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Comparative characterization of blood cells and hematological parameters between the mature and immature Caspian Vimba, *Vimba vimba persa* (Teleostei, Cyprinidae)

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Abstract. The aim of the present study was to obtain a basic knowledge of the hematology of Caspian Vimba and comparison hematological parameters between immature and mature specimens. Lymphocytes, monocytes, heterophils and eosinophils, were distinguished and characterized. Hematological indices (RBC, WBC, HCT, Hb, MCV, MCH, MCHC and leucocyte differential count) were measured in one blood sample from 81 *Vimba vimba persa* captured in South coasts of the Caspian Sea and in River-mouth of the Sefid-roud River in North of Iran. Twenty nine and fifty two of the specimens were mature and immature, respectively. The mean of counted red blood cell in immature fish was $1.80\pm0.22 \times 10^6$ μl^{-1} and in mature fish was $1.69\pm0.21 \times 10^6$ μl^{-1}. The mean of white blood cell in immature fish has been counted $5.36\pm0.78 \times 10^3$ μl^{-1}, and in mature fish was $6.02\pm0.96 \times 10^3$ μl^{-1}. Neutrophil in immature fish was 11.17±2.55 percent and in mature was 13.19±2.52 percent, and no basophils cell has been observed. The rates of clot time in mature and immature fishes were 125.38±24.94 and 186.03±25.82 S, respectively. Significant differences were observed in RBC, WBC, neutrophil and the rate of clot time between the mature and immature of the Caspian Vimba. Statistical analysis revealed that differences in hematological parameters between male and female fish were not significant. Compared to other Cyprinids, this species has higher mean values for HCT and Hb and similar value for RBC, and the percent of heterophils was found to be low in relation to the percent of lymphocytes. High lymphocyte counts occurred in *V. vimba persa*, as compared to these found in other species.

Key Words: hematology, Caspian Sea, HCT, Hb, RBC, WBC.

Introduction. The Caspian Vimba, *Vimba vimba persa* (Linnaeus, 1758) (Family: Cyprinidae) is distributed from the southern Caspian Sea and Anzali wetland, North of Iran (Abdoli & Naderi 2009). It is of economic importance as food fish in freshwater and brackish water capture fisheries.

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 (Blaxhall 1972; Wedemeyer et al 1983; Golovina & Trombicky 1989; Zhiteneva et al 1989; Bielek & Strauss 1993; Golovina 1996; Luskova 1997; Vosyliene 1999; Hrubec et al 2001). 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 (Gabriel et al 2004). 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), haematocrit (HCT or PCV), hemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), white blood cell differential count and the evaluation of stained peripheral blood films (Campbell

2004). The haematological profile of some cyprinid species are well documented in literature (Groff & Zinkl 1999; Orun & Erdemli 2002; Garavini & Martelli 1981; Page & Rowley 1983) but the haematological factors of most of them have not been reported. This study reports for the first time, the 'normal' haematological profile of mature and immature *Vimba vimba persa*, and compares it with that of other freshwater fish species. The purpose of this study was to obtain a basic knowledge of the hematology of *V. vimba persa*. Assessment of hematological parameters in *V. vimba persa* 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 in this species, which is representative of the ichthyich fauna in the southern Caspian Sea.

Material and Method

Sampling. Mature and immature specimens of *Vimba vimba persa* used in this study were captured from river-mouth of the Sefid-rud in the southern Caspian Sea, Guilan province, North of Iran (37°28'3.77" N, 49°56'26.15" E) (Figure 1).

