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Comparative Study on the Hematological Parameters and Nutritional Composition of *Heteropneustes fossilis* Cultured in RAS and Pond in Relation to Water Quality

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ABSTRACT

This study compared the effects of Recirculatory Aquaculture Systems (RAS) and traditional pond systems on the hematological parameters and nutritional composition of *Heteropneustes fossilis*, emphasizing the influence of water quality parameters. Hematological analyses showed that RAS-reared fish had higher red blood cell (RBC) counts (3.67×10^6/µL for males, 2.65×10^6/µL for females) and hemoglobin levels (9.0 g/dL for males, 8.61 g/dL for females), reflecting an enhanced oxygen transport efficiency. Conversely, pond-reared fish exhibited elevated white blood cell (WBC) counts (7.18×10^4/µL for males, 6.67×10^4/µL for females) and blood glucose levels (127.96 mg/dL for males, 128.23 mg/dL for females), indicating higher stress responses. Nutritional analysis revealed higher crude protein content in RAS-produced fish (16.37% for males, 16.04% for females) and crude lipid levels (3.47% for males, 5.00% for females). In contrast, pond-produced fish had significantly higher carbohydrate content (3.30% for males, 3.97% for females). Moisture, ash, and crude fiber content showed no notable differences between the systems. Water quality assessments highlighted the advantages of RAS, with stable dissolved oxygen (8.03–8.83 mg/L), pH (7.90–8.4), and temperature (27.72–29.23°C) compared to the seasonal variability in the pond. These controlled conditions minimized stress and supported better fish health and growth in RAS. The study demonstrates the potential of RAS to produce healthier, nutritionally superior fish while mitigating environmental stressors common in pond systems. These findings underscore the value of RAS in sustainable aquaculture, addressing challenges associated with traditional systems, particularly in environmentally sensitive regions.

INTRODUCTION

Biological research is essential in fast-growing aquaculture since it provides a deeper understanding of the life processes, health, and nutritional needs of cultured aquatic species **(Mahmud & Haque, 2025)**. Through biological studies, researchers can identify optimal breeding, feeding, and disease management strategies that enhance growth, survival, and productivity. It also helps in understanding how fish and shellfish respond to different environmental conditions, which is crucial for developing sustainable aquaculture systems that minimize stress and improve animal welfare. The stinging catfish, *Heteropneustes fossilis*, is a highly valued freshwater species widely cultured in South and Southeast Asia, primarily due to its exceptional nutritional profile and adaptability to diverse environmental conditions **(Araf *et al.*, 2021; Mahalder *et al.*, 2025)**. Rich in protein and essential amino acids, along with significant levels of minerals such as calcium and phosphorus, *H. fossilis* serves as a critical source of nutrition, especially in developing countries where fish constitutes a major component of the daily diet **(Araf *et al.*, 2021)**. Beyond its nutritional significance, this species plays an important economic role by supporting small-scale fish farmers and providing a sustainable livelihood option **(Ali *et al.*, 2014)**. Notably, *H. fossilis* exhibits remarkable tolerance to low-oxygen environments and resistance to diseases, which makes it particularly suitable for aquaculture in resource-limited settings **(Yasmin *et al.*, 2024)**. These characteristics highlight its potential contribution to food security and economic stability in countries like Bangladesh, where aquaculture is integral to the daily life of millions of people. Given its growing importance, a detailed understanding of the hematological parameters and nutritional composition of *H. fossilis* is essential for optimizing aquaculture practices and ensuring fish health and quality. Hematological parameters are vital indicators of fish health, providing insights into physiological responses to environmental stress **(Cnaani *et al.*, 2004; Parrino *et al.*, 2018; Fazio, 2019)** and diseases **(Clauss *et al.*, 2008)**. At the same time, nutritional composition, including protein, lipid, carbohydrate, ash, and moisture content, reflects the quality of fish, directly influencing both market value and consumer preference **(Chandravanshi *et al.*, 2019)**. These parameters serve not only as indicators of internal health status but also as tools for evaluating the suitability of various aquaculture systems and farming practices **(Uiuiu *et al.*, 2021)**.

Despite the well-recognized importance of *H. fossilis,* comprehensive comparative studies examining how different aquaculture systems influence its hematological and nutritional characteristics are still lacking. To date, most research has focused on growth performance and feed efficiency, often neglecting how culture environments affect the overall health and nutritional quality of this species. For instance, traditional pond systems, characterized by natural fluctuations in temperature, dissolved oxygen, and other water quality parameters, may expose fish to environmental stressors that negatively impact their health and nutritional status **(Demeke & Tassew, 2016)**. In contrast, RAS offers a controlled environment with stable water quality, which can potentially reduce stress and enhance fish growth and health **(Ahmed & Turchini, 2021)**. However, a systematic comparison of the effects of RAS and pond systems on the hematological and nutritional profiles of *H. fossilis* has yet to be fully explored, leaving a critical knowledge gap necessary for improving aquaculture management. Addressing this gap is essential for sustainable farming and effective management of *H. fossilis*, a hardy catfish species with high market demand due to its nutritional and medicinal values. Understanding its hematological parameters is crucial for monitoring fish health, stress responses, and disease resistance under different cultured conditions. Likewise, insights into its nutritional composition, including key components like protein, lipid, and mineral profiles, are vital for formulating balanced feeds that promote growth and improve flesh quality. Therefore, this study aimed to investigate the effects of RAS and traditional pond systems on the nutritional composition and hematological parameters of *H. fossilis*. Specifically, it compares hematological indicators, including red and white blood cell counts, hemoglobin concentration, and glucose levels, to assess fish health as well as nutritional components such as protein, lipid, carbohydrate, moisture, ash, and fiber under different rearing environments. Additionally, the study evaluates the influence of water quality and climatic factors on fish health and quality within these systems. By providing a detailed comparative analysis, this research not only addresses the existing knowledge gaps but also offers practical insights into the advantages and limitations of traditional and modern aquaculture systems for *H. fossilis.* Such insights are vital for guiding aquaculture development strategies aimed at maximizing fish health, nutritional quality, and farmer profitability in diverse production environments.

