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EFFECT OF ALTITUDE ON HAEMATOLOGICAL PARAMETERS OF RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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Abstract

Rainbow trout (Oncorhynchus mykiss) is one of the extensively cultured fish species of fresh water all over the world. In Pakistan, it is cultured in cold regions at different altitude range. This study has been conducted to find out the influence of altitude on haematological parameters of Rainbow trout (O. mykiss) inhibiting different altitude range at Dir upper, Khyber Pakhtunkhwa, Pakistan. The research was carried in the month of July 2021. Healthy adult rainbow trout, of age three years, were caught from three different locations (Sheringal, Kalkot, Kumrat). Blood samples collection were made from the caudal vein using vacutainer system and transferred to sample tubes. Some haematological variables of rainbow trout were examined, using CBC test, and the results obtained, were evaluated among different altitude range. The blood samples were analysed for 8 biochemical indices. In the present study, it has been observed that Oncorhynchus mykiss show clear decrease in erythrocytes, Haemoglobin, and haematocrit (PCV) from lower to higher altitude. Similarly, a gradual decrease occurred in the thrombocytes of rainbow trout towards high altitude. Conversely, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were found to be increased with high altitude. However, White Blood Cell (WBC) were higher in lower altitude then decreases and again increases at height. This may be due to the water quality and stress condition, which effect the number of WBC. The results achieved from this study would be useful for future research in Trout fish blood.

INTRODUCTION

Rainbow trout (*Oncorhynchus. mykiss*) is one of the extensively cultured fish specie of fresh water all over the world (Talas and Gulhan, 2009). In Pakistan it is cultured in cold regions at different altitude range.

Differences in altitude range of an area result differences in local environmental circumstances (Rosidah *et al.*, 2017) such as temperature and air pressure. Temperature and air pressure effect the dissolved oxygen content in the water. At hight, temperature generally decreases which provide a good supply of Dissolved Oxygen in water. On the other hand at hight, po_2 decreases which lead to decrease in DO in water.so both these parameter influence the blood content of fish. This dissolved oxygen in water brings changes in blood content (erythrocytes, haematocrit, and haemoglobin) etc of fish.

Assessments of Erythrocytes, haematocrit and haemoglobin can be used as one of the benchmarks to decide about the health status of fish. To evaluate the physiological and health condition of fish, haematological parameters play an important role (De Pedro *et al.*, 2005; Khan & Zafar, 2005; Sheikh & Ahmed 2020). In aquaculture, the control of fish illness has created developing concern in the haematology of both marine and freshwater teleost. Among haematological records, blood cells are probably the excellent pointer of body wellbeing and are enormously vital in the immune system (Zexia *et al.*, 2007). Blood cells such as RBC, WBC and platelets have their own significant role.

Red blood cells (erythrocytes)

Erythrocytes in fish are of various sizes (100–800 fl) and life time duration of 13–500 days. It is ellipsoid shape and nucleated. It is the main carrier source of haemoglobin. Haemoglobin is the iron-containing oxygen-carrying metalloprotein in the erythrocytes that provides red blood cells their colour and works as transportation of oxygen from the lungs to tissues and carbon dioxide from tissues to the lungs to be respired (Malgorzata, 2013). this haemoglobin reacts with oxygen carried in the blood to form oxyhaemoglobin in the course of respiration (1996; Chineke, Johnston & Morris, 2006). According to Isaac *et al.*, (2013) erythrocyte is engaged in carrying of oxygen and carbon dioxide in the body. Thus, a decline of oxygen level would lead to reduce oxygen in the tissues along with reduce carbon dioxide returning to the lungs (Ugwuene, 2011; Isaac *et al*, 2013; Akinrinde, Soetan, & Ajibade, 2013;). Erythrocytes of fish contain tetrameric haemoglobins of different oxygen affinity – lower in species living in well-oxygenated water than in those that experience hypoxia. Frequently numerous haemoglobin isoforms of diverse oxygen affinity contain in the blood, which is regarded as an adaptation to flexible oxygen concentration in water. Morphological assessment of fish erythrocytes are used as an indicator of poisonousness or toxic condition because these cells are very sensitive to environmental. (Malgorzata, 2013). Production of erythrocytes occur from hematopoietic stem cells in the bone marrow (red) through a process called erythropoiesis. Erythrocytes are formed in the red bone marrow from hematopoietic stem cells in a process known as erythropoiesis. When there is too much scarcity of erythrocytes the state is called as anaemia, and having too much increase is polycythaemia. The percentage (%) of red blood cells in the blood is haematocrit or Packed Cell Volume (PCV) (Heller, 2003). Its function is the transportation of oxygen and absorption of nutrients (Isaac *et al.*, 2013). Increased PCV indicate an improved transportation and lead to an amplified primary and secondary polycythaemia (Maton *et al.*, 1993) except for the fish family (channichthyldae) (Sidell & O' Brien, 2006) and tissues of invertebrates. For oxidation of consumed food to deliver energy for other body functions, and to transport carbon dioxide out of the assemblage of the body, is done by haemoglobin which has the physiological capacity of shipping oxygen to tissues of the body (Ugwuene, 2011). According to Peters and Ikeobi 2011, previous reports stated that Packed Cell Volume, haemoglobin and mean corpuscular haemoglobin are major indices for assessing circulatory erythrocytes and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Atodo & Dzende, 2005; Chineke *et al.*, 2006). Below 22 % value of haematocrit in fish specifies anaemia. Moreover, Chineke *et al.*, (2006) profound that elevated Packed Cell Volume (PCV) value show either an increase erythrocyte or decrease circulating plasma volume. Blood condition can be obtained by mean corpuscular haemoglobin & mean corpuscular haemoglobin concentration. According to Aster, 2004, a decrease condition is a sign of anaemia.

White blood cell (leucocytes)

Leucocytes are cells of the defence system associated with protecting the body from both irresistible infection and unfamiliar materials. Production and derivation of White blood cells occur from hematopoietic stem cells (multipotent cells) in the bone marrow. Leukocytes (WBC) are present all over the body, both in the blood and lymphatic system. White blood cells have various kinds that have certain role in defence system of the body (Albert *et al.*, 2002). Leucocytes are classified into granulocytes and agranulocytes, differentiated via the absence or presence of granules in the cytoplasm. Granulocytes comprised of eosinophils basophils, neutrophils, and mast cells. While Agranulocytes include monocytes and lymphocytes. When the white blood cells are very less in number than normal case, this state is called leukopenia. And the condition of having very large number is called leucocytosis. (Kumar *et al.*, 2010).

The significant role of the white blood cells is to battle diseases, protect the system by phagocytosis against intrusion of unfamiliar life forms and do manufacturing and transportation of antibodies in immune response (Ballarin *et al.*, 2004). Thus, with low WBC, animals have high chances to get infection, whereas those with high tallies are fit for making antibodies during the process of phagocytosis and have more defence from infections (Soetan *et al.*, 2013). It also provides flexibility to nearby environmental and infection predominant conditions (Isaac *et al.*, 2013; Kabir, 2011).

Leucocyte types

Lymphocytes

Large amount of the blood leucocytes are lymphocytes and are presumably the best studied leucocytes cells. Fish lymphocytes is similar to mammalian in morphology. It has small cells of about 5-8 µm in size with a large nucleus cytoplasm ratio. Lymphocytes recognized by light microscopy can without much of a stretch be mistaken with few morphological various kinds of thrombocytes (Ellis, 1977).

Monocytes

Similar to mammals, the cells monocyte in fish is considered the circulating mononuclear phagocytes, whereas macrophages are static or itinerant cells in tissues (Rowley *et al.*, 1988).

