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Changes in the blood composition of some anurans

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Abstract. We examined some hematological parameters (red blood cell count, white blood cell count, haemoglobin concentration, hematocrit value, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and plasma total protein) on five anuran species of terrestrial (*Pseudepidalea viridis*, *Pelobates syriacus* and *Hyla arborea*), semi-aquatic (*Rana dalmatina*) and aquatic (*Pelophylax ridibundus*) nature from Çanakkale, Turkey. Differences between males and females in terms of haemoglobin, hematocrit and mean cell volume in *P. viridis* were statistically significant. The RBC count was higher in terrestrial and aquatic species than in semi-aquatic species. Haemoglobin concentration, hematocrit value, MCV and MHC were higher in terrestrial species than in semi-aquatic and aquatic species. The MCHC values were all similar to each other. The plasma total protein was higher in terrestrial species than in aquatic species. To sum up, variations were detected in the some hematological parameters under examination among the anuran species.

Keywords. Anura, some hematological parameters, terrestrial, semi-aquatic, aquatic.

INTRODUCTION

Amphibia and Reptilia species were affected by negative conditions of pollution in the environmental condition and destruction of biotopes. Blood parameters of Amphibia species were particularly affected by the negative environmental conditions. Amphibians are potentially reliable and efficient bioindicators (Welsh and Ollivier, 1998; Garg and Hippargi, 2007). They are very sensitive to even the slightest fluctuations in the environmental settings (Cunnigham and Saigo, 1999).

Amphibians are a heterogeneous group of vertebrates with regard to their blood cell count and size. However, the blood cell counts in Amphibia reported a wide individual variation and considerable interspecies differences (Hutchison and Szarski, 1965; Szarski and Czopek, 1966; Rouf, 1969; Sinha, 1983; Atatür et al., 1999; Cabagna et al., 2005) as

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well as in relation to body weight, age and sex (Arvy, 1947; Schermer, 1954; Goniaakowska, 1973; Sinha, 1983; Banerjee, 1988; Wojtaszek and Adamowicz, 2003), season (Zhukova and Kubantsev, 1979; Sinha, 1983; Wojtaszek et al., 1997; Arserim and Mermer, 2008) and altitudinal distribution (Arıkan, 1989; Ruiz et al., 1989).

Most studies on hematology in various species of Anura have dealt with blood cell counts (Alder and Huber, 1923; Arvy, 1947; Kaplan, 1952; Stephan, 1954; Schermer, 1954; Hutchison and Szarski, 1965; Arıkan, 1989) and cell sizes (Wintrobe, 1933; Foxon, 1964; Hartman and Lessler, 1964; Szarski and Czopek, 1966; Kuramoto, 1981; Stöck and Grobe, 1997; Atatür et al., 1999; Arıkan et al., 2003; Cabagna et al., 2005; Gül and Tok, 2009). Nevertheless, some hematological parameters of the anuran species, such as hematocrit value (PCV), haemoglobin concentration (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and plasma total protein concentration, are very scarce and individual studies (Prosser and Weistein, 1950; Haris, 1972; Carmena-Suero et al., 1980; Sinha, 1983; Ostejic et al., 2000; Wojtaszek and Adamowicz, 2003; Coppo et al., 2005; Arserim and Mermer, 2008; Dönmez, 2009). So, the possibilities of using quantitative and qualitative parameters of the blood in amphibians undoubtedly hold great interest (Zhelev et al., 2006).

In the clinical investigation, blood samples are of great diagnostic value and can easily be obtained (Frye, 1991). The normal reference ranges of hematological parameters are also important in assessing the status of the species population, and deviation from the expected values can assess the impact of stress on the species (Dickinson et al., 2002).

Therefore, the objective of this study was to determine some hematological parameters [red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb), hematocrit value (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and plasma total protein value] in some Anuran species (*Pelophylax ridibundus*, *Rana dalmatina*, *Pseudepidalea viridis*, *Hyla arborea* and *Pelobates syriacus*).