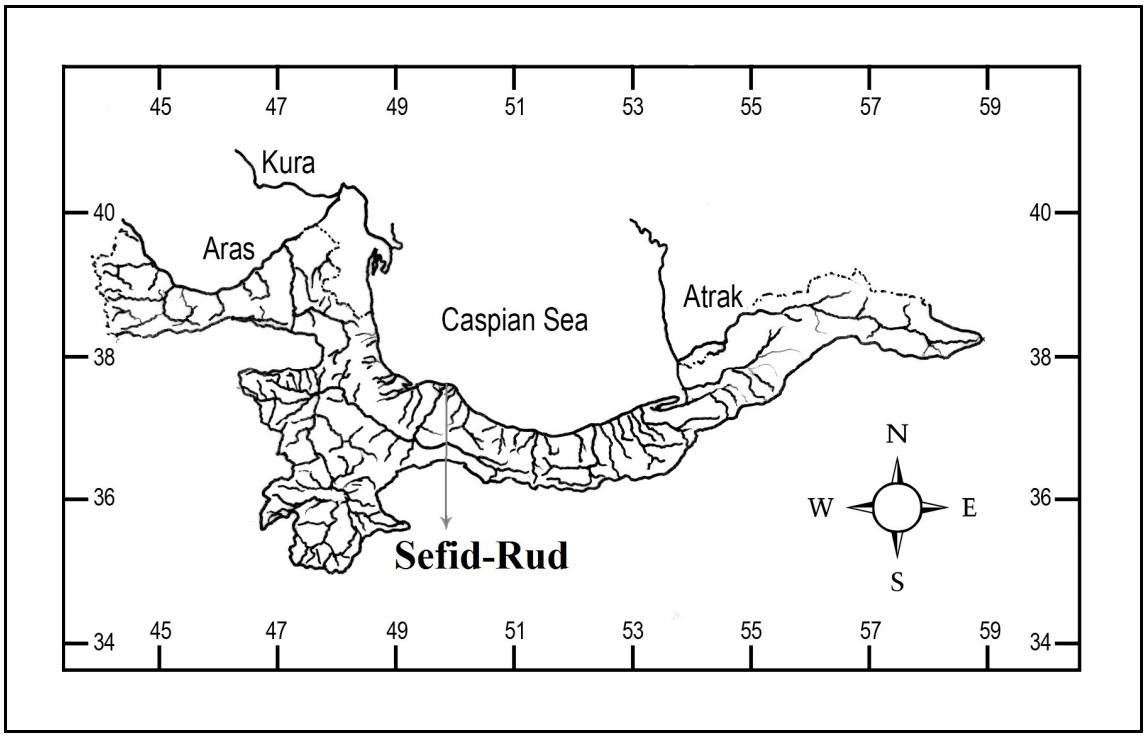

Figure 1. Map of Iranian parts of the southern Caspian Sea basin, showing some of the most important rivers system: Aras, Kura, Atrak and Sefid-Rud (the study area).

Eighty one fish were weighed and measured (34 females: 96.02±25.55 g, BW and 19.78±3.31 cm, TL; 18 males: 92.43±29.47 g, BW and 20.26±4.92 cm, TL; 29 juvenile: 64.43±14.4 g, BW and 15.95±2.20 cm, TL). Careful netting and handling was implemented to minimize stress. All fishes were considered healthy on the basis of their appearance and the absence of obvious signs of disease. The specimens were anesthetized with Fish Calmer (Clove powder), and the peripheral blood was collected by puncture of the caudal vein or heart (in small specimens) with a heparin-coated 25 gauge×0.5 in. needle, attached to an 1 ml syringe. Right after extraction, blood samples were processed for microscopy as follows: a blood smears form every fish was fixed in absolute methanol for 3 min at room temperature or formalin vapor at 37^{o}C for 1 h, and stained with 10% Giemsa in PBS, hematoxylin–eosin (Martoja & Martoja 1970; Pearse 1980).

Hematological indices. Within the first 2 h after each extraction, the blood samples were processed for RBC, WBC and HCT as follows: RBC (Kaplow 1955) and WBC (Natt &

Herrick 1952) were determined using a Neubauer hemocytometer. Differential white cell count was done on blood films stained with Giemsa. The different types of leucocytes 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) were calculated according to Seiverd (1964).

Statistical analysis. The data was expressed as mean±SD differences in hematological parameters between mature and immature specimens were statistically analyzed by Student's *t* test. The differences were accepted as significant if $p<0.05$.

Results

Morphology of the peripheral blood cells. Erythrocytes, heterophils and four types of leucocytes: lymphocytes, monocytes, neutrophils and eosinophils were distinguished by light microscopy (Figure 2).

