

THE DETERMINATION OF SOME SPERMATOLOGICAL AND HEMATOLOGICAL PARAMETERS OF SHABBOUT (*Barbus grypus*, H; 1843) IN ATATÜRK DAM LAKE, ŞANLIURFA**Zafer Doğu^{1*}, Faruk Aral², Erdinç Şahinöz¹**¹ Department of Fisheries and Aquaculture, Bozova Vocational High School, Harran University, Sanliurfa, Turkey² Department of Reproduction and Artificial Insemination, Bor Vocational High School, Niğde University, Niğde, Turkey

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Abstract: This study was carried out to determine some spermatological parameters and hematological characteristics of the *B. grypus* (H, 1843) in the spawning season. Investigation was performed using 20 *B. grypus* males captured from Atatürk Dam Lake. Milt and blood samples were collected and evaluated daily in sampling day. In collected milt, the mean values of milt volume (μL), the percentage of motile spermatozoa (%), duration of motility (s), spermatozoa concentration ($\times 10^9/\text{mL}$) and pH were 886.00 ± 78.76 , 71.25 ± 4.56 , 115.10 ± 6.19 , 10.61 ± 1.67 and 8.13 ± 0.45 , respectively. The blood parameters of *B. grypus* males were 30.01 ± 4.11 , 2.05 ± 0.07 , 7.72 ± 0.11 , 147.27 ± 4.93 , 38.98 ± 2.61 , 26.47 ± 0.84 and 29.45 ± 0.75 for WBC ($\times 10^3/\text{mm}^3$), RBC ($\times 10^6/\text{mm}^3$), Hb (g/dl), MCV (μm^3), MCH (pg), MCHC (%) and PCV (%), respectively. Milt volumes in 8 year old fish were significantly higher than those in 5, 6 and 7 year old individuals ($P < 0.01$). Also, mean PCV value was significantly higher ($P < 0.05$) in 8 year old males than the others. These results suggest that the milt volume and PCV value of the *B. grypus* (H, 1843) are influenced by age.

Keywords: *Barbus grypus*, Shabbout, Sperm, Blood

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Öz:

Atatürk Baraj Gölü'ndeki (Şanlıurfa) Şabut Balığının (*Barbus grypus*; H, 1843) Bazı Spermatolojik ve Hematolojik Özelliklerinin Belirlenmesi

Bu çalışma, üreme sezonundaki Şabut balığının (*B. grypus*; H, 1843) bazı spermatolojik ve hematolojik özelliklerinin belirlenmesi amacıyla yapılmıştır. Araştırma, Atatürk Baraj gölü'nden yakalanan 20 adet erkek *B. grypus* kullanılarak gerçekleştirildi. Sperma ve kan örnekleri günlük olarak alınıp değerlendirildi. Alınan spermalarda, ortalama sperma miktarı (μL), spermatozoa motilitesi (%), motilite süresi (s), spermatozoa yoğunluğu ($\times 10^9/\text{mL}$) ve pH değerleri sırasıyla ortalama 886.00 ± 78.76 , 71.25 ± 4.56 , 115.10 ± 6.19 , 10.61 ± 1.67 ve 8.13 ± 0.45 olarak bulundu. Erkek *B. grypus*'un kan parametreleri WBC ($\times 10^3/\text{mm}^3$), RBC ($\times 10^6/\text{mm}^3$), Hb (g/dl), MCV (μm^3), MCH (pg), MCHC (%) ve PCV (%) için sırasıyla, 30.01 ± 4.11 , 2.05 ± 0.07 , 7.72 ± 0.11 , 147.27 ± 4.93 , 38.98 ± 2.61 , 26.47 ± 0.84 ve 29.45 ± 0.75 oldu. Sekiz yaşındaki balıkların sperma miktarı 5, 6 ve 7 yaşındaki örneklerden daha yüksek bulundu ($P < 0.05$). Keza, 8 yaşındaki balıkların ortalama PCV değeri diğer yaşlardaki balıklardan daha yüksek oldu. Bu sonuçlar *B. grypus* (H, 1843) balıklarının sperma miktarı ve kandaki PCV değerlerinin yaş tan etkilendiğini göstermektedir.