In fish the identical role of monocytes has been revealed, as in the case of mammals, such as phygocytosis (Secombes & Fletcher, 1992), antigen presentation (Vallejo *et al*, 1992), the manufacturing of cytokines (Secombes, 1991), and immunostimulating aspects such as lipoxins and leukotrienes (Secombes & Fletcher, 1992). Similar to mammals, for speeding up the process of phagocytosis various kinds of receptors are recognized on microphages in the case of fish. According to Ozaki *et al*, 1983, lectin (receptors) was identified in (salmonids). The process by which foreign particles are removed out of the body (through phagocytosis) using opsonins was explained for rainbow trout (Honda *et al*, 1985, and Michel *et al*, 1990), that represent the complement receptors.

Platelets (Thrombocytes)

Ordinary fish thrombocytes intently similar to those present in birds and reptile species. they are little elongated cells with vibrant, uncoloured cytoplasm with an elongated compact nucleus at the middle. While studding blood smears, as in the haemocytometer, platelets of fish are sems to be in the elongated shape, rod-shaped, or rounded. These differences in morphology are because of the developmental stage of cell and degree of activation. Thrombocytes are frequently misunderstood with the lymphocyte of same size. This resemblance led to the wrong counting of white blood cell and to the differential. The vital characteristic that distinguishes the lymphocytes from thrombocytes, on the blood smear, are the cytoplasm colour (light blue vs colourless respectively) and the N:C ratio (higher in the lymphocyte). The transformation of prothrombin to thrombin is done by platelets that participate in the process of blood clotting. So, if the platelets intensity is more inside the blood, blood loss will be very less during damage and recovery will be fast and if they are low in quantity then more blood loss will be there due to prolonging clotting process.

Factors effecting haematological parameters of Fish:

Environmental changes affect Chemical and physical properties of fish blood (Hughes and Nemcsok, 1988)

and pollution also affect fish blood (Yanik & Atmanalp, 2001). For instance, aspects such as disease and stress can lead to remarkable deviations in fish blood parameters (Chen *et al.*, 2005; Cnaani *et al.*, 2004). Along with that, certain ecological aspects, for example diet, may also lead to biochemical changes in fish blood parameters (Rakovac *et al.*., 2005). Environmental aspects, such as oxygen, temperature, light, water density, and salinity have a great effect on haematological parameters of fish. Physiological aspects, like age, species and reproductive phase can alter changes in haematological parameters of fish. In conclusion, haematological boundaries work as an early sign of stress in fish, and they can add to the recognition of target organ poisonousness (Coşkun *et al.*,2015). Additionally, blood taking technique, laboratory practices and shipping could also affect haematological assessments (Sheikh & Ahmed 2016, Sharma *et al.*, 2017). For example, Anesthesia can increase the hematocrit (Phuong *et al.*,2017), whereas malnourishment, disease, or environmental pollutants led to reduction of hematocrit and haemoglobin values in fish (Witeska, 2015). Even the capturing, handling, and anesthitics involved in the blood sampling from fish has also profound effect on hemogram (Bolasina, 2006). The current survey is conducted to understand the impact of altitude on rainbow trout haematology.

Important species

Some important species of rainbow trout are,
Oncorhynchus mykiss mykiss
Oncorhynchus mykiss gairdneri
Oncorhynchus mykiss irideus
Oncorhynchus mykiss newberrii
Oncorhynchus mykiss aguabonita
Oncorhynchus mykiss Kamloops
Oncorhynchus mykiss nelsoni
Oncorhynchus mykiss aquilarum
Oncorhynchus mykiss stonei
Oncorhynchus mykiss chrysogaste
(Behnke, 2002).

Our focus is on Oncorhynchus mykiss mykiss.

Taxonomy of *Oncorhynchus mykiss*

Scientifically rainbow trout is known as Oncorhynchus mykiss. The name was given by Walbaum in 1792 in Siberia. Its first part (genus name) is derived from Greek word onchos which means spike or hook like and rynchos meaning nose. The reason behind this is that the male has a blended upward skype which show the maturity in reproductive stage (Behnke, 2002). The second part mykiss is a derivative form of the word mykizha which means fish in Kamchatkan resident language. Rainbow trout belongs to Actinopterygii class. The order, family, subfamily, Genus and specie is Salmoniformes, Salmonidae, Salmoninae, Oncorhynchus and mykiss respectively.

Golden rainbow trout is the mutated and intermediate form of Common and Golden Rainbow trout (FAQ, 2013).



Figure1. Rainbow trout (Oncorhynchus mykiss mykiss), a picture taken during sampling.

Morphology

Rainbow trout has commonly black spots at the upper dorsal side with greenish shiny background. Posteriorly and sidewise are covered with shady spots. Upper side of the head is also covered with these black spots. Mid side look like a band which is pink, red or purple in colour lower side is whitish in colour with completely white underneath. The dorsal, faty and caudal blades are light olive to golden set apart with dim hued spots. Lower blades are ordinarily pale shades of golden, orange, red, purple or dark and the fins at the anal and pelvic section are regularly brownish or whitish.

Life span and Reproduction

Trout becomes sexually mature at 2 to 3 years of age and is subject to many variables, species, yet additionally natural factors, for example, water temperature, sort of food, supply quality, development rate or latitude (Bieniarczyk and Epler, 1991). Maturation starts at the age of one year with normal life span of 4 to 5 years. Survived up to 11 years (Rossi *et al.*, 2007), however 7-year olds are normally the oldest in most of the ponds. Normally spawning occur all through the winter and spring. Once the water temperature reached between 11°C to 16°C, the process of spawning increases. In Dir Upper spawning begins from November to April. The spawning processes reached to its peak when the length of the day increases. Similarly increase in water flow and temperature also boost up the spawning. Spring water is best for spawning which balance water temperature between 13 and 15°C. Incubation period vary with water temperature, 13 to 15°C gives best result.

Size

Average size of freshwater trout is about 0.6kg to 2.5 kg, whereas those found in ocean, or an open lake sometimes reach to 10 kg. According to Behnke, 1992 rainbow trout of weight 17 to 23 kg had also been caught in Kooteney lake, Columbia. On August 19, 1995, Lloyd Bull caught a lake trout in Great Bear Lake, Canada, that weighed 32.65 kg. (IGF Association. 2015).

Habitat and Distribution:

Rainbow trout is very sensitive fish, that spend its life in cold, clear, and oxygen abounded water. Rainbow trout are viewed as local in Pacific Ocean at Northern region and related seepages, Asia, Australasia, Europe, Russia, and North America (Hardy, 2002). The introduction of rainbow trout has been made to farms in 1870 in different countries and set the production achievements in 1871. Its production achievement has been successful in water bodies all over the world, apart from Antarctica (Fuller, 2000). In Pakistan, work was made in this regard since 1923 at different cold and high-altitude northern regions such as Kashmir, Kagan, Dir, Gilgit Baltistan, and Bahrain (Swat). This research study has been done in Dir upper.

Nutrition:
The preferred nutrition included both plant and animals such as is small plant substance, green algal stuff, insects, and small creatures present in water. Usually, ideal feeding is premature and adult form of insects of order trichopteran and true flies. Sometimes take teloses eggs, hymenopterans, coleopterans, and grasshoppers. It also eats meat or fat which is provided to it in the ponds (Thomas, 2021).

