR, implying better nutrient assimilation and reduced feed wastage. These outcomes are consistent with previous studies by Santo *et al.* (2020) and Ahmed *et al.* (2026), which documented improved growth performance, feed efficiency, morphometric stability, and broodfish condition under recirculating aquaculture systems. The exponential growth curve observed throughout the study demonstrates that tilapia performance benefits significantly from stable rearing environments, where energy expenditure for homeostasis is minimized. Conversely, the variable physicochemical conditions in pond systems can lead to periodic physiological stress, reducing appetite and growth potential. Similar observations were reported by Martins *et al.* (2010), who emphasized that aeration and controlled recirculation improve fish welfare and productivity. Overall, the results show higher growth performance metrics in *O. niloticus* under the stable water quality parameters of the RAS environment, reflecting differences in environmental conditions and energy allocation compared to the pond system. This supports the evaluation of RAS as a technically stable production model that minimizes exposure to ambient environmental fluctuations, offering an alternative management option for intensive tilapia culture in regions characterized by high seasonal ecological variability (Bremer *et al.,* 2016; Sen *et al.,* 2020).

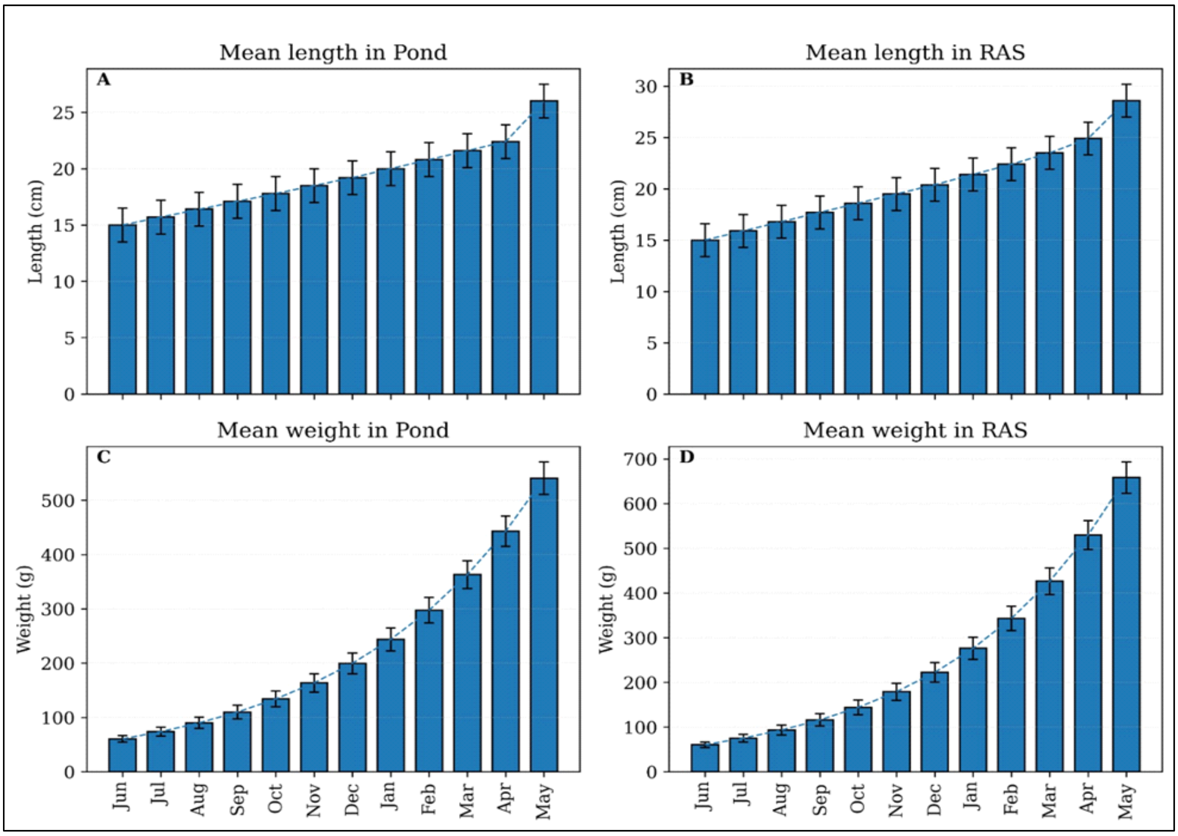
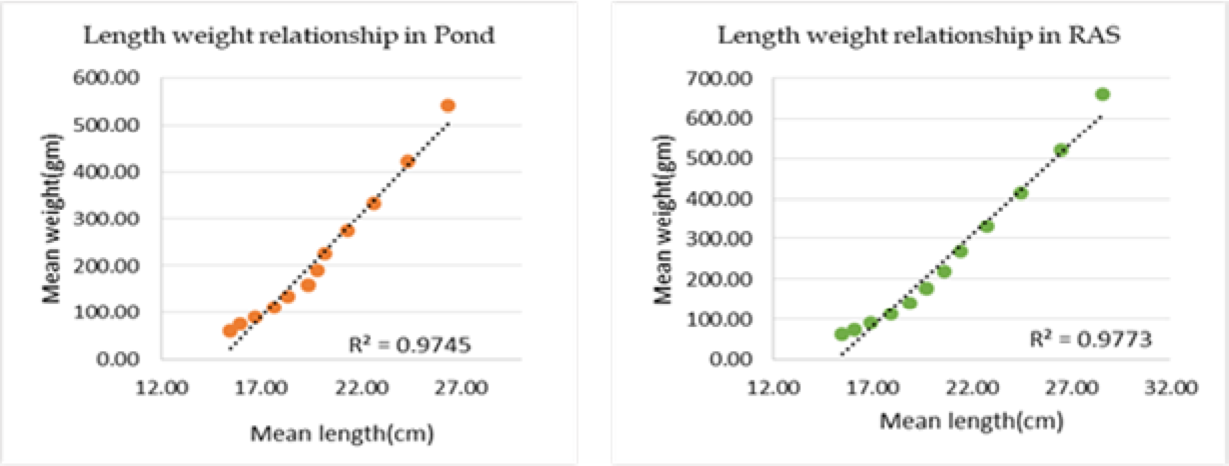


