4.3 Seasonal variations in the haematological parameters of *Garra gotyla gotyla* (Tables 4-9 and Figures 13-26)

A characteristic feature of fish, like other ectothermic vertebrates, is a wide physiological range of blood composition and a large individual variation, resulting from greater effect of environment (Homatowska et al., 2002). Their homeostatic system and mechanism of control is much less specialized compared to mammals and hence fishes find it difficult to maintain some of their parameters including haematological ones within a narrow range of physiological standard.

Haematological assessment of peripheral blood is important in evaluating the health of many organisms, including fish. Several factors can influence the quantitative and qualitative picture of circulating blood in fish, such as species and strain (Langston et
al., 2002 and Filiciotto et al., 2012), environmental stress (Hickey, 1982 and Solomon and Okomoda, 2012), temperature (Langston et al., 2002; Raina, 2011; Sachar, 2011 and Gupta, 2012), malnutrition (Casillas and Smith, 1977), gender (Siddiquie and Nasim, 1979 and Sachar, 2011), fish size (Garcia et al., 1992 and Sachar, 2011), seasonal difference and breeding (Cech and Wohlschlag, 1981; Sachar, 2011 and Gupta, 2012). In order to determine haematological values of fish, some characteristics of fish and its environment should be taken into consideration. Such information shall be of considerable help in determining level of variations in different parameters of fish blood.

Presently studies on seasonal changes in temperature and dissolved oxygen of Jhajjar stream have been monitored so as to assess their influence on the seasonal variations in different haematological parameters of fish Garra gotyla gotyla for a period of two years i.e. November 2009 to October 2011.

Results

The data on water temperature and DO and different haematological parameters have been depicted in Tables (4 & 7). The results obtained very clearly reveal a significant rise (P<0.01) in RBC dependent parameters viz. TEC, Hb and Hct from spring (February-March) onwards till late summer (June) (Tables 4 & 7 and Figures 23 & 25). From here onwards a significant decline (P<0.01) in the values of these parameters have been noticed during monsoon (July-August) till winter season (November-January). Tables (4 & 7) further reveals at all of the observed parameters record maxima (TEC: 3.04×10^6/cmm, Hb: 9.4% and Hct: 43.4%) during late summer (June) and minima (TEC: 2.02×10^6/cmm, Hb: 7.4% and Hct: 38.4%) during late winter (January).

Calculated indices of RBC include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). MCV and MCH have been observed to exhibit increase during monsoon through autumn till winter. Decline in their values was observed during spring and summer. Both MCV and MCH exhibited maxima (MCV: 191.0fl, and MCH: 36.4pg) during winter and minima (MCV: 142.7fl and MCH: 30.8pg) during summer season. Contrary to MCV and MCH, MCHC exhibited a decline in their values during monsoon till winter and increase during spring and summer. Lowest values of MCHC (19.2%) were recorded during winter and highest (21.5%) during summer (Tables 4 & 7).
Total leucocyte count (TLC) which comprises of both agranulocytes (lymphocytes and monocytes) and granulocytes (neutrophils, basophils and eosinophils) have been observed to witness significant increase (P<0.01) during spring and summer season. From here onwards a significant decline in all these parameters was noticed during monsoon till winter. Maxima of TLC have been observed during summer and minima during winter. Thrombocytes, which are the equivalent of mammalian blood platelets, depicted an appreciable increase in their values during monsoon till winter and exhibit decrease in their number during spring and summer season. Lowest values of thrombocytes (18.9%) were recorded during summer and highest (50.5%) during winter season (Tables 5 & 8 and Figures 24 & 26).

**Discussion**

**RBC dependent parameters during Spring and Summer season:**

It is clearly evident from the Tables (4 & 7) that RBC dependent parameters viz. TEC, Hb and Hct of *Garra gotyla gotyla* showed significant increase (P<0.01) during spring and summer season with an increase in water temperature and decrease in DO contents of Jhajjar stream. Maxima (TEC; 3.04×10⁶/cmm, Hb: 9.4% and Hct: 43.4%) of TEC, Hb and Hct was recorded during the month of June (summer) when water temperature was at its highest peak (30.3°C). Similar to present findings worker like Khan (1977), Joshi (1980), Tun and Houstan (1986), Jamalzedah and Ghomi (2009), Raina (2011), Sachar (2011), Singla *et al.* (2012) and Gupta (2012) also reported/observed an increase in RBC dependent parameters viz. TEC, Hb and Hct during spring and summer season with maxima during summer season.