Economic importance of Oncorhynchus mykiss:
Due to adoptability, high spawning rate, and good temperature range, rainbow trout is largely practice specie everywhere in the fresh water. (Talas & Gulhan, 2009) and is very valuable in diet and profitable for culturists (Coşkun *et al.*,2015). Trout meat is a source of valuable and highly digestible protein, phospholipids and unsaturated fatty acids, and also vitamins and minerals. Consuming rainbow trout provides a large dose of vitamins – including vitamins A, B and C vitamins. It is also a good source of minerals – phosphorus, magnesium, calcium, iron, potassium, and microelements such as: manganese, zinc, copper. These species have high requirement in marketplace because of its lovely taste, good and healthy nutritional benefits and persisting marketing every month of the year. Rainbow trout meat is a decent wellspring of fat, protein and a wellspring of nutrients and amino acids. Dishes ready from it are low in calories. The market comes in a variety of forms, adjusted to the developing requirements of the buyers. It is also an excellent export product valued in the European Union. (Magda *et al.*,2019).

Important disease
On of the reason of Decline in rainbow population is some fatal diseases due to viruses and bacteria. The main bacterial infections detailed as vibriosis, motile Aeromonas septisemia, lactococococosis, yersiniosis, and flavobacteriosis. In the evaluated report Flavobacterium psyrophilum were found the most important root of mortality in hatcheries (Ulukoy, 2016).
Flavobacterium psychrophilum bacterium is cold-loving. It is gram-negative rod shape bacterium. Its average size is 4×10^{3} mm and have its place in phylum bacteroidota. It was first evaluated in 1948 in Oncorhynchus kisutch and is the contributing factor of infection named BCWD (Bacterial Cold Water Disease) (Starliper, 2011). It inhibited very cold water of rivers streams and lakes with temperature range 15-17°C.locomotion is ensured without using locomotory structure (Hesami *et al.*, 2011). It is seen normally on external and internal sites of the host (fish) such as skin surface, ascites, wound, mucus, gills, brain, spleen, kidney, newly hatched eggs and sperm of grown up specie (Barnes & Brown, 2011), their population can be recognised by prominent snowy growth area on skin of host (Hesami *et al.*, 2011). *Flavobacterium psychrophilum* has rigidly aerobic metabolism uptakes but instead of carbohydrates, it produces amino acids and peptides for energy source. It is responsible for the bacterial cold-water disease. A prominent snowy growth area on the upper side of skin of host show that the fish has been affected, then eventually the discolouration saturate the whole body. This infection occurs among individuals of the same generation through water and interaction, as well as from mother to their offspring, because of its contact in first developmental phases. The resistance capacity of this bacterium to lysozyme is much higher than the spawning eggs so the transmission can be made without any confrontation. Treatment for bacterial cold-water disease is possible in early stages. oxytetracycline or quaternary ammonium show effectiveness and often bath treatment is done in such cases, however this should be done before the advent of infection saturation, otherwise it will be fruitless if the infection reached to the final stage. but such treatments are fruitless once the abrasion of the peduncle and caudal fin become apparent. If the bacterium has got less resistance, treatment can be done successfully by means of amoxicillin, florfenicol and oxytetracyline. At the present time the vaccination trail has not been made yet (Cipriano & Holt, 2005).

Literature review:
In a study conducted by Coşkun *et al.*, 2015 after data analysis have been done between different study areas no deviations, on seasonal base, were found in haematological values of fish blood. Similarly, no such differences were found while studying sampling of river. However, non suitable environmental variations

can bring fluctuations in biochemical properties of fish blood (Atamanalp *et al.*, 2002) such as variations in temperature, feeding, oxygen, water density, salinity, temperature and light (Coşkun *et al.*,2015). In winter higher cold stress may be the cause of increased formation of erythropoietin hormone, which might be responsible for higher RBC count during winter (Jelkmann, 2011). In addition, blood collection method, stage of fish, diet, and the place where the fish is culturing have imprint on the blood parameters at some degree (Sakomoto *et al.*, 2001).

On exposure to cobalt chloride the fish show increase in red and white cells, platelets, hemoglobin and MCHC values. While decrease was found in the parameters of HCT, MCV and MCH (Atamanalp *et al*,.2010).

In a study conducted by Johanne & Patrick in 2012 increases in the temperature, starts release of cells from spleen, causes the blood to carry more oxygen to tissues, which speed up the process of premature erythrocytes and boost up the production of new erythrocytes by means of erythropoiesis.

According to Rambhaskar & Rao 2006, Blood cells, Haemoglobin and Haematocrit values are decreasing due to the decline of water temperature.

In Carp fish variation in altitude brings changes in Haematocrit, leucocytes, and Haemoglobin indices. (Rosidah *et al.*, 2017). In most of the land animals, erythrocytes, Haemoglobin, and haematocrit level increases form low to high altitude as observed by Powell & Hopkins, 2010 and Storz *et al.*, 2010.

According to Suleimman & Alhaj,2012 & Lehmann *et al.*, 2005 towards high altitude, a regular decline can be seen in the thrombocytes of rainbow trout. As a result of hight a decrease of 20 percent in platelet count happens compared to low altitude. Gray *et al.*,1975 also stated that, platelets count decreases with high altitude. However according to Hartmann *et al.*,2005; Hudson *et al.*,1999 & Sharma, 1981 spending 3 or more days at high altitude were noted to cause increase in thrombocytes. While Sharma,1980 found that there is no consistent changes in platelets at high altitude.

Many researchers have performed studies on fish blood parameters in freshwater bodies of Pakistan but until now work has not been done on altitude effect of Rainbow trout blood at District Upper Dir, Khyber Pakhtunkhwa Pakistan. Hence, this research is conducted for the first time in Dir Upper, Khyber Pakhtunkhwa, Pakistan to know the blood parameters changes in rainbow trout with altitude.

Objectives:

1) To scrutinise various indices of blood in healthy Rainbow trout cultured in farms in district Dir upper.
2) To find the effect of altitude and sampling location on blood parameters of trout fish.

Methodology:

Research methodology of this search is given below.

Sampling Area

District Upper Dir is surrounded by Chitral to the North Side, to the Western side by jandul (part of Lower Dir) and Afghanistan, Dir Lower to the Southern side and Swat to Eastern side. Collection survey was carried out at three locations (Thal Kumrat farm, Kalkot hatchery and Sheringal university farm), of Dir upper. Their geographical coordinates are 35.486982, 72.233647, 35.414305, 72.178581, 35.277317, 72.006846 and altitudes are 2550m, 1900m, and 1370m respectively (Figure.2).

Materials required during collection

Materials used during collection were, catching net, clove oil as anaesthetics, water tank(15L), surgical gloves, EDTA tubes, ice box and vacutainer system.

For temperature and Dissolved oxygen, the used materials were Thermometer, Biological oxygen demand (BOD) bottle, pipette, glass flask, manganese sulphate, alkali iodide azide, conc. sulphuric acid, sodium thiosulphate, starch solution (1gm starch powder in 100 ml of distilled water), and white paper.

Site selection and sampling

In this study healthy female rainbow trout (Oncorhynchus mykiss) (3 years of age; 1.2kg weight) were taken from three designated locations (Thal Kumrat farm, Kalkot hatchery and Sheringal university farm), Dir upper in the month of July 2021. 3 sampling were made in each area so overall 9 samples have been taken. Fish were anesthetized with clove oil (at 60g/L of water) in 15L plastic tank prior to blood collection. A total of 9 samples were

collected. 2ml of blood were collected in EDTA tubes within 30 second from the caudal vein of anesthetized fish using vacutainer with 22-gauge needle. After each collection samples were shaken well to prevent form clotting and were kept in ice box. The samples were examined after bringing to the lab.

