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The studies on impact of variation in physicochemical properties of water on Haematological Parameter of Fresh Water Fishes from Mula river of Pune.

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Abstract;

The freshwater fishes are key and low prize source of protein of people. The amount and quality of proteins present in bodyis related to the health of fish.Fish health is subject to the environmental factors like pollutants, temperature of water, salinity and availability of food in water bodies. The blood parameters were used to known the health and quality of fish**.** The ecological conditions like seasonal differences, feeding, sex and size of fish, as well breeding efficiency can modify the haematological parameters of fishes.Haematological parameters reflect the healthy condition of fish than other parameters. In present investigation haematological parameters were studied in adult fishes of *Channamarulius*(Hamilton-Buchanan), *Oreochromismossambica*(Peters) and ***C****alriusgarpiens (Burchell, 1822),* in three seasons. During summer season, *Channamarulius* shows highest count of Red blood corpuscles (RBC), Haemoglobin, Packed cell volume (PCV), and Glucose. While *Orechromismossambicus* shows highest count of White Blood Corpuscles (WBC), Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC). The highest amount of Protein observed in *Clarius gariepinus*. In winter season, highest amount of RBC, Haemoglobin, MCHC and Glucose was observed in *Channamarulius*and highest amount of WBC, MCV, MCH was observed in *Orechromismossambicus*and highest amount of protein observed in *Clarius gariepinus*. However in Rainy season, highest amount of RBC, Haemoglobin, PCV and Glucose was observed in *Channamarulius*, while highest amount of WBC, MCV, MCH, MCHC was observed in *Orechromismossambicus*. And highest amount of protein was observed in *Clarius gariepinus*.

INTRODUCTION:

The freshwater fishes are major and low cost source of protein of low income group people. The nutritional quality of fishes are depends on amount and quality of proteins present in body of fishes, is directly proportional to the health of fish.Health of fish is depends upon the environmental factors like pollutants, temperature of water, salinity and availability of food in water bodies.The blood parameters have been commonly used to observe and follow the quality of fish**.** The environmental conditions, feeding, sex and size of fish, as well as seasonal differences and breeding efficiency can altered the haematological parameters of fishes.Haematological parameters reflect the healthy condition of fish more quickly than other commonly measured parameters and since they respond quickly than other commonly measured parameters. Also they respond quickly to changes in environmental conditions, so they have been widely used for the description of fish health in stress responses and for predicting the physiological adaptations of fish and provide a future understanding of ecological impacts. Since last few years, fishes have been extensively used as a protein rich diet for human consumption in India thus, it contribute a lot to its economy. In India, the fish management has occupied an important place value especially, in the agricultural economy.

TheHaematological parameters are frequently used as an essential diagnostic device to evaluate the wellbeingcondition of fishes due to the increasing emphasis on pisciculture and greater awareness towards the anthropological pollution of natural freshwater resources in the tropics.These studies aregenerally used as an operative and sensitive key to monitor the physiological and pathological changes in fishes[1].The haematological parameters of fish are being used to study the increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological alterations in fishery management disease investigations[2].The studies on haematological parameters provide a valuable diagnostic tool in assessing fish quality.[3,4 and 5].

The physiological standards of haematological parameters are species specific and age dependant[6].The quantitative and qualitative variation in haematological parameters includes red blood corpuscles (RBC) and white blood corpuscles (WBC) numbers.Amount of haemoglobin and size and number of RBC and WBC are most significant findings as regards diagnosis[7.]

The haematological parameters like haematocrit, erythrocytes count and haemoglobin concentration, packed cell volume, protein content and glucose content,Mean corpuscular haemoglobin (MCH),Mean corpuscular volume (MCV), Mean cell Haemoglobin

Concentration (MCHC) have been studied.Among different haematological parameters, haematocrit value is not easily altered as other parameters and should be used in conjunction with erythrocyte and leucocyte count, haemoglobin content,Most of the several contributions towards a better understanding of fish haematology. Variations in haematological parameters of fishes arecaused by environmental stress[8], malnutrition[9], gender[10, 11], fish size[12], seasonal differences and breeding efficiency[13].Haematological tests arebeneficial in detection and analysis of metabolic disorders and illnesses in fishes. The ecological influence on blood parameters of fishes generally been used as an effective and sensitive index to monitor the physiological and pathological changes in fish[14].The physiological values of haematological parameters are species specific and age dependant[6]

In presentinvestigationthree freshwater fishes*Channamarulius*(Hamilton- Buchanan), *Oreochromismossambica*(Peters) and ***C****alriusgarpiens (Burchell, 1822),* were used for experiments; these are commercial fishes in India with maximum market value and acceptability as food by the consumers due to their taste and flesh. Fishes contribute a major portion to the fresh water fish production. This study is performed in three seasons that is summer, rainy and winter.This study is comparative study of these three fresh water fishes.Use of haematological parameters as indicators of stress can provide valuable information concerning the physiological reaction of fish in a changing environment and it provides an ideal tool for toxicological studies. Blood is a pathophysiological indicator of the body as it is highly susceptible to internal and external environmental fluctuations in stress conditions.

