

## Hematological Profile of the Mature *Rutilus frisii kutum* (Cyprinidae) Migrated to the Tajan River in the Southern Caspian Sea

<sup>1</sup>Zahra Rashidi, <sup>1</sup>Hossein Khara and <sup>2</sup>Hamed Mousavi-Sabet

<sup>1</sup>Department of Fishery and Aquaculture, Faculty of Natural Resources,  
Islamic Azad university, Lahijan Branch, Lahijan, Iran

<sup>2</sup>Fisheries Department, Faculty of Natural Resources, University of Guilan,  
P.O. Box 1144, Sowmeh Sara, Guilan, Iran

**Abstract:** The main aim of this study was to obtain a basic knowledge of the hematology of mature Kutum in migrating time and comparison hematological parameters between male and female specimens. Lymphocytes, monocytes, neutrophils, eosinophils and myelocytes, were distinguished. Hematological indices included Red Blood Corpuscles (RBCs), White Blood Corpuscles (WBCs), Hematocrit (HCT), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and leukocyte differential count were measured in one blood sample from 30 *Rutilus frisii kutum* captured in south of the Caspian Sea, in river-mouth of the Tajan River, north of Iran. Eighteen and twelve of the specimens were male and female, respectively. The mean of counted red blood cells was  $1.81 \pm 0.22 \times 10^6$  per  $\text{mm}^3$  blood. The mean of counted white blood cell was  $16.07 \pm 2.38 \times 10^3$  per  $\text{mm}^3$ . The average of hematocrit,  $53.09 \pm 4.75$  percent was calculated. The average of hemoglobin values was  $16.66 \pm 2.31$ . Blood indices; the average of MCV  $294.46 \pm 65.85$  FL, the average of MCH  $92.38 \pm 11.82$  Pg and the average of MCHC  $36.40 \pm 3.63$  percent, were calculated. Differential WBC; Mean Lymphocyte and Monocyte cells were  $11.81 \pm 0.20$  and  $0.11 \pm 0.35 \times 10^3$  per  $\text{mm}^3$ , respectively, Neutrophil and eosinophil were  $2.09 \pm 0.08$  and  $0.25 \pm 0.42 \times 10^3$  per  $\text{mm}^3$ , respectively and Myelocytes was  $0.33 \pm 0.19 \times 10^3$  per  $\text{mm}^3$ , no Basophil cells have been observed. Significant differences were observed in RBC and differential WBC counts between the male and female Kutum. Compared to other Cyprinids, this species has higher mean values for Hematocrit and Hemoglobin and high value for erythrocyte indices.

**Key words:** Hematology % Sex % Hematocrit % Hemoglobin % RBC % WBC

### INTRODUCTION

The purpose of this study was to obtain a basic knowledge of the hematology of *Rutilus frisii kutum*. The Caspian Kutum, *Rutilus frisii kutum* (Kamenskiy, 1901) (Family: Cyprinidae) is distributed from the southern Caspian Sea and around the Anzali wetland, North of Iran [1] and is rarely found in the middle and northern parts of the Caspian Sea [2]. This fish is of economic importance as food fish in capture fisheries, with approximately 4-5 thousand total tons landing in Iran each year [1]. Kutum are usually found in deep waters in autumn, migrating toward estuaries and coastal waters in late winter [1]. As water temperatures warm to  $12-18^\circ\text{C}$ , kutum begin to migrate into the southern rivers of the Caspian Sea for spawning in early March [2].

Hematological indices are important parameters for the evaluation of fish physiological status. Their changes depend on the fish species, age, the cycle of sexual maturity and health condition [3-11]. Hematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the hematological characteristics [12]. These indices have been employed in effectively monitoring the responses of fishes to the stressors and thus their health status under such adverse conditions. They can provide substantial diagnostic information once reference values are established under standardized conditions. Evaluation of the hemogram involves the determination of the total erythrocyte count (RBC), total white blood cell count (WBC), hematocrit (HCT or PCV), hemoglobin

concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), white blood cell differential count and the evaluation of stained peripheral blood films [13]. The hematological profile of some cyprinid species are well documented in literature [5, 14-17] but those of them have not been reported. This study reports for the first time, the 'normal' hematological profile of male and female *Rutilus frisii kutum* and compares it with that of other Cyprinid species. Assessment of hematological parameters in *Rutilus frisii kutum* might provide some useful information for other researchers that could be used as a biomarker associated with stressors agents or as an available tool to diagnose and monitor disease.