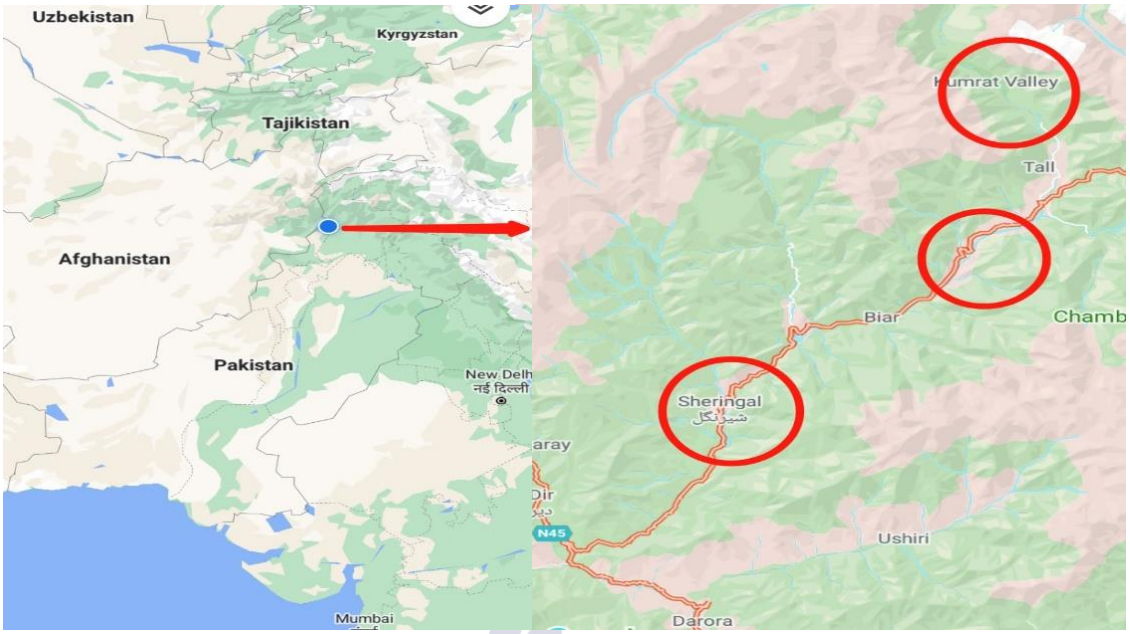


Figure.2 Three sites for blood sampling are Tal Kumrat, Kalkot, and Sheringal, Dir upper, KP, Pakistan.

Haematological parameters

Haematological indices like haemoglobin (Hb), Erythrocytes, haematocrit (Hct), Leucocytes count, thrombocyte count (TC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were determined through automated haematology analyser and then manually.

For haemoglobin assessment the cyanmethemoglobin technique were followed. The values of RBC and WBC were gain from studying haemocytometer under microscope with 10x and 40x lens using dilution fluids. Similar procedure was done for platelets with platelets -diluting fluid. Haematocrit values were obtained after micro heparinised capillary centrifugation process that was done at a rate of 12000 rpm up to 2 minutes. The remaining indices such as MCH, MCHC, and MCV were calculated from primary indices.

Physio chemical parameters

For temperature estimation, digital thermometer was used. For correct estimation the Thermometer were submerge into the water for about 1 minute to adjust the accurate value.

For Dissolved oxygen (DO), Winkler technique was used as given below

Water sample was taken in 300ml BOD bottle in each location. With the help of pipette 2ml of manganese sulphate was added then introduce 2ml of Alkali iodide azide and shake well. After mixing a brown yellowish precipitate was formed which indicate the presence of DO in the sample. After settling down the bottom, 2ml of conc. Sulphuric acid was added to dissolve the precipitate. Then in a flask 20ml of the sample was taken and titrate with sodium thiosulphate to reach to a pale straw colour. After that 2ml starch solution was mixed to give a blue colour. and finally, titration was made using a calibrated pipette by slowly dropping sodium thiosulphate until the colour became disappeared. Each ml of used sodium thiosulphate indicate 1mg DO in the sample.

Results

Altitude, temperature, RBC, WBC, Hb, Hct, MCV, MCH, MCHC, lymphocytes, neutrophils, valves of Oncorhynchus mykiss collected from three different

altitudes are presented in Table1. Altitude, Dissolved oxygen, and temperature comparison has been made in Table 2.

In Figure 3, 4, 5, 6, 7, 8, 9, and 10 individual parameters comparison has been made at different altitude range. In addition, pictures of erythrocyte, and leucocytes obtained from this trout species are shown in Figure 11.

Table.1 Mean Haematological parameters of rainbow trout at different altitude of Dir Upper.

Parameters	Kumrat	Kalkot	Sheringal
Altitude (m)	2550	1900	1370
RBC (× 10^6/µL)	1.16	1.24	1.54
WBC (× 10^3/µL)	59.2	54.6	69.95
Lym	96.1%	96.9%	95.6%
MID	2.5%	2%	2.55%
Neut	1.4%	1.1%	1.85%
Thrombocytes (× 10^3/µL)	47	54	74
Haemoglobin (g/dL)	11.2	11.4	13.1
PCV (%)	33.6	34.2	39.3
MCH (pg)	96.5	91.93	85.475
MCV (fL)	289.6	275.8	256.425
MCHC (g/dL)	570	116.6	78.35

Table.2 Physical and DO parameters of water at three different altitude of Dir Upper.

Parameters	Kumrat	Kalkot	Sheringal
Altitude (m)	2550	1900	1370
Temperature (^{0}C)	14.2	16.7	20.5
Dissolved oxygen (mg/l)	9.06	8.91	8.53

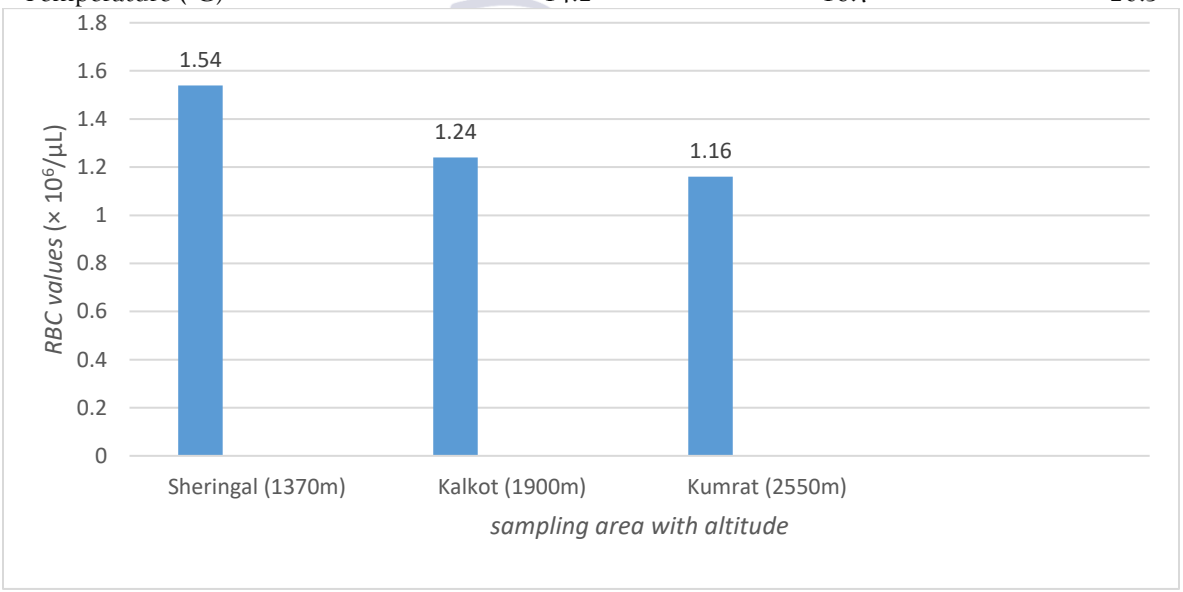


Figure.3 RBC value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) show a clear increase down the altitude.