In addition, this study aims to investigate differences in the some hematological parameters of anuran species of terrestrial (*P. viridis*, *P. syriacus* and *H. arborea*), semi-aquatic (*R. dalmatina*) and aquatic (*P. ridibundus*) nature.

MATERIAL AND METHODS

Specimens of the different Anuran species used in this study (*P. viridis*: n =10, *H. arborea*: n = 15, *P. syriacus*: n = 13, *R. dalmatina*: n = 15 and *P. ridibundus*: n = 10) were collected from their natural habitat and various localities of Canakkale, Turkey. Studies were carried out in the reproductive period from February to April between 2008 and 2010. Blood samples of the live specimens were obtained in the laboratory within one day of their capture. The blood samples were taken from the etherized frogs by means of ventriculus punctures, via heparinized hematocrit capillaries (Arıkan et al., 2003). The quantity of blood taken out from each specimens were 0.15 ml.

The blood cell counts were performed utilizing a Neubauer hemocytometer, and the standard Hayem's solution was used as diluting solutions for erythrocytes by Thoma pipettes, while for the leukocytes, the method of Jerrett and Mays (1973) (which is a slight modification of Blain's method-Sturkie, 1954) was utilized; i.e. a 1:1 mix of neutral red diluted to 1/5000 with 0.07% physiological saline and 12% formaline prepared with 0.07% physiological saline was used.

Blood from each frog or toad was placed into heparinised hematocrit capillaries and used to determine the hematological parameters. Hematocrit value (PCV) was determined by the micro-hematocrit method. The tubes were then spun in a micro-hematocrit centrifuge for 5 min at 12000 rpm, and the hematocrit value (PCV) was calculated with a hematocrit reader. Hemoglobin concentration (Hb) was measured with a Sahli's hemometer. The mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated mathematically from the above results. MCV was calculated by dividing hematocrit per liter of blood by total RBC count (Tanyer, 1985). The blood samples were centrifuged at 3000 rpm for 10 min and it was ensured that the plasma section be isolated from the blood cells. The plasma total protein quantity in the plasma was measured using a Refractometer. Non-parametric tests and descriptive statistics were calculated using SPSS (v10.0). Mann Whitney U test was applied between males and females.

RESULTS

Hematological parameters investigated in the present study are represented in Table 1. There were some significant differences in some parameters when comparisons were made between male and female data for one species. Differences between males and females in terms of hemoglobin, hematocrit and mean cell volume in *P. viridis* were statistically significant ($P < 0.05$).

When the hematological values of anuran species were examined, the highest RBC count, WBC count, hemoglobin concentration and hematocrit value were found in *Pseudepidalea viridis*, whereas the lowest RBC count, WBC count, hemoglobin concentration and hematocrit value were found in *R. dalmatina*. The mean cell volume was detected to be the highest in *H. arborea* and the lowest in *R. dalmatina*. The mean cell hemoglobin was found to be the highest in *H. arborea* and the lowest in *P. ridibundus*. The mean cell hemoglobin concentration was found to be the highest in *Pseudepidalea viridis* and the lowest in *P. ridibundus*. The plasma total protein was found to be the highest in *P. viridis* and the lowest in *P. ridibundus*. The hematological values of anuran species are given in detail in Table 1.

DISCUSSION

Several authors, who worked on different species of *Anura*, mentioned individual variations concerning both the RBC and WBC counts (Alder and Huber, 1923; Klieneberger, 1927; Schermer, 1954; Hutchison and Szarski, 1965; Cabagna et al., 2005; Chiesa et al. 2006). In the present study, significant differences were found between males and females only in *P. viridis* specimens in terms of hemoglobin, hematocrit and mean cell volume. The RBC count was higher in males than in females of species *P. ridibundus*, *R. dalmatina*, *H. arborea* and *P. syriacus*. Similar observations were found by Arvy (1947) in *R. temporaria*, where males (450000) showed a higher RBC count than females (330000). Even Sinha (1983) stated a higher count in males (320000) than in females (250000) in *R. esculenta*. Wojtaszek and Adamowicz (2003) have reported a higher number of RBC in males (340000) than in females (290000) of *Bombina bombina*. However, the RBC count of *P.*

Table 1. Red blood cell counts (RBC) (N/μL), white blood cell count (WBC) (μL), haemoglobin concentration (Hb) (g/dL), hematocrit value (PCV) (%), mean cell volume (MCV) (fL), mean cell haemoglobin (MCH) (pg/cell), mean cell haemoglobin concentration (MCHC) (%) and plasma total protein (PTP) (g/L). F: female, M: male, T: total; n: number of specimens, SD: standard deviation.

