ISSN 2250-3153

Seasonal Variations in Haematological Parameters of Golden Mahseer, Tor *putitora*.

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***Abstract*-** Present study was designed to investigate any seasonal (spring, summer, monsoon, autumn and winter) fluctuations in haematological parameters in the blood of *Tor putitora*. Significant seasonal variations in the number of white and red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration have been observed. In general, higher values of RBC dependent parameters ;viz, TEC, Hb, PCV were recorded during spring, summer, monsoon and autumn, and lowest during winter. White blood cells on other hand, exhibited gradual decline during monsoon and autumn upto winter only to rise again in spring and summer. The proportions of different leucocytes though variable but lymphocytes have been observed to be the chief contributor in TLC fluctuation. Thrombocyte count was significantly higher during autumn and winter season. Relation between seasonal changes in environmental factors such as temperature and dissolved oxygen with various blood parameters have been discussed.

***Index Terms*-** Seasonal, haematological parameters and *Tor putitora*.

I. Introduction

Tor putitora is an economically valuable freshwater fish. This cyprinid, indigenous to South Asian countries is popular with consumers as a highly esteemed food fish besides its role in sport fishery. However due to overexploitation and rise of growing number of hydroelectric projects in its natural habitat (Nautiyal and Singh, 1989), mahseer population has recorded a rapid decline over last few decades and thus have been accorded 'threatened' status in India (Khan and Sinha, 2000). This prompts us to work on its various biological aspects including haematology. Haematological parameters by acting as an early biomarker gives an insight of the health status the fish (Jawad et al, 2004). Moreover, haematological tests have prved useful in detection and diagnosis of metabolic disturbances and disease in fishes (Aldrin et al, 1982). To investigate fish blood factors and their changes in response to xenobiotics, normal range of these factors must initially be measured in healthy fishes. In light of the above, presently an attempt has been made to undertake a seasonal study on some haematological parameters of *Tor putitora* so as to generate referral values as well as their variations in the environmental conditions associated with season.

II. Materials and Methods

Study Area and Data collection:

Adult *Tor putitora* (30-35 cm in length and 405-460 gm in weight) were caught from tributaries of river Tawi in sunderbani area of jammu region. Sampling period was from February 2009 to January 2010 and every month a total of 10 fish were captured using cast net. Immediately after capturing, 2 ml blood was taken by cardiac puncture using heparanized syringe.

Haematological Analysis:

TEC and TLC was made using an improved Neubauer haemocytometer (Shah and Altindag, 2004). Hb was estimated by Sahli's haematin method. PCV was estimated by Wintrobe tube method. MCV (fl), MCH (pg) and MCHC (%) were calculated using following formulae:

$$MCV = PCV \times 10 / RBC\ count$$
$$MCH = Hb / RBC\ count$$
$$MCHC = Hb \times 100 / PCV$$

Determination of DO and Water temperature:

Water temperature values were recorded by mercury bulb thermometer. DO of the water was determined by sodium azide modification of winkler's method (A.P.H.A, 1985).

Determination of Gonadosomatic index:

G.S.I was calculated as- G.S.I= Gonad weight/Fish weight×100

Statistical Analysis:

Statistical analysis was performed with SPSS version 10.0 for windows (SPSS,1996). Data was presented as Mean±Standard deviation (S.D) of the mean and analyzed by one way analysis of variance (ANOVA).

III. Results and Discussion

Erythrocytes-

The results obtained (tab. 1) very clearly indicate rise in RBC dependent parameters; viz, TEC, Hb and PCV from spring season onwards till monsoon which are consequently followed by their decline during late autumn till winter season. Concerning calculated values, while MCH and MCHC were lowest during monsoon and highest during winter season, MCV was lowest during autumn and highest during winter.