MATERIALS AND METHODS

Ethical statement

This study was conducted in compliance with ethical guidelines for the care and use of animals in research, ensuring the welfare of all *H. fossilis* specimens. The research protocol was reviewed and approved by the Ethical Standard of Research Committee of the Bangladesh Agricultural University (BAU) with the reference code BAURES/ESRC/FISH-11/2022. All procedures, including handling, anesthesia, blood sampling, and euthanasia, were performed with minimal stress and pain. Anesthesia was induced using a 0.5% clove oil solution, and euthanasia followed approved humane methods.

Study site and culture systems

The experiment was conducted at the Laboratory of Climate Research for Fishes (LCRF), Faculty of Fisheries, which houses a RAS and a traditional pond adjacent to the LCRF at Bangladesh Agricultural University (BAU), Mymensingh. Hematological analyses were performed in the Aquaculture Systems Laboratory, while the nutritional composition was assessed in the Nutrition Laboratory of the Department of Aquaculture, BAU. Fish were cultured in two distinct systems. RAS consisted of circular concrete tanks (10000L capacity) equipped with mechanical and biological filtration units, UV sterilization, and continuous water circulation, while the earthen pond, measuring 4.57m ×

3.04m × 1.06m, was maintained to simulate natural conditions, with water levels controlled by a submersible pump. The same environmental parameters were monitored to ensure consistency with the RAS.

Fish stocking and feeding

Fish were carefully selected for uniformity, with males averaging 12g and females 28g. Stocking densities were 50 fish/m³ both in the RAS and the pond, maintaining a balanced sex ratio. Before stocking, fish were disinfected with potassium permanganate and acclimated to reduce stress. In the pond system, fish were fed a commercial diet (Mega Feed, containing 35% protein) at a rate of 3–5% of the total biomass, administered twice daily. In contrast, the RAS fish were provided with a combination of floating feed, also containing 35% protein, and live tubificid worms at 1–1.5% of the biomass. The inclusion of live worms in the RAS was intended to replicate the natural feed availability found in pond environments, thereby mimicking the fish's ecological dietary habits. Feeding rates in the RAS were carefully adjusted according to water temperature, fish size, and observed feeding behavior to avoid overfeeding and to maintain optimal water quality.

Hematological parameters analysis

Hematological parameters were analyzed to evaluate the physiological health and immune status of *H. fossilis* under different culture conditions. Blood samples (0.5 to 1.0 mL) collected from the caudal fin were processed for multiple analyses. Red and white blood cell counts were performed by diluting the blood samples (RBC at 200× and WBC at 20× dilution) and enumerating the cells using a hemocytometer under a compound microscope at 40× magnification. Hemoglobin concentration was determined through Sahli's acid hematin method, with measurements recorded in grams per deciliter (g/dL). Additionally, blood glucose levels were assessed rapidly and accurately using a portable glucometer, providing insights into the metabolic responses of the fish to the different aquaculture environments. These hematological parameters served as critical indicators of fish health, stress levels, and overall physiological condition throughout the study period.

Proximate composition analysis

The nutritional quality of *H. fossilis* was evaluated through proximate composition analysis. Following sampling, the fish were thoroughly cleaned by washing with distilled water to remove any external contaminants. They were then anesthetized using a 0.5% clove oil solution to minimize stress and to ensure humane handling. The samples were immediately processed or stored at -20°C in airtight containers to prevent degradation of nutritional components until analysis could be performed. For the analysis, moisture content was determined by oven-drying the samples at 105°C until a constant weight was achieved, following the **AOAC (2000)** guidelines. Crude protein content was measured using the Kjeldahl method, where nitrogen content was quantified and converted to protein by applying a conversion factor of 6.25. Crude lipid extraction was conducted using a

Soxhlet apparatus with ether as the solvent, allowing for efficient separation and quantification of fats. Ash content was assessed by incinerating the samples in a muffle furnace at 550°C to isolate the inorganic mineral residues. Carbohydrate content was calculated by difference, subtracting the sum of protein, lipid, ash, and moisture percentages from 100%. To ensure the reliability of results, all analyses were performed in triplicate, maintaining strict quality control throughout the process.

Water quality monitoring

Water quality was rigorously monitored in both systems to assess its impacts on the health and growth. Key parameters included temperature (measured with SMART Sensor AR 867), pH (pH-107 meter), dissolved oxygen (Lutron DO-5509), total dissolved solids (TDS-3 meter), and alkalinity (HI3811 test kit) were taken. In the RAS, mechanical and biological filtration maintained stable conditions, while the pond relied on natural processes and manual adjustments. Deviations from optimal ranges were promptly addressed through aeration, feeding adjustments, or water exchanges.