		Terrestrial											
	Sex	*Pseudepidalea viridis*				*Pelobates syriacus*				*Hyla arborea*			
		n	Mean	SD	Range	n	Mean	SD	Range	n	Mean	SD	Range
RBC	F	6	937666	212943	720000-1300000	11	765909	191818	560000-1200000	11	733636	261965	340000-1290000
	M	4	975000	94339.81	840000-1040000	2	652500	31819.80	630000-675000	4	647500	212818	400000-920000
	T	10	952600	168908	720000-1300000	13	748461	180444	560000-1200000	15	710666	245516	340000-1290000
WBC	F	6	5900	1391.40	4500-8000	11	2600	1798.33	1200-7400	7	3602	652.83	2000-4900
	M	4	6775	1117.66	5700- 8200	2	3250	353.55	3000-3500	2	2800	1131.37	2000-3600
	T	10	6250	1302.34	4500-8200	13	2700	1662.82	1200-7400	9	3602	604.40	3100-4900
Hb	F	6	15.76	0.79	14.60-17.00	11	10.38	2.46	6.00-12.80	11	12.21	1.42	10.00-13.80
	M	4	12.95	0.91	12.00-14.20	2	9.30	3.53	6.80-11.80	4	11.82	1.22	10.40-13.40
	T	10	14.64	1.65	12.00-17.00	13	10.21	2.50	6.00-12.80	15	12.11	1.34	10.00-13.80
PCV	M	6	58.50	6.94	52.00-70.00	5	40.00	0.08	27.00-50.00	11	50.18	8.64	40.00-67.00
	F	4	43.75	3.30	40.00-47.00	2	38.00	0.11	30.00-46.00	3	45.00	10.00	35.00-55.00
	T	10	52.60	9.40	40.00-70.00	7	40.00	0.08	27.00-50.00	14	49.07	8.81	35.00-67.00
MCV	M	6	638.63	96.05	538.46-793.06	5	468.20	83.07	366.67-595.24	11	758.10	285.86	456.59-1411.76
	F	4	450.18	34.10	403.85-476.19	2	577.35	143.06	476.19-678.52	3	878.72	437.86	546.88-1375.00
	T	10	563.25	122.40	403.85-793.06	7	499.39	104.15	366.67-678.52	14	783.94	308.21	456.59-1411.76
MCH	M	6	174.18	33.94	130.77-213.89	11	138.59	35.34	100.00-228.57	11	186.89	77.06	106.98-382.35
	F	4	133.93	17.53	115.38-152.38	2	141.37	47.28	107.94-174.81	4	194.08	48.48	144.57-260.00
	T	10	158.08	34.27	115.38-213.89	13	139.02	35.04	100.00-228.57	15	188.81	68.97	106.98-382.35
MCHC	M	6	27.24	3.10	23.03-30.77	5	28.86	2.73	25.60-31.90	11	24.68	2.91	20.24-31.16
	F	4	29.67	1.94	27.65-32.00	2	24.21	2.19	22.66-25.76	3	26.19	6.87	18.91-32.57
	T	10	28.21	2.86	23.03-32.00	7	27.53	3.30	22.66-31.90	14	25.00	3.77	18.91-32.57
PTP	M	5	8.16	1.36	6.00-9.50	4	7.12	1.65	5.00-9.00	11	7.47	1.56	6.00-10.00
	F	4	7.50	0.57	7.00-8.00	2	6.50	0.70	6.00-7.00	2	7.25	0.35	7.00-7.50
	T	9	8.00	1.14	6.00-9.50	6	6.91	1.35	5.00-9.00	13	7.44	1.43	6.00-10.00

Table 1. Continued.