The haematological response in Indian major carps in relation to supplementary feeding, they reportedthat fish production was highest in artificial feed compare with other feed, growth response in the same and high quantity of crude protein with low cost level[15].Seasonal variation in certain haematological parameters of *Catlacatla*was observedshows highest count during summer season due to highest ambient temperature[16].The experiment on haematology and biochemical parameter of different feeding behaviour of teleost fishes from velar estuary, India three fishes of different habitat was taken into consideration i.e. Carnivore, Herbivore, and Omnivore. They found that highest RBC count observed in *Calcifer*(Carnivore), and highest Hb count was observed in *L.calcifer* (Carnivore) and *Chanas*(Herbivore) [17]. Investigation on Seasonal Variation on Haematology parameter of Golden Mahseer, *Tor puritora*, they found that WBC decline

during monsoon and autumn season. And these factors were affected by endogenous and exogenous factors such as reproduction, water temperature and dissolved oxygen[18].

Thehaematological changes in fish *Mastacembelusarmatus* (Laacepede) of Song River they observed significant change in WBC in winter and rainy season[19].The studieson *Clarius gariepinus* exposed to sublethal concentrations of Mercuric chloride shows a substantial a reduction in total erythrocyte (RBC), haemoglobin (Hb), Packed cell volume (PCV) and mean corpuscular volume (MCV) values, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) with increase in time as compared to the control fish. However, total White Blood Corpuscle count is a considerably increased with increase period of exposure and concentration of Mercuric chloride. During this study it has been observed that number of mononocytesis increased at higher concentration of Mercuric chloride. White blood cells have a main role in the defence mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to recover fragments from damaged tissue and lymphocytes produce antibodies. The increase in WBC could be attributed to a stimulation of the immune system in response to tissue damage caused by mercuric chloride. The stimulation of the immune system causes an increase in lymphocytes by an injury or tissue damage. The mean corpuscular haemoglobin is decreased with increase time and concentration; it clearly indicates that the concentration of haemoglobin in the red blood cells is more reduced in the treated fish than in the control fish. All these factors indicate unhealthy condition of fish due to alteration in concentration of pollutions[20].

Table-1.Seasonal variation inPhysico-chemical characteristics of water of Mula River at the sampling station in Year-2018.

Parameters	Unit	Seasons		
		Manson	Winter	Summer
Temperature	(^{o}C)	28.0	22.3	31.5
pH		7.28	6.98	7.92
Dissolved Oxygen	Mg/litre	5.6	5.4	5.2
Free CO_2	Mg/litre	8.3	10.2	11.5
Acidity	Mg/litre	156	180	190
Total Alkalinity	Mg/litre	320	350	340
Biological Oxygen Demand	Mg/litre	3.3	3.5	3.7
Chemical Oxygen demand	Mg/litre	40.32	49.184	50.9
Nitrates	Mg/litre	0.282	0.114	0.17
Phosphates	Mg/litre	85.0	118	125

Materials and Method:

For the present investigation, fish samples were collected from the Government fish seed farm, Hadpsar Pune and Kothrud fish market, in each season like monsoon, winter and summer etc. This area and pond was chosen considering tropical climate. Threeindividualsof *Channamarulius*and*Oreochromismossambica* were collected from Mula river of Pune in the three seasons. However, *Clarius gariepinus* werecollected from fish market. Blood sample were collected from these fishes by caudal puncture using disposable plastic syringe with needle which is moisturised with EDTA andexpelled into separate EDTA vials immediately on ice[21&22].

Haemoglobin is estimated by acid–haematin method by using Sahlis haemometer[23]. Total RBCs and WBCs count were determined by using improved Neubauerhaecytometer[3], using Hayem's diluting fluid for red blood corpuscles. Total WBCs count by using improved Neubauerchamber by using Turck's diluting fluid. It is calculated by using proper formula. It is calculated in number/ cubic millimetre. Packed cell volume or haematocrit value is determined by Wintrobe's apparatus[24].It is calculated by formula ht= L1/L2 .

Estimation of protein was done by Lowry method. Optical density was taken at 740 nm. Estimation of blood glucose was followed by Folin–Wu method. Optical density was taken at 420nm. Erythrocytic indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)were calculated as per formula.

MCV = PCV × 100 / erythrocyte count in cubic microns[25].