Statistical analyses

The collected data were statistically analyzed to evaluate the effects of different culture systems on water quality parameters, hematological indices, and nutritional composition. Before analysis, the normality of data distribution was verified using the Shapiro-Wilk test. For normally distributed data, independent samples t-tests were employed to compare means between the RAS and pond culture systems. In cases where data did not meet normality assumptions, the non-parametric Mann-Whitney U test was utilized. All statistical tests were performed with a significance threshold set at $P< 0.05$. The analyses were conducted using SPSS software (version 23.0), while data visualization was accomplished through Microsoft Excel and Origin Pro (version 2024b).

RESULTS

Water quality parameters

The water quality analysis between the RAS and pond revealed distinct seasonal trends and variations across temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), and alkalinity. The temperature in the pond experienced a higher average than the RAS, especially during the summer months. For instance, in April, the pond temperature was recorded at 32.38°C compared to the RAS temperature of 29.23°C. This trend continued throughout the warmer months, with the pond averaging above 30°C from April to September, while the RAS temperature remained relatively stable, ranging from 27.72°C to 29.23°C. The cooler months showed a convergence in temperature, with January showing the lowest values of 19.09°C in the pond and 28.35°C in the RAS, indicating that the RAS maintained a more stable thermal environment year-round, which can be beneficial for species sensitive to temperature fluctuations.

RAS consistently showed higher levels of DO than the pond, reflecting better oxygen management typical of closed systems. In May, for example, the DO in the pond reached 7.42 mg/ L, significantly lower than the RAS's 8.14 mg/ L. Throughout the year, the RAS maintained DO levels between 8.03 mg/ L and 8.83 mg/ L, while the pond fluctuated more dramatically, from a low of 4.85 mg/ L in April to a peak of 8.23 mg/ L in July. This higher and more consistent DO level in the RAS was crucial for supporting high stocking densities, minimizing stress, and promoting better fish health. The pH levels displayed noticeable differences between the two systems. The pond generally exhibited a higher and more variable pH, with values ranging from 8.03 to 8.88, with the highest pH recorded in June (Fig. 1). In contrast, the RAS maintained a more stable pH, ranging from 7.90 to 8.40, with fewer fluctuations. For instance, the pond pH reached 8.82 in May, while the RAS pH in the same month is 8.01. The stable pH in the RAS was beneficial for fish health, as it minimizes stress associated with pH fluctuations. However, the higher pH in the pond reflected the natural buffering provided by soil and organic processes, which were less pronounced in the closed RAS environment.

In April, RAS TDS started at 278 mg/ L and fluctuated slightly across the year, ranging from a high of 280 mg/ L in September to a low of 254 mg/ L in February. Conversely, TDS in the pond displayed more noticeable fluctuations, from a low of 183.5 mg/ L in December to a peak of 236.8 mg/ L in August. This consistent difference may be due to the RAS's closed system, where controlled water chemistry adjustments are maintained to ensure optimal conditions, particularly with limited water exchange. The more variable TDS in the pond reflects natural influences like rainfall, evaporation, and interactions with soil and organic matter, leading to seasonal changes in water composition. The alkalinity levels in the RAS and pond exhibit distinct seasonal patterns, with the pond alkalinity showing greater variability. In the RAS, alkalinity remained relatively stable throughout the year, ranging from 180 mg/ L in April and September to 210 mg/ L in December (Fig. 1). This consistent level reflects the controlled environment of the RAS, where water chemistry adjustments help maintain stability. In contrast, pond alkalinity fluctuated more significantly, likely influenced by natural factors such as rainfall, soil interactions, and organic matter. Alkalinity in the pond varied from a high of 225 mg/ L in April to a low of 125 mg/ L in June. The pond displayed multiple peaks and troughs throughout the year, indicating less buffering stability compared to the RAS. The variability in the pond system suggests a natural buffering effect that is less predictable than the RAS, highlighting the differences between controlled and open water systems in terms of water quality stability.