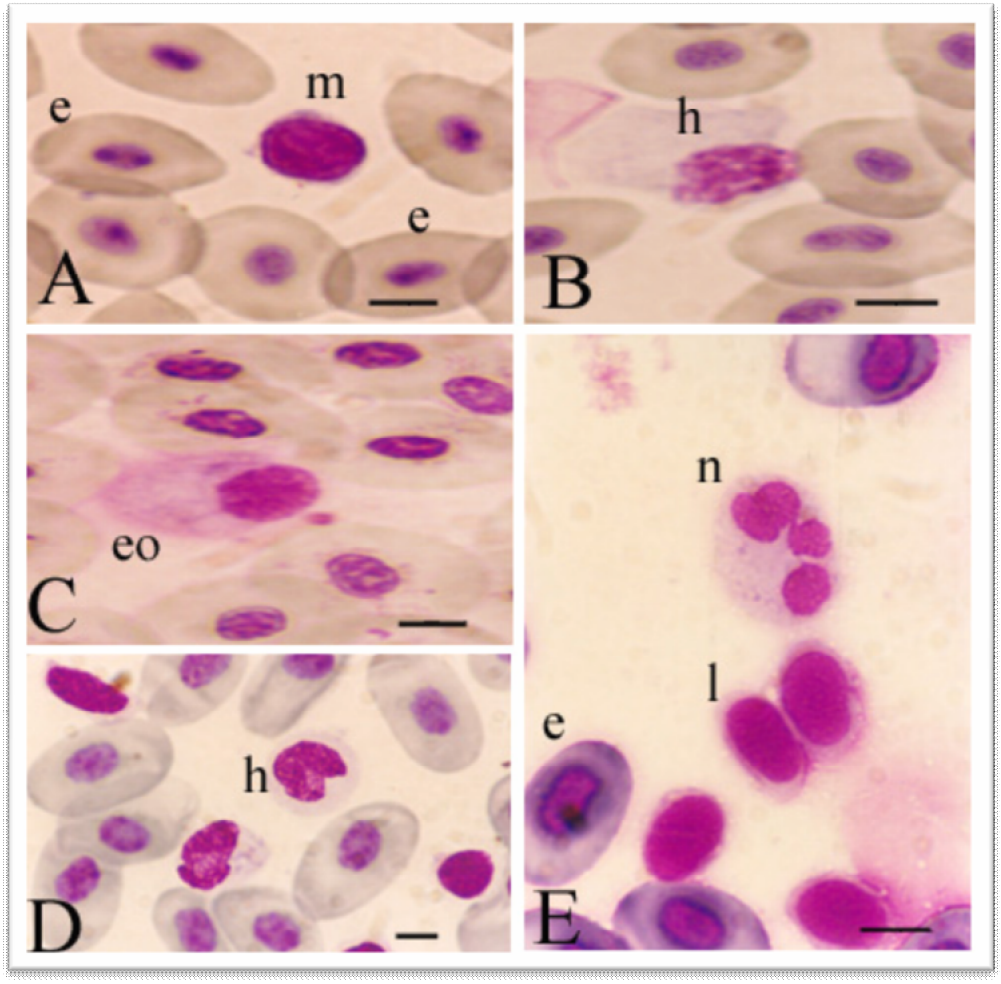

Figure 2. Light microscopic micrographs of peripheral blood. (A) e, erythrocyte; m, monocyte. (B) h, heterophile. (C) eo, eosinophile. (D) h, heterophilic granulocyte. (E) l, lymphocyte. Scale bars = 5 µm.

Hematological analysis. Results of hematological analysis are shown in Table 1, which includes the mean for each of the different parameters evaluated.

Table 1
Hematological parameters in the immature and mature (male and female) Caspian Vimba *V. vimba persa*, mean±SD

Hematological parameters	*Immature*	*Female*	*Male*
RBC ($\times 10^6$ μl^{-1})	1.80±0.22	1.70±0.24	1.69±0.19
WBC ($\times 10^3$ μl^{-1})	5.36±0.78	6.12±0.89	5.93±1.04
Hematocrit (%)	40.58±6.70	39.55±4.98	39.83±5.78
Hemoglobin (g/dl)	11.59±1.08	11.58±0.98	11.97±1.06
MCV (fl)	226.75±54.10	237.60±48.29	235.56±40.37
MCH (pg)	67.13±11.48	69.26±11.13	71.89±11.99
MCHC (g/dl)	29.97±4.55	29.63±3.74	30.20±4.35
Lymphocytes ($\times 10^3$ μl^{-1})	79.00±6.89	79.31±8.17	78.45±6.75
Monocytes ($\times 10^3$ μl^{-1})	0.14±0.30	0.12±0.32	0.14±0.34
Neutrophils ($\times 10^3$ μl^{-1})	11.17±2.55	12.45±2.75	12.45±2.64
Eosinophils ($\times 10^3$ μl^{-1})	0.34±0.46	0.33±0.47	0.36±0.57
Clotting time (s)	186.03±25.82	142.84±34.95	153.79±44.35

A high count of lymphocytes is observed. The percent of monocytes and eosinophils are found to be high in relation to the percent of lymphocytes in male specimens. Statistically analysis (*t*-test, $p<0.05$) reveals that hematological parameters are not significantly different between male and female specimens. Mean erythrocyte counts obtained for mature and immature *V. vimba persa* were 1.64±0.23 and 1.80±0.22×10^6 μl^{-1}, respectively, mean of the WBC counts were 6.45±0.80 and 5.36±0.78×10^3 μl^{-1} in mature and immature specimens respectively, mean of the neutrophil counts were 13.19±2.53 and 11.17±2.55 percent, and the mean of clotting time were 125.38±24.95 and 186.03±25.82 second for mature and immature *V. vimba persa*, respectively (Table 1). Significant differences were observed in RBC, WBC, neutrophils and the clotting time between mature and immature specimens ($p<0.05$). There was no significant difference between mature and immature of *V. vimba persa* in other hematological indices and between the male and female specimens ($p\geq0.05$).