Figure 6. Exponential growth trends of fish, presented as monthly mean ± standard deviation (SD): (a) mean length (cm) in pond culture, (b) mean length (cm) in RAS, (c) mean weight (g) in pond culture, and (d) mean weight (g) in RAS. Error bars indicate SD.



7(a)

7(b)

Figure 7. Length-weight linear relationship in (a) Pond (r=0.974) and (b) RAS (r=0.977).

Determination of Hematological Parameters of *O. niloticus* Cultured in Pond System and RAS

Throughout the study, significant variations in the hematological profiles of *O. niloticus* cultured in pond and RAS environments were observed. The mean white blood cell (WBC) count was 389.188 ± 8.82 × 10³/mL in the pond culture system and 359.862 ± 6.54 × 10³/mL in the RAS. WBC values in the pond ranged from 344.80 × 10³/mL to 441.07 × 10³/mL, whereas RAS values varied between 330.40 × 10³/mL and 403.73 × 10³/mL. The first and third quartiles were higher for the pond than for RAS, indicating that most WBC values were generally elevated in the pond environment. For both male and female fish, WBC counts were consistently higher in the pond system than in RAS (Figure 8a).

In contrast, the mean red blood cell (RBC) count was 1.375 ± 0.03 × 10v /mL in the pond and 1.512 ± 0.04 × 10v /mL in RAS. RBC values in the RAS ranged from 1.36 × 10v /mL to 1.75 × 10v /mL, higher than those in the pond (1.26 × 10v /mL to 1.53 × 10v / mL). The first and third quartile values of RBC were greater in RAS, with higher erythrocyte indices recorded within this cohort under the observed system parameters. Similarly, both male and female tilapia exhibited higher RBC counts in RAS than in the pond (Figure 8b). The average blood glucose concentration also differed significantly between systems. Glucose levels in the pond fluctuated widely from 105.3 mg/dL to 138.6 mg/dL, whereas RAS values remained within a narrower range (83.42 mg/dL to 98.83 mg/dL). The mean glucose level was 122.67 ± 3.11 mg/dL in the pond and 91.359 ± 1.32 mg/dL in the RAS, indicating that pond-reared tilapia experienced elevated glucose levels (Supplementary Table S2). Male and female tilapia from the pond both showed higher glucose values than their RAS counterparts (Figure 8c). Temporal analyses further revealed that in the pond system, WBC counts fluctuated in tandem with temperature changes, reflecting increased immunological activity during warmer periods. RBC counts also varied significantly, suggesting physiological adjustments to shifting oxygen levels. Glucose levels peaked during high-temperature months, indicating stress-induced hyperglycemia (Figure 9(a). In contrast, fish within the RAS cohort exhibited more uniform hematological profiles across months, with less pronounced variations in WBC, RBC, and glucose levels