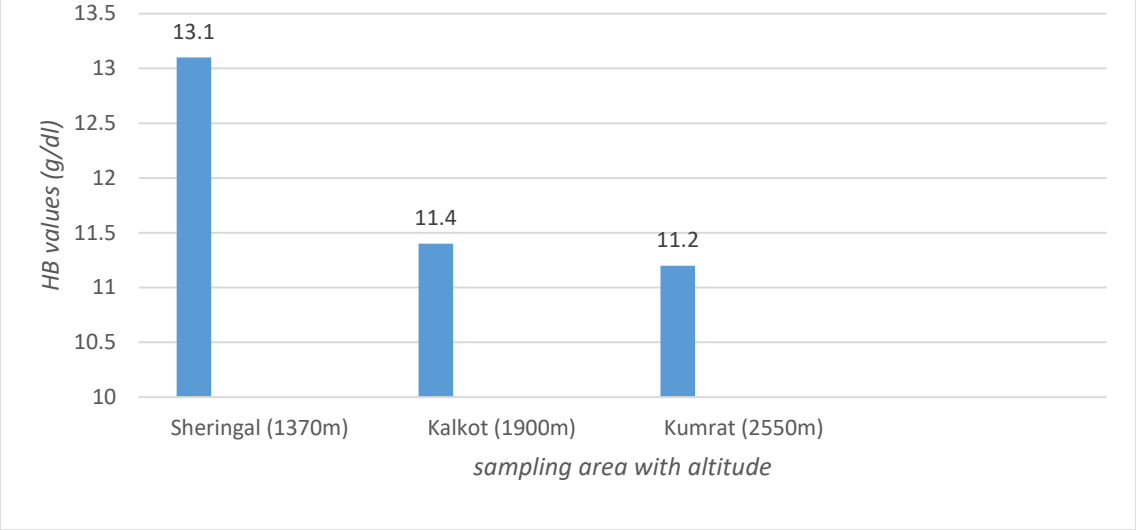


Figure.4 Hb value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) show a clear increase down the altitude

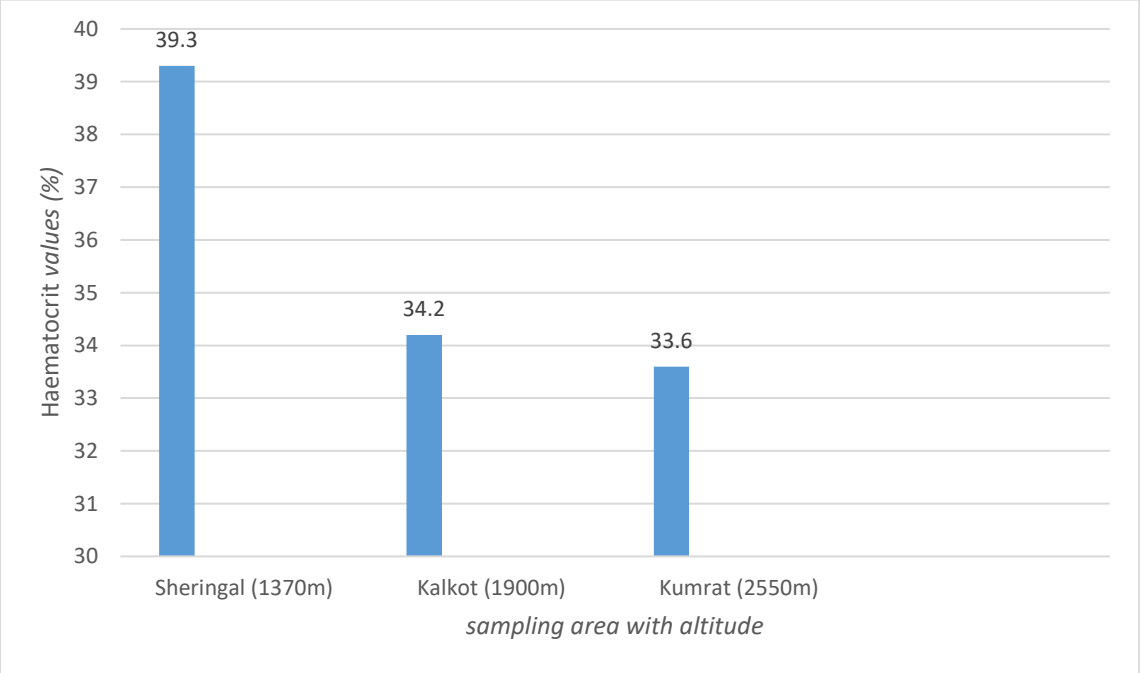


Figure.5 Hct (PCV) value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) show a clear increase down the altitude

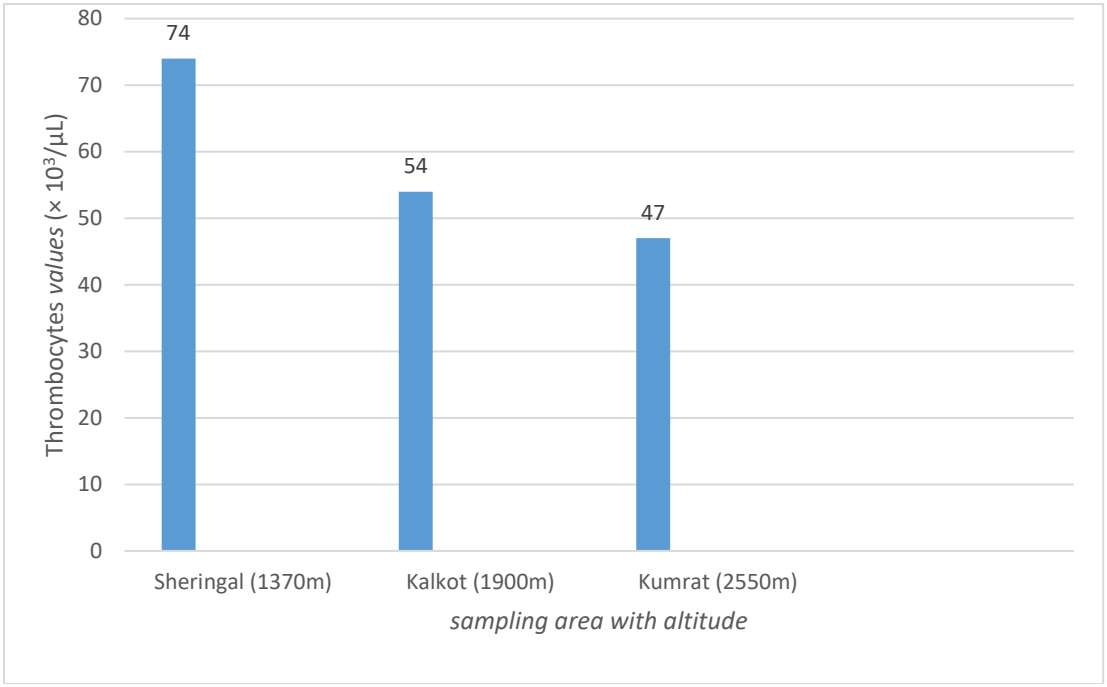


Figure.6 Thrombocytes value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) show a clear increase down the altitude

