

Seasonal Variations in Haematological Parameters and Body Composition of *Labeo rohita* (Rohu) and *Cirrhinus mrigala* (Mrigal Carp) in River Indus, District Dera Ismail Khan, Pakistan

Syed Sikandar Habib¹, Francesco Fazio^{2,*} , Saira Naz³, Francesca Arfuso², Giuseppe Piccione², Hameed Ur Rehman⁴, Wali Muhammad Achakzai⁵, Muhammad Nazir Uddin⁶, Khalid Hussain Rind⁷, Nadir Ali Rind⁷

¹University of Sargodha Punjab, Department of Zoology, Pakistan

²University of Messina, Department of Veterinary Sciences, Polo SS Annunziata, 98168 Messina, Italy

³University of Lahore Sargodha Campus Punjab, Institute of Molecular biology and Biotechnology, Pakistan

⁴Kohat University of Science and Technology, Department of Zoology, KP, Pakistan

⁵University of Baluchistan, Department of Zoology, Baluchistan, Pakistan

⁶University of Swat, Center of Biotechnology and Microbiology, KP, Pakistan

⁷Shaheed Benazir Bhutto University, Department of Molecular Biology and Genetics, Sindh, Pakistan

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Corresponding Author

Tel.: +390906766516

E-mail: ffazio@unime.it

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Abstract

The seasonal effect on haematology and body composition of Rohu and Mrigal carp were evaluated. Fifty-four fish of each species (Rohu length and weight 28.00 ± 0.34 cm and 385 ± 3.40 g; Mrigal carp length and weight 29.48 ± 0.24 cm and 393 ± 5.48 g) were collected from the Dera Ismail Khan. The values of WBCs (9.31 and 8.41×10^6 mm⁻³), RBCs (2.37 and 2.12×10^6 mm⁻³), Hb (9.58 and 8.78) and HCT (38.06 and 36.04 %) of Rohu and Mrigal carp were higher in summer whereas declined in winter ($P < 0.05$). The MCV, MCH and MCHC of both species were higher ($P < 0.05$) in winter, and decreased during summer. The moisture content of both Rohu and Mrigal carp was higher during winter followed by spring and lower in summer season. Lipid and protein content of both species were higher ($P < 0.05$) in summer and lower in winter. The moisture content, proteins and lipid were found higher in Rohu than Mrigal carp. According to the findings, it was concluded that the water quality parameters vary seasonally which in turn impacts on body physiology of fish species.

Introduction

From the very ancient time fish is considered one of the best source of protein that can be easily digested and have many therapeutics properties against many health issues like heart diseases, muscular degeneration and many problems associated with mental health (Begum *et al.*, 2013; Dhaneesh *et al.*, 2012). Proximate analysis of the fish shows that their body composition varies with the season, environment, age, sex, feeding habits and even with sexual changes and maturity (Roy & Lall, 2006; Norouzi & Bagheri, 2015).

Two important ecological parameters that are DO (Dissolve Oxygen) and the water temperature have a great influence on the body composition of the fish

(Mizanur *et al.*, 2014). Understanding the seasonal effects on the composition and quality of the fish can help the consumer to select better fish species. Besides this, fish health can be assessed by evaluating the blood parameters of the fish (Fazio *et al.*, 2016). Several studies have been carried out to assess the effect of the ecological change on the haematological profile of the fish. The low temperature can depress the immune system of the fish. Hence, their blood cell count and many other important characteristics vary with the seasonal change (Bett & Vinatea, 2009).

The current study aimed to determine the variation in haematological parameters and body composition of fishes according to seasonal change.

Materials and methods

Water Parameters and fish sampling

The water quality parameters including temperature, dissolved oxygen (DO) and pH were measured on monthly basis with different instruments such as temperature was measured with the help of a mercury thermometer, DO was measured using DO meter (Hanna Instrument HI9132) and pH was measured with a pH meter (Hanna Instrument HI221). The mean water quality of the site is given in Table 1.

Two commercially essential and widely cultured species of Pakistan, *Labeo rohita* (Rohu) and *Cirrhinus mrigala* (Mrigal carp) were selected for experiment and their sampling was done from the Indus River at Dera Ismail Khan District of Pakistan on monthly basis.

In each survey, six fish of each species (54 each; total 108) were captured with the help of local fishermen. Sampling was carried out from December 2018 to August 2019 covering three seasons including summer, spring and winter.