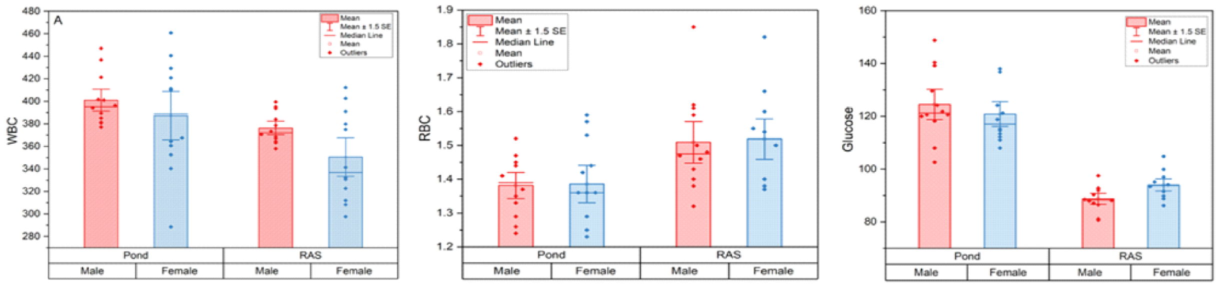


Figure 8. Comparison of a) White Blood Cell b) Red Blood Cells c) blood glucose level of male and female tilapia between pond and RAS systems.

(Figure 9b), coinciding with the stable environmental parameters observed in the indoor recirculating system. Elevated WBC counts in pond fish indicate an immune response to environmental stressors, such as elevated ammonia, fluctuating dissolved oxygen levels, and temperature extremes. However, elevated WBC levels may also be associated with inflammation, immune stimulation, or possible infection and therefore should not be interpreted solely as indicators of stress. In the present study, fish appeared physically healthy based on external observation, including normal movement and absence of visible disease signs; however, no laboratory-based pathogen or disease diagnostic assessment was performed. Similar findings were reported by Acar *et al.* (2018) and Islam *et al.* (2020), who linked increased leukocyte activity to chronic stress and pathogen exposure in fluctuating aquatic environments. The higher RBC count observed in the RAS compared to the pond system is consistent with the different environmental regimes of the two production setups, reflecting system-specific variations in baseline physical conditions(Bittencourt *et al.,* 2003; Colt, 2006; Martins *et al.,* 2010).

The higher RBC levels observed in the RAS system showed a measurable association with enhanced oxygen transport capacity and physiological adaptation to culture conditions. However, elevated RBC values should not be interpreted solely as indicators of improved health status, as they may also reflect compensatory physiological adjustments in response to environmental or metabolic demands. Blood glucose served as a reliable indicator of acute stress. The elevated glucose levels in pond fish likely resulted from cortisol-mediated metabolic responses to environmental challenges such as high temperature and low DO, as noted by Lermen *et al.* (2004) and Hamid *et al.* (2013). The glucose peaks observed during warmer months correspond to heightened physiological stress, aligning with observations in other tropical aquaculture species including striped catfish and pangasius (Hasan *et al.,* 2021). Furthermore, sex-based differences were evident, with male fish generally exhibiting higher WBC counts than females, consistent with Kefas *et al.* (2015) who attributed this to hormonal modulation of immune responses. Understanding such dimorphic physiological patterns can help optimize management strategies and improve stock performance in both systems (Haque *et al.,* 2014). Overall, the hematological findings demonstrate that fish reared in RAS exhibited more stable hematological and metabolic profiles compared to those cultured in open pond systems. These findings indicate that the indoor RAS environment effectively minimized the transmission of ambient meteorological fluctuations to the culture medium, preventing the stress-induced physiological shifts observed in the open pond system. The comparatively stable WBC, RBC, and glucose values observed in RAS suggest that controlled indoor water conditions were associated with reduced environmental variability and more consistent physiological responses in cultured tilapia (Bremer *et al.,* 2016; Sen *et al.,* 2020).