## MATERIALS AND METHODS

Female and male specimens of *Rutilus frisii kutum* used in this study were captured from river-mouth of the Tajan River in the southern Caspian Sea, Mazandaran province, North of Iran (36°48'47.24" N, 53°6'55.37" E). Thirty mature fish were weighed and measured (12 females: 835.83±204.38 g, BW and 46.69±4.41 cm, TL; 18 males: 817.22±187.67 g, BW and 45.26±3.00 cm, TL). Careful netting and handling was implemented to minimize stress. The specimens were anesthetized with Fish Calmer (Clove powder) and the peripheral blood was collected by puncture of the caudal vein with a heparin-coated 25 gauge×0.5 in. needle, attached to a 1 ml syringe. Right after extraction, blood samples were processed for microscopy as follows: a blood smears from every fish was fixed in absolute methanol for 3 min at room temperature or formalin vapor at 37 °C for 1 h and stained with 10% Giemsa in PBS, hematoxylin-eosin [18, 19]. Within the first 2 h after each extraction, the blood samples were processed for RBC, WBC and HCT as follows: RBCs [20] and WBCs [21] were determined using a Neubauer hemocytometer. Differential white cell count

was done on blood films stained with Giemsa. The different types of leukocytes were determined on each blood smear and a mean relative percent calculated. Replicate counts were made for each blood sample. Hematocrit value was determined by the standard microhematocrit method and expressed in percentage. Duplicate blood samples were loaded into standard heparinized capillary tubes, spun in a microhematocrit centrifuge at 12,000 rpm for 5 min and measured on a microcapillary reader. The Hb was determined using the cyanmethaemoglobin method. The following indices: mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) was calculated according to Seiverd [22]. The data was expressed as mean ± SD. Differences in hematological parameters between male and female specimens were statistically analyzed by Student's *t* test. The differences were accepted as significant if  $P < 0.05$ .

## RESULTS

**Erythrocytes, Myelocytes, Neutrophils and Three Types of Leukocytes:** lymphocytes, monocytes and eosinophils were distinguished by light microscopy (Fig. 1). Results of hematological analysis are shown in Table 1, which includes the mean for each of the different parameters evaluated. A high count of lymphocytes is observed in males. The percent of monocytes is found to be high in relation to the percent of lymphocytes in male specimens. Statistically analysis (*t*-test,  $p < 0.05$ ) reveals that some hematological parameters are significantly different between male and female specimens. According to Table 1, significant differences were observed in RBC and differential WBC counts, MCV and MCH between male and female specimens ( $p < 0.05$ ). Mean erythrocyte counts obtained for male and female *R. frisii kutum* were  $1.97 \pm 0.22$  and  $1.65 \pm 0.23 \times 10^6 \mu\text{L}^{-1}$ , respectively, mean of

Table 1: Hematological parameters in the male and female Kutum *Rutilus frisii kutum*, (mean ± SD)

Hematological parameters	Male	Female
RBC ( $\times 10^6 \mu\text{L}^{-1}$ )	$1.97 \pm 0.22$	$1.65 \pm 0.23$
WBC ( $\times 10^3 \mu\text{L}^{-1}$ )	$18.19 \pm 2.91$	$13.96 \pm 1.86$
Hematocrit (%)	$54.94 \pm 5.37$	$51.25 \pm 4.13$
Hemoglobin (g/dl)	$17.31 \pm 2.51$	$16.02 \pm 2.12$
MCV (fl)	$278.88 \pm 63.25$	$310.04 \pm 68.46$
MCH (pg)	$87.86 \pm 11.45$	$96.90 \pm 12.23$
MCHC (g/dl)	$31.50 \pm 4.17$	$31.30 \pm 3.10$
Lymphocytes ( $\times 10^3 \mu\text{L}^{-1}$ )	$13.62 \pm 0.17$	$10.07 \pm 0.23$
Monocytes ( $\times 10^3 \mu\text{L}^{-1}$ )	$0.17 \pm 0.37$	$0.05 \pm 0.33$
Myelocytes ( $\times 10^3 \mu\text{L}^{-1}$ )	$1.94 \pm 0.54$	$2.14 \pm 0.61$
Neutrophils ( $\times 10^3 \mu\text{L}^{-1}$ )	$2.44 \pm 0.68$	$1.70 \pm 0.48$
Eosinophils ( $\times 10^3 \mu\text{L}^{-1}$ )	$0.28 \pm 0.45$	$0.21 \pm 0.39$

