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Short Communication

Temperature-driven shifts in haematology and erythrocyte morphology of Nile tilapia *Oreochromis niloticus*

A. Sen[1], S. K. Nath[1], T. J. Abraham[1*], R. Das[1], P. Sinha[1], A. Ghorai[1], and P. K. Patil[2]

[1]Department of Aquatic Animal Health, Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata - 700 094, West Bengal, India; [2]Aquatic Animal Health and Environment Division, ICAR- Central Institute of Brackishwater Aquaculture, Raja Annamalai Puram, Chennai - 600 028, Tamil Nadu, India

Abstract

The present study investigated the haematological responses of *Oreochromis niloticus* to seasonal temperature variations, critical to understanding their physiological health under changing environmental conditions. Fish were acclimatised in controlled laboratory settings, and blood samples were analysed during the summer and winter seasons. The results indicated significant reductions in total erythrocyte counts (7.22%), total leukocyte counts (14.28%), haemoglobin (20.20%), haematocrit (8.84%), mean corpuscular volume (2.04%), mean corpuscular haemoglobin concentration (12.38%), and mean corpuscular haemoglobin (14.24%) during the winter compared to summer. Additionally, erythrocyte cellular and nuclear changes, such as crenation, tear-drop shaped, sickle-shaped and elongated cells, notched nucleus and vacuolation in cytoplasm, were observed, indicating low temperature induced stress and bioresponses. The results emphasised the necessity of monitoring haematological parameters as vital indicators of fish health and environmental stress.

Keywords: Aquaculture, Erythro-morphology, Haemogram, Temperature stress

Highlights

- Low temperature altered the haematology and erythro-morphology of tilapia
- It emphasised the need for routine haematological monitoring to ensure fish health
- Understanding fish physiology is crucial for developing effective management strategies

Globally, Nile tilapia, *Oreochromis niloticus*, is a commercially important fish species due to its rapid growth and nutritional value. It accounts for 80% of total tilapia production across 140 countries. As the third most farmed fish group after carps and catfish, tilapia holds a 10.6% share in global aquaculture (Food and Agriculture Organization [FAO], 2024). Its adaptability to a wide temperature range (22-30°C) makes *O. niloticus* ideal for studying thermal effects on fish physiology, particularly haematological parameters. Tilapias typically struggle to survive for more than a few days when exposed to ≤10°C, and their growth is notably hindered at ≤16°C and as high as 40°C (Abd El-Hack et al., 2022). Haemato-biochemical parameters are sensitive indicators of fish health, which enable a comprehensive understanding of the physiological and pathological state of fish in response to environmental stressors (Islam et al., 2020). Several fish, including *O. niloticus*, demonstrated varying haematological responses to environmental stressors, underscoring the link between water quality and fish health (Shahjahan et al., 2020; Witeska et al., 2023). These results confirmed the need for more investigation to clarify the intricate relationships between fish health and temperature. The current study evaluated the systematic changes in haematological responses and erythro-morphology of *O. niloticus* to the variations in water and ambient temperatures during the summer and winter seasons.

Healthy *O. niloticus* (15.50±0.78 g; 8.78±0.39 cm) were netted from a grow-out farm in Sonarpur, South 24 Parganas district and shifted to the Faculty of Fishery Sciences, Kolkata, West Bengal, in aerated double-layered bags. Fish were acclimatised in 500-L circular tanks with aeration for 15 days and stocked at 50 per tank, in triplicate, during the summer and winter experimental trials. A commercial floating pellet feed (Cargil India Pvt Ltd, India) was offered thrice daily at 2% body weight in equal rations. At 3-day intervals, nearly half of the tank water was changed. With minimal handling stress, three tilapias/tank were netted and anesthetised using clove oil at 40 μL/L water, and blood was drawn from the caudal vein using a 2 mL syringe (0.55×25 mm needle). Approximately

[*]Corresponding Author, E-mail: abrahamtj1@gmail.com

0.50±0.06 mL of blood/fish was collected and pooled/ replicates. A 5% ethylene diamine tetra acetic acid (EDTA) solution was used as an anticoagulant during blood collection. Blood samples were immediately transferred into 2 mL micro centrifuge tubes pre-rinsed with EDTA to prevent coagulation, and then used for haematological analysis at a 7-day interval during the summer (April-May) and winter (January-February) trials for 28 days each (Bardhan et al., 2022; Das et al., 2022). The fish behaviour, feed intake, mortalities, and biomass were recorded regularly. Ambient and rearing water temperatures were recorded daily using an Oakton multi-parameter tester 35 series (68X441601), and the average daytime temperature was used for the data analysis.