Anahtar Kelimeler: *Barbus grypus*, Şabut, Sperm, Kan

Introduction

The shabbout, *Barbus grypus* (H, 1843) is one of the most important fishes of *Cyprinidae* that widely distributed in the Euphrates and Tigris Rivers in Turkey, Iran, Syria and Iraq (Kuru, 1979; Coad, 1996). *Barbus grypus* is a vagile species that prefers rivers but is also found in estuaries. It is commercially fished and can reach nearly two meters and over 50 kg (Coad, 1996). Its growth, sexual maturity characteristics, and reproductive biology have been studied (Al-Hakim *et al.*, 1981; Pyka *et al.*, 2001). Spawning generally occurs from May to mid June (Geldiay and Balik, 1988). The spawned eggs are scattered above aquatic plants and cling to the vegetation (Geldiay and Balik, 1988). Also, the aquaculture industry in Turkey is continually evaluating new candidates and systems to diversify its production as effectively as possible. Therefore, it seems necessary to study about reproduction of *B. grypus* in order to provide knowledge for development of aquaculture.

Gamete quality is especially important in the examination of the male reproductive system. Evaluating their biological quality is significant particularly with regard to culture programs for commercially important species. Artificially reproducing these fish species and culturing them under controlled conditions might be a successful method for preventing populations from becoming extinct. The availability of semen with desirable quality is one of the critical factors necessary to increase the efficiency of artificial fertilization of fish species (Rurangwa *et al.*, 2004).

The use of high quality gametes from captive fish broodstock is of great importance for ensuring the production of valuable offspring for aquaculture (Bromage and Roberts, 1995). Sperm quality of male broodstock affects the production of healthy larvae.

Another biomarker used in diagnoses is the hematological profile. For intensive rearing of fish with minimal losses, it is necessary to be aware of the health status of fish. Blood variables are useful criteria for showing physiological disturbances in intensively farmed fishes and can provide important information for diagnosis and prognosis of diseases. Dawson (1979) stated that hematology an important tool to study the speed and effect of the toxins without killing the animals. The changes in the fish blood prior to the onset of more striking morphological and physiological changes can be indicative of unfavourable aquatic medium (Eisler, 1967). For example, qualitative and quantitative variations in hematological parameters including the red blood cell (RBC) and white blood cell (WBC) numbers, hematocrit (HCT, also known as packed cell volume (PCV)), the amount of hemoglobin (Hb) are the most significant findings as regards diagnosis (Şahan *et al.*, 2007). Although comparison of spermatological and hematological properties of some fish species have been studied in few studies (Imanpoor and Farahi, 2011), there are no available data on *B. grypus* in Atatürk Dam Lake in Southeastern Turkey.

Former studies showed that fish age influences quality of gametes such as rainbow trout *Oncorhynchus mykiss* (Schmidt-Baulain and Holtz, 1991), striped bass *Morone saxatilis* (Vuthiphandchai and Zohar, 1999), turbot *Scophthalmus maximus* (Suquet et al., 1998) and koi carp ornamental carp, (Mordenti et al., 2003).

Despite the commercial and conservation importance of the species, information for spermatological and hematological parameters and its relationships between them in *Barbus grypus* is scarce. The aim of the present study is to evaluate the effect of age on gamet quality and hematological parameters. And also, to try establishing if there is any correlation between parameters of the spermatological and hematological characteristics.