		Semiaquatic				Aquatic			
		Rana dalmatina				*Pelophylax ridibundus*			
RBC	Sex	n	Mean	SD	Range	n	Mean	SD	Range
	F	9	716660	150997	550000-1020000	5	886000	82945.76	780000-980000
	M	6	648330	84241.71	490000-720000	5	762000	206567	650000-1130000
	T	15	689330	129475	490000-1020000	10	824000	162152	650000-1130000
WBC	F	2	2700	989.94	2000-3400	3	3106	1462.20	1600-4520
	M	4	2675	960.46	2000-4100	2	2550	1484.92	1500-3600
	T	6	2683	865.83	2000-4100	5	2884	1308.92	1500-4520
Hb	F	9	8.51	1.29	6.80-10.60	5	9.52	1.47	8.20-12.00
	M	6	8.20	1.21	6.60-10.10	5	8.66	1.94	7.40-12.10
	T	15	8.38	1.23	6.60-10.60	10	9.09	1.68	7.40-12.10
PCV	M	9	34.91	6.49	28.57-47.05	5	43.19	4.12	39.45-48.78
	F	6	30.26	2.64	26.80-33.30	5	38.19	13.48	23.52-59.25
	T	15	33.05	5.67	26.80-47.05	10	40.69	9.76	23.52-59.25
MCV	M	9	495.14	80.99	399.41-603.20	5	488.83	38.74	428.80-524.52
	F	6	472.77	67.83	402.39-591.83	5	499.71	105.91	336.00-621.79
	T	15	486.19	74.30	399.41-603.20	10	494.27	75.40	336.00-621.79
MCH	M	9	120.58	15.27	103.92-149.15	5	107.50	13.28	95.65-129.03
	F	6	129.09	30.18	92.95-181.63	5	114.69	8.07	105.71-124.24
	T	15	123.98	21.84	92.95-181.63	10	111.09	11.03	95.65-129.03
MCHC	M	9	24.61	2.36	19.97-27.00	5	21.95	1.66	20.39-24.60
	F	6	27.16	3.76	22.20-30.68	5	23.78	5.11	18.72-31.46
	T	15	25.63	3.14	19.97-30.68	10	22.87	3.71	18.72-31.46
PTP	M	4	5.75	5.75	5.00-6.00	3	5.50	0.50	5.00-6.00
	F	2	5.50	0.70	5.00-6.00	3	5.83	0.76	5.00-6.50
	T	6	5.66	0.51	5.00-6.00	6	5.66	0.60	5.00-6.50

viridis was higher in females than in males. Mahapatra et al. (2010) have reported a higher RBC count in females (530000) than in males (480000) of the *Polypedates maculatus*. A similar observation was found by Kaplan (1951, 1952) in *Rana pipiens* and by Arserim and Mermer (2008) in *R. macrocnemis*. Meanwhile, no sexual dimorphism was reported for the RBC count of *R. pipiens* (Rouf, 1969), *R. catesbeiana* and *R. calamitans* (Hutchison and Szarski 1965) (Table 2).

The WBC counts vary depending on Anuran species, season, sex, nutritional conditions and some physiological conditions, such as diseases and breeding (Rouf, 1969; Arıkan, 1989; Wojtaszek and Adamowicz, 2003). In the present study, significant differences were not found between males and females in all species in terms of WBC count. The WBC counts were higher in males than in females of species *P. ridibundus*, *R. dalmatina* and *H. arborea*. A similar observation was found by Kaplan (1951, 1952) in *R. pipiens* (16,134 in males; 14,134 in females) and by Wojtaszek and Adamowicz (2003) in *B. bombina* (9,734 in males; 7030 in females). However, they were higher in females than in males of other species (*P. syriacus* and *P. viridis*). Arserim and Mermer (2008) put forth that the WBC count in *R. macrocnemis* is higher in females (3613) than in males (3445, Table 2).