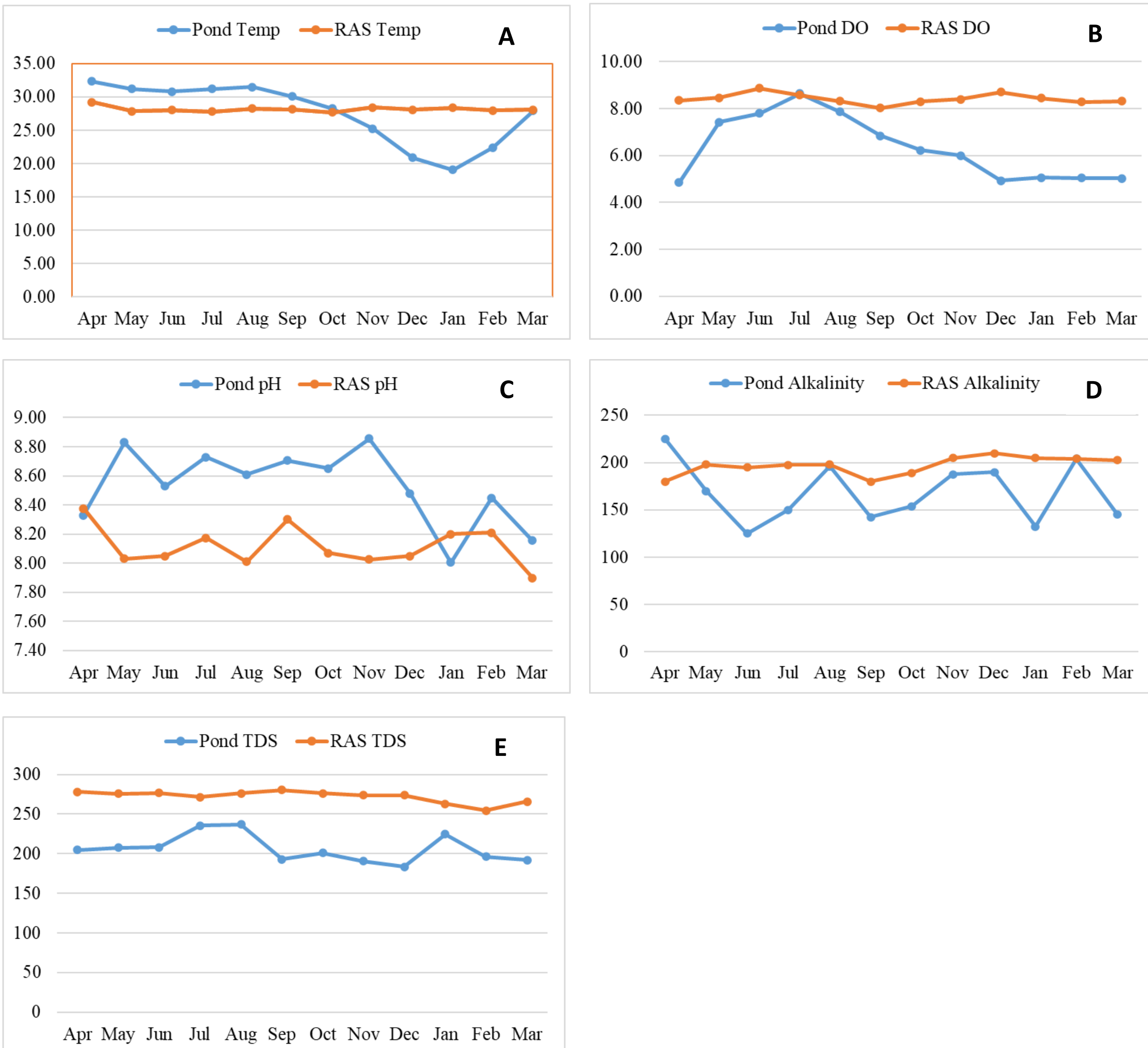

Fig. 1. Comparison of water quality parameters: (A) Temperature; (B) DO; (C) pH; (D) Alkalinity; and (E) TDS between the pond and RAS.

Hematological parameters

The hematological analysis revealed consistent advantages for RAS-reared *H. fossilis* across both sexes. Fish cultured in RAS exhibited a 10.5% higher mean RBC count in males (3.67 vs 3.32 × 10^6/µL) and 13.2% greater count in females (2.65 vs 2.34 × 10^6/µL), accompanied by 14.5% (males: 9.0 vs 7.86 g/dL) and 33.7% (females: 8.61 vs 6.44 g/dL) increases in hemoglobin levels, respectively (Fig. 2). These improvements in oxygen transport metrics contrasted sharply with the stress markers observed in pond system, where WBC counts were 21.9% higher in males (7.18 vs 5.89 × 10^4/µL) and 17.0% elevated in females (6.67 vs 5.70 × 10^4/µL). The most dramatic differences appeared in blood glucose levels, with pond-reared fish showing 26.7% (males: 127.96 vs 101.01 mg/dL) and 26.5% (females: 128.23 vs 101.36 mg/dL) higher concentrations compared to RAS specimens. These patterns, detailed statistically, demonstrate how RAS conditions promote hematological health while pond environments elicit measurable stress responses (Table 1).

Table 1. Comparison of hematological parameters in *H. fossilis* between pond and RAS

Hematological parameter	Male		*F-value*	*P- value*	Female		*F-value*	*P- value*
	Pond	RAS			Pond	RAS		
RBC ($\times10^6$/µL)	3.32 ± 0.03	3.67 ± 0.01	165.435	1.04E-11	2.34 ± 0.04	2.65 ± 0.01	50.010	4.29196E-07
WBC ($\times10^4$/µL)	7.18 ± 0.26	5.89 ± 0.20	14.971	0.000829	6.67 ± 0.30	5.70 ± 0.16	8.397	0.008348
Hemoglobin (g/dL)	7.86 ± 0.22	9.0 ± 0.17	16.712	0.000487	6.44 ± 0.06	8.61 ± 0.10	315.665	1.56E-14
Glucose (mg/dL)	127.96 ± 1.78	101.01 ± 1.19	158.231	1.6E-11	128.23 ± 1.11	101.36 ± 1.83	157.300	1.69E-11