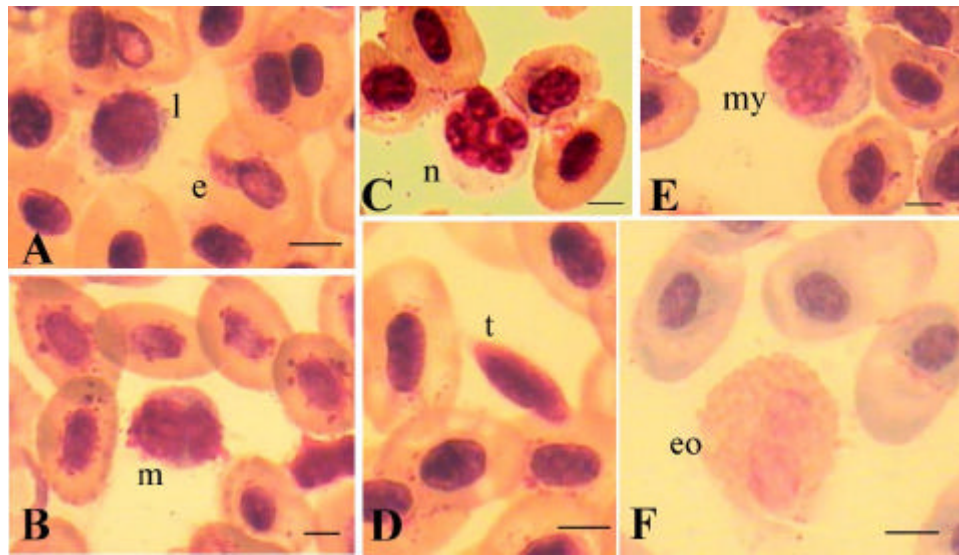


Fig. 1: Light microscopic micrographs of peripheral blood of *R. frisii kutum*.

(A) e: erythrocyte; l: lymphocyte (B) m: monocyte. (C) n: neutrophile. (D) t: thrombocyte. (E) my: myelocyte. (F) eo: eosinophil. Scale bars = 5  $\mu\text{m}$ .

the WBC counts were  $18.19 \pm 2.91$  and  $13.96 \pm 1.86 \times 10^3 \mu\text{L}^{-1}$  in male and female specimens respectively, mean of the MCV were  $278.88 \pm 63.25$  and  $310.04 \pm 68.46$  fl, the MCH were  $87.86 \pm 11.45$  and  $96.90 \pm 12.23$  pg, mean of the monocyte count were  $0.17 \pm 0.37$  and  $0.05 \pm 0.33 \times 10^3 \mu\text{L}^{-1}$  and mean of the Myelocyte counts were  $1.94 \pm 0.54$  and  $2.14 \pm 0.61 \times 10^3 \mu\text{L}^{-1}$  for male and female *R. frisii kutum*, respectively (Table 1).

## DISCUSSION

The knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes [23]. There is growing interest in the study of hematological parameters and structural features of fish blood cells regarded as important for health and aquaculture purposes. Erythrocytes are the dominant cell type in the blood of the vast majority of fish species. It is widely accepted that fishes, like most other vertebrates, have a common leukocyte pattern consisting of granulocytes, monocytes, lymphocytes and thrombocytes [3, 11, 14, 24-31]. Previous studies on fish hematology have revealed that interpretation of blood parameters is quite difficult since variations in the blood are caused by internal and external factors. In the present study, *Rutilus frisii kutum* blood cells were characterized microscopically and hematological indices were analyzed. Significant differences were observed in some hematological indices between the female and male Kutum (Table 1).