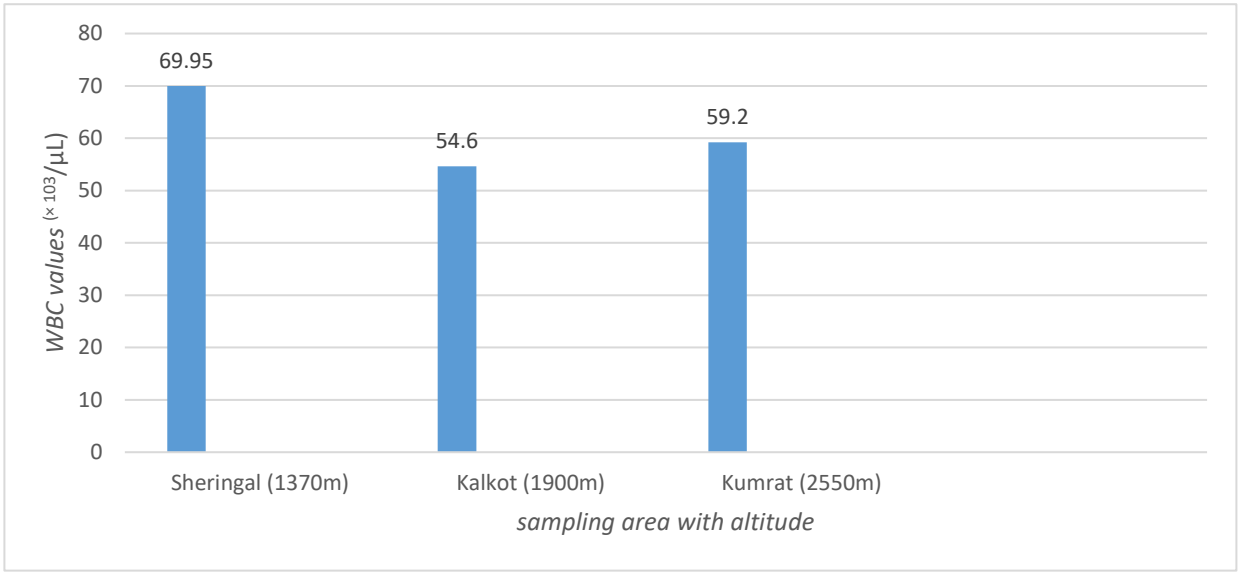


Figure.7 WBC value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) first show increase at hight in kumrat compared to Kalkot then show increase down the altitude at Sheringal.

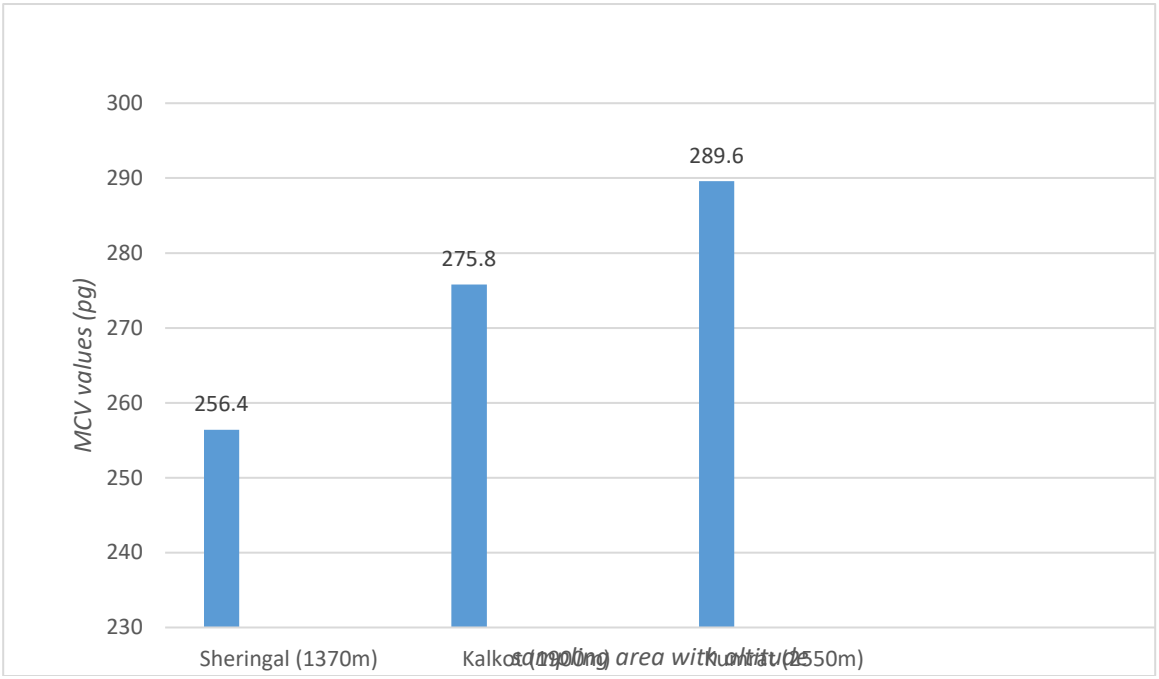


Figure.8 MCV value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) show a clear decrease down the altitude

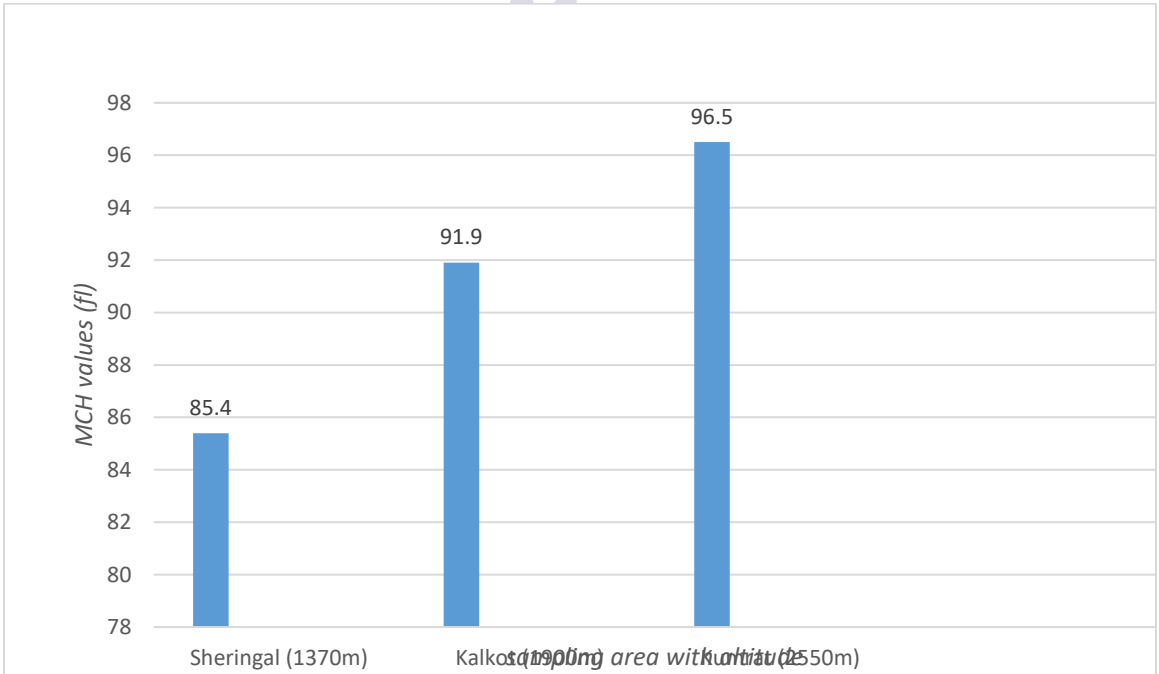


Figure.9 MCH value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) show a clear decrease down the altitude