MCH = Hb × 10 / erythrocyte count ingm%

MCHC = Hb × 100 / PCV count in %

Estimation of Red blood cells: -Red blood corpuscles were estimated by using Hayemfluid (1: 200)[25].Total number of RBC is calculated by using following formula.

Total number of RBC = $\frac{No.of\ cell \times Dilution\ \ factor \times Dept\,h\ factor}{Area\ \ counted}$

Estimation of White blood cell: Total number of white blood cell was estimated by using WBC diluting fluid(Turck's fluid**).** The number of WBCs per cubic millimetre was calculated as

Total number of WBC's= $\frac{No.of\ cell \times Dilution\ \ factor \times Dept\,h\ factor}{Area\ \ counted}$

Determination of haemoglobin:

Hb is estimated by using Sahli'shaemometer.

Estimation of packedcell volume was estimated by using Wintrobe tube. Ht= $\frac{L1}{L2}$

Where,

L1 = height of RBC column in mm,

L2 = the total length of column (RBC+WBC+plasma) in mm

Estimation of mean corpuscular volume (MCV) :

This is the average volume of red cells in cubic microns.

MCV= $\frac{Haematocrit \quad Ht\% \times 10}{RBC \ count \ in \ millions}$ µm³

Estimation of mean corpuscular haemoglobin (MCH) :

MCH is the average of Hb content of the red blood cell. MCH is influenced by the size of cell and concentration of Hb.

MCH= $\frac{Hb \ (hemoglobin \quad gram \ 100ml) \times 10}{RBC \ in \ millions}$ gm%

Estimation of mean cell hemoglobin concentration (MCHC) :

It is an expression of the average hb concentration per unit volume of packed cells .

MCVC = $\frac{\text{MCH}}{MCV} \times 100$

Estimation of Serumproteins :

Serum proteins were estimated as per method of Lowry[26].Bovine serum albumin was used as standard.

Estimation of blood glucose: Blood glucose level estimated by folin-wu method[27].

Result:

Table-1.Haematological parameters of *Clarius gariepinus*:

Sr. No.	Blood parameters	Rainy Season	Winter season	Summer Season
1)	RBC count (10^6 /cu mm)	2.4333 ±0.0736	1.86323 ±0.12885	2.49333 ±0.0656
2)	WBC count(10^3/cu mm)	1998.333 ± 846.069	2168.3666± 930.0971	21080.2355± 840.071
3)	Haemoglobin(%)	7. 9247 ± 2.51999	6.9825 ±2.818	8.4347 ±2.3718
4)	PCV(%)	28.36667 ± 1.427525	25.2467 ±1.4717	27.6667 ±1.7375
5)	MCV (µm³)	89.5922 ± 5.233097	83.4477 ±4.12372	88.8577 ±5.62473
6)	MCH (gm%)	32.2437 ± 7.076	31.832 ±6.287	32.88328 ±6.07322
7)	MCHC (g/dl)	35.2788 ±7.74361	33.3788 ±6.8732	37.8887 ±7.94565
8)	Glucose in blood (mg/100ml)	86.3333 ±6.5049	86.3321 ±7.0293	86.34167 ±5.02935
9)	Protein in blood (g/100ml)	69.6666 ±0.7371	68.666 ±0.857	63.76671 ±0.6711

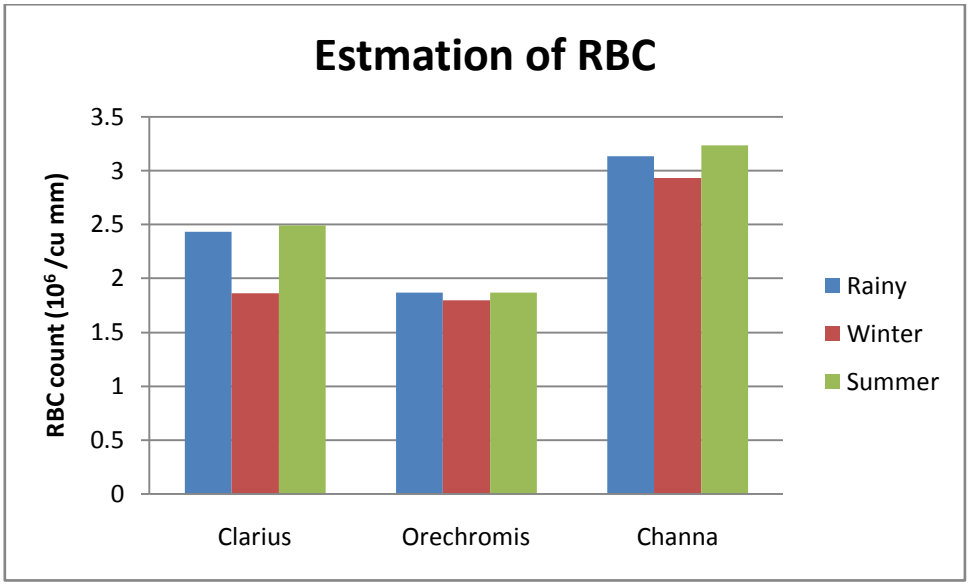
Table-2.Haematological parameters of *Oreochromismossabica*:

