Research Article

Comparative Hematological Profile of Three Species of *Schizothorax* Group Found in the Streams of Himalayan Region

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ABSTRACT

Assessing hematological parameters offers a comprehensive evaluation of the health condition of fish. The present study evaluated the variations in hematological parameters, including Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC), in three species of *Schizothorax* fish *(S. richardsonii, S. plagiostomus, and S. progastus*). The hematocrict values of the three species differed significantly (*S. richardsonii* 25.5±2.3 has the lowest hematocrit value compared to *S. progastus* 27.5±2.6 and *S. plagiostomus* 29±4.6). *S. plagiostomus* had considerably greater Hemoglobin (Hb) and Red Blood Cell count (RBC) levels compared to *S. richardsonii* and *S. progastus*. The results revealed significant ($p<0.05$) variations among the three species. The MCHC value ranged between 34.1%, 35.0%, and 35.9% indicating an insignificant ($p>0.05$) difference. Therefore, the baseline data produced during this study can be efficiently utilised to monitor the health condition of coldwater fishes in order to preserve them sustainably in aquatic systems under the scenario of climate change.

Keywords: Coldwater fishes, *S. richardsonii, S. plagiostomus, S. progastus*, Health status Hematological parameters.

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INTRODUCTION

Aquaculture, commonly referred to as fish farming, is increasingly becoming popular and significantly influencing the world economy. People from diverse backgrounds, regardless of their money, age, or health, consume fish because of its exceptional nutritional value and biologically active chemicals that have beneficial impacts on human health.[1] The world's population can be fed by aquaculture in the future, but this depends on the industry's capacity to reduce the dependence on wild fish, on ecosystem productivity changes brought about by climate change, and on aquaculture's ability to develop and grow sustainably. Aquaculture now accounts for 50% of global fish consumption, and it is expected that aquaculture will be the primary supplier of fish by 2030, due to consumer demand and the depletion of wild catch fisheries. Efficient husbandry and overall management are vital for the production of aquatic animals in intensive culture farms, where fish species are raised at high density. In order to boost fish production, it is imperative to employ and authorise standardised, non-lethal, and cost-effective strategies for assessing fish health.[2] *Schizothorax* spp., which are found in almost over the entirety of the upper belt of the Himalayan area in India, and are among the most significant fish species due to their tasty flavor, strong nutritional value, and reasonable availability all year long, with high market demand for these species. Thus, they are highly valued fish in the Himalayas both

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commercially and economically.[3,4] because they satisfy the expanding fish demand of a large population. The Himalayan foothills are covered with abundance of different species of *Schizothorax* group and commonly known as snow trout. Among the different species of this group, three species viz. *S. richardsonii, S. plagiostomus and S. progastus* are abundant and dominant species. These are freshwater fish and found mainly in rivers and streams of the hills, belonging to the family Cyprinidae and subfamily Schizothoracinae. All these three species are extremely sensitive and prefer to live in cold, clean waters with good oxygenation. They mainly consume algal debris, plant materials, detritus, and the associated invertebrate fauna, suggesting that they are omnivores that inhabit the bottom of aquatic environments. They breed across the upper portion of the river and migrate downstream after breeding.

A valuable technique for assessing the health of diverse aquatic creatures, including farmed and wild fish, is the hematological and biochemical profile of fish.[5,6,7,8,9,10,11] Due to the fact that these indices provide valuable information regarding fish reactions to stress, pollutants, hypoxia, nutrition, habitat, ecological conditions, and physiological factors, they are utilised extensively.[12,13,14,15,16] Hematopathology tests can be greatly impacted by a number of factors, including age, diet, sex, fish species and strains, sexual maturity cycle, stocking density and feeding schedule, seasonal variations, photoperiod, nutritious state, location, disease, physicochemical variations, temperature and salinity, sampling conditions of use, type of anaesthesia, laboratory techniques, handling and transport, blood collection, blood sample storage duration, and anticoagulant use.[17,18,19,20,21,22,23,24,25] Hematological analytical evaluation will improve fish farming by enabling early diagnosis of stressful events and/or diseases that could have an impact on production efficiency.[26,27] The objective of this investigation is to give an elementary knowledge of the hematological parameters and erythrocyte morphology of three essential food fish species. (*S. richardsonii, S. plagiostomus and S. progastus*) which were taken under varied climatic conditions and feeding regimes, enabling a more precise analysis of fish blood parameters.