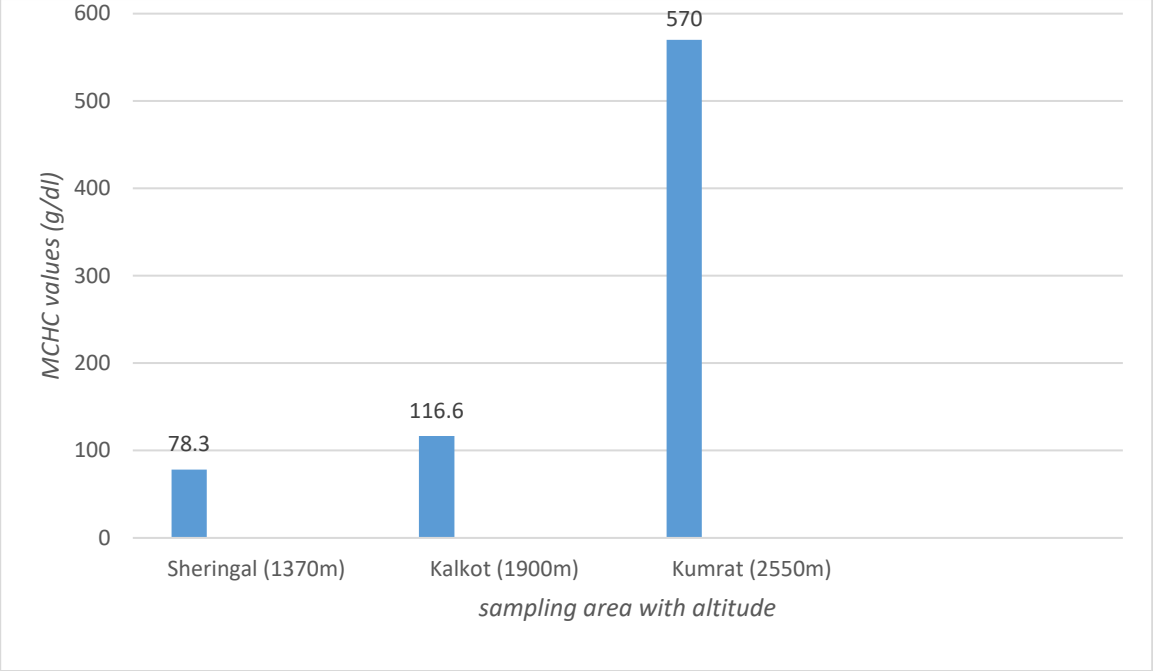


Figure.10 MCHC value at three different altitude (Kumrat 2250m, Kalkot 1900m and Sheringal 1350m) show a clear decrease down the altitude

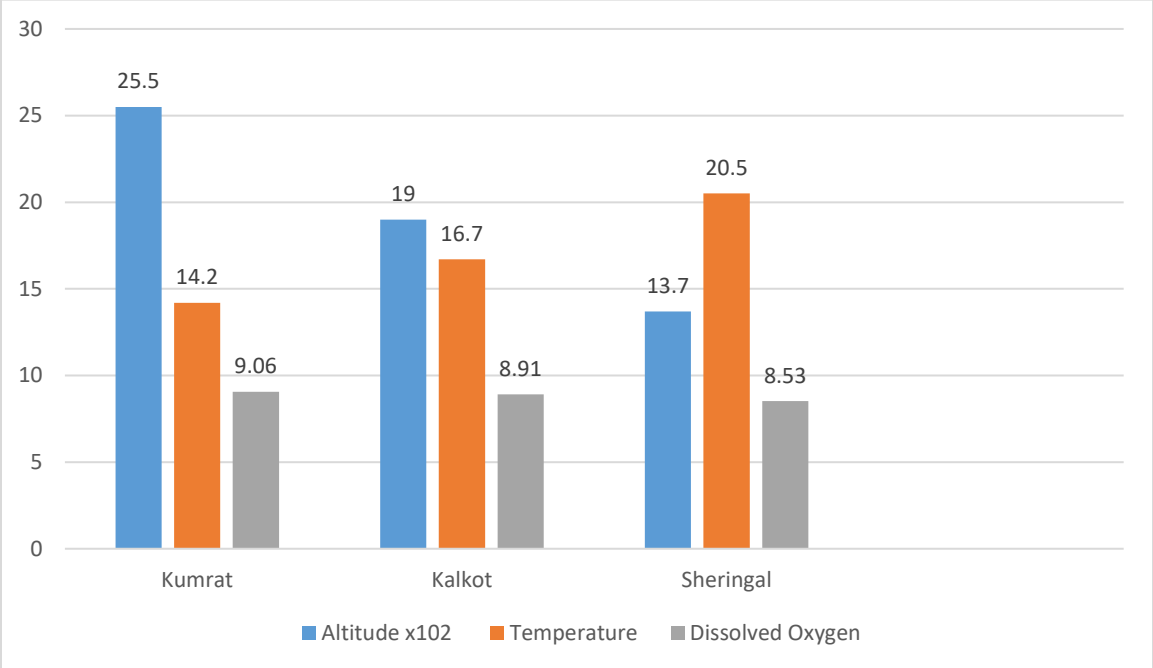


Figure.11 Altitude, Temperature, and Dissolved oxygen comparison

If we investigate figure 11. We can see the direct relation between the dissolved oxygen and hight. On the other hand, an inverse relation between the temperature and height, which actually determine the effect on blood.

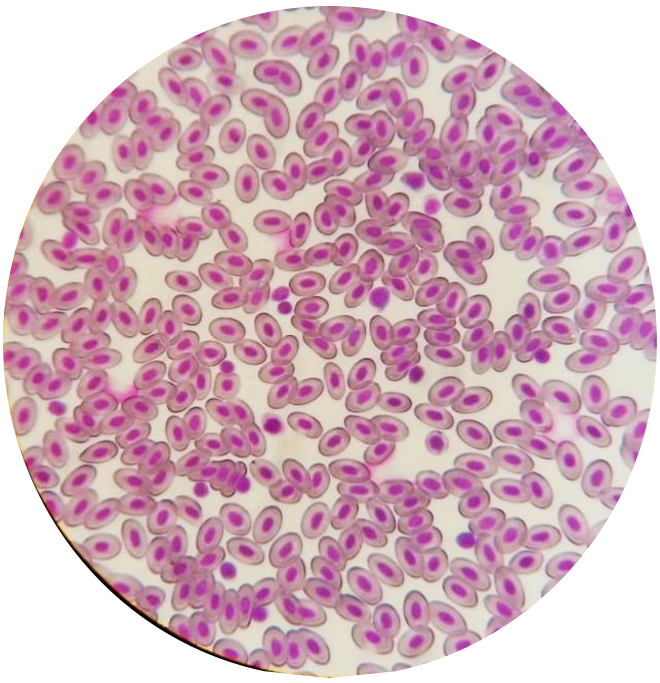


Figure.11 RBC and WBC of rainbow trout under microscope.