Indepth study of the table further details that from January onwards, as temperature starts rising, TEC, Hb and PCV undergo gradual increase through spring and summer. Further, it is also evident from the results that inspite of the declining temperature a consistent increase in their numerical value was observed till early autumn when they reach maxima in the month of September. From here onwards, a sharp drop in these values was observed with approaching low temperature in winter season. In this regards, present results though in agreement with these of Preston (1960), Joshi (1980), Syrov (1970), Radzinskaya (1966), Khan (1977), Jamalzadeh and Ghomi (2009) but partially. Similar to present findings, these workers reported an increase in blood parameters but only during spring and summer against the presently recorded increment upto early autumn. Winter decline, however, is in accordance with the op.cit workers. It appears that the rise in TEC, Hb and PCV values during spring and summer may be an outcome of the adaptive response to the respiratory stress caused by increasing water temperature related low DO. The fish perhaps tend to combat the conditions of high oxygen demand either by an increase in TEC, Hb or PCV or all of these parameters. That it is so gets strengthened by the findings of Di Prisco and Tamburrini (1992) who held water temperature to affect various blood parameters through its direct influence on the haemoglobin-oxygen binding properties and thus on oxygen transport. Guijarro et al (2003) and Jamalzadeh and Ghomi (2009) too explained temperature related seasonal variability to be the key factor responsible for increase in RBC dependent parameters during summer.

Positive correlation between feeding activity and temperature in *Tor putitora* (Malhotra, 2005) reflects the augmented nutritional status of the fish. Enhanced food consumption it appear also increases the rate of RBC formation through the process of erythropoiesis by making availability of required micronutrients to fishes. Rich RBC values and energy build up during these months also seems to be an adaptation on the part of presently studied fish, *Tor putitora* since they are in process of preparing them for the most important reproductive events. The present viewpoint gets corroborated from the previous reports wherein suggestions have been made that the contents of fish blood gets altered with season associated reproductive cycles (Kori-Siakepere (1985) and RBC dependent values elevate considerably prior to spawning (Bidwell and Heath, 1993). A consistent rise in TEC, Hb and PCV has been observed till September, which happens to be a protracted breeding season of the fish and is examplified by the rising GSI (tab. 3) during these months. Such rise can be very safely attributed to otherwise high energy demand of fishes during this period. Tor, which is known to show upstream breeding migrations, shows high rate of physical activity while paving their way to upper reaches of the river. For such strenous physical activity, apart from increased ATP production, a corresponding increase in the capacity for oxygen transport too is needed, which can probably be met by erythropoiesis. Thus inspite of low temperature and high DO during monsoon and autumn months, an increase in TEC along with Hb and PCV gets very clearly explained. Findings of Gallaugher and Farrel, (1998), that acute increase in haematocrit of exercising fish by release of erythrocytes from spleen lends a substantial support to present observation. The constant rise in TEC, Hb and PCV from spring (February) to early autumn (September) thus plausibly appears to be an adaptive response of the fish as a consequence of which they 1) can tide over the period of low DO from April to June very smoothly and 2) prepare them for intense physical activity during upstream migration for breeding events from June to September.

Winter decline in the TEC, Hb and MCV presently appear to be the result of hike in DO due to sharp drop in temperature. Much more oxygen available to the fish lowers the demand of oxygen carrier molecule; haemoglobin. Also, due to lack of food availability in general in the ecosystem and reduced food consumption by fish *Tor putitora* during this period (Malhotra, 2005) perhaps seems to be another causative factor for lower RBC dependent values in winter.

MCV, a reflection of TEC maintain an inverse relation with it. This inverse relationship between TEC and MCV justifies the decline in MCV in the period (March to September) during which TEC exhibits gradual increase and vice-versa. Increased erythropoiesis during spring and summer months seemingly may also contribute to lower MCV values. Prevalence of large number of young erythrocytes which are otherwise smaller in size than mature RBC's however result in lowering of overall corpuscular volume. It is so has earlier also been reported by Blaxhall and Daisley (1973) who observed lower MCV values at those times of year when erythropoiesis is greatest.