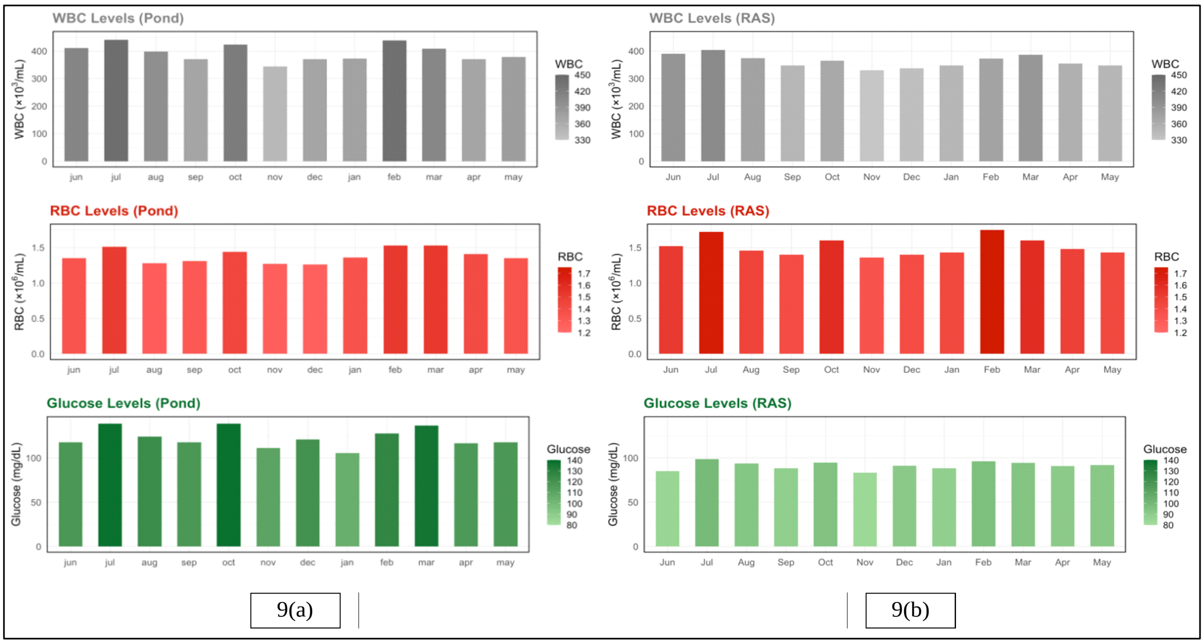


Figure 9. Monthly variation of WBC, RBC and glucose levels in (a) Pond and (b) RAS systems.

Principal Component Analysis (PCA) of Water Quality Parameters and Hematological Parameters of *O. niloticus* in Pond and RAS

The Principal Component Analysis (PCA) illustrated in Figure 10 demonstrates the multivariate distribution of 12-month average values for water quality and hematological parameters across two aquaculture systems: Pond and Recirculating Aquaculture System (RAS). The PCA biplot clearly separates the two culture systems, with blue squares (Pond) clustering to the right side of the PC1 axis, and red circles (RAS) clustering distinctly to the left. The observed segregation underscores strong system-level differences in environmental stability and fish physiological responses. The first principal component (PC1) accounted for 40.2% of the total variance, while the second component (PC2) explained 20.68%, jointly representing 60.88% of the total variability in the dataset.

The Pond system samples (blue squares) displayed a broader dispersion ranging approximately from 0 to 4 along PC1 and –2 to 2 along PC2, signifying greater monthly variability in both water quality and hematological indicators. This dispersion reflects the high environmental fluctuation typical of open pond systems, where factors such as temperature, rainfall, and dissolved oxygen vary in response to external climate (Sen *et al.*, 2020). In contrast, the RAS samples (red circles) formed a tight cluster between –2 and 0 on both PC1 and PC2 axes, indicating stable and consistent conditions across the measured parameters.

The PCA ellipses further emphasize these differences: RAS observations were concentrated toward negative PC1 values and narrowly aligned along PC2, forming a compact confidence ellipse. This pattern indicates low multivariate dispersion, suggesting that optimized and regulated conditions in RAS minimized variability in both water quality and physiological blood responses (WBC, RBC, glucose). In contrast, the pond system's broad scatter toward positive PC1 and PC2 values reflects greater environmental heterogeneity, corresponding to fluctuations in oxygen availability, ammonia buildup, and stress-related hematological responses (Martins *et al.,* 2010; Larsen *et al.,* 2011; Khangembam *et al.,* 2018).