The haemoglobin concentration (Hb) and hematocrit values (PCV) were found to significantly differ between males and females in only *P. viridis*. Similar observations were found by Sinha (1983) in *R. esculenta*, by Wojtaszek and Adamowicz (2003) in *B. bombina*, by Arserim and Mermer (2008) in *R. macrocnemis* and by Dönmez et al. (2009) in *Bufo bufo*. Kaplan (1954) found statistically significant sexual differences in the hematocrit values of *R. pipiens*. The hemoglobin concentration of *P. maculatus* was higher in females (7.80 g/100 ml) than in males (6.56 g/100 ml) (Mahapatra et al., 2010). Arserim and Mermer (2008) put forth that the hematocrit values in *Rana macrocnemis* are higher in females (35.00) than in males (32.00). Also, a similar observation was found by Haris (1972) (Tables 1 and 2).

The values of MCV were found to significantly differ between males and females in only *P. viridis*. In other species, there were no significant differences between males and females. Generally, the MCV values were higher in females than in males of *H. arborea*, *P. syriacus* and *P. ridibundus*. Similar MCV observations were found by Dönmez et al. (2009) in *B. bufo*. Sinha (1983) and Arserim and Mermer (2008) reported that the MCV value in *R. esculenta* and *R. macrocnemis* is higher in females than in males. MCH values were higher in females than in males of *P. syriacus, H. arborea, R. dalmatina* and *P. ridibundus*. Sinha (1983) reported that the MCH value in *R. esculenta* is higher in males than in females. MCHC values were higher in females than in males of *P. viridis*, *H. arborea, R. dalmatina* and *R. ridibunda*. Mahapatra et al. (2010) has also reported a higher MCHC value in females than in males of *P. maculatus*. However, Dönmez et al. (2009) stated a higher MCHC value in males than in females of *B. bufo* (Tables 1 and 2).

Plasma total protein values were higher in males than in females of *P. viridis, P. syriacus* and *H. arborea* (Table 1).

Atatür et al. (1999) determined differences in the size of erythrocytes from several species of Anura in Turkey and looked for the reasons in the different environmental conditions of the biotopes. Therefore, several researchers (Atatür et al., 1999; Zhelev et al., 2006) have found that aquatic anurans have larger erythrocytes than semi-aquatic and terrestrial species.

Table 2. The some hematological parameters in different Anura species refered by various authors.

	Sex	RBC	WBC	Hb	PCV
Alder and Huber (1923)					
Hyla arborea		674000	29000		
Arvy (1947)	M	450000			
Rana temporaria	F	330000			
Rouf (1969)					
Rana pipiens		319400		6.75	24.65
Harris (1972)	M	160000-540000	9600-38000	2.00-9.50	8.00-41.50
Rana pipiens	F	110000-450000	7850-25150	2.90-12.70	17.00-47.50
Carmena-Suero et al. (1980)					
Hyla septentrionalis				6.20	22.40
Rana catesbeiana				9.50	40.40
Sinha (1983)	M	320000		7.20	21.80
Rana esculenta	F	250000		5.80	19.80
Arıkan et al. (1989)					
Rana ridibunda		326620	3142		
Ostojic et al. (2000)					
Bufo spinulosus limensis		634000			39.53
Arıkan et al. (2003)					
Pelodytes caucasicus		776000	2560		
Wojtaszek and Adamowicz (2003)	M	340000 (190000-465000)	9734	7.44 (4.99-12.20)	13.70-26.20
Bombina bombina	F	290000 (240000-355000)	7030	6.78 (3.38-8.31)	12.00-23.30
Coppo et al. (2005)					
Rana catesbeiana					30.10 (25.00-39.00)
Arserim and Mermer (2008)	M	506250 (280000-940000)	3445 (2600-4200)	8.12 (5.60-12.10)	32.00 (19.00-42.00)
	F	524000 (320000-900000)	3613 (2800-5200)	8.07 (6.20-11.00)	35.00 (16.00-46.00)
Rana macrocnemis	T	514839 (280000-940000)	3527 (2600-5200)	8.10 (5.60-12.10)	34.00 (16.00-46.00)
Dönmez et al. (2009)	M	900000 (880000-920000)		11.80 (11.20-12.40)	42.36 (41.53-43.20)
Bufo bufo	F	870000 (840000-900000)		10.10 (9.40-10.80)	33.08 (28.57-37.60)
Gül and Tok (2009)					
Rana ridibunda		320500 (200000-650000)	2536 (800-4520)		
Rana dalmatina		415750 (310000-550000)	2683 (2000-4100)		
Bufo viridis		721750 (450000-900000)	1646 (900-2500)		
Bufo bufo		534722 (453000-703000)	2325 (1100-3800)		
Hyla arborea		579583 (405000-703000)	2980 (460-4900)		
Pelobates syriacus		657100 (543000-793000)	1216 (600-2500)		
Mahapatra et al. (2010)	M	480000 (370000-580000)	14628 (12400-16200)	6.56 (5.20-8.40)	28.65 (18.51-37.60)
Polypedates maculatus	F	570000 (400000-710000)	16642 (14000-20000)	7.80 (6.40-9.00)	23.80 (16.00-32.07)