Materials and Methods

Broodstocks and Samples Preparation

The fish were caught with gill nets (45 mm x 45 mm and 55 mm x 55 mm) at 5th June in Ataturk Dam Lake (37°23'29''03''N, 38°34'38''05''E) in 2012. During the study, physico-chemical parameters of the sampling areas were measured with YSI Environmental (YSI 85). Samples obtained were moved to the laboratories of Harran University Bozova Vocational School. The scales and otoliths were examined under a stereomicroscope for age determination (Nikon SMZ 2Tstereo) (Baker and Timmons, 1991).

The fish caught were grouped by age into four different groups. The milt was taken from 20 fish in 4 groups; 7 in the first group (age 5), 6 in the second group (age 6), 4 in the third group (age 7) and 3 in the fourth group (8 years) respectively.

Spermatological Parameters Measurement

After cleaning the genital area with fresh water and thoroughly drying to avoid contamination of samples with faeces, urine and lakewater, milt was collected in a graduated tube after applying gentle abdominal pressure to unanesthetised males. After collections, milt samples were transported to the laboratory under cold conditions (7–10 °C). In collected milt; sperm volume (µL), spermatozoa motility (%), duration of spermatozoa motility (s), spermatozoa concentration (x10⁹/mL) and sperm pH were determined.

Milt volume was determined by the measuring pipette and expressed as µL. Motility was assessed by a procedure similar to that of Aas et al. (1991) and expressed as a percentage of motile spermatozoa. Briefly, ten seconds after activation, sperm motility was evaluated under a light microscope by placing a 10 µL drop of diluted semen (5 µL of milt was mixed with 5 mL of activating solution in a tube) on a slide covered with a glass coverslip (22 mm x 22 mm). An activation solution, 0.3% NaCl was used for estimating motility rate. Motility evaluation was performed by focusing the binocular light microscope at the centre of the coverslip at 400x magnification at 25°C. The motility rate was determined visually by estimating the proportion of motile and non-motile cells, in triplicate. The duration of sperm motility was subjectively evaluated as the time elapsed from activation until 5% of the spermatozoa maintained forward swimming activity. Sperm motility observations were done at room temperature (25°C). Same person conducted all the sperm motility observations, in order to decrease the degree of variation among observers. Spermatozoa concentration was determined by using haemocytometer and expressed as x10⁹/mL. Milt was first diluted in a 10-mL test tube by adding 10 µL of milt to 9990 µL of a distilled well water and then mixed on a vortex mixer, and counting the number of milt cells in a known haemocytometer volume (Thoma chamber, American Optical, Buffalo, NY) viewed with a light microscope (Tvedt *et al.*, 2001). The pH of whole milt was measured in triplicate on freshly collected milt using pH indicator strips (pH: 0–14; Merck, Germany) with checked by Model GLP 21 pH meter (Crison, Barcelona).

Hematological Analysis

In this study, the blood samples were taken by cut off caudal vein method into 2 mL vacutainer tube containing heparin sodium shook for two minutes gently and stored in refrigerator prior to hematological analysis. After collections, blood samples were transported to the laboratory under cold conditions (7–10°C). The indices used to evaluate the hematological profile were included; hematological parameters; White Blood Cell (WBC) (x10³/mm³), Red Blood Cell (RBC) (x10⁶/mm³), Hemoglobin (Hb) (g/dl), Mean Corpuscular Volume (MCV) (µm³), Mean Corpuscular Hemoglobin (MCH) (pg), Mean Corpuscular Hemoglobin Concentration

(MCHC) (%) and Hematocrit (PCV) (%) were determined (Houston, 1990).

Statistical Analysis

Statistical data was conducted using SPSS 10.0.1 (SPSS Inc. 1999). Descriptive analysis was carried out to determine mean and standard error on milt volume, sperm motility percentage, duration of sperm motility, sperm concentration, milt pH and blood parameters used in the present study during spawning season. All values are expressed as mean \pm standard error (S.E.M.). The correlation between spermatological characteristics and blood parameters were analyzed using the bi variate correlation coefficients of Pearson (SPSS, ver. 10.05; SPSS, Chicago, IL). Statistical comparisons of sperm and blood traits were

done by using One way ANOVA followed by Tukey's post hoc test as appropriate. Significance was taken at $P < 0.05$.