Sr. No.	Blood parameters	Rainy Season	Winter season	Summer Season
1)	RBC count (10^6 /cu mm)	1.8666 ±0.0351	1.800 ±0.0244	1.8866 ±0.029612
2)	WBC count(10^3/cu mm)	2800 ±676.074629	2755 ±675.09528	3020 ±788.0522
3)	Haemoglobin(%)	6.96666 ±2.71538	6.4000 ±0.2024	6.75666 ±2.85389
4)	PCV(%)	25.6666 ±1.19303	24.8000 ±0.25981	27.9000 ±0.3598
5)	MCV (µm³)	46.4433 ±4.9146	42.2100 ±4.99408	45.2200 ±4.91458
6)	MCH (gm%)	45.7666 ±4.42010	49.0100 ±0.2598	42.7967 ±5.45071
7)	MCHC (g/dl)	45.07 ±4.697	43.000 ±0.202	47.08 ±4.773716
8)	Glucose in blood (mg/100ml)	82.21 ±16.2491	79.20 ±14.2966	80.25 ±15.2967
9)	Protein in blood (g/100ml)	53.333 ±2.96090	49.666 ±3.4006	52.66667 ±1.91116

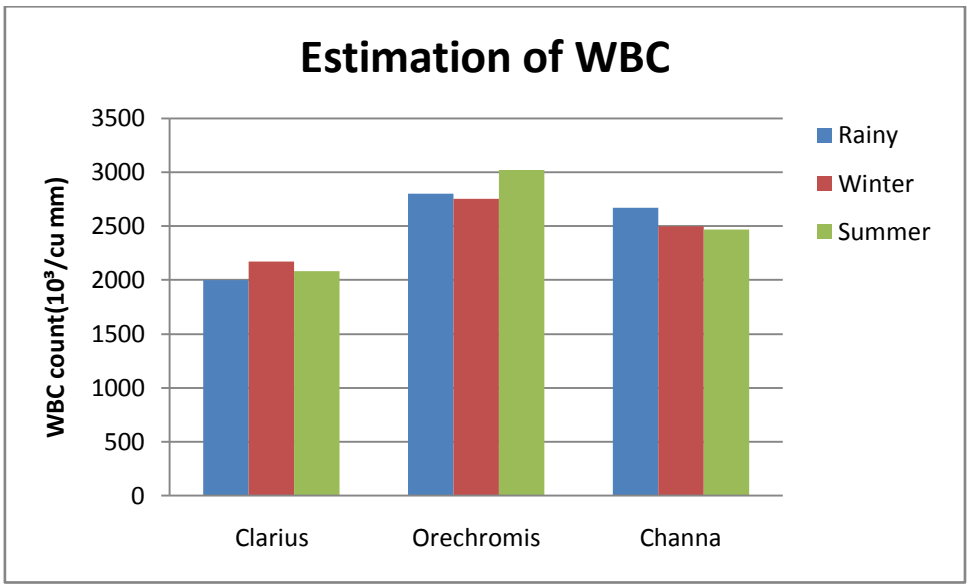
Table-3.Haematological parameters of *Channamarulius*:

Sr. No.	Blood parameters	Rainy Season	Winter season	Summer Season
1)	RBC count (10^6 /cu mm)	3.1333 ± 0.224	2.9333 ±0.1846	3.23333 ±0.2246
2)	WBC count(10^3/cu mm)	2366.666 ±1205.5424	2496.6665 ±1005.4264	2467.6555 ±1200.5326
3)	Haemoglobin(%)	9.7666 ± 1.7161	9.00717 ±0.00767	9.8666 ±1.69614
4)	PCV(%)	28.9666 ±3.25166	26.9697 ±2.1322	29.9667 ±3.27166
5)	MCV (µm³)	73.1166 ±9.5308	85.3133 ±3.7314	95.3433 ±3.8324
6)	MCH (gm%)	25.51466 ±4.74451	24.4166 ±3.3451	29.4266 ±3.4351
7)	MCHC (g/dl)	39.31666 ±5.1200	38.3066 ±4.9200	39.4167 ±5.12000
8)	Glucose in blood (mg/100ml)	80.87666 ±10.7530	82.8666 ±20.7531	83.87777 ±22.78315
9)	Protein in blood (g/100ml)	52.6666 ±4.0692	50.666 ±5.9692	59.7776 ±3.9796782