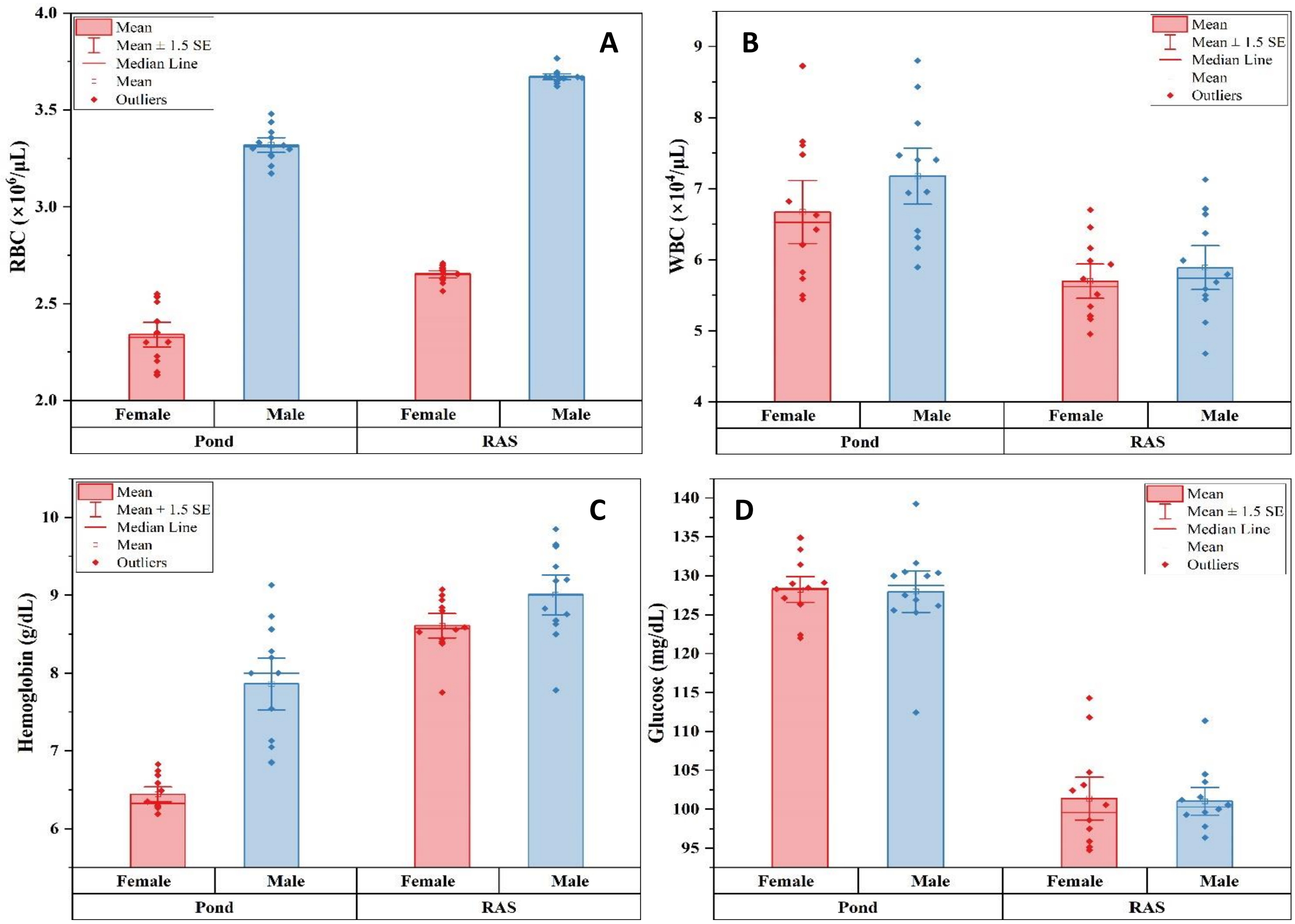

Fig. 2. Box plots showing: (A) RBC counts; (B) WBC counts; (C) Hemoglobin level; and (D) Blood glucose level comparison of pond and RAS of *H. fossilis.*

Nutritional composition

The nutritional analysis revealed significant differences in key components between rearing systems (Table 2). RAS-cultured *H. fossilis* exhibited enhanced protein content, with males showing a 3.3% relative increase (16.37% vs. 15.85%) and females an even greater 8.2% increase (16.04% vs. 14.83%). Lipid content followed a similar trend, particularly among females, where RAS rearing resulted in a 19.6% increase (5.00% vs. 4.18%, $P < 0.0247$). Although males also showed a 9.5% higher lipid content under RAS conditions, the difference was not statistically significant ($P = 0.0516$). In contrast, pond-reared fish accumulated substantially more carbohydrates, 46.0% higher in males (3.30% vs. 2.26%) and 69.7% higher in females (3.97% vs. 2.34%), both statistically significant. Moisture, ash, and crude fiber content remained relatively stable between systems, with no significant differences observed ($P > 0.05$). These patterns suggest that RAS environments promote greater protein and lipid retention (Fig. 3), while pond conditions favor carbohydrate accumulation, likely reflecting metabolic adaptations to differing rearing conditions.

