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Variation in Hematological Parameters of Snow Trout (*Schizothorax richardsonii*) During Gonadal Maturity and Breeding

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Abstract: This study was focused to investigate comparative hematological changes in *Schizothorax richardsonii* in relation to reproductive activities. The values of Red blood cells (RBC) were significantly higher in both the sexes during the immature stage as compared with maturity phase. Female brooders reflect higher count of WBC in females during both the phases. However, no significant difference was observed in White blood cell (WBC) count during immature and mature stage. Mean corpuscular volume (MCV) and Mean corpuscular hemoglobin (MCH) showed significant difference ($P < 0.05$) in both the phases of maturity. The values of MCH were at higher side in males during the maturity phase. Hematological values of Hematocrit (Hct), Total erythrocyte count (TEC)/Red Blood Corpuscles and Mean corpuscular hemoglobin concentration (MCHC) were significantly higher in post-spawning phase than the pre-spawning phase. MCHC values of males are in higher side during pre-spawning phase than the post-spawning period, while higher values of MCHC were observed in females during post-spawning phase. Present study reveals that MCV is at higher side in female during pre spawning phase expressing that it is due to lower Hct in females as MCV reflects the volume occupied by a single RBC. Present findings reflect the major factors responsible for the variation in hematological parameters during reproduction of fish.

Keywords: Hematology, Spawning, *Schizothorax richardsonii*, Pre-spawning, Post-spawning

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Introduction

Fish play an important role for the nutritional security on global level having source of energy, long-chain omega 3 fatty acids, vitamin A and several micronutrients. Hence, fisheries and aquaculture is a fastest growing food producing sector over the past three decades and plays a

key role in meeting the rising demand for fish and fishery products (Sawin *et al.*, 2007). Like other animals, fish also enjoy a good environment and has a relationship with their environment with respect to habitat and different biological needs (Guerriero *et al.*, 2003;

Borkovic *et al.*, 2008; Acharya and Mohanty, 2014; Giannetto *et al.*, 2014; Maisano *et al.*, 2016) Fish pathology which is a major component of aquaculture production has generated growing interest in the hematology of fish (Zexia *et al.*, 2007). Among hematological indices, blood cells are one of the best indicators of body health. They are extremely important in the immune system (Zexia *et al.*, 2007). The study of fish hematology has contributed significantly to our understanding of the comparative physiology, phylogenetic relationships, habitat, habit, food selection, and other significant eco-biological parameters of fishes (Ayotunde and Ochang 2006). Being aquatic vertebrate, hematological profile of fish is influenced by both intrinsic and extrinsic factors (De Pedro *et al.*, 2005; Motlagh *et al.*, 2012). The influences of divergent environmental conditions and feeding habits on fish blood parameters have also recently been reported in two fish species, *Mugil cephalus* and *Carassius auratus*, using flow cytometry techniques (Parrino *et al.*, 2018). Luskova *et al.* (1995) observed variations of some parameters in *Chondrostoma nasus*, coincident with both spawning and water temperature. Bridges *et al.* (1976) had demonstrated significant seasonal differences in red blood corpuscles, occurring with relation to the reproductive activity of *Pseudopleuronectes americanus*. Therefore, the study of hematological parameters is not only useful in monitoring the health status of fish, but is also useful in understanding the relationship of fish physiology with different environments. Bastami *et al.* (2009) carried out a study to obtain a basic knowledge of the hematology and the influence of sex on some blood parameters of wild carp (*Cyprinus carpio*) spawners. Jawad *et al.* (2004) analyzed the relationship between hematocrit and body length, sex and reproductive state in the Indian *Tenualosa ilisha*.

Snow trout is an indigenous coldwater fish (family Cyprinidae; subfamily Schizothoracinae) and is endemic to the Himalayas. Their distribution is mainly restricted to mountain regions of Asia-- mainly the highlands (above 670 masl) of Himalaya and Central Asia (Mirza and Saeed, 1988). The spawning of *Schizothorax richardsonii* inhabiting in various zones of Himalayas mainly depend on the thermal regime of each drainage and flood conditions created in mountain streams due to snow melting (Jhingran and Sehgal, 1978). Though, information on hematological profile of this species is very limited, however, comparative study of sex related hematological parameters of *Schizothorax richardsonii* (Snow trout) in wild and farmed raised stock has been done by Uzma *et al.* (2020). Effects of experimental infection with *Aeromonas hydrophila* on different blood parameters and hematopoietic tissue in *Schizothorax richardsonii* was carried out by Uzma *et al.* (2020). Changes in hematological parameters of *Schizothorax richardsonii* (Gray, 1832) infected with saprolegniasis in farmed condition was also reported by (Uzma *et al.*, 2020). The purpose of this study was to know the basic knowledge of the hematological parameters in relation to reproductive activities with the aim to set the baseline data regarding the health status of brood fish which can be used to assess any impact of stress on breeding behaviour of the fish.