From the Tables (6 & 9) it is also clearly evident that temperature exhibited significant negative correlation (P<0.01) with dissolved oxygen (DO) but a significant positive correlation (P<0.01) with TEC, HB and Hct. Presently observed increase in RBC dependent parameters viz. TEC, Hb and Hct during spring and summer season can be safely attributed to significant increase in water temperature and marked decrease in DO contents of waterbody.

Increase in temperature during spring and summer season by increasing the rate of evaporation from the waterbody, seemingly appears to reduce the oxygen carrying
capacity of water and creates stressful conditions in waterbody. To cope up such stress created due to deficiency of oxygen, the hill stream fishes including presently studied *Garra gotyla gotyla* tends to increase their red blood cell count which then seemingly results in an increase in its respiratory pigment ‘Hb’ and also in its Hct. Present findings are in accreditation to the work of Raizada *et al.* (1983), Wells (1999), Jawad *et al.* (2004), De-Pedro *et al.* (2005), Raina (2011), Sachar (2011) and Gupta (2012) who also reported an increment in the values of RBC, Hb and Hct during spring and summer season and proposed temperature related deficiency of oxygen to be a major factor contributing toward increase of these parameters.

It is on record (Sharma, 1984; Rauthan and Agarwal, 1996; Gupta, 2012 and Singla *et al.*, 2012) that hill stream fishes demand more oxygen as well as higher RBC, Hb and Hct for higher metabolic activities to survive and stay against the turbulent force of hill stream water as compared to calm flowing waters of plains. In tune to above workers, presently also remarkably higher values of RBC (3.04×10⁶/cmm), Hb (9.4 %) and Hct (43.4%) were observed in fish *Garra gotyla gotyla* inhabiting hilly waters of Jhajjar stream located at an elevation of 1476 feet from minimal sea level (Tables 4 & 7). Similar to present findings Rauthan and Agarwal (1996) and Gupta (2012) also recorded higher values of TEC, Hb and Hct being TEC: 2.89×10⁶/cmm; Hb: 10.4% and Hct: 32.0% (Ruthan and Agarwal, 1996) and TEC: 3.15×10⁶/cmm; Hb: 9.6% and Hct: 44.6% (Gupta, 2012) in fish *Garra gotyla gotyla* which they obtained from waterbody at an altitude of 3556 feet and 2077 feet respectively from minimal sea level. Sharma (1984) and Gupta (2012) too reported higher values of TEC, Hb and Hct in hill stream fish *Tor putitora*.

Among different environmental parameters, temperature is one of the important factor known to influence the nutritional budget of aquatic ecosystem on which depends the health of biotic organisms including fish. Aras *et al.* (2008) reported temperature related increase in feeding activity and metabolic rate of fish during spring and summer season. Malhotra (2005), Raina (2011), Sachar (2011) and Gupta (2012) too reported increase in RBC and its dependent parameters viz. Hb and Hct with increased feeding intensity during spring and summer season. The highest values of TEC, Hb and Hct recorded during spring and summer season in fish *Garra gotyla gotyla* according to present author too indicates increased feeding intensity of fish during this period. By making availability of required micronutrients, increased food consumption seemingly
appears to increase the rate of erythropoiesis (Tables 4 & 7). Therefore increase in RBC dependent parameters TEC, Hb and Hct during spring and summer is justifiable. Salami et al. (1993) also documented an increase in RBC and its related parameters Hb and Hct to enhanced food consumption.

Like temperature, photoperiod is another important environmental factor that affects the haematological parameters of fish. After February onwards till June (Spring-Summer) as the day length increase, photoperiod also increases and a corresponding increase in the haematological parameters i.e. TEC, Hb and Hct of fish Garra gotyla gotyla has been observed. During this very time period fishes usually observed an increase in feeding regime too. Same viewpoint has been given by Valenzuela et al. (2008) who also recorded an increase in erythrocyte count during long photoperiod of spring and summer season in fishes in their natural environment as also under laboratory conditions.

Besides environmental factors (temperature and photoperiod), breeding related stress is also known to influence the blood parameters of fish (Sachar, 2011 and Gupta, 2012). Breeding period of fish can be determined with the help of gonadosomatic index (GSI). Presently, GSI has been observed to increase during spring and summer season and exhibit maxima during the month of May and after that a decline has been observed in the following months. As GSI increases during spring and summer so does the RBC dependent parameters of Garra gotyla gotyla (Table 12).