Table 2. Nutritional Composition of *H. fossilis* between pond and RAS

Nutritional component	**Male**				**Female**			
	Pond	**RAS**	***F-value***	***P- value***	**Pond**	**RAS**	***F-value***	***P- value***
Moisture (%)	72.81±0.32	73.47±0.10	3.826	0.0632648	72.08±0.25	71.75±0.24	0.892	0.355315
Crude Lipid (%)	3.17±0.12	3.47±0.08	4.235	0.0516494	4.18±0.30	5.00±0.15	5.811	0.024721
Crude Protein (%)	15.85±0.20	16.37±0.13	4.901	0.0375154	14.83±0.18	16.04±0.08	36.758	4.21E-06
Ash (%)	3.62±0.13	3.35±0.15	1.756	0.1987511	3.68±0.15	3.80±0.08	0.519	0.479064
Crude Fiber (%)	1.25±0.04	1.08±0.09	3.676	0.0682936	1.26±0.04	1.07±0.09	3.676	0.068294
Carbohydrate (%)	3.30±0.33	2.26±0.19	7.604	0.0114913	3.97±0.25	2.34±0.19	26.262	3.9E-05

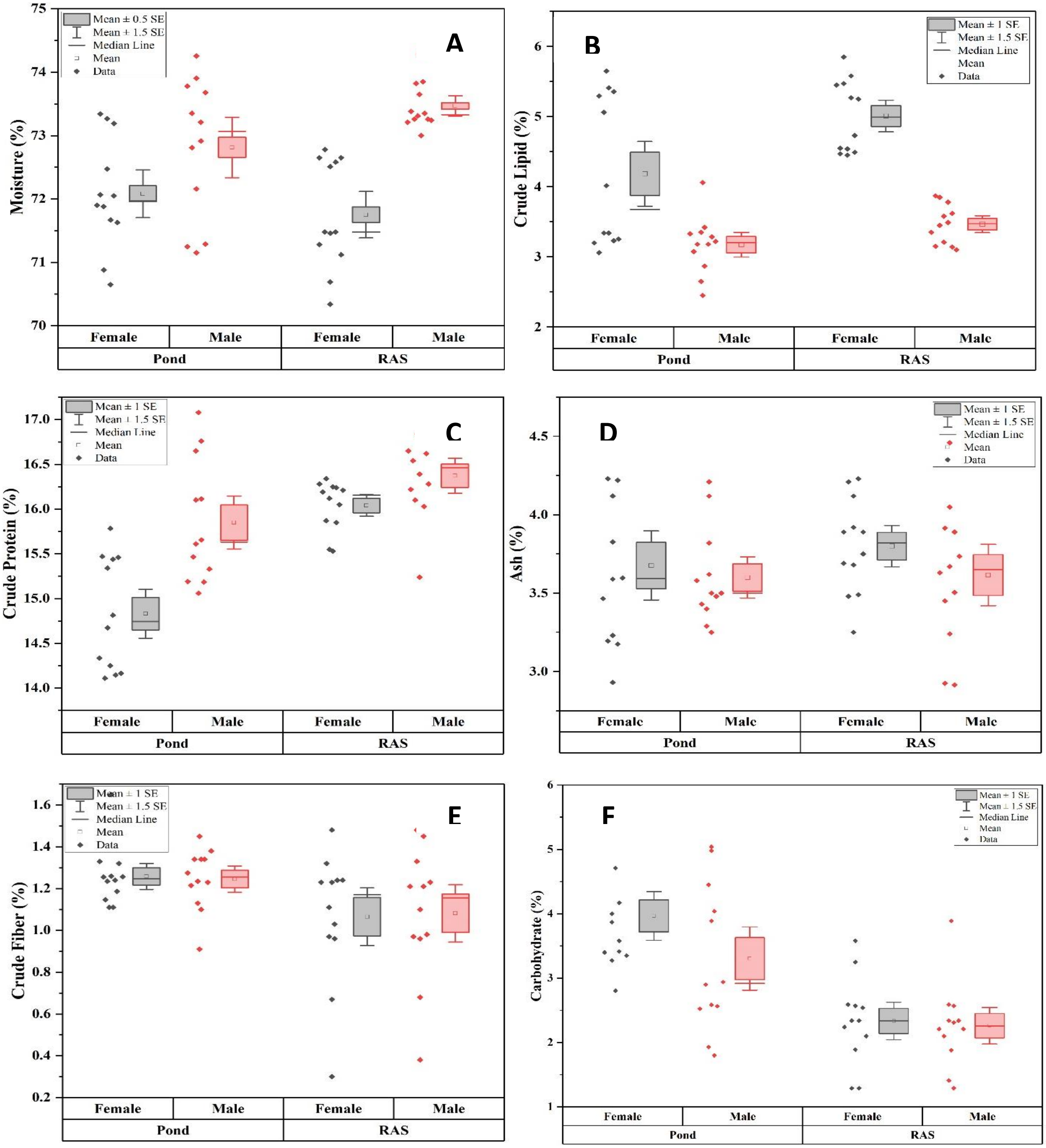

Fig. 3. Box plots showing: (A) Moisture (%); (B) Crude lipid (%); (C) Crude Protein (%); (D) Ash (%); (E) Crude fiber (%); and (F) Carbohydrate (%) of *H. fossilis.*