The enumeration of total erythrocyte counts (TECs), total leucocyte counts (TLCs), and thrombocyte counts (TCs) of fish blood was made in Neubauer's counting chamber using Hayem's fluid, Shaw's WBC diluting fluid, and Rees-Ecker diluting fluid, respectively. Haematocrit (Ht) was measured via micro capillary centrifugation (7 cm × 11 mm capillary tubes; HiMedia, India) at 10,000 rpm for 5 minutes, and haemoglobin (Hb) was determined using Sahli's acid-hematin method in a haemometer. Subsequently, these blood parameters were utilised in the computation of haematological indices like mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) (Das et al., 2022). Blood smear slides were prepared following the general protocol. The methanol fixed slides were Giemsa-stained, allowed to set for 5 minutes, washed with phosphate buffer and air-dried. The Giemsa-stained blood smears were qualitatively examined under the immersion oil lens in a microscope (Bardhan et al., 2022). Six blood smear slides were prepared for each sampling day, and the entire fields were observed from each slide. Descriptive cell visualisation was facilitated by an advanced trinocular research microscope (Olympus, Japan, Model: BX51) equipped with an SCO-LUX camera of 16 MP, which was connected to both the microscope and a computer. The data on haematology and haematological indices, in mean±standard deviation, were analysed by Student's t-test. The statistical analyses were performed using the Statistical Package for Social Sciences (IBM-SPSS), version 22.0, considering the probability level of $p<0.05$.

During the summer, the average water and ambient temperatures were 27.0±1.8°C and 30.5±1.2°C, respectively. While in winter, the respective water and ambient temperatures were 17.0±1.2°C and 18.9±1.4°C. In summer, the fish demonstrated active and aggressive feeding behaviour and were evenly distributed throughout the water column without displaying any abnormal actions. In winter, feed intake declined by 5±1%, and the fish were observed to remain at the surface with noticeably reduced activity during daytime. The recorded mortalities were 0.66±0.57% and 2.00±0.00% in summer and winter, respectively (Fig. 1). The increment in tilapia biomass was markedly less in winter compared to summer. The TECs and TLCs were significantly declined by 7.22% and 14.28%, respectively, in winter ($p<0.05$), while the reduction in TCs (2.26%) was insignificant ($p>0.05$). The levels of Hb and Ht were significantly decreased by 20.20% and 8.84%, respectively ($p<0.05$). The indices like MCV, MCHC and MCH experienced a decline of 2.04%, 12.38% and 14.24%, respectively, in winter

Table 1. Variations in the haematological parameters and indices of Nile tilapia *Oreochromis niloticus* due to temperature

Haematological parameters and indices	Summer (27.0±1.8°C)	Winter (17.0±1.2°C)	Mean decrement (%)
Total erythrocyte counts (TECs) ($\times10^6/\mu L$)	1.73±0.06	1.61±0.06*	7.22
Total leucocyte counts (TLCs) ($\times10^3/\mu L$)	1.26±0.12	1.08±0.08*	14.28
Thrombocyte counts (TCs) ($\times10^6/\mu L$)	3.09±0.05	3.02±0.08	2.26
Haemoglobin (g/dL)	5.89±0.52	4.70±0.45*	20.20
Haematocrit (%)	23.88±2.28	21.77±2.01*	8.84
Mean corpuscular volume (MCV) (fL)	13.80±0.90	13.52±0.88	2.04
Mean corpuscular haemoglobin concentration (MCHC) (%)	24.65±1.08	21.60±0.98*	12.38
Mean corpuscular haemoglobin (MCH)(pg)	34.04±0.23	29.19±0.37*	14.24

Each value represents an average of at least 8 observations. The winter data with an asterisk (*) for a specific parameter differed significantly ($p<0.05$)

compared to summer (Table 1).