Breeding period is high energy demanding period and creates a stressful condition due to the increased physical activity and metabolic demands in fish (Wang et al., 2003; Sachar, 2011 and Gupta, 2012). This energy demand of fish can be fulfilled by increased ATP production, which is generally met by corresponding increase in the capacity for oxygen transport. Such high O₂ demand can be met only from increased rate of RBC production. Khan (1977), Pandey (1977), Putman and Freel (1978), Jawad et al. (2004), Sachar (2011) and Gupta (2012) also recorded an increment in haematological parameters viz. RBC, Hb and Hct during breeding period which is high energy demanding phase of reproductive cycle.

Moreover, an increase in the values of RBC, Hb and Hct during spring and summer can also be attributed to phenomenon of haemoconcentration due to increased
rate of erythropoiesis. Preston (1960) also attributed process of haemoconcentration for an increase in the values of RBC during spring and summer season.

Thus the rise observed in the RBC dependent parameters during spring and summer appear to be an effort on the part of fish to cope with natural stress caused by an increase in water temperature, photoperiod and low dissolved oxygen content (environmental factors) in aquatic environment. It also seems to prepare fish for the breeding phase which is a period of greater physical activity and high energy demand (physiological factor). Further, it also help these hilly fishes to stay in water column while struggling the turbulent forces of water.

**RBC dependent parameters during Monsoon, Autumn and Winter:-**

Perusal of Tables (4 & 7) clearly reveals that after exhibiting an increase during spring and summer season, RBC dependent parameters viz. TEC, Hb and Hct tend to fall during monsoon to acquire minima during winter (January). From July onwards as temperature of waterbody decreases, an increase in DO contents has been observed which present author proposes results in a decrement in the values of haematological parameters viz. TEC, Hb and Hct. Such decrement in the values of TEC, Hb and Hct during monsoon-winter can be very safely attributed to temperature related increase in DO contents of waterbody as during this period, DO retaining capacity of water has increased. Hence a subsequent decline in the values of TEC, Hb and Hct seemingly may fulfill the low oxygen demand of fish *Garra gotyla gotyla* due to availability of plenty of oxygen in the waterbody during this period. Franjioni *et al.* (1997) also observed marked decline in the circulatory erythrocytes, Hb and Hct during this period of low temperature in Cyprinid *Phrealichthys andruzzii*. Singla *et al.* (2012) too, have reported a decline in the values of TEC and related Hb and Hct during winter in different species of hill stream fishes. Gupta (2012) also reported such decline in the Values of TEC, Hb and Hct in fish *Garra gotyla gotyla* from Sunderbani region of Rajouri district, J & K.

Reduction in TEC from monsoon onwards till winter seemingly appear to directly influence the Hb and Hct. Present author proposes that the declining number of RBCs in the circulation leads to haemodilution of blood and hence decrement in the values of Hb and Hct gets explained. Similar to present viewpoint Schaefer (1925) and Preston (1960) earlier too held process of haemodilution to occur during autumn and winter. Hence
haemodilution is another important factor responsible for the observed decline in the values of TEC, Hb and Hct.

In natural environment starvation like condition usually are known to exist during the winter season which also leads to a decline of RBC dependent parameters. Lack of sufficient food which decreases during this period due to short photoperiod may also cause deficiency of essential nutrients to fishes. Moreover during this period, fishes remain metabolically inactive, as breeding period (high energy demanding period) is over and thus a subsequent decline in the values of TEC, Hb and Hct observed during this period appears justifiable. Similar to present viewpoint, Gupta (2012) also reported existence of starvation like condition in waterbody of Sunderbani while studying the haematological parameters of *Garra gotyla gotyla* during winter period and attributed it for decline of RBC dependent parameters during this period.