MATERIALS AND METHODS

Location of the study site and Experimental animal

A total of 25 adult of each species (*S. richardsonii, S. plagiostomus and S. progastus*), were collected from local streams of Kumaun region of Uttarakhand (29º 21'N and 79º 34'E, Altitute 1370 msl) and maintained in the captive condition at ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand.

Blood sample collection

The fish was initially gently raised to a comfortable handling posture with one hand and a towel; the fish should be upside down, but its tail should be closer to the person performing the procedure. On the other hand, the caudal vein, which is located directly ventral to the spinal cord, was venipunctured using a sterile plastic disposable syringe and a needle with a size 24 gauge. The blood was then collected in a vial containing anticoagulant EDTA (2 ml). The caudal vein is approached ventral or lateral at an angle of 45º to collect blood. As the needle approached the vertebral column, it was retracted slightly, allowing the blood artery covering the vertebral column to be sampled simply and quickly. The blood was immediately transferred to the EDTA Vials. For the present study following hematological parameters were analyzed viz. Hemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Hematocrit (Hct), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Mean Corpuscular Volume (MCV) respectively[4]

Statistical Analysis

The data gathered was analysed statistically using one-way Analysis of Variance (ANOVA).

RESULTS

Observed values of hematological parameters of all the three species (*S. richardsonii, S. plagiostomus and S. progastus*) are summarized in (Table 1 and Figure 1). In the study, almost all of the hematological parameters reflect the significantly difference in observed values. *S. plagiostomus S. richardsonii* and *S. progastus* may have different degrees of physiological activity and variations in the oxygen content of their individual habitats, which may account for the greater erythrocyte count and Hb concentration observed. Erythrocytes and hemoglobin content are known to have significant roles in influencing the blood's ability to carry dissolved oxygen.[13,15,28,29] Although the hematocrict values of the three species differed significantly (*S. richardsonii* 25.5±2.3 has the lowest hematocrit value compared to *S. progastus* 27.5±2.6 and *S. plagiostomus* 29±4.6). *S. plagiostomus* had considerably greater Hemoglobin (Hb) and Red Blood Cell count (RBC) levels compared to *S. richardsonii* and *S. progastus* that may have arise from various physiological activity levels and variations in

Table 1: Comparative haematological parameters of *Schizothorax sps.*

Parameters/ Species	*S. richardsonii* Mean±SE	*S. progastus* Mean±SE	*S. plagiostomus* Mean±SE
Hemoglobin (g/dl)	8.7±0.8*	10.0±0.5	10.7±1.3*
Hematocrit (%)	25.5±2.3*	27.5±2.6*	29±4.6*
RBC (10^6/µl)	1.2±0.1	1.6±0.3	1.6±0.4**
WBC (10^3/µl)	19.1±2.3	28.8±3.4*	27.4±2.9*
MCV (fl	212.5±17*	178.1±13.1	168.7±15.4
MCH (pg)	72.5±5.5*	62.5±29.8*	60.6±56.7*
MCHC (%)	34.1±0.2	35.0±5.6	35.9±6.2

*Shows significant difference (p<0.05)
**shows increased significant difference (p<0.05).