The drop in MCHC and MCH was significant ($p<0.05$). The tilapia blood samples in summer had normal elliptical erythrocytes with intact nuclei (Fig. 2A). However, the erythrocytes recorded morphological changes in winter (Figs. 2B-G). Erythrocytes' altered structure included crenation at the cell wall's margins and sickle-shaped cells (Fig. 2B), crenations on cells, teardrop-shaped cells, elongated cells, vacuoles inside of cells, hypertrophied nuclei, blebbed nuclei, notched nuclei, and binucleated cells (Figs. 2C-G).

Haematological parameters, particularly Hb and TECs, are essential for evaluating the oxygen-carrying capacity of blood and the fish's overall health (Witeska et al., 2023). Thermal stress outside the optimal range (24-30°C) can significantly alter these parameters, by causing hypoxia or anoxia, reducing Hb and TECs in *O. niloticus* (Islam et al., 2020), disrupting erythropoiesis (Panase et al., 2019) and suppressing erythropoietin production (Qiang et al., 2013). Optimal temperatures, therefore, enhance oxygen availability and metabolism, supporting higher Hb and TECs. Our study revealed a significant decline in Hb and TECs in *O. niloticus* at ≤17°C, likely due to cold-induced physiological stress impairing haematopoiesis. Also, the TLCs are key indicators of fish immune health, enhancing innate or nonspecific immunity, reflecting responses to biological (Ndong et al., 2007) and environmental stressors (Gao et al., 2021). Under optimal temperatures, TLCs support immune functions like phagocytosis and cell-mediated immunity, but deviations can suppress immunity (Gao et al., 2021; Witeska et al., 2023), as was recorded in this study with a 14.28% decline in TLCs in winter. The current findings align with Slater and Schreck (1998), who suggested that acute temperature shifts can suppress immune function. In *O. niloticus*, TCs remained relatively stable across seasons, with only a 2.26% decrease in winter, unlike the more temperature-sensitive TECs and TLCs. This stability was relatively resilient as TCs play critical roles in immune responses and haemostasis (Jerônimo et al., 2011) and may reflect an adaptive response to reduced metabolic and immune activity at lower temperatures. The Ht showed a significant seasonal variation, with levels decreasing by 8.84% in winter, possibly attributable to decreased metabolic rates (Gabriel et al., 2015), reflecting impaired oxygen transport and physiological stress (Witeska et al., 2023). In contrast, no significant change in Ht with temperature was reported in other fish (Mulhollem et al., 2015). The reduced winter-feeding rates, as observed in this study, can further affect energy reserves and haematological health (Nyauchi et al., 2025). The decline in MCV (2.04%) suggested reduced erythrocyte size and maturation, likely linked to seasonal temperature stress (Bardhan et al., 2022). The more pronounced decrease in MCHC (12.38%) indicated a reduction in Hb relative to the volume of TECs, potentially reflecting impaired Hb synthesis due to metabolic or nutritional stress. The observed 14.24% reduction in MCH may be due to seasonal temperature variations and their effects on nutrition, as environmental influences on metabolic rates can lead to substantial changes in blood parameters (Witeska et al., 2023).Thus, maintaining optimal temperature is vital for fish welfare and effective aquaculture management.

In the present study, summer temperature favoured a normal elliptical erythrocyte with the intact nucleus, characteristic of healthy *O. niloticus*. The low temperature stress significantly affected erythrocyte morphology and haematopoiesis, similar to Shahjahan et al. (2020), which may lead to compromised oxygen transport and overall fish health. The winter exposure illustrated the direct impact of temperature on erythrocyte structure, notably cell crenations, where the cell membranes appeared scalloped at the margins, and the formation of sickle-shaped cells indicated dehydration or osmoregulatory challenges, and disruption in the osmotic balance within the bloodstream. The alterations in the cellular membrane can lead to aberrations in the normal shape of erythrocytes, converting them into crenated forms (Wong, 2013). Cooler temperatures can also reduce the fish's metabolic rates, leading to ionic imbalance within the blood, subsequently affecting osmotic pressure and osmotic fragility of erythrocytes (Lu et al., 2016). This creates a condition where erythrocytes may lose water to a hypotonic extracellular environment, resulting in crenation (Magill & Sayer, 2004). The presence of notched nuclei and elongated cells indicated structural compromise due to stress responses. The erythrocytes with vacuoles and hypertrophied nuclei suggested cellular damage due to the heightened metabolic stress under cold conditions. The appearance of binucleated cells and blebbed nuclei could signify an upregulation of cell turnover processes in response to environmental stressors (Seebacher & Kazerouni Ghanizadeh, 2021). The presence of elongated and teardrop-shaped erythrocytes emphasised the adverse physiological responses likely driven by low temperature exposures, highlighting potential weakened defence capabilities against pathogens during colder months (Lahnsteiner, 2020).

