E.Ü. Su Ürünleri Dergisi 2008
E.U. Journal of Fisheries & Aquatic Sciences 2008
Cilt/Volume 25, Sayı/Issue (1): 39–46

ISSN 1300 - 1590
http://jfas.ege.edu.tr/

Hematology of the Uludağ Frog, *Rana macrocnemis* Boulenger, 1885 in Uludağ National Park (Bursa, Turkey)

*Suha Kenan Arserim, Ahmet Mermer

Ege University, Faculty of Science, Biology Department, Zoology Section, Bornova, İzmir, Türkiye
**E mail: suhaarserim@hotmail.com*

Özet: ***Uludağ Milli Parkı'nda uludağ kurbağası,*** **Rana macrocnemis** ***Boulenger, 1885'in hematolojisi (Bursa, Türkiye).*** Uludağ Milli Parkı'ından, 30 (15 ♂♂, 15 ♀♀) ergin *Rana macrocnemis* örneğinde bazı hematolojik parametreler ve periferal kan hücrelerinin (eritrosit, lökosit, trombosit) morfolojisindeki mevsimsel değişimler istatistiksel ve histolojik yöntemlerle incelenmiştir. 1 mm³ kandaki eritrosit sayısı, 514,839 (280,000 – 940,000); lökosit sayısı, 3,527 (2,600 – 5,200); trombosit sayısı, 31,069 (20,000 – 50,000); hemoglobin miktarı (Hb), 8.10 (5.60 – 12.10) g/dl; hematokrit değeri (HCT), 0.34 (0.16 – 0.46) L/l; ortalama hücre hacmi (MCV), 694.54 (420.45 – 1105.26) fl; ortalama hücre hemoglobini (MCHb), 167.88 (87.78 – 257.89) pg; ortalama hücre hemoglobin konsantrasyonu (MCHbC), 251.43 (160.87 – 387.50) g/l olarak hesaplanmıştır. Eritrosit sayısı ile ilgili parametreler açısından (Hb, HCT, MCV, MCHb ve MCHbC) mevsimsel değişimler saptanmıştır. Bu değerler açısından cinsiyetler arasında da küçük farklılıklar olsa da istatistiki açından önemsizdir. Buna karşın, lökösit ve trombosit sayısı değerleri açısından gerek mevsimsel ve gerekse cinsiyete bağlı varyasyonlar gözlenmemiştir. Hücre büyüklükleri açısından küçük mevsimsel farklılıklar tespit edilmiştir. Fakat bunların bireysel varyasyonlar olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Uludağ Milli Parkı, Türkiye, *Rana macrocnemis*, Hematoloji, Kan Hücreleri.

Abstract: Seasonal change of some hematological parameters and cytomorphometry of blood cells (erythrocyte, leukocyte and thrombocyte) were studied statistically and histologically in a total of 30 (15 ♂♂, 15 ♀♀) adult individuals of *Rana macrocnemis* from Uludağ National Park. In 1 mm³ of blood, erythrocyte count was estimated as 514,839 (280,000 – 940,000), leukocyte count as 3,527 (2,600 – 5,200), thrombocyte count as 31,069 (20,000 – 50,000), hemoglobin amount (Hb) as 8.10 (5.60 – 12.10) g/dl, hematocrit value (HCT) as 0.34 (0.16 – 0.46) L/l, mean cell volume (MCV) as 694.54 (420.45 – 1105.26) , mean cell hemoglobin (MCHb) as 167.88 (87.78 – 257.89) pg and mean cell hemoglobin concentration (MCHbC) as 251.43 (160.87 – 387.50) g/l. Seasonal changes were detected in erythrocyte count and related parameters (Hb, HCT, MCV, MCHb, and MCHbC). There are small differences between sexes in terms of these values although they are statistically insignificant. Nevertheless, neither seasonal nor sex-based variations were observed in leukocyte and thrombocyte count values. Small seasonal differences are observed in terms of cell sizes although they are concluded to be individual variations.

Key Words: Uludağ National Park, Turkey, *Rana macrocnemis*, hematology, blood cells.

Introduction

Erythrocyte counts in blood circulation of amphibians are varied significantly among species and individuals of same species (Hutchinson and Szarski 1965, Szarski and Czopek 1966, Rouf 1969, Kaloustian and Dulac 1982, Sinha 1983). These differences were depended on body mass, age, sex (Arvy 1947, Goniakowska 1973, Sinha 1983, Choubey et al. 1986, Banarjee 1988), environmental conditions (Ruiz et al. 1983, 1989) and season (Zhukova and Kubantsev 1979, Sinha 1983, Samantaray 1985, Wojtaszek et al. 1997). In addition, some variations are occured in cell morphometry, chemistry and ultrastructure (Sinha 1983, Barni et al. 1992, Wojtaszek et al. 1997). It is stated that similar differences were distinguished also in leukocytes, but investigations that carried on this subject were not in detail when compared to the studies focused on the erythrocytes (Schermer 1954, Foxon 1964, Nano et al. 1991).

The hematological studies were carried out on various *Rana* species are also limited and mostly performed on the blood cell counts (e.g. Alder and Huber 1923, Klieneberger 1927, Arvy 1947, Kaplan 1951, 1952, Schermer 1954, Stephan 1954, Hutchinson and Szarski 1965, Arıkan 1989) and measurements (Atatür et al. 1999, Arıkan et al. 2001). Only a few researchers have examined hematological parameters such as the blood volume, hematocrit value (Prosser and Weinstein 1950), fragility and pH value (Rouf 1969).