Table 2. Continued.

	Sex	MCV	MCH	MCHC	PTP
Alder and Huber (1923)					
Hyla arborea					
Arvy (1947)	M				
Rana temporaria	F				
Rouf (1969)					
Rana pipiens					
Harris (1972)	M				
Rana pipiens	F				
Carmena-Suero et al. (1980)					
Hyla septentrionalis				27.70	
Rana catesbeiana				23.50	
Sinha (1983)	M	707.00	246.00	33.00	
Rana esculenta	F	840.00	250.50	29.50	
Arıkan et al. (1989)					
Rana ridibunda					
Ostojic et al. (2000)		621.90	172.65	28.06	
Bufo spinulosus limensis					
Arıkan et al. (2003)					
Pelodytes caucasicus					
Wojtaszek and Adamowicz (2003)	M	411.70-757.70	158.30-268.10	290.60-554.00	
Bombina bombina	F	363.30-916.60	145.30- 320.30	189.00-604.10	
Coppo et al. (2005)		709.00 (505.00-788.00)	157.00 (121.00-197.00)	23.30 (20.20-31.40)	4.34 (3.05-5.65)
Rana catesbeiana					
Arserim and Mermer (2008)	M	674.07 (425.53-1000)	170.48 (91.49-229.41)		
Rana macrocnemis	F	716.39 (420.45-1105.26)	165.10 (87.78-257.89)		
	T	694.54 (420.45-1105.26)	167.88 (87.78-257.89)		
Dönmez et al. (2009)	M	470.74 (469.56-471.93)	131.02 (127.27-134.78)	27.83 (26.97-28.70)	
Bufo bufo	F	378.90 (340.12-417.70)	115.95 (111.90-120.00)	30.81 (28.72-32.90)	
Gül and Tok (2009)					
Rana ridibunda					
Rana dalmatina					
Bufo viridis					
Bufo bufo					
Hyla arborea					
Pelobates syriacus					
Mahapatra et al. (2010)	M	582.01 (474.57-700.00)	137.51 (119.29-146.66)	26.05 (20.57-32.34)	
Polypedates maculatus	F	419.56 (360.57-462.16)	135.82 (125.00-160.00)	33.49 (28.06-40.00)	

In this study, the RBC counts were higher in terrestrial (*P. viridis*, *P. syriacus* and *H. arborea*) than in semi-aquatic (*R. dalmatina*) species. There were variations in WBC count of all species. Hemoglobin concentration, hematocrit value, MCV and MHC were higher in terrestrial (*P. viridis*, *H. arborea* and *P. syriacus*) than in semi-aquatic (*R. dalmatina*) and aquatic (*P. ridibundus*) species. The MCHC values were all similar to each other. The plasma total protein was higher in terrestrial species than in semi-aquatic species.

In summary, it was reported that blood cell counts and sizes in anuran species displayed considerable individual variations and interspecies differences. Furthermore, in this study, individual and interspecies variations were observed in the some hematological parameters examined on the anuran species.

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