The clear separation of clusters along PC1, which explained the largest proportion of total variance (40.2%), suggests that system management and environmental control were the dominant drivers of variability in the measured parameters. This separation aligns with the conclusion that RAS ensures improved environmental regulation and fish physiological stability, while pond systems remain vulnerable to environmental and water quality fluctuations (Abdel-Tawwab & Ahmad, 2009; Yu *et al.,* 2021). An independent-samples t-test confirmed significant differences between the pond and RAS systems for multiple parameters, including water temperature, dissolved oxygen, pH, ammonia, total dissolved solids, and hematological measures (WBC, RBC, glucose) (Table 3). The Group Statistics summary highlights these distinctions: RAS maintained higher dissolved oxygen (8.536 mg/L), zero ammonia, and a stable pH (7.963), demonstrating efficient aeration and biofiltration processes. TDS was slightly higher in RAS (242.301 ppm) due to limited water exchange and solute recirculation, though still within acceptable limits (Siddique *et al.,* 2026b). In contrast, pond systems exhibited greater variability in temperature and physicochemical parameters, reflected by higher standard deviations and reduced overall stability. Hematological data also support these environmental findings. Fish reared in RAS had higher RBC counts (1.512 × 10v /mL vs. 1.375 × 10v /mL) and lower glucose levels (91.359 mg/dL vs. 122.675 mg/dL) than pond fish, indicating enhanced oxygen transport and reduced physiological stress. Conversely, elevated WBC counts in pond fish (389.188 × 10^3/mL vs. 359.862 × 10^3/mL) signify immune activation due to environmental stressors such as fluctuating DO and ammonia (Acar *et al.,* 2018; Islam *et al.,* 2020). The PCA and t-test analyses together confirm that RAS provides a more controlled and homogenous aquatic environment, promoting optimal physiological responses. At the same time, open pond systems are inherently more variable and stress-prone. These findings align with prior research emphasizing that RAS enhances fish growth, immune stability, and welfare under climate-induced variability (Kubitza *et al.,* 2000; Santo *et al.,* 2020; Sen *et al.,* 2020). Ultimately, the multivariate differentiation observed through PCA and the statistical validation by t-tests collectively demonstrate that system design and environmental regulation are pivotal determinants of water quality, fish physiology, and overall aquaculture sustainability.

However, the present study demonstrated that RAS achieved comparatively better outcomes under the specific experimental conditions evaluated in this work. However, these findings should be interpreted cautiously, as the observed effects may also be influenced by environmental conditions, management practices, and other biological factors. Rather than indicating universal superiority, the results suggest that RAS may provide certain advantages within the context of the current study. Further investigations under diverse culture conditions and larger-scale applications can be considered as part of future studies to validate and expand upon these findings.