Uludağ frog, *Rana macrocnemis,* is distributed in forest and subalpine belt at high altitudes in Caucasus, Turkey and Iran (Başoğlu and Özeti 1973, Tarkhnishvili and Gokhelashvili 1999). There is still a lack of sufficient information about hematology of *R. macrocnemis*. The only on this subject is carried out by Arıkan *et al*. (2001) about the erythrocyte morphology in Anatolian mountain frogs (*R. macrocnemis, R. holtzi* and *R. camerani*). The objectives of this study were to determine some hematological parameters and cytomorphometry of circulating blood in Uludağ frog, *Rana macrocnemis,* related to season.

Materials and Methods

The study was conducted in Sarıalan creek in fir (*Abies bornmuelleriana*), forest Uludağ National Park, Bursa (40˚ 07' 964'' N, 29˚ 06' 753'' E, 1617 m). A total of 30 (15 ♂♂, 15

♀♀) adult individuals were captured in May, July and September 2006. The specimens reached sexual maturity, which were similar to each other in terms of size and weight are prefered. The Ethics Board for Experimental Animals, Faculty of Pharmacy, Ege University approved the animal handling and laboratory methodology.

The specimens were studied within 4 hours at the latest after having been caught. Snout-vent length and weight were measured initially in living specimens caught. Blood samples were sampled from heart ventricle of individuals after anesthetized in ether and were counted manually by Neubauer hemocytometer. As diluting solution, Hayem's solution was used for erythrocytes, Turck's solution for leukocytes and Rees and Ecker's solution for thrombocytes. Dilution was carried out for 200 times for erythrocyte count and for 20 times for leukocyte count. Thrombocyte count was done using the erythrocyte dilution pipette according to Rees and Ecker's method (Seiverd 1964).

In measuring morphology and size of blood cells, blood smears, which were prepared by Wright's stain, were used. Blood cells were measured using MOB-1-15x micrometric ocular. Length (L) and width (W) of 40 randomly-chosen erythrocytes as well as nucleus length (NL) and nucleus width (NW) were measured on each blood smear. Erythrocytes (S) and their nucleus sizes (NS) were calculated according to formula of LWπ/4 and NLNWπ/4. Cell and nucleus shapes were compared according to L/W and NL/NW ratios while nucleus/cytoplasm comparison was made according to nucleocytoplasmic ratio (N/C). Moreover, leukocytes and thrombocytes (TL, TW) on blood smears of each individual were measured and their sizes were detected.

Sahli device (Satake et al. 1986, Reddy and Bashamohideen 1989), based on the acid hematine method, was used for detecting hemoglobin amount (Hb). 5 % of HCl solution was put in sahli tube until 2 line, 20 µl of blood sample obtained by sahli pipette was added in this solution and it was homogenized by a glass mixing stick. Distilled water was added until filter color was achieved and the value obtained was read from the scale on the tube and recorded in g/dl (Kocabatmaz and Ekingen 1984, Satake et al. 1986, Reddy and Bashamohideen 1989). Microhematocrit pipettes of 1.1 mm in diameter and 75 mm in length were utilized in detecting hematocrit (HCT) (Conroy 1972, Sniezsko 1960). Blood was centrifuged for 5 minutes using a 3000 rpm constant-rotation centrifuge in thin-walled capillary tubes and the value obtained was read from the scale and recorded in L/l (Blaxhall and Daisley 1973, Jones and Pearson 1976). The mean cell volume (MCV), mean cell hemoglobin (MCHb) and mean cell hemoglobin concentration (MCHbC) were calculated according to the Wintrobe's formula (1933).

Statistical analyses were performed by SPSS (vers. 10.00) statistical package program. Since the distribution of data was not significantly different from the normal distribution (Kolmogorov-Smirnov test, $P>0.05$), averages were compared with parametric *t* test and one-way ANOVA. The significance level was $P\leq0.05$.

Results

In the specimens examined, snout-vent length was measured to be an average of 57.96±1.11 (51.43 – 62.87) mm in males and 61.46±1.83 (53.79 – 76.24) mm in females. The weight of the experimental animals was measured to be 20.35±1.22 (14.20 – 28.30) g in males and 21.94±1.87 (14.70 – 33.10) g in females.

The mean erythrocyte count in 1 mm^3 of blood was 514,839±33.63 (280,000 – 940,000) (Table 1). Although small differences were observed between males and females in terms of erythrocyte count, this difference was statistically insignificant (t= 0.260, df= 29, P= 0.797). However, some differences were recorded according to the studied months (F= 13.446, df= 28, P= 0.000). Erythrocyte count was increased especially in May (Table 2). Moreover, the mean erythrocyte count of females (810,000) was higher than that of the males (636,000) also in May.

The mean leukocyte count in 1 mm^3 of blood was found to be 3,527±11.10 (2,600 – 5,200) (Table 1). Although the leukocyte count is less in females than in males, it is not statistically significant (t= 0.747, df= 29, P=0.461). No seasonal variations were observed in terms of leukocyte count (F= 1.036, df= 28, P= 0.368) (Table 2).

The mean thrombocyte count in 1 mm^3 of blood was 31,069±15.62 (20,000 – 50,000) (Table 1). No sex-based variations were detected in terms of thrombocyte count (t= 1.474, df= 29, P= 0.151). The highest thrombocyte count was detected in July (Table 2). No seasonal variations were observed in terms of thrombocyte count (F= 0.490, df= 28, P= 0.618).