DISCUSSION

Water quality

The observed differences in water quality parameters between RAS and pond environments reflect the distinct nature of open and controlled systems, which have implications for fish health, growth, and overall system efficiency. The more stable temperature profile in the RAS, which ranged from 27.72 to 29.23°C throughout the year, contrasts with the seasonal fluctuations in the pond, where temperatures reached as high as 32.38°C in summer and dropped to 19.09°C in winter. Temperature stability in RAS systems is known to be beneficial for fish health, particularly for species sensitive to sudden temperature changes **(Avnimelech, 2006)**. Such stability can reduce stress, prevent thermal shock, and support optimal metabolic rates, thus enhancing growth and survival **(Ebeling & Timmons, 2012)**. The consistently higher DO levels in the RAS, which remained between 8.03 and 8.83 mg/ L, are advantageous, as sufficient oxygen availability is critical in high-density systems. Closed systems like RAS allow for precise DO regulation, essential for maintaining adequate oxygen levels despite high stocking densities **(Martins *et al.*, 2010)**. By contrast, the pond DO fluctuates more widely, reflecting natural variations influenced by seasonal temperature changes and biological activity. Studies have shown that DO levels below 5mg/ L can lead to stress and reduced fish performance, highlighting the importance of consistent oxygen levels in intensive aquaculture **(Boyd & Tucker, 2012)**. The higher and more consistent DO in RAS likely contributes to better growth rates and lower mortality, aligning with findings from previous studies on closed system advantages **(Ebeling & Timmons, 2012)**.

The observed pH differences further underscore the contrast between controlled and natural environments. While the pond exhibited higher variability in pH (8.03 to 8.88), the RAS maintained a stable range (7.9 to 8.40). Maintaining a stable pH is crucial in aquaculture, as fluctuating pH can stress fish and affect physiological processes **(Ekubo & Abowei, 2011)**. The buffering stability in RAS is facilitated by regular water quality adjustments, while pond pH variability is influenced by external factors such as soil interactions, organic matter, and seasonal changes. The stable pH in RAS aligns with recommendations for aquaculture systems, as it provides an environment less prone to pH-induced stress, supporting healthier fish **(Martins *et al.*, 2010)**.

Total Dissolved Solids (TDS) levels were consistently higher in the RAS, with minor seasonal variations, while the pond exhibited greater fluctuations due to natural influences. High TDS levels can indicate better nutrient control in closed systems like RAS, where water chemistry is managed to enhance growth conditions **(Boyd, 2019)**. In the pond, however, TDS varied more significantly, suggesting the influence of rainfall, evaporation, and soil interactions, which align with findings by **Campanati *et al.* (2022)** on seasonal impacts on open systems. Higher TDS stability in RAS contributes to a more controlled environment, promoting steady fish growth by minimizing stress factors linked to fluctuating water quality **(Rakocy, 2012)**.

The alkalinity differences observed between the RAS and pond also highlight the stability of controlled systems. The relatively stable alkalinity in RAS (180–210mg/ L) reflects regular adjustments to maintain water quality, whereas pond alkalinity fluctuated significantly (125–225mg/ L) in response to natural factors such as rainfall and organic matter decomposition. Alkalinity is essential in aquaculture as it stabilizes pH and contributes to the overall buffering capacity of water **(Boyd, 2019)**. The greater variability in pond alkalinity aligns with observations by **Boyd and Tucker (2012)**, who noted that open systems often experience broader fluctuations due to environmental interactions. These findings reinforce the advantage of RAS in providing a controlled environment that mitigates the effects of external variables, leading to more predictable water quality conditions **(Clay, 2008)**.

The RAS environment demonstrated significant advantages in maintaining stable water quality parameters, particularly in terms of temperature, DO, pH, TDS, and alkalinity. These results are consistent with previous research emphasizing the benefits of closed aquaculture systems in achieving more controlled and optimal conditions for fish growth and health. However, the pond's natural buffering and seasonal variability reflect a less predictable but ecologically relevant system, indicating that each rearing environment has unique attributes that can impact aquaculture outcomes.

Hematological parameters

The hematological analysis of *H. fossilis* revealed distinct physiological responses between RAS and pond environments. Fish reared in RAS exhibited significantly higher RBC counts (males: $3.67 \pm 0.01 \times 10^6/\mu L$; females: $2.65 \pm 0.01 \times 10^6/\mu L$) and hemoglobin levels (males: 9.0 ± 0.17 g/dL; females: 8.61 ± 0.10 g/dL) compared to pond-reared fish. These findings reflect the stable oxygen availability in RAS, which supports erythropoiesis and enhances oxygen transport efficiency **(Hrubec *et al.*, 2000)**. The controlled conditions of RAS likely reduce environmental stress, allowing for optimal hematological development **(Docan *et al.*, 2018)**.

Conversely, pond-reared fish displayed elevated WBC counts (males: $7.18 \pm 0.26 \times 10^4/\mu L$; females: $6.67 \pm 0.30 \times 10^4/\mu L$), suggesting heightened immune activity due to variable water quality and potential pathogen exposure **(Burgos-Aceves *et al.*, 2019)**. Such fluctuations are characteristic of open systems, where natural stressors can stimulate leukocyte production as a defense mechanism **(Anderson, 1996)**. Blood glucose levels were markedly higher in pond fish (males: 127.96 ± 1.78 mg/dL; females: 128.23 ± 1.11 mg/dL) than in RAS fish (males: 101.01 ± 1.19 mg/dL; females: 101.36 ± 1.83 mg/dL). This disparity aligns with the role of cortisol in mobilizing glucose under stress **(Mommsen *et al.*, 1999)**, further implicating pond conditions as a source of metabolic strain. The lower glucose levels in RAS-reared fish underscore the system's capacity to minimize stress and maintain physiological homeostasis **(Segner *et al.,* 2012)**. Collectively, these results highlight the advantages of RAS in promoting hematological health through stable conditions, while pond systems elicit measurable stress responses linked to environmental variability.