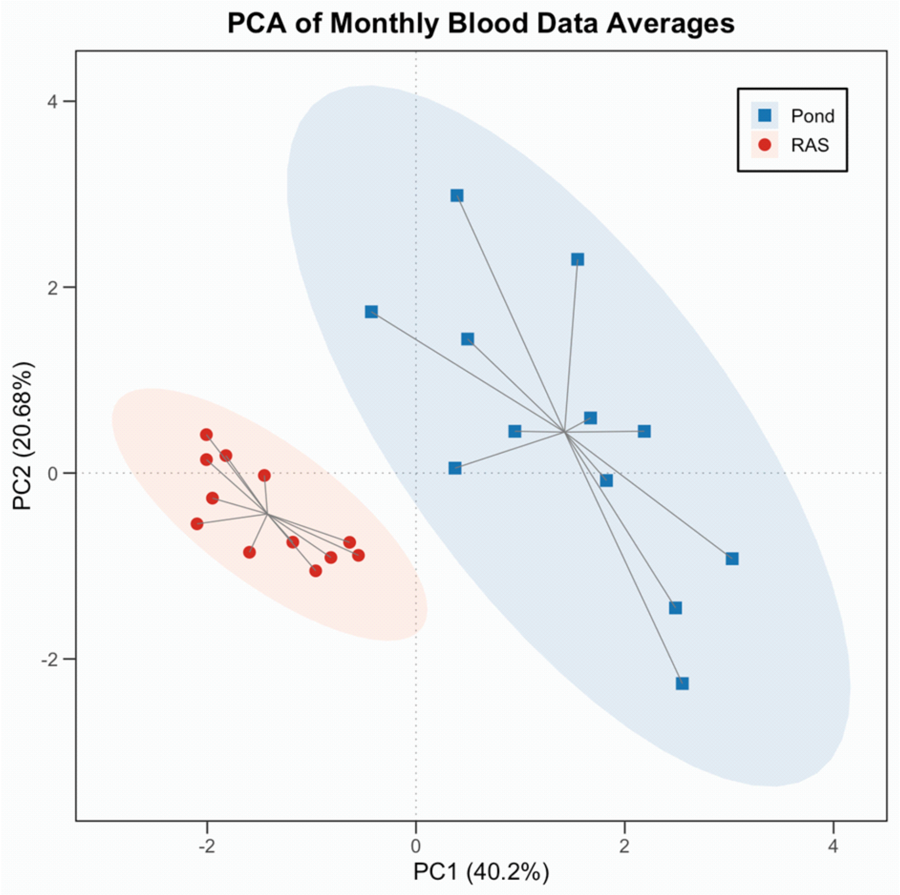


Figure 10. Principal Component Analysis (PCA) of Water Quality Parameters and Hematological Parameters of *O. niloticus* in Pond and Recirculatory Aquaculture System. Independent Samples t-test of Hematological and Water Quality Parameters Between Pond and Recirculatory Aquaculture System.

Table 3. Group statistics table of Hematological and water quality parameters in the pond and RAS system

Group Statistics					
	System	N	Mean	Std. Deviation	p - value (two tailed)
W.temp	Pond	12	27.606	4.2745	0.567
	RAS	12	28.301	.6070	
DO	Pond	12	7.032	.4004	<0.001
	RAS	12	8.536	.2926	
pH	Pond	12	7.728	.3603	0.903
	RAS	12	7.963	.3008	
Ammonia	Pond	12	0.125	.0479	<0.001
	RAS	12	0.000	.0000	
TDS	Pond	12	235.102	5.1338	0.034
	RAS	12	242.301	9.4594	
WBC	Pond	12	389.188	30.5380	0.011
	RAS	12	359.862	22.6704	
RBC	Pond	12	1.375	.1016	0.008
	RAS	12	1.512	.1282	
Glucose	Pond	12	122.675	10.7662	<0.001
	RAS	12	91.359	4.5661	

Finally, we would like to acknowledge some limitations of this study. The experiment involved limited culture units and monthly aggregated data, which may have influenced the broader interpretation of the findings. In addition, cortisol measurement, pathogen screening, and comprehensive immune assessments beyond WBC analysis were not included. Therefore, further studies with larger-scale trials and more detailed physiological and immunological evaluations are recommended to strengthen and validate the present findings.

CONCLUSION

This study mainly highlights the profound impact of environmental factors and water quality parameters on the hematological responses of Nile tilapia (*O. niloticus*) reared in two distinct aquaculture systems traditional earthen ponds and Recirculatory Aquaculture Systems (RAS). Findings revealed that tilapia in RAS exhibited distinct hematological profiles, including higher RBC counts and lower stress indicators (WBC and glucose levels), under more stable water quality conditions. The higher RBC values recorded within the RAS cohort reflected a distinct physiological baseline compared to the pond cohort under their respective environmental regimes. In contrast, pond systems were more susceptible to fluctuations in temperature, pH, and other water quality parameters driven by external environmental variability, which contributed to physiological stress in fish. . This study highlights the advantages of RAS in mitigating climate-induced stressors through greater environmental control, suggesting that such systems offer a promising and sustainable approach for aquaculture. By minimizing stress and promoting better fish health, RAS not only enhances overall productivity but also supports the development of high-quality broodstock, which is crucial for long-term sustainability. These insights are especially relevant in the face of climate change, as aquaculture systems must evolve to ensure resilient, efficient, and eco-friendly fish production practices.

Data availability statement

The data presented in this study are available upon request.

Conflict of interest

The authors declare no conflict of interest to anybody or any organization.

Author contribution

Nushrat Jahan: Overall data analysis & presentation and writing the original draft; Mohammad Abu Baker Siddique: Overall data analysis & presentation and writing the original draft; Imran Bin Younos: Overall data analysis, Methodology & Editing the draft; Ilias Ahmed: Overall data analysis & Presentation and Editing the draft; Md. Shiekh Tauhiduzzaman Shimul: Editing the draft; Safiara Nusrat Nova: Editing the draft; Balaram Mahalder: Editing the draft; Mohammad Mahfujul Haque: Review & editing the draft; Mariom: Editing the draft; A. K. Shakur Ahammad: Concept development, validation, overall supervision and editing the draft.

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