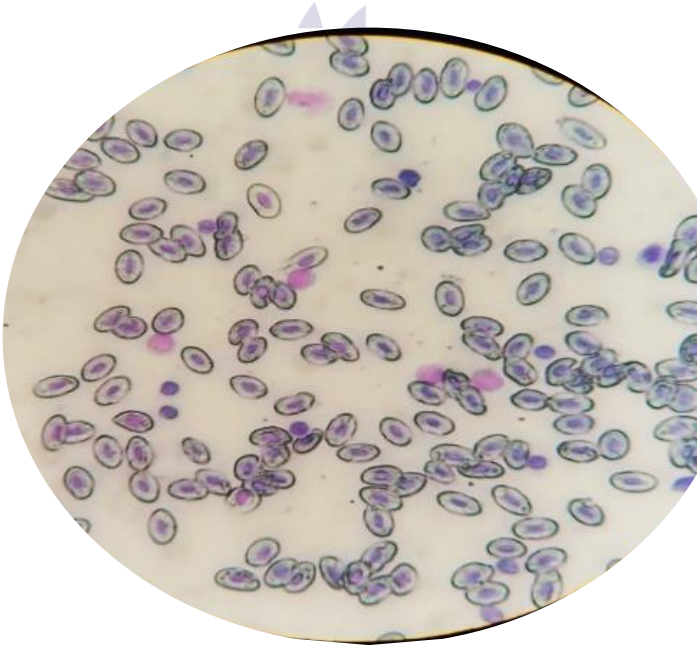


Figure.12 RBC slide after applying RBC solution

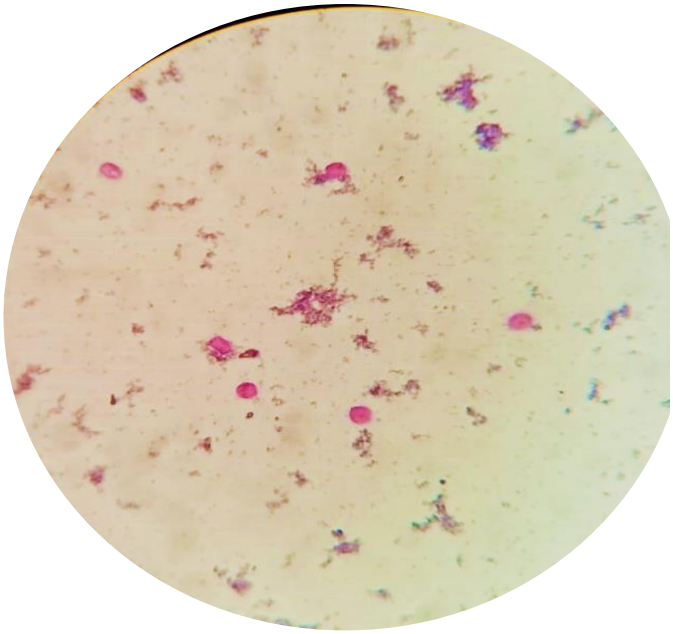


Figure.13 WBC slide after applying WBC solution

Discussion:

In this study as shown in Table 2 when we move from high towards lower altitude, the temperature increases continuously which tend to decrease the dissolved oxygen level continuously, this is because the temperature show inverse relation to the dissolved oxygen. So, this whole set up brings up changes in blood indices of fish. The temperature in Kumrat, Kalkot, and Sheringal is 14.2^{0}C, 16.7^{0}C and 20.5^{0}C and the Dissolved oxygen is 9.06mg/l, 8.91mg/l, and 8.53mg/l respectively.

Erythrocytes, Haemoglobin, and Haematocrit

In most of the land animals, erythrocytes, Haemoglobin, and haematocrit level increases from low to high altitude as observed by Powell & Hopkins, 2010 and Storz *et al.*, 2010. this is due to the decrease in temperature at high altitude which leads to hypoxia, that stimulate erythropoietin to produce more erythrocytes to fulfil the needs of oxygen in tissue level. but conversely, it has been observed that *Oncorhynchus mykiss* (fish) table.1 show clear increase in erythrocytes, Haemoglobin, and haematocrit (PCV) from higher to lower altitude. This is because at lower altitude the temperature of water is high compared to higher altitude specially in the present study area, this acute thermal stress causes reduction of dissolved oxygen in the water as shown in table 2, the DO in Sheringal is 8.53 gm/l which is lower than Kalkot 8.91 gm/l and in Kumrat the value is highest 9.06 gm/l. Being poikilotherms, the body temperature of fish varies with that of its surrounding which causes hypoxia. To cope with this problem, rainbow trout respond by starting release of cells from spleen, causes the blood to carry more oxygen to tissues, which speed up the process of premature erythrocytes and boost up the production of new erythrocytes by means of erythropoiesis (Murad *et al.*,1990 and Lewis *et al.*, 2010). WBC, Hb, Hct decreases whenever the temperature of water goes down and other analysts supported this outcome (Leard *et al.*, 1998 and Rambhaskar & Rao, 2006).

Thrombocytes

It can be observed from table1. That a gradual decrease occurs in the thrombocytes of rainbow trout towards high altitude which is similar to the results of Suleimman & Alhaj,2012 & Lehmann *et al.*,2005 that due to hight a decrease of 20 percent in platelet count happens compared to low altitude. Gray *et al.*,1975 also stated that, platelets count decreases with high altitude. However according to Hartmann *et al.*,2005; Hudson *et al.*,1999 & Sharma 1981, spending 3 or more days at high altitude were noted to cause increase in thrombocytes. While Sharma,

1980 found that there are no consistent changes in platelets at high altitude.

Leucocytes

Whenever a strange matter or bacteria tries to enter the body, the cells of leucocytes engulf and restrain that infectious matter and serve as guard of the body immune system. Leukocytes contributing as a one of the recognition factors in case of infection. The production of leucocytes increases in response of upcoming external danger (Dianti *et al.*,2013). Based on the table shown above WBC values found in Sheringal was 69.95 (×103/µL) which was higher than Kalkot 54.6, but in Kumrat its vales increased back to 59.2. This increase may be due to the water quality which was polluted in Sheringal and Kumrat in sampling month, as stated by Paulo *et al.*, 2009, WBC increases in numbers with stress and bad water quality. WBC number is also affected by the age, and sex.

MCV, MCH and MCHC

In this study Mean corpuscular volume increases (256.425fl, 275.8fl, 289.6fl) with high altitude which show similarity with Suleimman & Alhaj, 2012 study which also show increase in MCV with high altitude. MCH and MCHC values were also found to be increased (85.475pg, 91.93pg, 96.5pg and 78.35g/dl, 116.6g/dl, 570g/dl) with high altitude. MCH mean average haemoglobin concentration in the RBC in the body. Low MCH values may specify the presence of iron deficiency anaemia. Iron is important for the production of haemoglobin. If MCHC is high, it means there is an increased concentration of Hb inside red blood cells. Additionally, condition where Hb is present outside of the red blood cell due to the RBC destruction or fragility can also produce high MCHC values.

In the earlier studies, amplified metabolic activity, condensed oxygen level and higher energy demand, as of increased water temperature, are testified to be the leading cause of alteration in haematological indices (Bitmis & Yılayaz, 2002; Akmirza and Tepecik, 2007). As we know that these parameters changes with altitude so in the present study altitude show direct or indirect effect on fish haematology.

Conclusion

From the results it is cleared that Rainbow trout blood parameters changes with differences in altitude which is specifically due to changes in water qualities such as temperature and dissolved oxygen. This is because when going towards sea level, the temperature increases which lead to decrease dissolved oxygen in the water body. And this increase or decrease of dissolved oxygen has great effect on fish blood.

For the same haematological parameters other fish species should also be studied for the same purpose and the results should be compared with the rainbow trout in the same area and altitude to explore the findings

A baseline indices is important to be established for haematological indices and the need of performing further research to find out the effects of such indices on these parameters. For example, It is important to determine whether it is possible to predict the threshold values of Dissolved oxygen at which it becomes necessary to have an increased or decreased haematological parameters specifically haemoglobin-oxygen carrying capacity.

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