Nutritional composition

The proximate analysis of *H. fossilis* revealed system-dependent variations in nutritional quality. RAS-reared fish demonstrated superior protein content (males: 16.37%; females: 16.04%) compared to pond systems (males: 15.85%; females: 14.83%), consistent with the 15.56-16.96% range reported by **Erfanullah and Jafri (1999)** for protein-optimized diets. This enhancement reflects RAS's stable conditions that improve feed conversion efficiency, as demonstrated in biofloc systems by **Nguyen *et al.* (2021)**. The protein values align with **Ali *et al.* (2010)**, who recorded 15.85-16.75% in controlled environments.

Lipid profiles showed sexual dimorphism, with RAS females accumulating significantly more lipids (5.00% vs 4.18%, $p = 0.0247$). These values fall within the 3.48-7.8% range documented by **Ali *et al.* (2010)**, suggesting RAS conditions promote lipid deposition by reducing stress-related energy demands **(Li *et al.,* 2023)**. The male lipid difference (3.47% RAS vs 3.17% pond) showed marginal significance ($P = 0.0516$), possibly due to sex-specific metabolic responses. Carbohydrate content was markedly higher in pond fish (males: 3.30%; females: 3.97%) versus RAS (males: 2.26%; females: 2.34%), supporting findings by **Abdel-Tawwab *et al.* (2019)** that environmental stressors trigger carbohydrate mobilization. Moisture levels (72.08-73.47%) matched the 70-80% range typical for fish **(Rahman *et al.,* 1982)**, while ash content (3.35-3.80%) remained stable across systems, consistent with **Akand *et al*. (1989)**, who reported 2.44-3.00% ash regardless of diet.

These results demonstrate that RAS enhances protein and lipid retention while minimizing stress-induced carbohydrate accumulation. The moisture and ash stability **(Hossain *et al.,* 2016)** confirms these components are less responsive to rearing environments, emphasizing RAS's advantages for producing nutritionally optimized *H. fossilis*. Among the fish species in aquaculture in Bangladesh, *H. fossilis* is particularly important. While various research studies have been carried out over the years focusing on aquaculture production, policy, and climate change-related issues **(Haque *et al.,* 2014; Bremer *et al.,* 2016; Alam *et al.,* 2019; Aziz *et al.,* 2021; Haque *et al.,* 2021; Aziz *et al.,* 2022; Siddique *et al.,* 2022; Aziz *et al.,* 2024)**, biological research on this species remains relatively limited. Nevertheless, such studies have the potential to play a vital role in its sustainable development. Therefore, greater emphasis should be given to this area of research in the future.

One limitation of this study is that while water quality parameters were rigorously controlled and analyzed, the potential influence of dietary factors on hematological and nutritional outcomes in *H. fossilis* was not independently assessed. Although both systems received nutritionally balanced feeds (35% protein), subtle differences in feed intake, nutrient assimilation, or the inclusion of live tubificid worms in the RAS may have contributed to physiological variations. Given the established role of diet in fish health, future studies should incorporate standardized dietary controls or nutrient tracers to disentangle its effects from water quality. This limitation underscores the need for cautious interpretation of our findings while highlighting a critical avenue for further research in aquaculture optimization.

CONCLUSION

The study presents a comprehensive comparison of the nutritional composition and hematological parameters of *H. fossilis* cultured in RAS and traditional pond systems, emphasizing the influence of water quality and environmental stability on fish health and nutritional outcomes. The findings underscore the advantages of RAS, where controlled conditions foster superior water quality, evidenced by stable temperature, dissolved oxygen, and pH levels, as well as consistent alkalinity and TDS. These optimal environmental conditions contributed to enhanced physiological and metabolic performance in RAS-reared fish compared to their pond-reared counterparts.

Key hematological parameters, including higher red blood cell counts and hemoglobin levels in RAS fish, highlight improved oxygen-carrying capacity and metabolic efficiency. Conversely, elevated WBC counts and blood glucose levels in pond fish suggest heightened stress and immune responses due to fluctuating environmental conditions. Such physiological stressors in pond systems likely stem from seasonal temperature variations and dissolved oxygen levels, as well as increased exposure to potential pathogens. Nutritional composition analyses revealed significant differences between the two systems. RAS-reared fish exhibited higher crude protein and lipid levels, reflecting efficient nutrient utilization and reduced stress. In contrast, pond-reared fish demonstrated elevated carbohydrate levels, likely resulting from stress-induced glucose mobilization. Moisture, ash, and crude fiber content showed no significant variations, indicating their relative stability across both rearing environments.

The findings underscore the advantages of RAS in promoting fish welfare, optimizing growth, and producing nutritionally superior fish. By minimizing environmental stressors and enhancing water quality, RAS enable consistent production of high-quality fish, suitable for both commercial and sustainable aquaculture. The study highlights the potential for RAS to address challenges associated with traditional pond aquaculture, particularly in regions vulnerable to environmental fluctuations and climate variability.

This research validates the benefits of controlled aquaculture environments like RAS in enhancing fish health and quality while mitigating the adverse effects of variable pond conditions. These insights provide valuable guidance for aquaculture practitioners seeking to optimize production systems and meet the growing demand for sustainable, high-quality fish products.

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