Blood Sampling

To determine the influence of seasonal variation on haematological parameters, of fish's blood sampling was performed by the caudal peduncle from each fish. Blood was poured into vials containing ethylene-diamine-tetra-acetic acid (EDTA) as an anticoagulant agent. During sampling, fishes were not anesthetized because it may influence the blood indices (Torrecillas *et al.*, 2011).

Haematological Parameters

The WBC and RBC count was performed through a Neubauer counting chamber according to the method of Schaperclaus *et al.* (1991). Haemoglobin (Hb) concentration was estimated according to the cyanmethemoglobin procedure proposed by Goldenfarb *et al.* (1971). In this procedure, a 0.02cc blood sample (non-clotted) was taken and dilute with 5cc of Drabkin solution and let stand for 10 minutes at 25°C.

The amount of haemoglobin was calculated (against hemoglobin Standard) by reading absorbance at 540 nm. Further, Hematocrit (HCT) was estimated through the process of sedimentation: briefly, 50µl of the heparinized blood was taken in the micro-

hematocrit capillary and spun through the micro-hematocrit centrifuge for five minutes at 12000 rpm. Then hematocrit reader was used to count the value of HCT. Further, the indices of the erythrocytes (MCH, MCHC and MCV) were calculated according to the procedure of the Dacie & Lewis (1991).

Formula used for each indices was as follows:

$$MCH = \frac{Hb \left(\frac{g}{L} \right) \times 10 \text{ microgram}}{RBC \left(\frac{\text{millions}}{\text{microlitre}} \right)}$$

$$MCHC = \frac{Hb \left(\frac{g}{L} \right) \times 100 \text{ ml}}{HCT \%}$$

$$MCV = \frac{HCT\% \times 10 \text{ cubic microns}}{RBC \left(\frac{\text{millions}}{\text{microlitre}} \right)}$$

Fish body composition

Average total lengths of the sampled fish were noted, and they were within the range from 25-32 cm and their average weight was within the range of 320-450 g. Fish proximate composition (moisture contents, protein concentration, lipid, ash content and dry matter contents) was determined by the standard method of AOAC (Association of Official Analytical Chemists, 2000) in triplicate to identify fish nutritional value with changing season.

Fishes were washed with distilled water and slaughtered. Scales and visceral organs were removed. To obtain a small piece of fillet fish was cut in length with the help of a sharp knife so that maximum fillet without backbone can be obtained.

Moisture determination

Moisture content was determined by drying the sample in the oven overnight. For this first petri dish was measured and then 5 g of wet sample was put in a petri dish and dried overnight in an oven at 105°C. The next day sample weight was measured again and the process of drying in the oven repeat several times until a uniform weight is achieved.

The final weight was calculated with the help of the following formula (Haider *et al.*, 2016).

$$\text{Moisture} = W1 - W2 / W1 \times 100$$

W1 = initial weight of the sample

W2 = final weight of the sample

Table 1. Mean values (\pm Standard Deviation) of water quality parameters measured during study period

Seasons	Water quality parameters		
	pH	Temperature (°C)	Dissolve oxygen (mg L ⁻¹)
Summer	7.1 \pm 0.058	30 \pm 0.48	6.5 \pm 0.18
Winter	7.9 \pm 0.065	21 \pm 0.50	7.8 \pm 0.17
Spring	7.0 \pm 0.025	26 \pm 0.78	7.0 \pm 0.35

Protein determination

In order to determine the protein content, 5g of oven-dried sample were mixed with potassium sulphate + copper (digestion mixture). And then add 200 mL of concentrated H₂SO₄.

The mixture was heated on heating blocks white fumes formation id stop and solution became transparent. This is the indication that the digestion process is complete. Then after cooling the solution 60 mL of distilled water was added. Then with the help of the 45% NaOH the pH was increased from 6.5 to 7. After adding 6 drops of indicator into the solution and were mixed evenly, the flask was joined and combines with the condenser. And the tip of the condenser was dipped into the HCl and the solution was heated to evaporate the NH₃. Then the final solution was titrated against sodium hydroxide (Pearson, 1999). Protein contents were identified by using the following formula:

$$\text{Protein} = (A - B) \times N \times 14 \times 6.25 / W$$

A = volume of 0.2 N HCl used in sample titration

B = volume of 0.2 N HCl used in blank titration

N = normality of HCl

W = sample weight

14 = atomic weight of nitrogen

6.25 = constant for nitrogen calculation

Ash determination

An amount of 5g sample was taken into a crucible (already measured) and kept in the furnace for 6 hours at 550°C. After the color change into white, the sample was measured again and the content at the bottom of the crucible is ash (Haider *et al.*, 2016).