Erythrocyte counts are used as indicators for anemia. Mean erythrocyte counts obtained for the *Rutilus frisii kutum* was  $1.81 \pm 0.22$  (Table 2) and was more than those reported for *Cyprinus carpio* [14], *Carasius auratus* [14], *Capoeta trutta* [15] and *Vimba Vimba persa* [17] (Table 2). Collated with other Cyprinids, *R. frisii kutum*, presents higher mean values for RBCs, HCT and Hb (Table 2). It is observed a high range of HCT and Hb in *R. frisii kutum* and *V. Vimba persa*, which are brackish water species, than others which are freshwater species. Blaxhall and Daisely [3] noted that fish biologists rely more on hematocrit and hemoglobin concentration estimates as indicators of anemia and these indices may be different in different habitats and conditions.

Generally, leukocyte counts are useful as indicators of disease condition or response to infection and significantly elevated or depressed values are obtained in abnormal conditions. Mean WBCs ( $\pm$ SD) obtained for the kutum was  $16.07 \pm 2.38 \times 10^3 \mu\text{L}^{-1}$  (Table 1). This value was comparable to the reported for *Capoeta trutta* [15], but was much lower than values reported for *Cyprinus carpio* [14] and *Carasius auratus* [14] and was higher than value reported for *Vimba Vimba persa* [17]. There are wide variations in the leukocyte counts reported for various freshwater and brackish water Cyprinids (Table 2). Several authors have demonstrated a special interest in the leukocytes of teleost fishes with regard to their morphology and absolute values. Their investigations revealed a great diversity of morphological aspects and values in some types of leukocytes [3, 31-37].

Table 2: Comparison of the normal hematological indices between the Kutum *R. frisii kutum* and some other Cyprinids

Parameters	Species				
	<i>R. frisii kutum</i>	<i>V. vimba persa</i>	<i>C. carpio</i>	<i>C. auratus</i>	<i>C. trutta</i>
RBC ( $\times 10^6 \mu\text{L}^{-1}$ )	1.81 $\pm$ 0.22	1.69 $\pm$ 0.21	1.67 $\pm$ 0.08	1.61 $\pm$ 0.81	1.74 $\pm$ 0.12
WBC ( $\times 10^3 \mu\text{L}^{-1}$ )	16.07 $\pm$ 2.38	6.02 $\pm$ 0.96	37.8 $\pm$ 2.88	52.3 $\pm$ 4.88	17.65 $\pm$ 2.15
HCT (%)	53.09 $\pm$ 4.75	39.69 $\pm$ 5.38	33.4 $\pm$ 1.51	22.3 $\pm$ 1.04	26.05 $\pm$ 2.38
Hb (g/dl)	16.66 $\pm$ 2.31	11.77 $\pm$ 1.02	8.2 $\pm$ 0.36	6.7 $\pm$ 0.25	7.9 $\pm$ 0.24
MCV (fl)	294.46 $\pm$ 65.85	236.58 $\pm$ 44.33	202 $\pm$ 5.5	137 $\pm$ 2.6	149.71 $\pm$ 2.28
MCH (pg)	92.38 $\pm$ 11.82	70.57 $\pm$ 23.12	49.1	42 $\pm$ 1.4	45.4 $\pm$ 1.8
MCHC (g/dl)	36.40 $\pm$ 3.63	29.91 $\pm$ 4.04	-	-	30.32 $\pm$ 0.8(%)
L ( $\times 10^3 \mu\text{L}^{-1}$ )	11.84 $\pm$ 0.20	4.24 $\pm$ 0.16	32.26-35.15	26.7 $\pm$ 2.89	13.1 $\pm$ 0.01
M ( $\times 10^3 \mu\text{L}^{-1}$ )	0.11 $\pm$ 0.35	0.13 $\pm$ 0.33	0.19-0.76	0.2 $\pm$ 0.1	1.9 $\pm$ 0.07
N ( $\times 10^3 \mu\text{L}^{-1}$ )	2.07 $\pm$ 0.58	0.75 $\pm$ 0.49	-	-	-
E ( $\times 10^3 \mu\text{L}^{-1}$ )	0.25 $\pm$ 0.42	0.34 $\pm$ 0.52	0.19-0.38	0.1 $\pm$ 0.1	0.075 $\pm$ 0.005
H ( $\times 10^3 \mu\text{L}^{-1}$ )	-	2.74 $\pm$ 0.61	1.13-3.78	2.3 $\pm$ 0.56	2.56 $\pm$ 0.12
My ( $\times 10^3 \mu\text{L}^{-1}$ )	2.04 $\pm$ 0.57	-	-	-	-
References	Present study	[17]	[14]	[14]	[15]

-Data are represented as mean  $\pm$  SD

-(L: Lymphocytes; M: Monocytes; N: Neutrophils; E: Eosinophils; H: Heterophils; My: Myelocytes).