Discussion. 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 leucocyte pattern consisting of granulocytes, monocytes, lymphocytes and thrombocytes (Watson et al 1963; Hartman & Lessler 1964; Conroy 1972; Blaxhall & Daisley 1973; Javaid & Akhtar 1977; Nakamura & Shimozawa 1984; Rowley et al 1988; Groff & Zinkl 1999; Hrubec et al 2000; Esteban et al 2000; Ueda et al 2001). 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 (Kori-Siakpere et al 2005). 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. 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 (Ezzat et al 1973; Denton & Yousef 1975; Fourtie & Hatting 1976; Van Vuren & Hattingh 1978; Hardig & Hoglung 1984; Wilhem et al 1992; Orun & Erdemli 2002; Arnold 2005; Kori-Siakpere et al 2005).

In the present study, *V. vimba persa* blood cells were characterized microscopically and hematological indices were analyzed. No statistically significant variations were found in the level of these parameters between sexes, but significant differences were observed in some hematological indices between the mature and immature specimens (Table 1).

Erythrocyte count. Generally, erythrocyte counts are used as indicators for anaemia. Mean erythrocyte counts (±SD) obtained for the mature *V. vimba persa* was 1.64±0.22 (Table 2), and were comparable to those reported for *Cyprinus carpio* (Groff & Zinkl 1999) and *Carasius auratus* (Groff & Zinkl 1999), but was less than values of 1.74±0.12 reported for *Capoeta trutta* (Orun & Erdemli 2002) (Table 2). Collated with other species, *V. vimba persa* presents similar mean values for RBC and higher for HCT and Hb (Table 2). Blaxhall & Daisely (1973) noted that fish biologists rely more on haematocrit and haemoglobin concentration estimates as indicators of anaemia.

Leucocyte count. Leucocyte counts are useful as indicators of disease condition or response to infection, and significantly elevated or depressed values are obtained in abnormal conditions. Mean WBC (±SD) obtained for the mature *V. vimba persa* was $6.45\pm0.80\times10^{3}$ μl^{-1} (Table 1). This value was much lower than values reported for *C. carpio* (Groff & Zinkl 1999), *C. auratus* (Groff & Zinkl 1999) and *C. trutta* (Orun & Erdemli 2002). May be attributed/related to the conditions in the habitat or the general well-being of the fishes and habitats differences. There are wide variations in the leucocyte counts reported for various freshwater and brackish water Cyprinids (Table 2). Several authors have demonstrated a special interest in the leucocytes 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 leucocytes (Srivastava 1968; Blaxhall & Daisley 1973; Ezzat et al 1973; Ellis 1976; Ferguson 1976; Imagawa et al 1989; Ueda et al 1997; Veiga 1999). In addition, the same type of leucocyte has been described with different names by different authors. In *V. vimba persa*, lymphocytes are more abundant than in other species (Table 2). The nomenclature used to describe monocytes in fishes is variable. Monocytes have been termed hemoblasts and macrophages (Barber et al 1981), while other authors have been unable to find monocytes (Blaxhall & Daisley 1973). There are relatively few morphological studies on fish monocytes (Cannon et al 1980; Page & Rowley 1983; Ueda et al 2001; Valenzuela et al 2003). 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 occurence of eosinophils, and in particular basophils, is often questioned (Ellis 1977; Rowley et al 1988). In the blood of *Oreochromis niloticus*, Ueda et al (2001) identified all three types of granulocytes, however Rodrigues Bittencourt et al (2003) failed to find basophils and eosinophils in this tilapia under semi-intensive culture conditions. In *V. vimba persa*, we report the presence of heterophilic and eosinophilic granulocytes, while basophilic granulocytes have not been found. Garavini & Martelli (1981) stated that two types the granules are formed in immature and mature goldfish heterophils. In *C. carpio*, the substructure of heterophil granules is similar to that of the eosinophil granules of *C. auratus* (Page & Rowley 1983). The most abundant types of leucocytes found in the peripheral blood of *Vimba vimba persa* were the lymphocytes and heterophils (Table 2), as demonstrated in *Pimelodus maculatus* (Ribeiro 1978), *Synbranchus marmoratus* (Nakamoto et al 1991), *Mugil platanus* (Ranzani-Paiva 1995) and *Oncorhynchus mykiss* (Ranzani-Paiva et al 1998). A similar pattern was reported in *C. carpio* and *C. auratus* (Groff & Zinkl 1999), and *C. trutta* (Orun & Erdemli 2002), although the relative ratio changed according to the species (Table 2). High lymphocyte count occurred in *V. vimba persa* compared to that found in other species (Table 2).