Both MCHC and MCH have been observed to behave in similar way and attain their maxima in the winter season (January) when Hb otherwise declined exhibiting an inverse relation between them. Possibly this inverse relationship observed presently finds its association to the nutritional status of the fish. High altitude water bodies usually are nutrient deficient more so during winter season and for most part of winter fish remain underfed. This natural phenomenon seems to be responsible for decline observed in TEC, Hb and PCV. These unfavourable conditions perhaps provoke the fish to increase haemoglobin concentration per cell and hence the swollen MCH and MCHC values can be taken as an adaptation to cold environment. Influx of young RBC's (erythroblasts), which are otherwise deficient in haemoglobin, in the circulation during summer months further contribute to the lowering of MCH and MCHC. In the months of August and September however increase in MCH and MCHC was found to be in accordance with increasing Hb which can be explained by the high energy requirement of fish for breeding purpose during this period.

Leucocytes:

Leucocytes by acting as first line of defence against any type of infection/pathogen makes an organism immune enough to fight any possible stress. This holds true for fishes also but due to less developed mechanisms of specific immunity, fishes rather depend on the non-specific resistance system (which include increase/decrease in number of leucocytes and their products; lysozymes, interferons, lysins etc (Sahoo et al, 2005). Also during times of stress, be it natural or antropogenic, fish responds by change in number and proportion of leucocytes (Christensen and Faindt-Poischi, 1978). Fluctuations in TLC observed presently during different seasons (tab. 2) also indicate that this may be a response to fluctuating weather conditions and/or some environmental stress encountered frequently in the water bodies now a days. TLC rise during hot part of the year; spring and summer with a peak in the month of June seems to be a response to elevated water temperature which by acting as natural stressor probably stimulates fish to increase those formed elements as leucocytes which prepare them to become immunologically strong enough to face any unfavourable condition. Guijjaro et al (2003) attributed increased TLC values to the poor water quality of water during summer season. Moreover, enhanced prevalence of opportunistic pathogens and various type of infection during this time of the year can also trigger immunostimulatory response of the fish.

TLC decline initialized during monsoon and autumn culminates by reaching ever low values in winter (January). Immunodepression reported by early workers seemingly appear to be triggered by lower water temperature during winter hence is responsible for the low count of leucocytes. Sahoo et al (2005) too proposed lower temperature to adversely affect both cellular and humoral immune response. Earlier reports (Avtalion, 1981; Durborow and Crusboy, 1988 and Bly and Clem, 1991) that poikilothermic animals including fish suffer from immunodepression due to low temperature aptly substantiate the present findings. Starvation like condition during these months because of low availability of food, makes the fish deficient of important nutrients which ultimately may appear to be another reason for the lower TLC values encountered during colder months. DLC exhibited an increase in both granulocytes (monocytes and lymphocytes) and agranulocytes (neutrophils, eosinophils and basophils) and hence contribute in the elevated total leucocyte count during spring and summer. Decline in lymphocytes to the tune of 26% during winter season very safely credit them to be the chief contributor for decrementing in TLC during winter period.

Thrombocytes, on the other hand observed significant increase during autumn and winter and decrement right through spring, summer upto monsoon. Similar results were also reported by Joshi and Sharma (1991) while studying seasonal fluctuations in *Nemacheilus rupicola*. Since winter season appear to be immunodepressant for fishes, elevated thrombocyte count may rather be a sort of defence on the part of fish to tide over the immunologically weakened period. Thus it can be inferred that as a part of compensatory mechanism, these defence molecules might be playing a role to protect fish in general and *Tor putitora* in particular from either temperature related changes or pathogen attacks at various periods of the year.

IV. Conclusion

From the overview of present results, it can be concluded that all the haematological parameters were affected by endogenous and exogenous factors such as reproduction, water temperature and DO. This investigation may be helpful as a tool to monitor the health status of this and other related fish species since evaluation of haematological parameters definitely guarantees early detection of clinical pathology as well as the presence of any stressor in the environment.

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Tables:

Table 1. Seasonal variations in haematological values of *Tor putitora* in relation to DO and Water temperature. All values are mean±S.D for 10 fishes (5 males and 5 females) in each month.