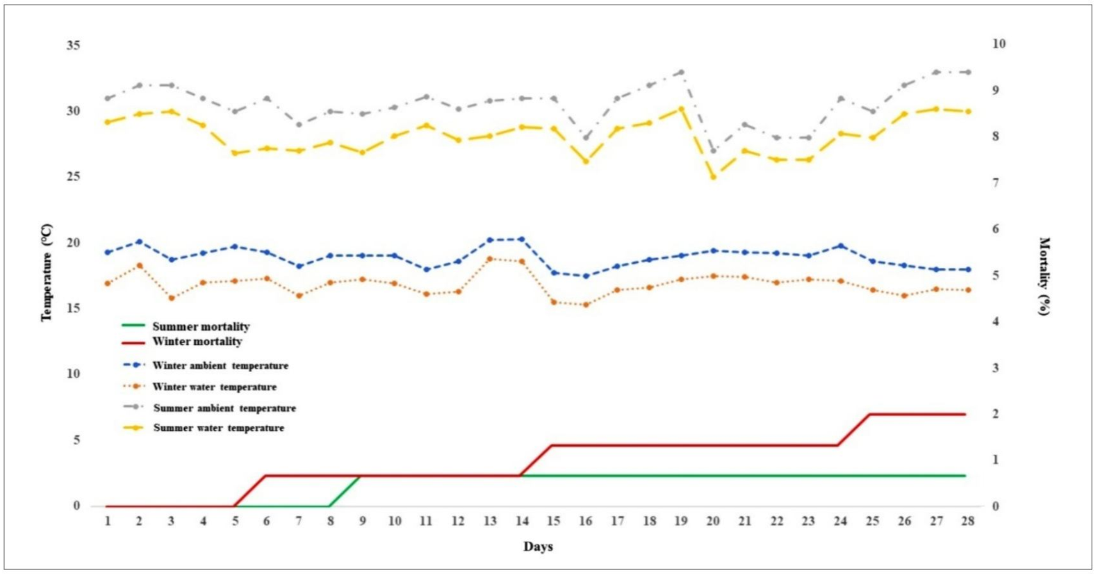

Fig. 1. Mortalities and temperature variations observed during the experimental period