Ash content was measured by the following formula:

$$\text{Ash\%} = \text{Weight of ash} / \text{Weight of sample} \times 100$$

Lipids determination

Lipid content was determined by using the soxhelt apparatus (ST243 Soxtec, FOSS, Hillerød, Denmark) following the method of the Bligh & Dyer (1959).

Flask was filled with petroleum ether. After placing 5g of sample in thimble the apparatus was run for 17 hours. After completion of the process, the flask was removed gently and dried on the heater.

When the sample starts oily smell the content was weighted while the fat content was measured by using the following formula:

$$\text{Fat} = \text{Weight off} / \text{Weight of sample} \times 100$$

Statistical analysis

The values are given as Mean \pm Standard Error of the mean (SEM).

Findings were checked for normal distribution with normality plots. One-way analysis of variance (ANOVA). After applying one-way ANOVA, the Tukey's HSD test was applied. P values <0.05 are considered statistically significant.

Results

Effect of seasonal changes on haematological parameters of *Labeo rohita* and *Cirrhinus mrigala*

The haematological parameters such as White Blood Cells (WBCs), Red Blood Cells (RBCs), hemoglobin (Hb), haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were examined and the detail results are depicted in Table 2. The value of WBCs, RBCs, Hb and HCT was observed higher in the summer season followed by spring season respectively, and are significantly different (P<0.05) in both species (*Labeo rohita* and *Cirrhinus mrigala*). While in between the species these values were higher in *Labeo rohita* as compared to *Cirrhinus mrigala*. While the values of MCV, MCH and MCHC were higher (P<0.05) in winter season in both species followed by spring respectively. However, these values were observed higher in *Cirrhinus mrigala* than *Labeo rohita*.

Effect of seasonal changes on biochemical parameters of *Labeo rohita* and *Cirrhinus mrigala*

The biochemical constituents such as moisture, protein, lipid, ash contents and dry matter were recorded seasonally and the result is represented in Table 3. The moisture content of both species was recorded higher (P<0.05) significantly in winter followed by spring season respectively. However, the moisture content of the body was more in *Labeo rohita* than *Cirrhinus mrigala*. Apart from this, the protein and lipid content of the body were significantly higher (P<0.05) in the summer season followed by the spring season in both species, but their concentration was more in *Labeo rohita*. It was recorded that the body ash content of both species does not show any significant difference (P > 0.05) and was the same in all the seasons.

Discussion

Deep knowledge of the fish body composition is advocated to utilize the better fish. Indeed, along with haematology, proximate is also considered as a valuable tool to assess the health and physiological condition of the fish (Silva & Chamul, 2000; Fazio *et al.*, 2019). The shallow water fishes are more prone to environmental dynamics as compared to deep water. Because the deep-water condition is more stable and constant as compared to shallow that rapidly changed due to environmental conditions which result in variation in temperature. Temperature is one of the most crucial

factor of the environment, particularly for the aquatic organisms that change their physiological activities under different environmental stress (Wing *et al.*, 2006; Pratt & Fox 2002).

In the fish body, the moisture content is the most essential variable which varies seasonally. The variation of moisture content in the body of fish is linked to other biochemical variables such as lipid content. In all living bodies, the fluid is playing an essential role in transporting metabolites, nutrients etc. in the body parts. In the current study, the results revealed that the moisture content changes seasonally and was observed within a range from 70.81- 74.76% in *Labeo rohita* and was 69.81- 72.83% in *Cirrhinus mrigala*. However, the highest moisture content was observed in the winter season (*Labeo rohita* and *Cirrhinus mrigala*) and fat content was observed to be decreased in the winter season which showed an inverse relationship. This might be due to a decrease in temperature, low feeding rate and more demand for energy to keep the body temperature constant and to handle the food shortage in the winter season. Dinakaran *et al.* (2009) recorded similar findings while working on the *Macrobrachium idea*. In another study conducted by Bakhtyar & Langer