Results and Discussion

The values of mean water temperatures were 22.70 ± 1.80 °C, while dissolved oxygen and pH were 8.72 ± 0.40 mg/l and 8.45 ± 0.20 respectively. Mean weight and length of the captured twenty fish were 3120.33 ± 90.37 g and 69.73 ± 4.92 cm respectively. The ages of the samples ranged between 5 to 8 year. Also, the total weight (TW) and total length (TL) of *B. grypus* are shown in Table 1. Overall mean values of some milt properties and blood parameters in *B. grypus* in the spawning season are presented in the Table 1 and 2.

Table 1. The general spermatological properties of *B. grypus* in 5, 6, 7 and 8- year- old fish (n=20)

Spermatological Properties	N	Age	Mean \pm S.E.	Minimum	Maximum
Milt Volume (μ L)	7	5	720.71 \pm 60.88	450.00	954.00 ^a
	6	6	829.83 \pm 44.24	657.00	960.00 ^a
	4	7	1013.50 \pm 93.04	785.00	1200.00 ^a
	3	8	1217.33 \pm 183.23	874.00	1500.00 ^b
	2	-	886.00 \pm 78.76	450.00	1500.00
	0	-			P<0.01
Motility (%)	7	5	77.14 \pm 9.31	30.00	95.00
	6	6	65.83 \pm 5.06	50.00	85.00
	4	7	72.50 \pm 6.29	60.00	90.00
	3	8	66.66 \pm 14.52	40.00	90.00
	2	-	71.25 \pm 4.56	30.00	95.00
	0	-			
Duration of Motility (s)	7	5	99.14 \pm 15.53	45.00	140.00
	6	6	103.83 \pm 15.58	45.00	150.00
	4	7	149.00 \pm 17.54	120.00	200.00
	3	8	130.33 \pm 3.17	125.00	136.00
	2	-	115.10 \pm 6.19	45.00	200.00
	0	-			
Sperm Concentration ($\times 10^9$ /mL)	7	5	10.06 \pm 6.58	8.60	13.92
	6	6	10.01 \pm 6.03	8.42	12.12
	4	7	10.10 \pm 8.21	9.18	13.12
	3	8	10.11 \pm 13.42	9.42	13.76
	2	-	10.61 \pm 1.67	8.42	13.92
	0	-			
pH	7	5	8.10 \pm 0.10	7.80	8.50
	6	6	8.20 \pm 0.02	8.10	8.30
	4	7	8.05 \pm 0.11	7.80	8.30
	3	8	8.13 \pm 0.12	7.90	8.30
	2	-	8.13 \pm 0.45	7.80	8.50
	0	-			

The mean values of milt volume, the percentage of motile spermatozoa, duration of mo-

tility, spermatozoa concentration and pH in all ages were 886.00 ± 78.76 μ L, 71.25 ± 4.56 %, 10.06 ± 6.58 $\times 10^9$ /mL and 8.10 ± 0.10 respectively.

115.10 ±6.19 s, 10.61 ±1.67 x10⁹/mL and 8.13 ±0.45, respectively.

The semen volume of 8-year-old fish (1217.33 µL), were determined significantly higher with respect to the fish aged between; 5-7 (720.71-1013.50 µL) (P<0.01). The average total weight and total length of the fish caught were determined as 3120.33 ±90.37 g and 69.73 ± 4.92 cm, respectively.

It was found that age has significant effects on total weight (P<0.001) and total length (P<0.01). Total weight (3833.33 g) and length (79.33 cm) of 8-year-old fish were determined higher than the total weight (2714.28-3050.00 g) and length (66.85-70.25 cm) of 5 to 7-years-old fish.