Haematocrit. Mean haematocrit values (±SD) obtained for the mature *V. vimba persa* was 39.01±4.27%. Values reported for haematocrit of other Cyprinids are usually between 20 and 35% (Table 2). In present study the mean of haematocrit value in immature specimens were higher than this range. Haematocrit is important as an indicator of the percentage of packed red blood cells, and the colour of the plasma layer above the packed cells, and could be used to detect haemolysis (Archer & Jeffcott 1977). There is hence the possibility of using haematocrit as a tool in aquaculture and fisheries management for checking anemic condition in fishes.

Haemoglobin concentration. In fish blood, oxygen is carried in physical solution and also in combination with haemoglobin. Haemoglobin is crucial for the survival of the fish as its role is directly related to the oxygen-binding capacity of blood. Mean haemoglobin concentration values (±SD) obtained for the mature *V. vimba persa* was 11.91±0.98 g/dl. The low values of haemoglobin concentration are reported of other Cyprinids (Table 2). And reflects high oxygen carrying capacity of the blood, which is consistent with the correlation of haemoglobin concentration with fish activity as suggested by Lenfant & Johansen (1972).

Mean corpuscular haemoglobin concentration (MCHC). Mean corpuscular haemoglobin concentration values for the mature *V. vimba persa* was 29.80±3.63 g/dl. The MCHC values reported for *C. trutta* was similar to the results of present study (Orun & Erdemli 2002).

Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). There are wide variations in both the mean corpuscular volume and mean corpuscular haemoglobin values reported in literature for various freshwater and brackish water Cyprinids (Table 2).

Table 2

Comparison of the normal hematological indices between the mature Caspian Vimba and some other Cyprinids, mean±SD or reference interval

Parameters	Species			
	Vimba vimba persa	*Cyprinus carpio*	*Carasius auratus*	*Capoeta trutta*
RBC ($\times 10^6$ μl^{-1})	1.69±0.21	1.67±0.08	1.61±0.81	1.74±0.12
WBC ($\times 10^3$ μl^{-1})	6.02±0.96	37.8±2.88	52.3±4.88	17.65±2.15
Hematocrit (%)	39.69±5.38	33.4±1.51	22.3±1.04	26.05±2.38
Hemoglobin (g/dl)	11.77±1.02	8.2±0.36	6.7±0.25	7.9±0.24
MCV (fl)	236.58±44.33	202±5.5	137±2.6	149.71±2.28
MCH (pg)	70.57±23.12	49.1	42±1.4	45.4±1.8
MCHC (g/dl)	29.91±4.04	-	-	30.32±0.8 (%)
Lymphocytes ($\times 10^3$ μl^{-1})	78.88±7.46	32.26–35.15	26.7±2.89	13.1±0.01
Monocytes ($\times 10^3$ μl^{-1})	0.13±0.33	0.19–0.76	0.2±0.1	1.9±0.07
Neutrophils ($\times 10^3$ μl^{-1})	12.45±2.69	-	-	-
Eosinophils ($\times 10^3$ μl^{-1})	0.34±0.52	0.19–0.38	0.1±0.1	0.075±0.005
Heterophils ($\times 10^3$ μl^{-1})	2.74±0.61	1.13–3.78	2.3±0.56	2.56±0.12
References	Present study	Groff & Zinkl (1999)	Groff & Zinkl (1999)	Orun & Erdemli (2002)

The erythrocyte indices (MCV and MCH) have a wide range of physiological variation. The MCV of *V. vimba persa* is similar to that of *C. carpio* and higher than *C. auratus* and *C. trutta*, while the MCH is higher than all of them (Table 2). Arnold (2005) argued that in elasmobranches, manual RBC lacks the precision necessary for the accurate assessment of anemia or for calculating accurate MCV and MCH values. Blaxhall & Daisley (1973), 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.

Conclusions. The results of our research provide a contribution to the knowledge of the characteristics of blood cells and hematological parameters of the cyprinid fish, *V. vimba persa*, under the normal conditions employed in this study. This investigation may be helpful as a tool to monitor the health status of this and other related fish species. The evaluation of hematological parameters will grant early detection of clinical pathology as well as the presence of disturbance in the environment.

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