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Comparative study of sex related hematological parameters of *Schizothorax richardsonii* (Snow trout) in wild and farmed raised stock

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Abstract
An attempt has been made to assess the hematological profile of snow trout *Schizothorax richardsonii* a cyprinid fish of different sex, male and female. For this purpose specimen from the wild as well as the farm-raised stock has been used. Hematological studies provide diagnostic keys of various diseases and are valuable indicators of the physiological state of the organism. The value of WBC was 20.4±5×103/µl, for males and 26.4±3.7×103/µl for females with highly significant difference (p<0.05). The value for hemoglobin (Hb) for males was 8.7±1. 7(g/dl) and for females 8.4±1. 5(g/dl), which was non-significant different, and a similar trend was observed for the hematocrit (Hct) values i.e. 25.4±5.4% for males and 24.8±4.8% for females with a non-significant difference. MCH value observed in this study for male and female is 130.63±13.94pg and 77.60±8.80pg. respectively in farmed condition and 37.3±14.4pg and 49.4±16.4pg in wild stock and this might be due to anemic condition in the farmed raised female. All the parameters in wild as well as the farmed condition were in the same trend of increasing hematological parameters in the present study, though the values differ in wild and farmed raised stock of snow trout *Schizothorax richardsonii* due to the different environmental conditions.

Keywords: Snow trout, anemic, hemoglobin, farmed raised wild stock

Introduction
The term 'coldwater' generally refers to the aquatic ecosystem, which maintains thermal and oxygen levels for the well-being of trout, mahseers, snow trouts, and other minor species. In the Indian subcontinent, coldwater fishes are generally confined to the Himalayan and sub-Himalayan zones in the north and watersheds draining the southern slope of the Deccan plateau [26]. These water resources harbor about 272 fish species belonging to 21 families and 76 genera in the country of which 203 are recorded from the Himalaya while 91 from the Deccan Plateau [24]

Snow trout is an indigenous cold water fish and is endemic to the Himalayas that belong to the family Cyprinidae and subfamily Schizothoracinae. Their distribution is mainly restricted to mountain regions of Asia-mainly the highlands (above 670 masl) of Himalaya and Central Asia [18].

Blood acts as a vehicle for quickly mobilizing defense against trauma and diseases. Since fishes differ considerably in their activity patterns and respond to the pollutant. Blood is a fluid connective tissue circulating in the body. It provides one of the methods of communication between the cells of different parts of the body. Similar to other vertebrate fish blood consists of plasma and cellular components. [9] Suggested that the differences in hematocrit other hematological indices among sex might be due to higher metabolic rate and hormonal activity of male fish as compared to the female fish. [12] found males showed a higher hematocrit value than females in Indian shad fish and are in agreement with results from other fish species *Cyprinus carpio Tilapia zilli*. [2, 8, 22] considered that the differences might be due to the higher metabolic rate of males compared to females.

Materials and Methods
Wild stock from nearby stream and farm-raised fish from DCFR Bhimtal, Uttarakhand was used for the observation of hematological characteristics. A total of 200 *S. richardsonii* from wild (50 male and 50 female) and (50 male and 50 female) from farmed raised stock. The wild stock was acclimatized for two days and then the blood sampling was done.

Blood sampling

The blood samples were collected using a sterile plastic disposable syringe of needle size 20 and 24 gauge already moistured with EDTA (2ml) by venipuncture of caudal vein, which lies just ventral to the spinal cord, the blood is collected either by the lateral or ventral approach of the caudal vein. The blood was immediately transferred to the anticoagulant vial (EDTA Vials). After the collection of blood following hematological parameters were analyzed for the present study, Hemoglobin (Hb), Total Erythrocyte Count(RBC), Total Leukocyte Count(WBC), Hematocrit (Hct), Mean Corpuscular Hemoglobin(MCH), Mean Corpuscular Hemoglobin Concentration(MCHC), and Mean Corpuscular Volume(MCV) respectively. Simultaneously the water parameters were also analyzed during the study period under the captive condition. Peripheral blood film (PBF) tests were performed on thin blood smears fixed in methanol and stained with Leishman's stain.