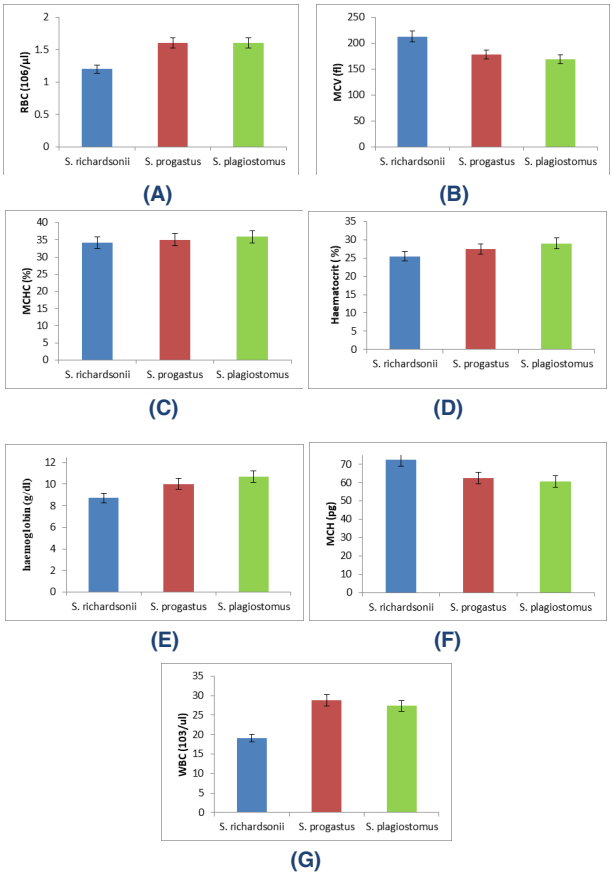

Figure 1: Assessmentof haematological parameters, (A) RBC, (B) MCV, (C) MCHC, (D) Hematocrit, (E) Hemoglobin, (F) MCH and (G) WBC in *schizothorax sps.*

the oxygen composition of the surroundings that each species inhabits. *S. progastus* had considerably higher WBC than the other two species. The WBCs are the body's defence cells.[15] WBC levels influence immunological responses and fish ability to resist illness.[30] White blood cells, which include granulocytes, monocytes, lymphocytes, and thrombocytes, play a vital function in the fish's defence system. The numbers of WBC and TLC can alter in response to environmental elements or triggers such as infection, as well as a variety of other factors ranging from fish age to species features or nutritional differences[31] The results revealed significant (p<0.05) variations among the three species hematological parameters. Red blood cell size is determined by MCV, hemoglobin content per red blood cell by MCH, and hemoglobin content per unit volume by MCHC. The value of MCH also demonstrated the noteworthy variation. During the study period, the MCHC value ranged between 34.1%, 35.0%, and 35.9% indicating an insignificant (p>0.05) difference between the three fish. When compared to *S. progastus* and *S. plagiostomus*, MCV value in *S. richardsonii* was found to be greater with a significant difference (p<0.05).

DISCUSSION

A wide range of environmental factors, including stress, nutritional status, seasons, and reproduction, have been found to exert significant effects on the hematological parameters of fish.[32] Fish can adapt to differences in ambient temperature due to their ectothermy; warm water can stimulate metabolic activity. The measurement of hematological parameters is commonly employed to assess the presence of health issues in fish[10] Additionally, it has also been found to be useful in analysing the influence of habitat alterations on fish biology.[12,17,20] Also, the normal blood parameters of some fish species living in different environments remain unknown. In the current investigation, the hematological profiles of *S. plagiostomus, S. progastus* and *S. richardsonii* were determined using standard methodology.As the temperature of the water fluctuates, the amount of oxygen in it drops. Due to the hypoxia/lower dissolved oxygen state, the fish exhibit an increase in erythrocyte number and hemoglobin content.[33,34] The diagnosis and prognosis of the sick condition of the fish population can be confirmed by the examination of hematological characteristics.[35] The elevated erythrocyte count and Hb concentration observed in *S. plagiostomus* in comparison to *S. niger* may be attributed to contrasting physiological activity levels and differences in the oxygen content of the species[36] which are in agreement with the current study. The higher the amount of Red Blood Cells (RBCs) in *S. plagiostmus* is a result of its smaller size and more active lifestyle, which is influenced by the quicker water flow in its habitat. Various fish species require specific levels of metabolic activity. This activity necessitates many physiological modifications that significantly affect the enhancement of blood

circulation to the muscle as a result of alterations within them. Hematocrit demonstrated a reliable correlation with fish activity.[27] Baseline information on hematological parameters amenities serve as a reference for assessment of diagnostic fresh water fish health. It has been demonstrated that the quantity of red blood cells and hemoglobin concentration vary with length and age[37] The determination of hematological parameters is greatly influenced by both external and internal variables, such as the time of year, sex, age, health status, stage of the reproductive cycle, insufficient feeding regimens, physiological factors, and individual genetics, which determine reference values for fish. Since fish have a direct relationship with their environment, water quality is a significant component that affects fish hematology. Microscopic analysis of blood smears found that red blood cells in *S. richardsonii* were more many but smaller in size, whereas erythrocytes in *S. plagiostomus* and *S. progastus* were less in number but larger in size. All of the species' erythrocytes under study had elliptical shapes, violet and purple colours, and oval nuclei in the middle. (Figure 2)