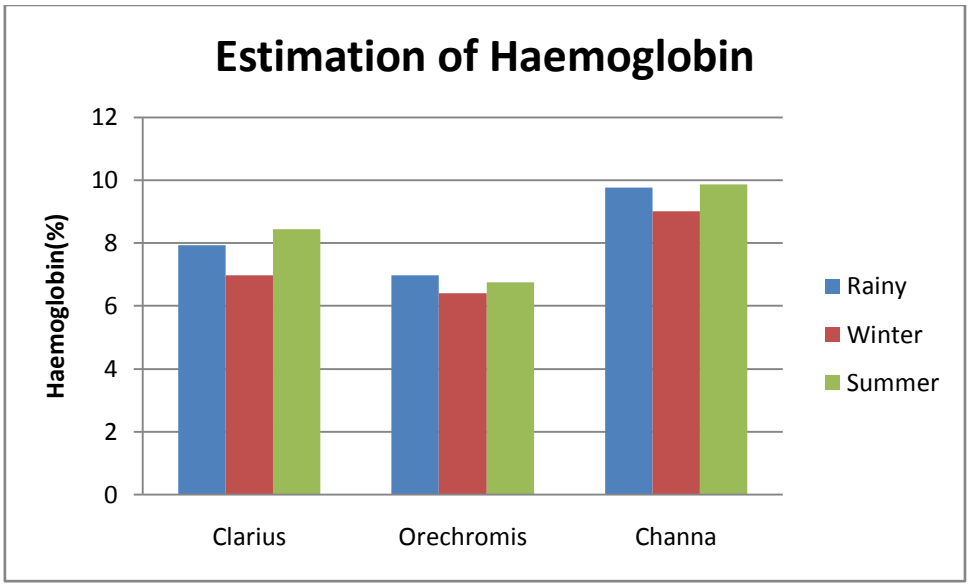
Graph-1:- Comparative of study of Red Blood cells of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*during monsoon, winter and summer season.



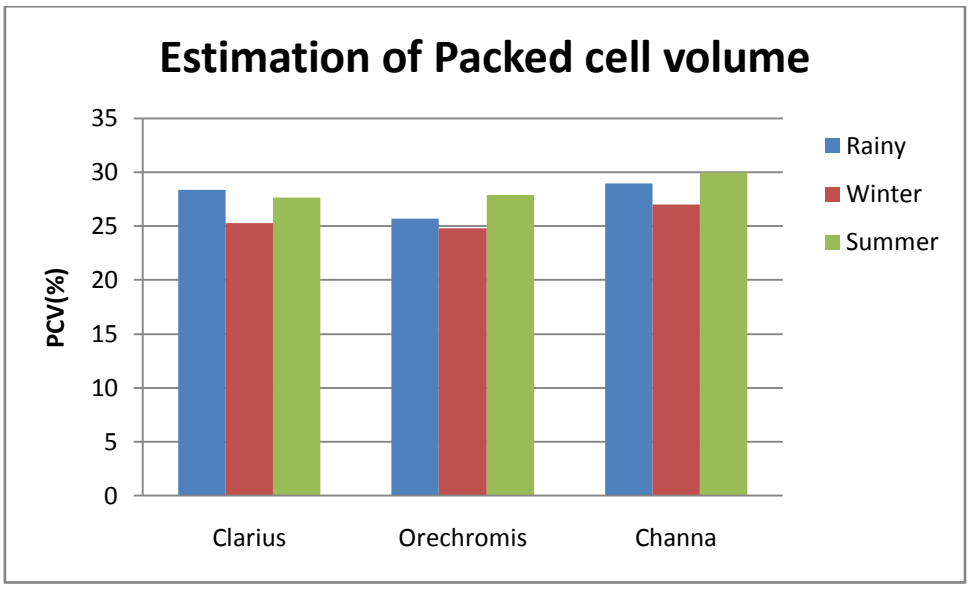
Graph-2:- Comparative of study of White Blood cells of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*during monsoon, winter and summer season.