Hemoglobin amount was detected to be 8.10±0.30 (5.60 – 12.10) g/dl (Table 1). No differences occurred between male and female individuals in terms of hemoglobin amount (t= 0.073, df= 29, P= 0.942). Due to the hemoglobin amount was higher in May and July (Table 2), a seasonal variation was detected (F= 4.858, df= 28, P= 0.015).

The mean hematocrit value was 0.34±1.35 (0.16 – 0.46) L/l (Table 1). No differences were observed between sexes in terms of hematocrit value (t= 1.139, df= 29, P= 0.264). However, some differences were observed among the periods studied (F= 8.598, df= 28, P= 0.001). The hematocrit value was found to be higher in May and July than in September (Table 2).

The mean cell volume was estimated to be 694.54±35.01 (420.45 – 1105.26) □ (Table 1). No sex-based differences existed in terms of mean cell volume (t= 0.598, df= 29, P= 0.555). However, a seasonal difference was observed (F= 6.019, df= 28, P= 0.007). The mean cell volume was observed to be higher in July than in the other months (Table 2).

The mean cell hemoglobin was 167.88±7.01 (87.78 – 257.89) pg (Table 1). No differences were detected between male and female individuals in terms of mean cell hemoglobin (t= 0.378, df= 29, P= 0.708). A seasonal difference was observed (F= 3.975, df= 28, P= 0.030). The amount was higher in July and September (Table 2).

The mean cell hemoglobin concentration was calculated to be 251.43±11.14 (160.87 – 387.50) g/l (Table 1). No differences were observed between sexes in terms of the mean cell hemoglobin concentration (t= 0.778, df= 29, P= 0.443). A seasonal difference was detected (F= 3.970, df= 28, P= 0.032). The mean cell hemoglobin concentration was high in September (Table 2).

The shape of the erythrocytes of *R. macrocnemis* is ovoid, like the other amphibians. The nuclei are also ovoid and oriented centrally. Their long axis is located in parallel to long cell axis. The erythrocytes are stained as pinkish while the nucleus is stained as dark purple with Wright's stain (Figure 1a). The mean erythrocyte length was measured as 22.66±0.05 (16.50 – 28.50) µm, width as 14.22±0.03 (12.00 – 17.50) µm and size as 278.23±0.90 (184.57 – 419.98) µm^2 (Table 3).

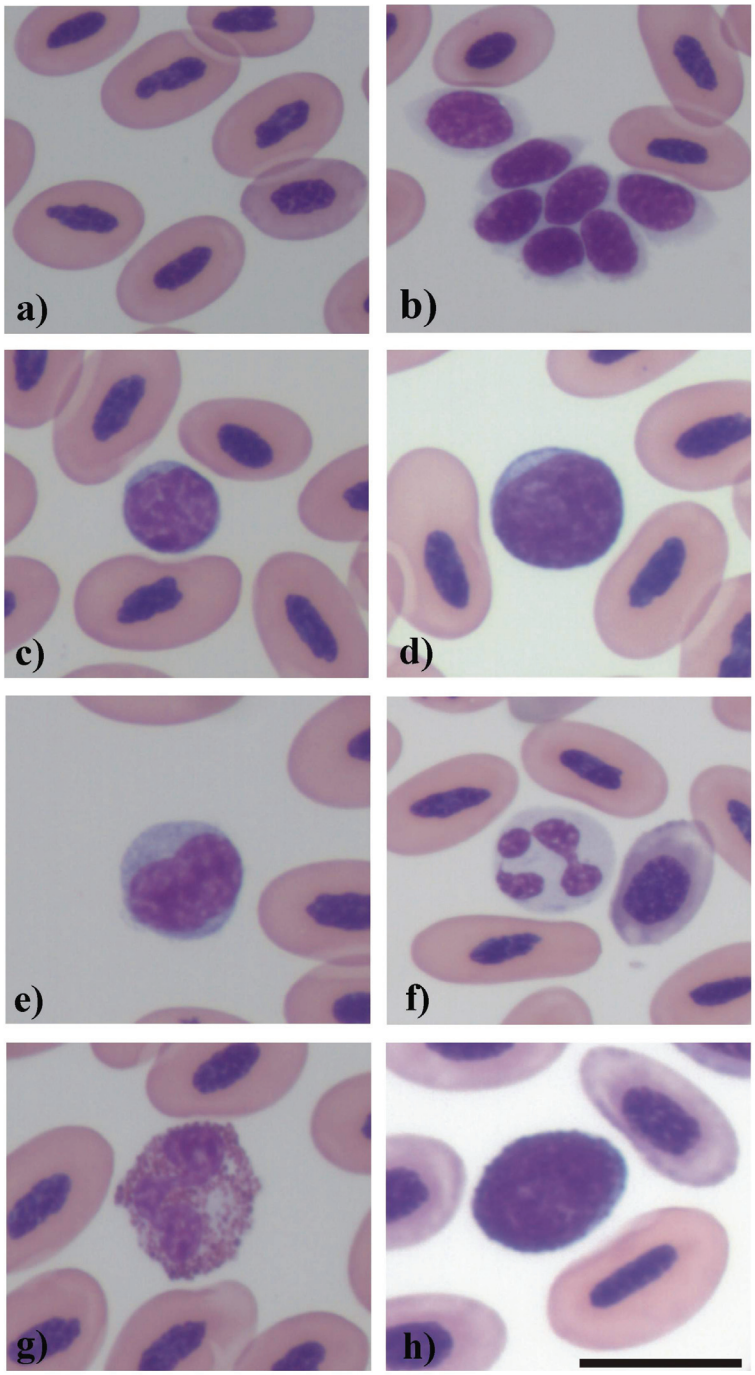

Figure 1. Blood cells of *Rana macrocnemis*. a) erythrocytes, b) a cluster of thrombocytes, c) small lymphocyte, d) large lymphocyte, e) monocyte, f) neutrophil, g) eosinophil, h) basophil. [scale bar is 20 µm].