Results and Discussion

Gender wise variation in hematological parameters

In captive-reared specimens, observed values of gender-wise hematological parameters in *S. richardsonii are* summarized in Table 1 and depicted in Fig.1. Observed data showed a highly significant difference ($p<0.05$) for total erythrocyte count between male and female *S. richardsonii*. A similar significant difference ($p<0.01$) was also observed for MCV and MCH. However, other parameters such as WBC and MCHC showed variation in hematological parameters but not significant on the statistical scale for the male and the female specimens.

Table 1: Gender wise hematological parameters in captive reared *S. richardsonii*.

Hematological parameters	Male Mean± SD	Female Mean±SD
Hb(g/dl)	8.74±0.33	8.15± 0.56
Hct (%)	24±1.65	25.88±0.95
RBC(×106/ µl)	1.21±0.10**	0.90±0.10**
WBC(×103/µl)	15.99±1.02	17.01±0.87
MCV(fl)	227.94±25.57*	366.56±42.44*
MCH(pg)	77.60±8.80*	130.63±13.94*
MCHC (%)	33.89±0.09	36.92±1.83

** showed difference at ($p<0.05$) level, * at ($p<0.01$) level

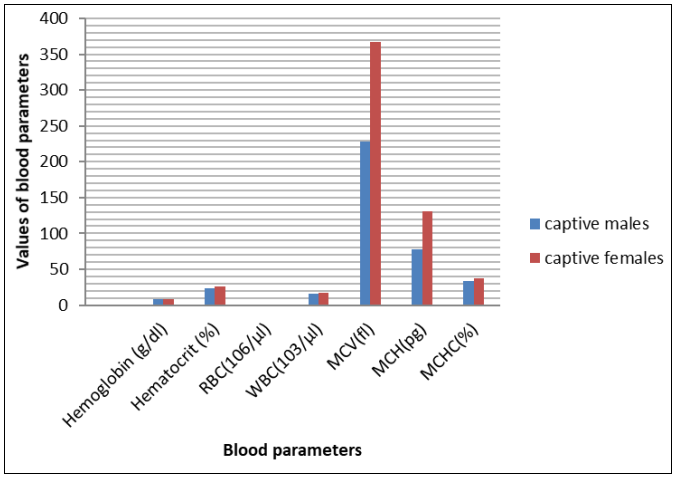

Fig 1: Gender wise comparative hematological parameters in captive reared *S. richardsonii*.

In wild specimen (Table 2, Fig. 2), the mean value of RBC for male and female was observed as 1.7± 0.6×106/µl and 0.90±0.4×106/µl respectively with no significant difference. The value of WBC was 20.4±5×103/µl, for males and 26.4±3.7×103/µl for females with highly significant difference ($p<0.05$). The value for hemoglobin (Hb) for male was 8.7±1.7(g/dl) and for females, 8.4±1.5(g/dl), which (showed) non-significant different and similar trend was observed for the hematocrit (Hct) values i.e. 25.4±5.4% for males and 24.8±4.8% for females with non- significant difference. MCV value showed higher variation between males and females and the observed values were 109±42.1fl and 146±49.1fl, respectively for males and females. A significant difference was also observed for MCHC as 34±0.8% and 33.9±0.9 % for males and females, respectively.

Table 2: Gender wise hematological parameters in wild *S. richardsonii*.

Hematological parameters	Male Mean±SD	Female Mean±SD
Hb(g/dl)	8.7±1.7	8.4±1.5
Hct (%)	25.4±5.4	24.8±4.8
RBC(×106/ µl)	1.7± 0.6	0.90±0.4
WBC(×103/µl)	20.4±5.0*	26.4±3.7*
MCV(fl)	109±42.1*	146±49.1*
MCH(pg)	37.3±14.4*	49.4±16.4*
MCHC (%)	34±0.8	33.9±0.9

* showed difference at ($p<0.01$) level

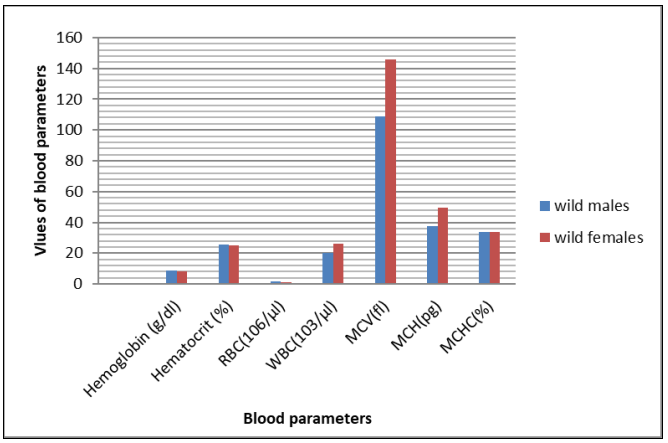

Fig 2: Gender wise comparative hematological parameters in wild *S. richardsonii*