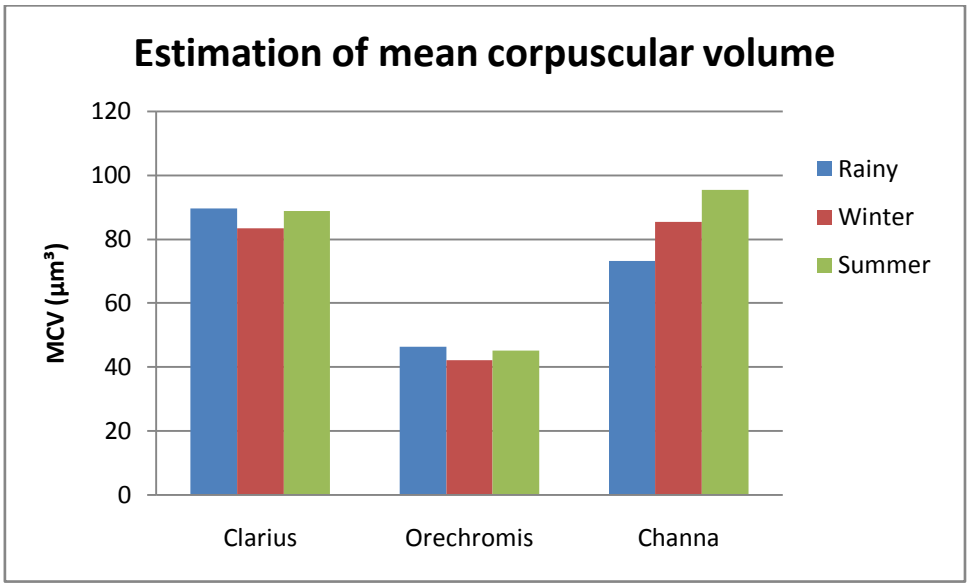
Graph-3- Comparative of study of Haemoglobin estimation of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*during monsoon, winter and summer season.



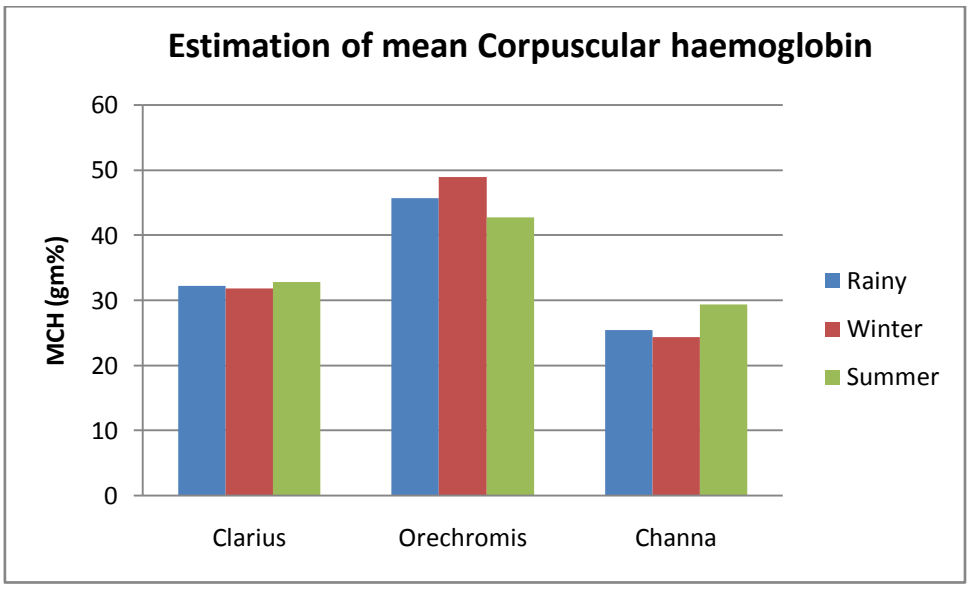
Graph-4- Comparative of study of packed cell volume of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*duringmonsoon, winter and summer season.



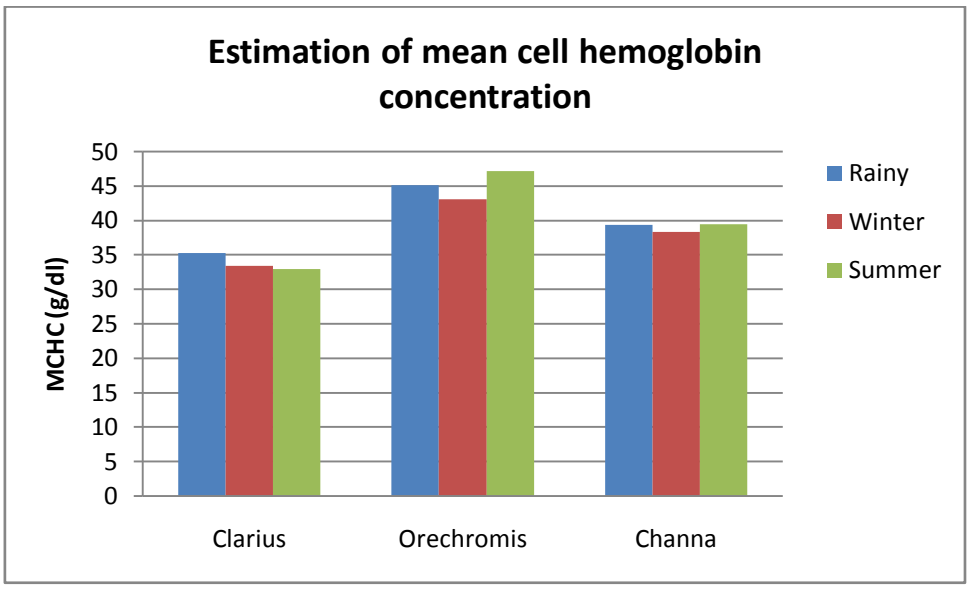
Graph-5- Comparative of study of Mean cell volume of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*duringmonsoon, winter and summer season.