Table 1. Hematological parameters of circulating blood of *Rana macrocnemis*. [EC: erythrocyte count, LC: leukocyte count, TC: thrombocytes count, Hb: hemoglobin, HCT: hematocrit, MCV: mean cell volume, MCHb: mean cell hemoglobin, MCHbC: mean cell hemoglobin concentration, n: sample size, SEM: standard errors of means].

Parameter	Sex	n	Mean	SEM	Range
EC (x10⁶/µl)	♂♂	15	506,250	45.63	280,000 – 940,000
	♀♀	15	524,000	51.16	320,000 – 900,000
	♂♀	30	514,839	33.63	280,000 – 940,000
LC (x10⁶/µl)	♂♂	15	3,445	13.2	2,600 – 4,200
	♀♀	15	3,613	18.4	2,800 – 5,200
	♂♀	30	3,527	11.10	2,600 – 5,200
TC (x10⁶/µl)	♂♂	15	33,257	21.74	20,000 – 50,000
	♀♀	15	28,735	21.57	20,000 – 40,000
	♂♀	30	31,069	15.62	20,000 – 50,000
Hb (g/dl)	♂♂	15	8.12	0.44	5.60 – 12.10
	♀♀	15	8.07	0.43	6.20 – 11.00
	♂♀	30	8.10	0.30	5.60 – 12.10
HCT (L/l)	♂♂	15	0.32	1.65	0.19 – 0.42
	♀♀	15	0.35	2.15	0.16 – 0.46
	♂♀	30	0.34	1.35	0.16 – 0.46
MCV (fl)	♂♂	15	674.07	44.72	425.53 – 1000.00
	♀♀	15	716.39	55.44	420.45 – 1105.26
	♂♀	30	694.54	35.01	420.45 – 1105.26
MCHb (pg)	♂♂	15	170.48	9.64	91.49 – 229.41
	♀♀	15	165.10	10.51	87.78 – 257.89
	♂♀	30	167.88	7.01	87.78 – 257.89
MCHbC (g/l)	♂♂	15	259.88	14.02	170.59 – 355.88
	♀♀	15	242.43	17.72	160.87 – 387.50
	♂♀	30	251.43	11.14	160.87 – 387.50

Table 2. Seasonal change on hematological parameters of circulating blood of *Rana macrocnemis* (♂♂/♀♀).

Parameter	Season	n	Mean	SEM	Range
EC (x10⁶/µl)	Spring	10	713,333	73.18	340,000 – 940,000
	Summer	10	450,000	20.66	340,000 – 620,000
	Autumn	10	390,000	40.24	280,000 – 520,000
LC (x10⁶/µl)	Spring	10	3,592	12.93	3,000 – 4,200
	Summer	10	3,613	19.53	2,600 – 5,200
	Autumn	10	3,200	11.54	2,800 – 3,600
TC (x10⁶/µl)	Spring	10	29,123	26.28	20,000 – 40,000
	Summer	10	32,565	23.23	20,000 – 50,000
	Autumn	10	30,000	36.51	20,000 – 40,000
Hb (g/dl)	Spring	10	9.10	0.45	7.60 – 11.00
	Summer	10	8.09	0.44	5.80 – 12.10
	Autumn	10	6.62	0.29	5.60 – 7.40
HCT (L/l)	Spring	10	0.37	0.01	0.33 – 0.42
	Summer	10	0.35	0.01	0.24 –0.42
	Autumn	10	0.24	0.05	0.16 – 0.46
MCV (fl)	Spring	10	569.00	14.43	420.45 – 970.59
	Summer	10	795.20	38.78	521.74 – 1105.26
	Autumn	10	614.62	67.52	470.59 – 884.62
MCHb (pg)	Spring	10	140.00	15.72	87.78 – 229.41
	Summer	10	180.97	7.73	140.91 –257.89
	Autumn	10	174.99	10.75	142.31 – 206.25
MCHbC (g/l)	Spring	10	247.00	13.57	195.24 – 291.89
	Summer	10	234.97	14.15	165.00 – 355.88
	Autumn	10	301.40	33.94	160.87 – 387.50

Lymphocytes are spherical cells. Their cytoplasm is stained as dark grayish blue while the nuclei stained dark purple with Wright's stain. The nucleus is as large as to fill almost the whole cell whereas the cytoplasm is observed as a thin ring. Small and large lymphocytes are encountered on the blood smears (Figures 1c and 1d). Although large lymphocytes look like monocytes in shape, they are distinguished easily due to their nucleus shapes and staining properties. The mean diameter is 9.75±0.18 (8.75 – 11.25) µm in small lymphocytes while it is 13.41±0.23 (11.50 – 18.50) µm in large lymphocytes (Table 4). Monocytes are also spherical in shape and their cytoplasm is stained as light

grayish blue while the nuclei as purple. The chromatin structure is not as dense as that of the lymphocytes. Kidney shaped nuclei is located on one side of the cell (Figure 1e). The mean diameter in monocytes has been measured to be 14.30±0.19 (10.00 – 18.50) µm (Table 4).