Materials and Methods

Location of the study and Experimental animal:

The study was conducted at ICAR-Directorate of Coldwater Fisheries Research (DCFR), Bhimtal (Latitude 29° 21'N, Longitude 79° 34'E, 1370 masl) located in Kumoun region of state Uttarakhand, India.

The study was basically focused on snow trout, *Schizothorax richardsonii* from two different

environment i.e. wild and farm raised. Specimens of more than 3 years age group (n=340) were collected during the month of August from the nearby stream and were transferred to FRP tanks having flow through system at hatchery complex. Feeding was stopped 6 h before taking the blood samples. The size of the specimen ranges from 120-230 g for males and 210-340 g for females. Sampling was done sex-wise for immature, mature, pre-spawned and post-spawned brood fish.

Blood sampling:

The blood samples were collected using a sterile plastic disposable syringe of needle size 26 gauge by venipuncture of caudal vein that lies just ventral to the spinal cord and immediately transferred into heparinized vials on ice.

Analysis of blood parameters:

For hemoglobin estimation the cyanmethemoglobin method was used (Lavanya *et al.*, 2011). The total erythrocyte and leukocyte counts were estimated using a Neubauer hemocytometer, Natt and Herrick's (1952) diluent fluid, and were calculated according to Blaxhall and Daisley (1973), Mgbenka and Oluah (2003) and Shah and Altindag (2005). Hematocrit (Hct %) was determined using heparinized microhematocrit capillaries, blood was centrifuged at 12,000 rpm for 5 min to obtain hematocrit, and values are expressed as percentages (Adebayo *et al.*, 2007). The secondary hematological parameters [MCH (pg), MCHC (%) and MCV (fl)] were obtained from hemoglobin, erythrocyte count, hematocrit data and calculated according to Dacie and Lewis (1975). Simultaneously, water quality parameters (water temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity, ammonia, nitrate-N and nitrite-N) were analyzed as per the standard methods (APHA, 1998). Peripheral blood film (PBF) test were performed on thin blood smears fixed in methanol and stained with Leishman's stain.

Statistical Analysis:

The unpaired t-test was used to determine significant ($P < 0.05$ and $P < 0.01$) differences between the two species in all the parameters measured. All the calculations were carried out using the statistical software SPSS 20.0.

Results and Discussion

Reproductive activities in fish affect physiological changes and metabolic rate of the fish. Efforts have also been done to explore the hematological variation in immature and mature stage. Hematological parameters of sexually immature and mature brood fish of the age of more than 3 years are presented in Table 1 and Figure 1, which shows the higher values of Hb and Hct in the males at immature stage. The values of RBC were significantly higher in both the sexes during the immature stage as compared with maturity phase. Female brooders reflect higher count of WBC during both the phases. However, no significant difference was observed in WBC count during immature and mature stage. WBC are the second major cells after erythrocytes in the blood of fish and are repeatedly used as indicators of health status so slightly higher trend was observed for WBC during maturity stage. Secondary blood indices MCV and MCH showed significant difference ($P < 0.05$) in both the phases indicating that high amount of energy is required for physiological activeness of male. The value of MCH was at higher side in males during the maturity phase, which refers to the average quantity of hemoglobin present in single red blood cell. As there is an inverse relation between RBC and MCV, the values of MCV were significantly ($P < 0.05$) lower in male and female at immaturity compared to maturity phase.

Orun *et al.* (2001) found comparatively higher total leukocyte, neutrophil and monocyte levels in female fish, especially in reproductive season than in male fish (Adebayo *et al.*, 2007; Swain *et al.*, 2007). Uzma *et al.* (2020) reported lower values during immature phase due to low metabolic rate.

Table 1: Hematological parameters of brood fish at immature and maturity stages

Hematological parameters	Immature stage Mean±SD		Maturity stage Mean±SD	
	MALE	FEMALE	MALE	FEMALE
Hb(g/dl)	9.7±1.1	8.4±0.8	8.7±0.8	8.5±0.9
Hct(%)	29±4.2	26.1±3.6	25.1±3.4	26.1±4.3
RBC($\times 10^6/\mu\text{l}$)	2.7±0.3*	1.6±0.3*	1.4±0.2	1.1±0.1
WBC($\times 10^3/\mu\text{l}$)	16.6±3.3**	23.6±4.5**	17.7± 3.2**	22.5±2. 3*
MCV(fl)	106.8±24.8*	165.3±26.2*	168.1± 32.2*	287.3±28.5*
MCH(pg)	35.5±3.4*	52.5±6.5*	56.8±11.2*	93.8±12.3*
MCHC (%)	34±3.2	32±3.7	34±5.6	36±4.8

**p<0.05; *p<0.01

Fig.1: Hematological parameters of brood fish at immature and maturity stages.