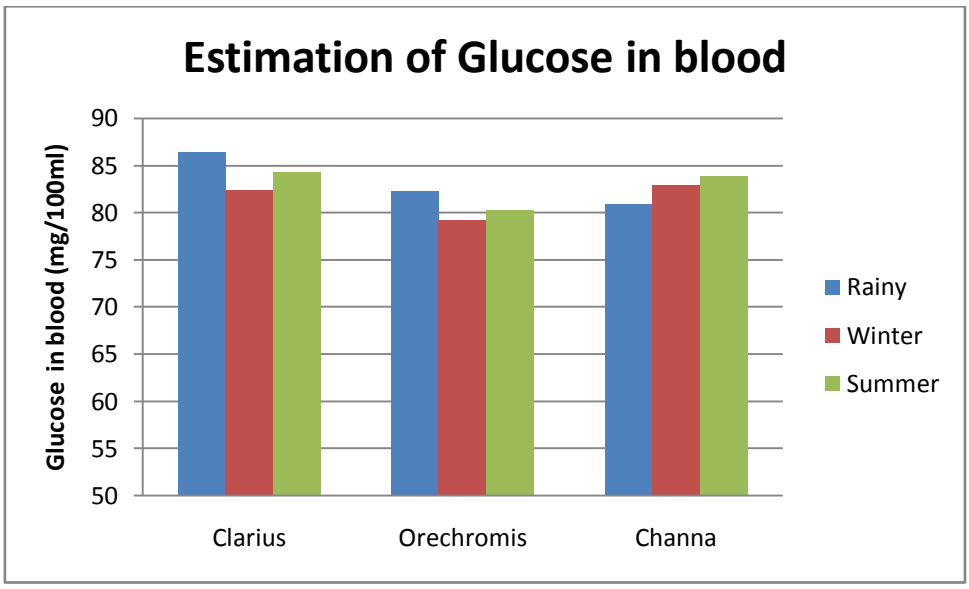
Graph-6- Comparative of study of mean Corpuscular haemoglobin of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*during monsoon, winter and summer season.



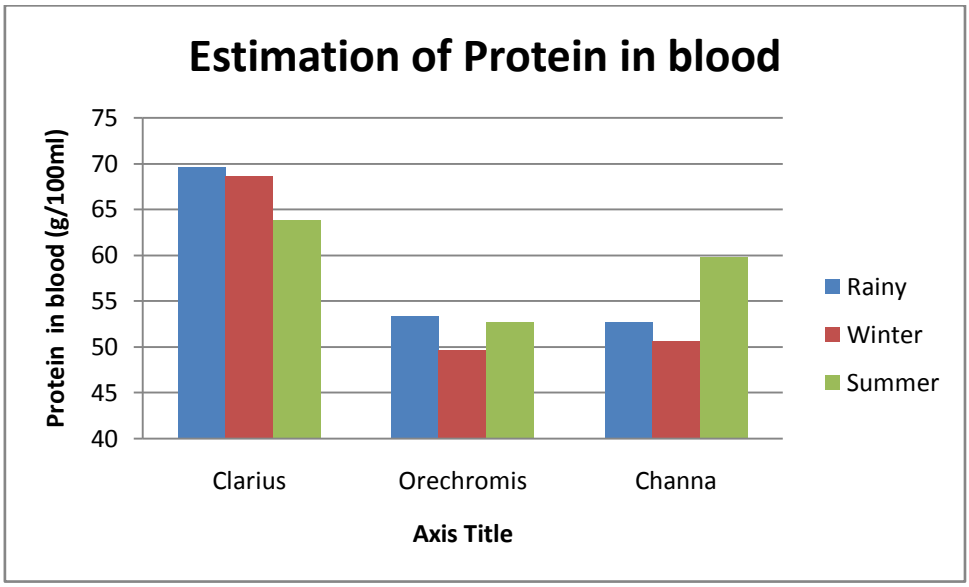
Graph-7- Comparative of study of mean cell haemoglobin concentration of*Clarius garpiens, Oreochromismossambica* and *Channamarulius*during monsoon, winter and summer season.