Neutrophilles are spherical, large and the most encountered cell type. They are easily distinguished from other cells due to their lobular or segmental nuclei shape and staining properties. The cytoplasm is stained as lightest blue, while the nuclei as light purple with Wright's stain (Figure 1f). Neutrophilles have a mean diameter of 16.98±0.22 (12.75 – 21.50) µm.

Eosinophilles are also spherical and have some large, reddish and shiny granules in their cyloplasm that can be seen easily with Wright's stain. Their nucleus has segments or lobes, like in neutrophilles, and they are purplish red with Wright's stain (Figure 1g). The mean diameter in eosinophilles is 16.30±0.21 (11.75 – 19.75) µm.

Basophiles are also small and spherical cells. Quite large and dark purplish black granules are masked in their cytoplasm with Wright's stain. The nucleus is slightly stained and it has larger and fewer segments (Figure 1h). The diameter was measured to be 13.69±0.15 (10.50 – 16.25) µm in basophiles (Table 4).

Thrombocytes are spindle-shaped, thin, long and oval cells. The nucleus is large and ovoid fill almost the whole cell. Their cytoplasm is stained as light bluish gray and the nucleus as dark purple with Wright's stain. It has been observed that the thrombocytes are distributed widely and form clusters on the blood smears (Figure 1b). The mean length was measured as 16.14±0.19 (12.50 – 19.75) µm and width as 8.26±0.10 (6.75 – 10.00) µm in trombocytes (Table 4).

Discussion

The erythrocyte count in 1 mm^3 of blood varies between 500,000 and 1,500,000 on average in anurans (Glomski et al. 1997). The highest values were detected in *Rana cyanophlyctis* (EC= 2,060,000) and *R. tigerina* (1,850,000) during the monsoon season (Samantaray 1985, Pai and Shanbhag 1988). Alder and Huber (1923) reported the erythrocyte count in 1 mm^3 as 408,000 in *R. temporaria* and as 324,000 – 800,000 in *R. esculenta*. Stephan (1954) detected the erythrocyte count as 400,000 in *R. temporaria*. This figure is between 180,000 and 590,000 among Anatolian populations of *R. ridibunda* (Arıkan 1989). In present study, mean erythrocyte count was calculated to be 514,839 (280,000 – 940,000) in *R. macrocnemis*.

Arvy (1947) stated that the erythrocyte count in males in *R. temporaria* (450,000) is higher than that of females (300,000). Besides, Kaplan (1951, 1952) reported that the erythrocyte count in females in *R. pipiens* (512,000) is higher than that of the males (480,000). In *R. pipens* (Rouf 1969), *R. catesbeiana* and *R. calamitans* (Hutchinson and Szarski 1965) no sexual dimorphism was reported for erythrocyte count. No noteworthy differences were detected between sexes also in Uludağ frog in terms of the annual mean erythrocyte count. This finding is in accordance with the report of Arıkan (1989).

Table 3. Erythrocyte and nuclei measurements in the circulating blood of *Rana macrocnemis*. [L: erythrocyte length, W: erythrocyte width, S: erythrocyte size, NL: nucleus length NW: nucleus width, NS: nucleus size, N/C: nucleocytoplasmic ratio]

Characters	Sex	n	Mean	SEM	Range
L (µm)	♂♂	15	22.32	0.07	16.50 – 28.50
	♀♀	15	23.03	0.06	18.75 – 27.50
	♂♀	30	22.66	0.05	16.50 – 28.50
W (µm)	♂♂	15	13.65	0.05	12.75 – 15.25
	♀♀	15	14.59	0.04	12.00 – 17.50
	♂♀	30	14.22	0.03	12.00 – 17.50
L/W	♂♂	15	1.43	0.01	1.08 – 1.92
	♀♀	15	1.48	0.03	1.16 – 2.02
	♂♀	30	1.46	0.02	1.08 – 2.02
S (µm²)	♂♂	15	274.68	1.38	184.57 – 419.98
	♀♀	15	282.03	1.14	188.40 – 382.20
	♂♀	30	278.23	0.90	184.57 – 419.98
NL (µm)	♂♂	15	9.80	0.04	5.00 – 12.75
	♀♀	15	10.37	0.04	7.75 – 12.75
	♂♀	30	10.08	0.03	5.00 – 12.75
NW (µm)	♂♂	15	5.90	0.03	3.00 – 8.75
	♀♀	15	5.91	0.03	4.25 – 8.25
	♂♀	30	5.91	0.02	3.00 – 8.75
NL/NW	♂♂	15	1.68	0.01	0.95 – 3.42
	♀♀	15	1.78	0.01	1.14 – 2.72
	♂♀	30	1.73	0.01	0.95 – 3.42
NS (µm²)	♂♂	15	35.51	0.31	15.80 – 47.27
	♀♀	15	37.09	0.26	28.26 – 44.31
	♂♀	30	36.76	0.21	15.80 – 47.27
N/C	♂♂	15	0.17	0.01	0.07 – 0.25
	♀♀	15	0.17	0.02	0.10 – 0.25
	♂♀	30	0.17	0.01	0.07 – 0.25

Table 4. Leucocytes and thrombocytes measurements in the circulating blood of *Rana macrocnemis*. [TL: thrombocyte length, TW: thrombocyte width]