**Calculated indices of RBC during Spring and Summer:**

A significant decline (P<0.01) in the values of MCV and MCH have been recorded during spring and summer (Tables 4 & 7 and Figures 23 & 25). This decline in the values of MCV and MCH has been attributed to an increase in the values of TEC during this period. MCV and MCH have been observed to exhibit an inverse relation with TEC and that is clearly depicted in the Tables (4 & 7). TEC exhibit maxima during late summer and contrary to it both MCV and MCH exhibit a minima during this period. Both MCV and MCH exhibit a significant negative correlation (P<0.01) with temperature as is clear from the Tables (6 & 9). It is evident from the Tables 4 & 7 that as temperature witnessed a hike during this period both MCV and MCH exhibit a significant decline (P<0.01). The possible reason for decline in the values of MCV and MCH with increase in temperature has been attributed presently to process of erythropoiesis through which young erythrocytes possibly gets added to general blood circulation of fish. These erythrocytes being smaller in size but larger in number significantly lower the MCV and MCH values during this period. Thus MCV and MCH showed a significant positive correlation (P<0.01) with each other (Tables 4 & 7). To support this contention findings of Blaxhall and Daisley (1973), Sachar (2011) and Gupta (2012) have been taken into consideration who observed that as temperature rises during summer, rate of erythropoiesis also increases significantly and MCV showed a sharp decline as young
erythrocytes are added which being smaller in size exhibit low corpuscular volume. These smaller erythrocytes being large in number ultimately increases the surface area that is prerequisite for proper gas exchange in lieu of stress caused by higher temperature and low oxygen.

Perusal of Tables (4 & 7) also indicates that MCHC record a gradual increase from monsoon onwards through spring and acquires maxima during summer (June) and depict a gradual increase during spring and summer. The possible reason for this significant increase (P<0.01) in MCHC during spring and summer season is possibly to meet energy demand during greater physical activity and higher metabolic rate of these fishes. During spring and summer MCHC increased with an increment in Hb content to carry more and more oxygen to tissues to compensate the stress caused by high temperature and low DO. Review of literature reveals that workers like Ranzani-Paiva (1995), Lochmiller et al. (1989), Guijarro et al. (2003) and Gupta (2012) also observed a positive correlation between Hb and MCHC.

Calculated values of RBC during Monsoon, Autumn and Winter:-

Both MCV and MCH depicted an increment (P<0.01) in their values during monsoon till winter as shown in Tables (4 & 7). The peak values of MCV and MCH were recorded in the winter season (January).

Both MCV and MCH have a negative correlation with TEC and temperature (as mentioned earlier), therefore during the period of low temperature when TEC count of fish is low, a marked increase in the values of MCV and MCH has been observed. This seems to be an adaptive strategy to meet the condition that arises from the low temperature and high oxygen holding capacity of water. During this period, present author proposes possibly haemopoietic machinery gets hampered due to non availability of essential nutrients in waterbody. This by affecting the rate of erythropoiesis (decline) then may result in an increment in the values of MCV and MCH.

Contrary to MCV and MCH, MCHC exhibit a significant decline (P<0.01) during monsoon through autumn till winter and exhibit minima during winter (January). Decline in the values of MCHC as observed by present author were also observed by Kakuno and
Koyama (1994), Raina (2011) who too attributed low oxygen demand by fish during this period to be possible reason for its decline.

**WBC dependent parameters during Spring and Summer:-**

Like other blood parameters total leucocyte count (TLC) of fish *Garra gotyla gotyla* has been observed to exhibit marked seasonal variations (Tables 4 & 7). Data clearly reveals a significant increase (P<0.01) during spring and summer. Remarkably high values of leucocytes (spring and summer) during present studies (Tables 4 & 7) are in accreditation to the earlier findings of Joshi and Sharma (1991), De-Pedro *et al.* (2005), Tavares-Dias and Moraes (2007), Raina (2011), Sachar (2011) and Gupta (2012). This increase in TLC during spring and summer finds a direct relation with increased water temperature and low DO of water during this period, which in turn affects the water quality. Both increased water temperature and poor water quality acts as natural stressor and creates a pathogenic condition in fish. To counteract such conditions fishes usually respond by altering their immune machinery by either increasing leucocyte number or their products. Thus an immunostimulatory response gets evoked in natural habitat to counter any type of stress. Collazos *et al.* (1996), Guijaro *et al.* (2003), De-Pedro *et al.* (2005) and Adewoye (2010) also advocated increased number of leucocytes during spring and summer to be an immunostimulatory response of fish they studied.

The prevalence of opportunistic pathogens and various types of infections during this period of year also stimulates the immunostimulatory response in fish. They seemingly appears to ultimately results in an increase in number of leucocytes. Sahoo *et al.* (2005) and Gupta (2012) have also put forth that fish in general respond by increasing number of leucocytes during unfavorable conditions.