In general, data reveal that male specimen have comparatively higher total erythrocyte count (RBC), higher level of hemoglobin (Hb), lower total leukocyte count(WBC), lower mean corpuscular hemoglobin(MCH) and lower level of mean corpuscular volume (MCV) in comparison to females.

Results of the present study are in conformity of [7] which believed that gender has a great influence on hematology of fish and included it among factors influencing fish hematology. Similar trends have also been reported by [15] in golden mahseer (*Tor putitora)*, and [25] in *Labeo rohita.* As per the results of present study, [8] also reported higher erythrocyte count in males than females in case of *Tilapia zillii.* [9] Advocated that variation in hematocrit value and other hematological parameters between sexes and diversity might be due to higher metabolic rate of males compared to the females. These differences in hematology between males and females may be related to differential oxygen demand by sex, which in turn can be linked to reproductive activity [27].

WBCs in female were higher than the levels measured in the

males, which indicate egg carriage stage, infection, or adverse condition in female but no significant difference was there according the present study. The other workers also correlated the differences in the WBC counts to biotic (age, maturity, sex, pathogens) and abiotic (water temperature, pH, dissolved oxygen content) factors and in particular to stress [21]. Probably due to the differential genetic makeup of male and female fish, female fish have higher WBC value throughout the different seasons; however, Leukocyte levels in the blood may also fluctuate according to environmental quality, nutritional status and the presence of infectious agents [16, 4, 17]. Apart from TLC considering the sexual characteristics, many workers demonstrated that male fish attributes higher values in approximately all hematological parameters in female fish [5, 19, 13] Observed sex related cyclic variation in blood of *H. fossilis*. Similar trend in haematological values were recorded for *Labeo rohita* [25] *Anguilla anguilla* [3] *Tor putitora* [15] and *Clarias gariepinus* [11] which are in agreement with the present study. [6] investigated the sex related variations of some blood parameters of an exotic fish, *Sarotherodon mossambica* and observed that the TEC, Hb and PCV values were highest in males compared to females.

In the present study, there is a comparison between the male and female *S. richardsonii* under farmed and wild condition, secondary blood indices (MCV, MCH) also showed wide range of physiological variation. However, MCHC values did not show any marked difference between sexes though, there was difference in MCHC but the difference was not statistically significant under farmed condition in our results during the study period in wild and farmed raised stock. MCV value reflects the size of red blood cells by expressing the volume occupied by a single red blood cell. The present study shows significantly higher value of MCV in female compared to male. MCH value observed in this study for male and female is 130.63±13.94pg and 77.60±8.80pg respectively in farmed condition and 37.3±14.4pg and 49.4±16.4pg in male and female wild stock and this might be due to anemic condition in farmed raised female. Higher MCH value in female than male indicates higher likelihood of occurrence of anemia in females than in males [23].

All the parameters in wild as well as farmed condition were in same trend of increasing hematological parameters in the present study, though the values differ in wild and farmed raised stock of snow trout *Schizothorax richardsonii* due to the different environmental conditions.

The hemotological profile of *Schizothorax richardsonii* in relation to sex is significant in diagnosis the health status of male and female fishes, as well. The same species such changes may be influenced by the breeding seasons or other intrinsic/extrinsic factors such as pollution or other stress conditions. Higher values of some hematological indices for male than females is due to the hormones Testosterones, which stimulate its production, the female hormones while estrogens, have a suppressing effect on erythropoietin, which is responsible for changes in some of blood parameters [20, 1, 10].

Conclusion

Results of the present study, which is a data inventory of the hematological parameters, can be used for developing a diagnostic methodology for direct assessment of the environmental and physiological stress on male and female population of fish. Though, the present findings provides baseline values, further, which can be used to draw conclusive remarks against the health status and wellness of *Schizothorax richardsonii*, for breeding performance.

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