Characters	Sex	n	Mean	SEM	Range
Small Lymphocytes (µm)	♂♂	15	10.13	0.22	8.75 – 11.00
	♀♀	15	9.78	0.13	9.25 – 11.25
	♂♀	30	9.75	0.18	8.75 – 11.25
Large Lymphocytes (µm)	♂♂	15	13.11	0.36	11.50 – 18.50
	♀♀	15	13.71	0.27	11.75 – 16.25
	♂♀	30	13.41	0.23	11.50 – 18.50
Monocytes (µm)	♂♂	15	14.18	0.31	10.00 – 17.75
	♀♀	15	14.43	0.23	12.25 – 17.25
	♂♀	30	14.30	0.19	10.00 – 17.75
Neutrophis (µm)	♂♂	15	16.59	0.29	12.75 – 20.25
	♀♀	15	17.36	0.32	13.50 – 21.50
	♂♀	30	16.98	0.22	12.75 – 21.50
Eosinophils (µm)	♂♂	15	16.44	0.36	11.75 – 19.75
	♀♀	15	16.15	0.23	12.75 – 18.25
	♂♀	30	16.30	0.21	11.75 – 19.75
Basophils (µm)	♂♂	15	13.50	0.21	11.25 – 15.75
	♀♀	15	13.88	0.21	10.50 – 16.25
	♂♀	30	13.69	0.15	10.50 – 16.25
TL (µm)	♂♂	15	16.11	0.28	13.50 – 19.25
	♀♀	15	16.17	0.27	12.50 -19.75
	♂♀	30	16.14	0.19	12.50 – 19.75
TW (µm)	♂♂	15	8.03	0.13	6.75 – 9.50
	♀♀	15	8.50	0.14	7.25 – 10.00
	♂♀	30	8.26	0.10	6.75 – 10.00

The erythrocyte count was quite high in *Rana macrocnemis* during spring when compared with that of summer and autumn. The mean erythrocyte count in females was higher than that of the males especially in spring. The highest value of the erythrocyte counted in spring is probably related to the increase of the metabolic activities at the end of hibernation. The metabolic activity of the females, would have to increase in order to hatch, consequently, the erythrocyte count would be reached to its maximum. Variations in the erythrocyte count and size based on the metabolic activity were reported in amphibians by various researchers (Goniakowska 1970, 1973; Kuramoto 1981). Some histological parameters that recorded in the liver (Barni and Bernocchi 1991) and bone marrow (Foxon 1964) were indicated on increase of the erythrocytes at the end of the hibernation period. This causes the highest erythrocyte count in the life cycles of individuals to be observed at the end of hibernation (Wojtaszek and Adamowicz 2003). Shermer (1954) stated that the erythrocyte count in *R. temporaria* and *R. esculenta* is lower in winter months (280,000 – 626,000 in winter; 166,000 – 200,000 in summer). It were also reported that some individual and seasonal differences in the erythrocyte count in *Rana* species (Alder and Huber 1923, Klieneberger 1927, Shermer 1954, Sinha 1983, Wojtaszek et al. 1997). In addition, some researchers stated that the differences in the erythrocyte count are caused by geographical variations (Hutchison and Szarski 1965, Rouf 1969). Arıkan (1989) noted that the erythrocyte count in Anatolian *R. ridibunda* populations is not affected by the factors such as elevation and season, but are related to geographical variation.

The leukocyte count varies depending on species, season, sex, nutritional conditions and some physiological conditions (e.g. diseases, breeding) (Arıkan 1989, Rouf 1969, Wojtaszek and Adamowicz 2003). The values in *R. temporaria* (25,000) by (Alder and Huber 1923) and in *R. pipiens* (16,134 in males; 14,134 in females) by (Kaplan 1951, 1952) are quite higher than the values we have found for *R. macrocnemis*. They appear to be similar to the values (1,700 – 5,000) found in *R. ridibunda* by Arıkan (1989). Shermer (1954) considered the leukocyte values in *R. temporaria* and *R. esculenta* as the summer period (4,900 – 7,300) and the winter period (1,100 – 2,100) and stated that a considerable decline took place in the leukocyte count in the winter. Kaplan (1951, 1952) put forth that the leukocyte count in *R. pipiens* is higher in males than in females. It was concluded that the small differences detected in *R. macrocnemis* in terms of the leukocyte count both of the sexes and seasons are statistically insignificant. Stephan (1954) calculated the ratio of leukocytes to erythrocytes as 1: 20 – 70. The mean leukocyte count in *R. macrocnemis* is 3,527 and this value remains below these limits.

The thrombocyte count has been examined by a few researchers. In addition, the use of different methods during counting and the formation of clusters by thrombocytes have led to achieving very different results. The thrombocyte count was found to be 15,270 in *R. esculenta* (Klieneberger 1927). Furthermore, Kaplan (1951, 1952) found as 880,000 in *R. pipiens*. The value found by Kaplan is almost 28 times higher than that of the Uludağ frog (31,069). Shermer (1954) reported that it is quite higher in summer (5,500 – 8,500) than in winter (500 – 1,200) in *R. temporaria* and *R. esculenta*. No sex-based or seasonal differences were detected in the thrombocyte count in *R. macrocnemis*.

The hemoglobin value was reported to be 6.75 g/dl (2.4 – 9.6 g/dl) in *R. pipiens* (Rouf, 1969). In *B. bombina*, this value is 7.44 g/dl in males and 6.78 g/dl in females (Wojtaszek and Adamowicz 2003). These values are quite close to the values detected for *R. macrocnemis*.

The hematocrit value was calculated as 0.25 L/l in *R. pipiens*, 0.20 L/l in males and 0.19 L/l in females in *B. bombina* (Rouf 1969, Wojtaszek and Adamowicz 2003). In his study on the Lake Sevan population of *R. macrocnemis* (2000 m a.s.l., Armenia) in July 1973, Ishchenko (1978) reported the hematocrit value as 0.53 (0.39 – 0.67) L/l in individuals with vertebral stripe and as 0.43 (0.36 – 0.55) L/l in individuals without vertebral stripe. These values are also in close to the values reported in present paper.