A significant increase in TLC during spring and summer has also been attributed to increased feeding regime (due to increased photoperiod) and feeding rate of fish *Garra gotyla gotyla*, which is also the breeding period of fish. In this context, Smirnova (1965) and Joshi (1980) observed a positive relation between TLC and feeding status of fish and noticed an increase in total leucocyte count during the hot period, which they added also happen to be the breeding period of fish under studies.
Pradhan (1961), Haws and Good Knight (1962) and Srivastava (1968) proposed that increased TLC is the usual outcome of activity status of fish and further added that sluggish fishes in general have more TLC compared to active fishes and same is true in *Garra gotyla gotyla* which being a hill stream fish remain hidden under stones and pebbles and live a sluggish life. Also during the experimental period it confined itself to the bottom of tubs (discussed elsewhere).

From the data given in Tables (4, 5, 7 & 8) and Figures (13-16 & 23-26) that apparently reveals increase in TLC during spring and summer finds a direct relation with corresponding increase in both agranulocytes and granulocytes. The most frequent white blood cells during this period are lymphocytes which as stated by Kaattari (1992), Raina (2011) and Gupta (2012) play an important and a key role in strengthening the immune system of fish and makes the fish strong enough to fight against any kind of stressor by producing antibodies. Joshi and Sharma (1991) and Gupta (2012) similar to present findings also reported higher number of lymphocytes during spring and summer and related it to increased antibody producing capacity of these cells during the period of elevated temperature.

Thrombocytes in fish are multifunctional cells, involved in eicosanoid release, blood coagulation besides their involvement in immune defense (Tavares-Dias and Mataqueriro, 2004 and Witeska, 2005). Therefore, as they are in constant movement in haemopoietic organs and systemic circulation, this might cause a variant change in its regular number (Witeska, 2005). During present investigation they also exhibit a marked seasonal variation and depict minima during summer season (Tables 5 & 8 and Figure 10). A significant (P<0.01) numerical decline in thrombocyte observed during spring and summer season finds a direct relation with increase in water temperature. Similar to present findings Joshi and Sharma (1991), Guijaro et al. (2003), Sachar (2011) and Gupta (2012) also recorded a decrease in thrombocyte count during spring and summer and related it to increased temperature during this period.

**WBC dependent parameters during Monsoon, Autumn and Winter:-**

Decline in TLC initiated during monsoon, through autumn has been observed to touch their lowest values during winter (Tables 4 & 7 and Figures 23 & 25). Significant decline (P<0.01) in TLC during this period appears to be an immunodepressive response
by fish at low temperature. Similar to present results workers like White et al. (1983), De-Pedro et al. (2005) and Bowden (2008) also held decline in leucocyte count during this period of year to be an immunodepressive response in fish they studied. Present findings also get a direct support from the work of Bly and Clem (1991) and Gupta (2012) who also reported state of Immunodepression in fishes in lieu of low temperature. Besides low temperature, decreased TLC values during winter season also find a direct relation to the feeding status of fish which is found at all time to be at low ebb during these months. Since starvation like conditions usually prevail during winter months in aquatic ecosystem therefore fishes suffer from deficiency of important nutrients. This ultimately can lead to lower production of TLC which in turn results in weakening of immune system of fish. Sala-Ranabal et al. (2003) also reported decline in TLC during winter due to combined effect of low temperature and starvation. Present author also put forth similar views on decline of TLC during winter and related it with temperature mediated response on one hand and scarcity of food on the other. Both agranulocytes and granulocytes also exhibit decline in their number during this period (Tables 5 & 8 and Figures 17-22 & 24-26).

Contrary to granulocytes and agranulocytes, thrombocytes exhibit a significant increase in their number during this period after exhibiting minima during late summer (Figures 17, 19 & 21). Increase in thrombocyte count during winter season according to present author appears to be a sort of defense on the part of fish to protect itself from any adverse condition arising during otherwise immunologically weakened period of the year which may make them prone to pathogenic attack during this period.

From above results and discussion it can be inferred that fish Garra gotyla gotyla like other hill stream fishes also exhibit seasonal variations in its haematological parameters throughout the year with corresponding variation in temperature, DO, photoperiod on one hand and feeding intensity and reproduction on other.