Jawad *et al.* (2004) reported a higher hematocrit value in males than the females in the Indian *Tenualosa ilisha*. These studies lend support to the observations of present investigation..

Hematological parameters during pre-spawning and post-spawning are illustrated in Table 2 and Figure 2. During post-spawning in *S. richardsonii*, as a result of high metabolic rate in females the hematological values of Hct, TEC and MCHC were significantly higher than the pre-spawning phase. These findings derive support from the reports of earlier investigators (Debayo *et al.*, 2007; Swain *et al.*, 2007). In this study the value of TEC is $2.7 \times 10^6/\mu\text{l}$ in male and $1.6 \times 10^6/\mu\text{l}$ in female during pre-spawning period and $2.1 \times 10^6/\mu\text{l}$ in male and $3.9 \times 10^6/\mu\text{l}$ in female

during post-spawning period. Similarly the TLC is $16.6 \times 10^3/\mu\text{l}$ in male and $23.6 \times 10^3/\mu\text{l}$ in female during pre-spawning phase and $15.8 \times 10^3/\mu\text{l}$ in male and $20.8 \times 10^3/\mu\text{l}$ in female during post-spawning phase. The observed TLC in female was significantly higher than the levels measured in the males, which indicate egg carriage, infection, adverse condition etc. (Smith, 1986). Spawning period is an important cyclical process in adult fish and blood parameters can change depending on the maturation of the gonads (Joshi, 1982; Ranzani Paiva and Godinho, 1985). From the above results it is obvious that in general, these blood parameters were higher during the post-spawning phase as compared to pre-spawning phase thus indicating that higher metabolism is

Table.2: Hematological parameters of brood fish at pre-spawning and post spawning stages

Hematological parameters	Mean±SD Pre-spawning	Mean± SD Pre-spawning	Mean± SD Post-spawning	Mean± SD Post-spawning
	MALE	FEMALE	MALE	FEMALE
Hb(g/dl)	9.7±1.5	8.4±1.3	11.3±1.7*	10.84±1.5*
Hct(%)	28.8±7.1	26.4±4.8	34.6±5.2*	32±4.8*
RBC($\times 10^6/\mu\text{l}$)	2.7±0.6**	1.6±0.4**	2.1±0.2***	3.29±0.3***
WBC($\times 10^3/\mu\text{l}$)	16.6±3.4*	23.6±3.1*	15.8±2.8	20.8±2.4
MCV(fl)	106.8±30.5**	165.2±50.5**	119.9±18.16	100.1±18.4
MCH(pg)	37.9±12.9**	53.5± 16.2**	42.4±13.3**	33.1±2.01**
MCHC (%)	32.4±2.2	31.8 ±3.0	33±2.7**	36±4.2**

*p<0.05; **p<0.01; ***p<0.001

Fig. 2: Hematological parameters of brood fish at Pre-spawning and post-spawning stages.

required in the post-spawning phase. Similarly, the MCHC values of males are higher during pre-spawning phase than the post-spawning period, which reflects higher metabolic activities of males during spawning. Higher values of MCHC in females during post-spawning phase might be due to higher metabolism. Sharma *et al.* (2017) found lower values of Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular volume (MCV) and Mean Corpuscular Haemoglobin (MCH) in the female *Cyprinus carpio* during post-spawning period. Variation in blood indices during pre-spawning period and post-spawning period might

be due to the activeness of male and relatively higher RBC counts as compared to female. Present study reveals that MCV is at higher side in female during pre-spawning phase expressing that it is due to lower Hct in females as MCV reflects the volume occupied by a single RBC. The present observations are in the conformity with Joshi (1982) and Jawad *et al.* (2004).

Conclusion

On the basis of above observations, it can be inferred that variation in various haematological parameters can be broadly correlated with reproductive activities performed by *Schizothorax*

richardsonii. Through this study, a general overview has been generated about the major factors responsible for the variation in haematological parameters during reproduction of fish. Haematological parameters have been acknowledged as valuable tools for monitoring fish health and their normal values are still undefined for the different species of cultured fish.

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