The mean cell volume was calculated to be 558.22 □ in males and 655.77 □ in females in *B. bombina* (Wojtaszek and Adamowicz 2003). Although the values are slightly high in *R. macrocnemis* (674.07 □ in males; 716.39 □ in females), no noteworthy differences take place. It is found out that the hemoglobin value and the mean cell volume in *R. esculenta* are higher in males than in females (Sinha 1983). Although the mean cell volume is a slightly higher in females in *R. macrocnemis*, it is not statistically important.

The mean cell hemoglobin was 167.88 pg in *R. macrocnemis*. It is stated by Wojtaszek and Adamowicz (2003) to be 206.49 pg in males and 230.61 pg in females in *B. bombina*. These values are a little higher than the values of *R. macrocnemis*.

The mean cell hemoglobin concentration was calculated to be 251.43 g/l in *R. macrocnemis*. In *B. bombina*, the mean cell hemoglobin concentration is given as 367.8 g/l (Wojtaszek and Adamowicz 2003). This value is slightly higher than that of the Uludağ frog.

It was reported that the erythrocyte size in amphibians has a relationship with the activity of the individuals and, depending on this; the erythrocytes of more active species are while the species characterized with low oxygen consumption exhibit large ones (Evans, 1939). It was reported by Atatür et al. (1999) reported that, aquatic species (*Rana ridibunda, Bombina bombina*) have larger erythrocytes while terrestrial species (*Bufo bufo, Bufo viridis, Hyla arborea* and *Pelobates syriacus*) have smaller. It was detected by Arıkan et al. (2001) that among the mountain frogs living in Turkey, the largest erythrocyte size is in *R. macrocnemis* while the smallest is in *R. holtzi*. The findings obtained in this study are in accordance with that of Arıkan et al. (2001).

In conclusion, hematology of *R. macrocnemis* appears to be similar to the other anuran species mentioned in the literature. The erythrocyte count and the related parameters (Hb, HCT, MCV, MCHb and MCHbC) vary depending on season. Although small differences are observed between sexes in terms of these values, they are statistically insignificant. No seasonal or sex-based differences have been observed in terms of the leukocyte and thrombocyte count. Even though small seasonal differences are observed in terms of cell sizes, they are concluded to be individual variations.

Acknowledgements

The Manuscript, based partly on the MSc thesis of S. K. ARSERİM, supervised by A. MERMER. Besides, it was supported by the project No. 2006/FEN/012 of E. U. Accounting Office of Funds. We thank Dr. Dinçer AYAZ and Kerim ÇİÇEK for significant help in the field studies and Prof. Dr. Hüseyin ARIKAN for his invaluable comments on an earlier version of the manuscript.

References

Alder, A., E. Huber. 1923. Unstersucheng über blutcellen bei Amphibien und Reptilien. Folia Heamatol. 29:1-22.

Arıkan, H. 1989. Investigation on *Rana ridibunda* (Anura, Ranidae) populations aspect of blood cells, (in Turkish). Turk. J. Zool. 13: 54-59.

Arıkan H., İ.E. Çevik, U. Kaya, and A. Mermer. 2001. Erythrocytes measurements on Anatolian mountain frogs, (in Turkish). Anadolu Ünv. Bilim ve Teknoloji Dergisi 2: 387-391.

Arvy, L. 1947. Le dimorphisme sexuel sanguin chez *Rana temporaria* L. et *Bufo vulgaris*. Laur. Compt. Rend. Soc. Biol. 141: 457-459.

Atatür, M.K., H. Arıkan, and İ.E. Çevik. 1999. Erythrocyte sizes of some Anurans from Turkey. Turk. J. Zool. 23: 111-114.

Banerjee, V. 1988. Erythrocyte related blood parameters in *Bufo melanostictus* with reference to sex and body weight. Environ. Ecol. Kalyani. 6: 802-806.

Barni, S., G. Bernocchi. 1991. Internalization of erythrocytes into liver parenchymal cells in naturally hibernating frogs (*Rana esculenta* L.). J. Exp. Zool. 258: 143-150.

Barni, S., R. Nano, V. Bertone, and E. Prosperi. 1992. Ultrastructure and cytochemistry of circulating erythrocytes during the annual cycle of *Rana esculenta* L. Comp. Haematol. Int. 2: 166-169.

Başoğlu, M., N. Özeti. 1973. The Amphibians of Turkey, (in Turkish). Ege Üniversitesi Fen Fakültesi Kitapları Serisi, No. 151, Bornova-İzmir. 155 pp.

Blaxhall, P.C., K.W. Daisley. 1973. Routine haematological methods for use fish with blood. J. Fish Biol. 5: 771-781.

Choubey, B.G., A. Shankar, and B.J. Choubey. 1986. Haematological investigation of Himalayan toad *Bufo melanostictus* Schneider in relation to sex and size. Biol. Bull. India. 8: 106-114.

Conroy, D.A. 1972. Studies on the haematology of the Atlantic Aalmon (*Salmo salar* L.). Symp. Zool. Soc. Lond. 30: 101-127.

Evans, G. 1939. Factors in fluencing the oxygen consumption of several species of plethodontid salamanders in aerial aquatic media. Ecology 20: 74-95.

Foxon, G.E.H. 1964. Blood and respiration, pp. 151-209. In J.A. Moore [ed.], Physiology of the Amphibia. Academic Press.