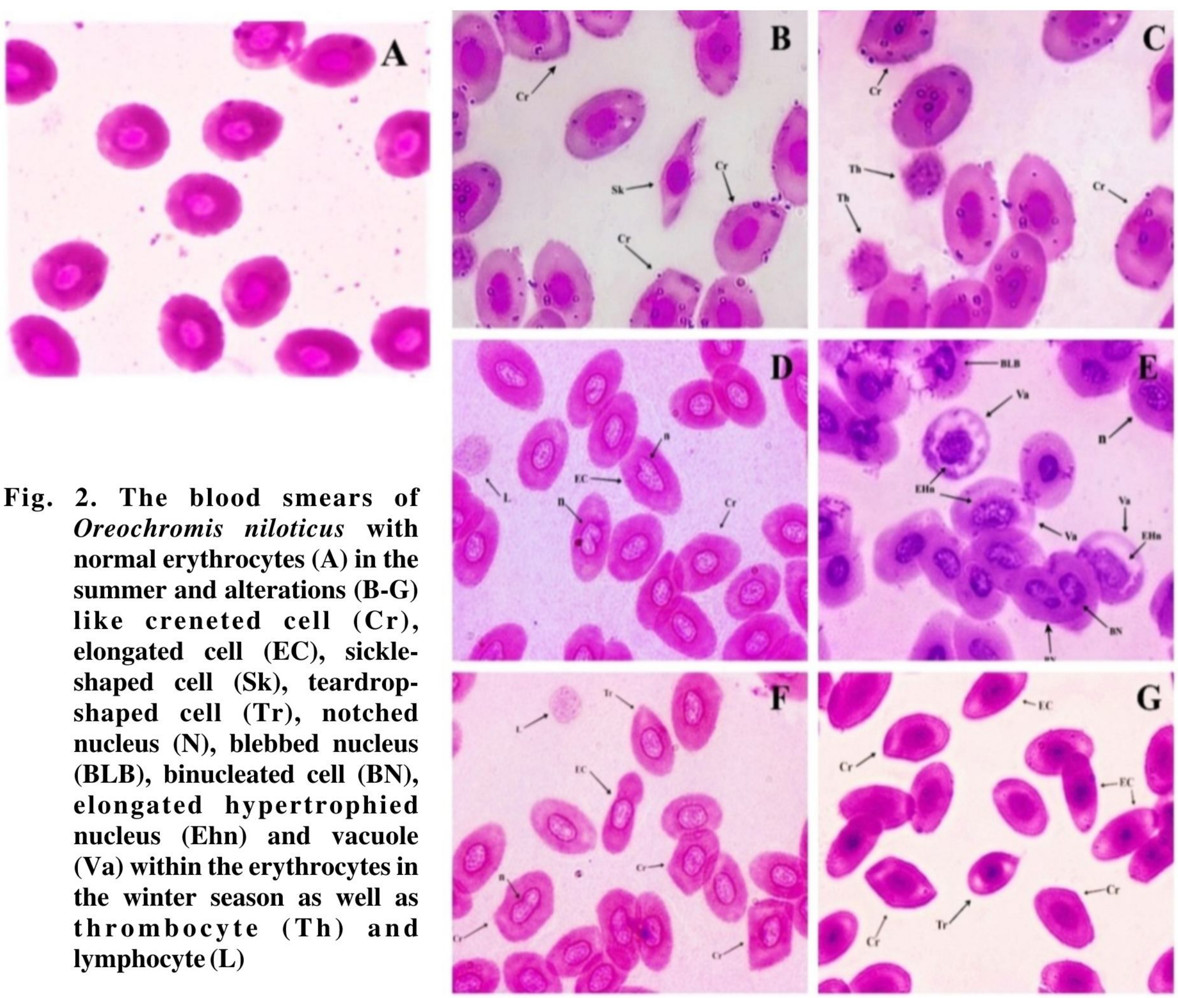

Fig. 2. The blood smears of *Oreochromis niloticus* with normal erythrocytes (A) in the summer and alterations (B-G) like creneted cell (Cr), elongated cell (EC), sickle-shaped cell (Sk), teardrop-shaped cell (Tr), notched nucleus (N), blebbed nucleus (BLB), binucleated cell (BN), elongated hypertrophied nucleus (Ehn) and vacuole (Va) within the erythrocytes in the winter season as well as thrombocyte (Th) and lymphocyte (L)

These alterations in erythrocyte morphology are indicative of impaired cellular functions.

Overall, temperature fluctuations significantly influenced the haematological parameters and erythrocytes of *O. niloticus*. Notable changes in Hb, Ht, TECs, TLCs, related indices and erythro-morphology under low-temperature exposure underscored the impact of thermal stress on haematopoiesis, oxygen transport, and overall resilience. These findings highlight the importance of monitoring blood parameters as indicators of fish health in aquaculture. Understanding these temperature-driven changes is vital for developing strategies to maintain fish welfare and productivity, especially amid climate change.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's contribution: AS, SKN, RD, PS, AG: Execution of wet laboratory experiments, laboratory investigations, formal analysis, generation of data, statistical analyses and drafting the manuscript; TJA: Conceptualisation, experimental design, data interpretation, writing, review and editing the manuscript; PKP: Resources, project administration and funding acquisition.

Data availability statement: The data supporting this study's findings are available on request from the corresponding author.

Ethical approval: The current study was performed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocols were approved by the ICAR, Government of India, New Delhi, under the All-India Network Project on Fish Health (F. No. CIBA/AINP-FH/2015-16 dated 16.7.2015).

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