(2016) worked on different sizes of *Macrobrachium dayanum* and their results revealed that the moisture in fish body increases in the winter season and is reverse of lipid content. A study reported on Atlantic bonito by Zaboukas *et al.* (2006) observed that with the maturation of gonads the moisture content increased in somatic tissues and was found lower in immature bonito. The protein was observed to increase during the summer season in both species followed by the spring season. The possible reason of the increased muscle protein might be due to the optimum range of temperature and more food availability as different zooplankton, phytoplankton and algal blooms. A similar study was reported on *Aristeus antennatus* and protein content was high in the summer and spring seasons (Rosa & Nunes, 2003). According to Nargis (2006), a high amount of muscle protein was observed in the medium size of *Anabas testudineus* and was recorded a decrease in fish of increased age. In our study, the lipid content was recorded higher in the summer season and lower in winter in both species (*Labeo rohita* and *Cirrhinus mrigala*). The lipid changes are due to environmental conditions and the availability of food. In previous studies (Nargis, 2006; Jonsson & Jonsson, 2005) it has

Table 2. Seasonal variation in haematological parameters of *Labeo rohita* and *Cirrhinus mrigala* collected from Indus River Dera Ismail Khan District

Species	Haematological parameters	Seasons		
		Winter	Spring	Summer
<i>Labeo rohita</i>	WBCs ($\times 10^6 \text{ mm}^{-3}$)	4.72 \pm 0.31 ^c	6.07 \pm 0.20 ^b	9.31 \pm 0.31 ^a
	RBCs ($\times 10^6 \text{ mm}^{-3}$)	1.25 \pm 0.04 ^c	1.71 \pm 0.07 ^b	2.37 \pm 0.08 ^a
	Hb (g dL ⁻¹)	7.20 \pm 0.15 ^c	8.69 \pm 0.18 ^b	9.58 \pm 0.51 ^a
	HCT (%)	25.27 \pm 0.57 ^c	31.16 \pm 1.02 ^b	38.06 \pm 1.08 ^a
	MCV (fL)	189.69 \pm 1.89 ^a	189.49 \pm 3.92 ^a	170.13 \pm 3.02 ^b
	MCH (Pg cell ⁻¹)	58.12 \pm 0.71 ^a	53.17 \pm 1.67 ^b	42.79 \pm 1.31 ^c
	MCHC (%)	28.75 \pm 0.41 ^a	26.89 \pm 0.61 ^b	24.31 \pm 0.35 ^c
<i>Cirrhinus mrigala</i>	WBCs ($\times 10^6 \text{ mm}^{-3}$)	4.11 \pm 0.11 ^c	5.09 \pm 0.80 ^b	8.41 \pm 0.51 ^a
	RBCs ($\times 10^6 \text{ mm}^{-3}$)	1.37 \pm 0.03 ^c	1.50 \pm 0.05 ^b	2.12 \pm 0.02 ^a
	Hb (g dL ⁻¹)	6.80 \pm 0.22 ^c	7.87 \pm 0.19 ^b	8.78 \pm 0.71 ^a
	HCT (%)	24.31 \pm 0.83 ^c	29.17 \pm 1.07 ^b	36.04 \pm 1.08 ^a
	MCV (fL)	198.68 \pm 1.91 ^a	196.38 \pm 3.81 ^a	180.11 \pm 3.01 ^b
	MCH (Pg cell ⁻¹)	58.39 \pm 0.82 ^a	54.16 \pm 1.92 ^b	44.61 \pm 1.42 ^c
	MCHC (%)	29.65 \pm 0.51 ^a	27.91 \pm 0.81 ^b	25.41 \pm 0.43 ^c

*Values in the same row of different superscript are significantly different (P < 0.05)

Table 3. Muscle composition of *Labeo rohita* and *Cirrhinus mrigala* collected from Indus River, Dera Ismail Khan District

Species	Muscle composition	Seasons		
		Winter	Spring	Summer
<i>Labeo rohita</i>	Moisture	74.76 \pm 1.21 ^a	72.09 \pm 1.68 ^b	70.81 \pm 2.07 ^b
	Protein	13.21 \pm 0.37 ^d	16.14 \pm 0.72 ^c	18.12 \pm 0.52 ^a
	Lipid	5.17 \pm 0.27 ^c	7.04 \pm 0.89 ^b	7.86 \pm 0.62 ^a
	Ash	4.50 \pm 0.41 ^b	4.71 \pm 0.61 ^b	4.22 \pm 0.29 ^b
<i>Cirrhinus mrigala</i>	Moisture	72.83 \pm 1.51 ^a	70.08 \pm 1.91 ^b	69.91 \pm 2.09 ^b
	Protein	13.10 \pm 0.77 ^d	15.18 \pm 0.91 ^c	17.13 \pm 0.71 ^a
	Lipid	5.19 \pm 0.39 ^c	7.06 \pm 0.41 ^b	7.91 \pm 0.41 ^a
	Ash	4.21 \pm 0.61 ^b	4.81 \pm 0.52 ^b	4.11 \pm 0.31 ^b