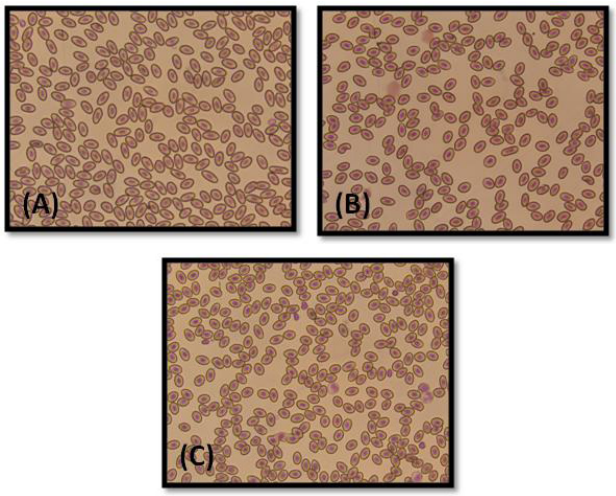

Figure 2: Blood smear of three species (A) *S. plagiostomus* (B) *S. progastus* (C) *S. richardsonii*

LIMITATIONS

The study of blood in fish is not as advanced as in other groups of vertebrates. However, analysing the blood can still provide valuable information regarding disease processes in bony fish and cartilaginous fish. Despite the challenge of limited reference values, the lack of information in interpreting fish hemograms gives an opportunity to further explore clinical pathology investigations in fish. The study provides a comprehensive analysis of the primary factors that contribute to the variability in haematological parameters of coldwater fish species. Hematological measures are recognised as helpful indicators for monitoring the health of fish, but the normal values for these parameters in different species of cultured fish have not yet been identified.

CONCLUSION

There has been an increasing interest with analysing the hematological parameters of fish blood in recent years. This can be explained since it is considered a crucial tool for aquaculture, as different fish species interact with various ecological components in their natural environments. Many factors can influence changes in hematological and biochemical parameters, including season, gender, nutritional status, age, stress, feeding regimens, and temperature. Baseline information on hematological parameters reference values is useful for diagnosing freshwater fish health conditions. It is required to understand the standard values and reference interval for blood parameters for a specific fish species in order to use these as biomarkers. The study reflects significant differences among the studied species for almost all the hematological parameters might be due to the different degrees of biological processes and variations in the oxygen content of their individual habitats. However, a baseline information on hematological parameters for three species of *Schizothorax* group have been generated that would serve as a reference to assess the identity as well as the health condition in wild condition.

RECOMMENDATION

The researchers recommends to study the hematological profiles of other related coldwater fish species found in different elevations of the regions within the Uttarakhand range.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

No ethical approval is needed.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Hb: Hemoglobin; **TEC:** Total Erythrocyte Count; **TLC:** Total Leukocyte Count; **Hct:** Hematocrit; **MCH:** Mean Corpuscular Hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **MCV:** Mean Corpuscular Volume.

SUMMARY

The Coldwater Fishery sector in India is currently undergoing a significant change and plays a crucial role in providing a means of subsistence for a substantial portion of the economically deprived population in the country. Aquaculture is one of the world's fastest expanding food producing areas. As a result of the expansion of aquaculture, the usage and validation of fish health monitoring techniques has become increasingly vital. Hematology is a branch of medicine that studies blood and blood problems. Blood parameters assessment of fish may be used as a rapid method for assessing fish health and normal values, which are still unknown for the many species of cultured fish. Hematological parameters provide useful information for understanding the link of blood properties to habitat and the species' adaptability to the environment. In ichthyologic study, hematology gives information about living habitat. It is also vital to measure the rate of feed intake and the status of fish stocks. When haematological procedures are employed as aids in fish illness diagnosis, economic losses can be avoided. As a result, hematology offers essential details for determining the taxonomic, ecological, physiological, and health conditions of fish.The study compared hematological parameters among three fish species (*S. richardsonii, S. plagiostomus, and S. progastus*). Significant differences were observed in most parameters, indicating potential variations in physiological activity and oxygen content in their habitats. *S. plagiostomus* showed higher hemoglobin and red blood cell counts, likely influenced by their habitat's oxygen levels. *S. progastus* had notably higher white blood cell counts, impacting their immune responses. While red blood cell size (MCV) and hemoglobin content per cell (MCH) varied significantly, hemoglobin content per unit volume (MCHC) showed insignificant differences among the species. *S. richardsonii* had a higher MCV compared to *S. progastus* and *S. plagiostomus*. Overall, the study highlighted distinct hematological profiles among these fish species, potentially linked to their environmental adaptations and physiological activities.

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