Graph-8- Comparative of study of estimation of blood glucose of *Clarius garpiens, Oreochromismossambica* and *Channamarulius*duringmonsoon, winter and summer season.



Graph-9- Comparative of study of estimation of blood Protein of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*duringmonsoon, winter and summer season.



Discussion:

The Fish species were influenced bynumerous factorssuch as varied water qualities, pollution, malnutrition, infection and disease and can adapt themselves altered environmental condition.All these above factors linked to fish health. It is essential to establish an identity the causes of disease in fish presents as a challenge for the associated person. Water quality is an important factor, which is responsible for variation in fish haematology[9].

In present work principallywe focused on haematological evidence in adult fishes of *Clarius garpiens*, *Oreochromismossambica* and *Channamarulius*. The haematological

parameters observed in this study showed marginal differences in comparison of these three fresh water fishes.

During summer season, *Channamarulius*shows highest count of Red blood corpuscles (RBC), Haemoglobin,Packed cell volume (PCV), and Glucose. While *Oreochromismossabica* shows highest count of White Blood Corpuscles (WBC), Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC). The highest amount of Protein observed in *Clarius gariepinus*. In winter season, highest amount of RBC, Haeamoglobin, MCHCand Glucose was observed in *Channamarulius*and highest amount of WBC, MCV, MCH was observed in *Oreochromismossambica*and highest amount of protein observed in *Clarius gariepinus*. Whereas in Rainy season, highest amount of RBC, Haemoglobin,PCVand Glucose was observed in *Channamarulius*, while highest amount of WBC,MCV,MCH,MCHC was observed in *Oreochromismossambica*. And highest amount of protein was observed in *Clarius gariepinus*.

This study further stated that during summer time body metabolic rate is high due to high body metabolic rate, which is correlated to high ambienttemperature. In this season most of haematological parameters shown higher value than other seasons.However, lowest values were observed in winter might be due to low ambient temperature and low metabolic rate. These result are supported by findings of[21,28& 29].

In fishes haematological parameters might be affected by deviations in water temperature and oxygen concentration[21].The fish haematology is extremelydependent on wellbeing, physiological process, habitation and the considerate of different ecological factors that gives reference ranges for ranges for haematological parameters like RBC, WBC numbers, amount of haemoglobin, amount of protein, amount of Glucose and other erythrocytic indices. More research will be necessary to determine the effects of microhabitat, environmental conditions, ambient temperature, nutritional status and possible seasonal fluctuations on the fish haematological parameters.

Many authors reported that there are differences in these blood indices that can be related to many factors such as age, size of fish, nutrition status, season, sex, and genetic variation.The number of blood cells and haemoglobin concentration tends to increase with age and length[30].

During the investigation it has been observed that there are no remarkable changes or differences in MCHC and MCH values.The Leucocytes are acting as a first line of defence against any type of infection and pathogen makes an organism immune enough to fight any

possible stress.However, in fishes specific immunity is less developed, so fishes depends on the non-specific resistance system which include increase/ decrease in number of leucocytes and their products; lysozymes, interferon's,lysins etc. Fishes respond by alteration in number and proportion of leucocytes.Fluctuations in TLC observed presently during different seasons also indicate that this may be a response to fluctuating weather conditions and some environmental stress which is encountered frequently in water bodies. TLC rise in summer season in response to increase in water temperature.It acts as a natural stress agent which stimulate fish to increase leucocytes count which prepare them immunologically strong enough to fight with these stress condition.Also the increased pathogen level and to the infection level during this time can also stimulate the immunologically strong response. Whereas TLC count is decline during monsoon than the summer. And it is lowest during winter due to low water temperature. Starvation like condition during these winter season is also the main reason for the declining TL count. Throughout starvation there is deficiency of important nutrient occur[31].

The elevated numbers of red blood cells and haemoglobin levels seem to be an indication of the increase in newly formed immature red blood cell population and shortening of the life span of mature red blood cells. Hence decrease in PCV towards the fish suffering from a physiological disorder due to water. Total count of cell (TC) is the defence cells of the body and is the diagnostic feature of many diseases. During stress condition due to the environmental condition which might have produced gill damage and fish becomes restless.MCV gives an indication of the status or size of RBCs. The increases in MCHC of the blood conform to the erythrocyte count and their production in the disorder. A decrease inMCV indicates that the erythrocytes have shrunk, either due to anaemia,stress or impaired water balance or a large concentration of immature erythrocytes that have been released Hb in tissue. MCH during low and high concentrations might be caused due to the above reason.

In present study decrease in MCV can lead to elevation in EC values observed in the fish. The MCHC is a good indicator of red blood cell swelling or shrinkage. The increase in MCHC values is the indication of the shrinking of red blood cells. These variations in blood parameter is directly or indirectly concerned with variation in physicochemical properties of water.

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