Glomski, C.A., J. Tamburlin, R. Hard, and M. Chainami. 1997. The phylogenetic odyssey of the erythrocyte. IV. the Amphibians. Histol. Histopathol. 12: 147-170.

Goniakowska, L. 1970. The respiration of erythrocytes of some amphibians in vitro. Bull. Acad. Polon. Sci., Sér. Sci. Biol. 12: 793-797.

Goniakowska, L. 1973. Metabolism, resistance to hypotonic solutions, and ultrastructure of erythrocytes of five amphibian species. Acta. Biol. Crac., Ser. Zool. 13: 225-236.

Hutchison, V.H., H. Szarski. 1965. Number of erythrocytes in some amphibians and reptiles. Copeia 3: 373-375.

Ishchenko, V. 1978. Dinamicheskij polimorfism burikh lyagushek fauni SSSR [Dynamic polymorphism of brown frogs of fauna of USSR], Nauka Publ. House.

Jones, B.J., W.D. Pearson. 1976. Variations in haematocrit values of successive blood samples from Bluegill. Trans. Am. Fish. Soc. 2: 291-293.

Kaloustian, K.V., R.W. Dulac. 1982. Relationship between red blood cell indices and the effect of thyroxine in three species of amphibians. Comp. Biochem. Physiol. 73A: 427-430

Kaplan, H.M. 1951. A study of frog blood in red leg disease. Trans. III. State Acad. Sci 44: 209-215.

Kaplan, H.M. 1952. Variations in white blood cells between normal and red leg frogs. Ibid. 45: 170-176.

Klieneberger, C. 1927. Die Blutmorphologie der laboratoriumstiere, Barth,

Liepzig.
Kocabatmaz, M., G. Ekingen. 1984. Taking blood sample in different fish species and standardization of hematological methods, (in Turkish). Doğa Bilim Dergisi 8: 149-159.
Kuramoto, M. 1981. Relationships between number, size and shape of red blood cells in amphibians. Comp. Physiol. 69: 771-775.
Nano, R., P. Griffini, and S. Barni. 1991. Morphohistochemical changes of the blood cells in the hibernating frog (*Rana esculenta* L.). Comp. Haematol. Int. 1: 220-223.
Pai, R.M., S.V. Shanbhag. 1988. On erythrocytes of certain Indian amphibians. Comp. Physiol. Ecol. 13: 103-110.
Prosser, C.L., J.F. Weinstein. 1950. Comparison of blood volume in animals with open or closed circulatory systems. Physiol. Zool. 23: 113-124.
Reddy, P., M. Bashamohideen. 1989. Fenvalarate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio*. Acta. Hydrochim. Hydrobiol. 17: 101-107.
Rouf, M.A. 1969. Hematology of the Leopard frog, *Rana pipiens*. Copeia 4: 682-687.
Ruiz, G., M. Rosenmann, and A. Veloso. 1983. Respiratory and hematological adaptations to high altitude in Telmatobius frogs from the Chilean Andes. Comp. Biochem. Physiol. 76A: 109-113.
Ruiz, G., M. Rosenmann, and A. Veloso. 1989. Altitudinal distribution and blood values in the toad, *Bufo spinulosus* Wiegmann. Comp. Biochem. Physiol. 94A: 643-646.
Samantaray, K. 1985. Studies on hematology of Indian skipper frog, *Rana cyanophlyctis* Schneider. Comp. Physiol. Ecol. 10: 71-74.
Satake, T., A. Nuti-Sobrinho, O.V. Paula-Lopes, R.A. Lopes, and H.S. Leme Dos Santos. 1986. Haematological study of Brazilian fish. III. Blood parameters in Armored Catfish *Hypostomus paulinus* I. Ars Veterinaria, Faculdade de Ciências Agrárias e Veterinárias "Campus" de Jaboticabal Unesp 2: 179-183.
Schermer, S. 1954. Die Blutmorphologie der laboratorium stiere, Barth.
Seiverd, C.E. 1964. Hematology for medical technologists, Lea and Febiger.
Sinha, R.C. 1983. Haematological studies on the prewintering and wintering frog, *Rana esculenta*. Comp. Biochem. Physiol. 74A: 311-314.
Snieszko, S.F. 1960. Microhaematocrit as a tool in fishery research and management. U. S. Wildl. Serv. Sci. Rep. Fish. 341: 1-5.
Stephan, F. 1954. Morphologie générale du systéme circulatoire, pp. 854–973. In P.P. Grasse (ed.), Traitéde zoologie, Masson.
Szarski, H., G. Czopek, 1966. Erythrocyte diameter in some Amphibians and Reptiles. Bull. Acad. Pol. Sci. Cl. 2 Ser. Sci. Biol. 14: 433-437.
Tarkhnishvili, D.N., R.K. Gokhelashvili. 1999. The Amphibians of the Caucasus, Pensoft Publications.
Wintrobe, M.M. 1933. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Haematologie 51: 32-49.
Wojtaszek, J., A. Adamowicz. 2003. Haematology of the fire-bellied toad, *Bombina bombina* L. Comp. Clin. Path. 12: 129-139.
Wojtaszek, J., M. Baranowska, M. Glubiak, and A. Dżugaj. 1997. Circulating blood parameters of the water frog, *Rana esculenta* L. at pre-wintering stage. Zool. Pol. 1–4: 117-126.
Zhukova, T.I., B.S. Kubantsev. 1979. Changes in the blood composition of salientians during hibernation. Sov. J. Ecol. 9: 379-382.