Month	Water temp	DO	TEC ($\times 10^6$/cmm)	TLC (/cmm)	Hb (gm%)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fl)
February	13.5	10	2.42±0.20	17265±309	8.8±0.85	36.1±3.2	36.1±3.20	23.4±0.65	149.46±11.05
March	16	9.4	2.60±0.20	17465±228	8.9±1.20	38.4±1.0	34.2±1.05	23.1±1.02	147.88±8.40
April	19	8.5	2.98±1.21	17854±236	9.3±0.68	39.2±2.1	31.2±0.75	23.6±0.58	131.77±5.62
May	23.5	8.7	3.10±0.85	18185±109	9.6±1.45	40.5±2.4	30.9±1.65	23.6±0.85	130.77±11.8
June	27	8.2	3.16±1.60	18650±203	9.8±2.30	41.0±0.2	30.9±2.50	23.8±1.30	129.90±10.2
July	26	8.3	3.36±1.32	18345±425	9.8±1.60	44.7±0.2	29.1±3.06	21.8±0.72	133.18±4.54
August	24	8.5	3.40±0.78	18048±235	10.0±2.15	45.0±2.7	29.4±2.55	22.2±1.15	132.23±10.0
September	21	8.8	3.42±1.40	17854±158	10.1±1.72	45.3±3.1	29.6±1.83	22.2±0.96	132.48±8.65
October	20	9.1	2.85±1.15	17670±324	9.5±0.10	41.0±2.6	33.2±2.70	23.1±0.15	144.03±4.00
November	17.5	10.0	2.61±0.62	17245±410	9.2±0.05	38.2±1.8	35.2±0.55	24.0±0.10	146.59±11.1
December	14	11.4	2.39±0.15	16985±265	8.6±0.90	35.7±2.3	35.9±0.50	24.0±0.86	149.45±3.53
January	11	13.6	2.31±0.14	16245±180	8.2±1.45	34.9±3.0	36.3±1.85	24.3±0.70	151.42±8.30

Table 2. Seasonal variations in Differential Leucocyte Count (%) of *Tor putitora*. All values are mean±S.D for 10 observations in each month.

Month	Lymphocyte	Monocyte	Neutrophil	Eosinophil	Basophil	Thrombocyte
February	31.1±2.3	1.5±0.4	23.0±2.6	0.5±0.2	0.8±0.3	38.6±5.2
March	33.3±3.4	1.6±0.7	23.3±2.4	1.2±0.4	1.5±0.6	35.3±4.8
April	33.9±2.8	1.8±0.6	24.7±3.2	1.4±1.0	1.7±0.7	34.4±3.6
May	34.6±4.6	2.0±1.2	25.4±2.7	1.5±0.8	2.1±1.2	33.8±4.0
June	35.1±5.0	2.5±0.8	27.0±2.5	1.6±0.5	2.4±1.3	32.1±3.5
July	35.9±2.5	2.3±1.4	26.8±3.8	1.6±1.1	2.0±0.9	30.4±2.8
August	33.3±2.8	2.2±1.5	25.5±4.5	1.3±0.9	1.7±1.1	31.5±2.5
September	30.1±3.1	1.9±1.0	26.6±3.6	1.3±0.8	1.4±0.8	32.5±3.4
October	28.4±4.5	1.7±0.8	24.0±4.0	1.2±0.6	1.0±0.6	35.7±4.2
November	27.6±3.4	1.6±1.2	23.5±3.5	0.8±0.7	0.8±0.2	36.6±3.5
December	27.8±3.5	1.4±0.6	23.2±3.0	0.7±0.4	0.8±0.3	36.8±2.0
January	26.3±2.8	1.2±0.5	22.9±2.8	0.6±0.3	0.7±0.1	37.5±3.2

Table 3. Seasonal variation in G.S.I of *Tor putitora*. All values are mean±S.D for 10 observations.

Month	Mean G.S.I
February	0.27±0.13

March	0.40±0.23
April	0.32±0.20
May	0.47±0.32
June	0.59±0.42
July	1.08±0.57
August	2.7±1.68
September	2.05±2.36
October	1.25±1.43
November	0.16±0.07
December	0.19±0.08
January	0.19±0.09