In addition, the same type of leukocyte has been described with different names by different authors. In *Rutilus frisii kutum*, lymphocytes are similar to reported for *Capoeta trutta* [15], but they are less abundant than in other Cyprinids except in *Vimba Vimba persa* [17] (Table 2). The nomenclature used to describe monocytes in fishes is variable. Monocytes have been termed hemoblasts and macrophages [38], while some authors have been unable to find monocytes [3]. There are relatively few morphological studies on fish monocytes [16, 31, 39, 40]. In fishes, granulocytes are of three types: heterophils and eosinophils are the most common, while basophils are much rare. Most authors recognize heterophils as the most frequent type of granulocyte. The occurrence of eosinophils and in particular basophils, is often questioned [29, 34]. In the blood of *Oreochromis niloticus*, Ueda *et al.* [31] identified all three types of granulocytes; however Rodrigues Bittencourt *et al.* [41] failed to find basophils and eosinophils in this tilapia under semi-intensive culture conditions. In *Rutilus frisii kutum*, basophilic granulocyte has not been found. Garavini and Martelli [8] stated that two types the granules are formed in male and female goldfish heterophils. In *C. carpio*, the substructure of heterophil granules is similar to that of the eosinophil granules of *C. auratus* [16]. The most abundant types of leukocytes found in the peripheral blood of *Rutilus frisii kutum* was the lymphocytes (Table 2), as demonstrated in other Cyprinids [14, 15, 17], *Pimelodus maculatus* [42],

*Synbranchus marmoratus* [28], *Mugil platanus* [43] and *Oncorhynchus mykiss* [44]. In differential WBC counts, a similar pattern was reported in *C. carpio* and *C. auratus* [14] and *Capoeta trutta* [15], although the relative ratio changed according to the species (Table 2). Low lymphocyte count occurred in *Rutilus frisii kutum* compared to that found in freshwater Cyprinids (Table 2).

Mean hematocrit value obtained for the *R. frisii kutum* was 53.09 $\pm$ 4.75 %. Values reported for haematocrit of other Cyprinids are usually between 20% and 40% (Table 2). In present study the mean of hematocrit value in male specimens were higher than this range. Hematocrit is important as an indicator of the percentage of packed red blood cells and the color of the plasma layer above the packed cells and could be used to detect hemolysis [45]. There is hence the possibility of using hematocrit as a tool in aquaculture and fisheries management for checking anemic condition in fishes.

Hemoglobin is crucial for the survival of the fish as its role is directly related to the oxygen-binding capacity of blood. In fish blood, oxygen is carried in physical solution and also in combination with hemoglobin. Mean hemoglobin concentration values obtained for the kutum was 16.66 $\pm$ 2.31 g/dl. The low values of hemoglobin concentration are reported of other Cyprinids (Table 2). And reflects high oxygen carrying capacity of the blood, which is consistent with the correlation of hemoglobin concentration with fish activity as suggested by Lenfant and Johansen [46], also may be attributed/related to the

conditions in the habitat or the general well-being of the fishes and habitats differences. However, it is well known that blood sampling, laboratory techniques, seasonal variations, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect hematological data [15, 23, 33, 47-60].

There are wide variations in both the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values reported in literature for various freshwater and brackish water Cyprinids (Table 2). The erythrocyte indices (MCV and MCH) have a wide range of physiological variation. The MCV and the MCH of *Rutilus frisii kutum* are higher than other Cyprinid species (Table 2). Arnold [52] argued that in elasmobranchs, manual RBC lacks the precision necessary for the accurate assessment of anemia or for calculating accurate MCV and MCH values. Blaxhall and Daisley [3], working with teleosts, also concluded that manual RBC counts were error proved and suggested that HCT and Hb may be better parameters for the assessment of anemia in fish.

Mean corpuscular hemoglobin concentration (MCHC) values for the *R. frisii kutum* was  $36.40 \pm 3.63$  g/dl. The MCHC values reported for *Capoeta trutta* and *Vimba vimba persa* were similar to the results of present study [15, 17].

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