*Values in the same row of similar superscript are insignificantly different (P > 0.05)

been shown that the lipid or fat content was lower in adult specimens (*Anabas testudineus* and *M. dayanum*) because the adults are involved in breeding as their feeding intensity is lower and their metabolic rate is higher. According to Devi *et al.* (2015), the lipid content is involved in the maturation of gonads in adult fishes. Ash content in the present study does not show any significant difference seasonally in both species and there is no direct connection of ash with the breeding and feeding activities. Varghese & Mathew (2016) conducted a study on *Heteropneustes fossilis* and *Channa striata* and observed no significant difference in ash content seasonally. A similar result was observed in another study while working on *Trachurus mediterraneus* (Tzikas *et al.*, 2007).

In the natural habitat, fish species are pact with different factors such as varied water qualities, pollution, malnutrition, infection and disease and can adapt themselves to such environmental conditions by changing their physiological activities. Haematological study of the fishes has proved as a perfect tool for physiological indicators, through which fish health status can evaluate easily (Fazio, 2019). The present study revealed the seasonal variation effect on blood parameters of *Labeo rohita* and *Cirrhinus mrigala*. The haematological parameters such as WBCs, RBCs, Hb and HCT were recorded higher in both species during the summer season. It might be due to increased metabolic rate, optimum temperature and breeding activities lead to increase haematological parameters. These results are according to other studies conducted in the past while working on *Capoeta trutta* (Orun & Erdemli, 2002), three Cyprinidae fish species (Orun *et al.*, 2003; Pradhan *et al.*, 2012) and *Pleuronectes platessa* (Preston, 1960). However, these haematological parameters were lower during the winter season which is due to decrease surrounding temperature and low food availability. Similar findings were observed by Adebayo *et al.* (2007) while working on *Parachanna obscura*. The RBC, Hb and HCT values in *Labeo rohita* and *Cirrhinus mrigala* were recorded higher in the summer season and are correlating with each other followed by spring and were found lower in the winter season. As we know, the fish is ectothermic and adapting the surrounding temperature of their habitat and at a higher temperature, their body metabolic activities increase and the amount of oxygen decreases in water at that period (Siddiqui and Nasim 1979) and due to hypoxia the RBCs and Hb in the body increases (Das *et al.*, 2006). Also during the winter season, the RBC, Hb and HCT values decrease because, the fish environmental temperature is lower and the oxygen concentration is higher (Guijarro *et al.*, 2003). In our study, WBCs count was recorded higher in the summer season and lower in winter. This indicates that in the summer season the WBCs are physiologically more capable of fish effective immunity (Santos *et al.*, 2009). The MCH, MCV and MCHC in the current study increase during the winter season, whereas a decline has been observed in summer, these

changes are probably related to environmental conditions and influence on body size. This is according to the results obtained by Orun *et al.* (2003).

Conclusion

According to the findings herein obtained it could be suggested that the exogenous factors especially seasonal variation affect the water parameters which in turn influence largely the haematological parameters of fish species investigated in the current survey. Also, the endogenous factors like reproductive period, fish length and weight affect the body composition of fishes like fat level, protein content etc. The blood profile and nutrients contents measured in both the species herein analyzed were significantly higher in the summer season, however, when comparing both species, it has been found that *Labeo rohita* had slightly higher protein and lipids content in summer as compared to any other season.

Ethical Statement

All applicable national guidelines for the care and use of animals were followed by the authors.

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Author Contribution

All authors have made substantial contributions to each step of experimental procedure and article preparation. The idea for the study was conceived by Syed Sikandar HABIB. The experiment was designed by Saira NAZ, Hameed Ur REHMAN, Wali Muhammad ACHAKZAI, Muhammad Nazir UDDIN, Khalid Hussain RIND, Nadir Ali RIND. The experiment was performed by Francesco FAZIO, Francesca ARFUSO and Giuseppe PICCIONE. The data were analyzed by Syed Sikandar HABIB, Saira NAZ, Hameed Ur REHMAN, Wali Muhammad ACHAKZAI, Muhammad Nazir UDDIN, Khalid Hussain RIND, Nadir Ali RIND. The article was written by Syed Sikandar HABIB. The last version of article was reviewed by Francesco FAZIO and Francesca ARFUSO.

Conflict of Interest

The authors declare that they have no conflict of interest.

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