The blood parameters of *B. grypus* males were 30.01 ±4.11 x10³/mm³, 2.05 ±0.07 x10⁶/mm³, 7.72 ±0.11 g/dl, 147.27 ±4.93 µm³, 38.98 ±2.61 pg, 26.47 ±0.84 % and 29.45 ±0.75 for WBC, RBC, Hb, MCV, MCH, MCHC and PCV, respectively.

The effect of age on the PCV were determined significant (P<0.05). The highest PCV (29.45%) was found at 8-year-old fish. PCV in the age group of fish at 5, 6 and 7- years- old was 28.28, 28.16 and 29.75% respectively and was found similar.

Relationships among sperm characteristics and blood parameters were determined in Table 3. In sperm parameters, a positive relationships (r=0.543, P<0.05) were detected between milt volume and the percentage of motile spermatozoa and also, the sperm pH and spermatozoa concentration (r=0.472, P<0.05). In hematological parameters, the highest correlation coefficients between PCV and MCHC (r= -0.705, P<0.01); Hb and MCHC (r=0.682, P<0.01); Hb and WBC (r= -0.755, P<0.01); RBC and MCV (r= -0.876,

P<0.01); RBC and MHC (r= -0.837, P<0.01) and MCV and MCH (r= 0.822, P<0.01).

And also, the correlation between some spermatological and hematological parameters of *B. grypus* are shown in Table 3. A negative relationship was found only between the percentage of motile spermatozoa and RBC (r=-0.45, P<0.05).

The study was carried out to determine some spermatological parameters and hematological characteristics of the Shabbout. Milt volume is one of the important characteristics of the fish sperm. The mean values of fish milt volume caught in Atatürk Dam Lake were 886.00 ±78.76 µL (Table 1). Policar *et al.* (2011) reported that the milt volume of the *B. barbuis* was 150 ±40.00 -420.00 ±80.00 µL. The milt volume of this study was found higher than Policar *et al.* (2011). On the contrary, the finding in present study is lower than reported between 5000-20000 µL in different *Cyprinidae* species (like *C. carpio*, *C. idella* etc.) (Horvath and Lukowicz, 1982). The milt volume in males affected from both the spawning period and day light changes during the spawning period (Campos-Mendoza *et al.*, 2004).

In our study, 8-years-old males showed higher milt volume (1217.33 ±183.23 µL) than in 5, 6 and 7 -years-old individuals (P>0.01). In addition, The semen volume of the samples were measured for 5, 6 and 7 -year-old samples as 720.71 ±60.88 µL, 829.83 ±44.24 µL and 1013.50 ±93.04 µL, respectively. This situation shows that age had a significant effect on semen volume. Similarly, Tekin *et al* (2003) reported that based on increasing age the semen volume increased significantly. Also, Rahbar *et al* (2012) stated that because of older fish have larger testes; the production of sperm volume will increase.

Table 2. The general hematological properties of *B. grypus* in 5, 6, 7 and 8-year- old fish (n=20)

Heamatological Properties	N	Age	Mean \pm S.E.	Minimum	Maximum
WBC ($\times 10^3/\text{mm}^3$)	7	5	21.35 \pm 3.17	12.00	37.12
	6	6	32.11 \pm 5.21	11.20	45.25
	4	7	36.00 \pm 1.62	13.20	84.26
	3	8	38.02 \pm 1.36	13.20	60.46
	20	-	30.01 \pm 4.11	11.20	84.26
RBC ($\times 10^6/\text{mm}^3$)	7	5	1.90 \pm 0.12	1.45	2.45
	6	6	2.09 \pm 0.15	1.35	2.33
	4	7	2.07 \pm 0.13	1.69	2.34
	3	8	2.32 \pm 0.17	1.98	2.55
	20	-	2.05 \pm 0.07	1.35	2.55
Hb (g/dl)	7	5	7.51 \pm 0.28	6.07	8.00
	6	6	7.88 \pm 0.04	7.70	8.00
	4	7	7.77 \pm 0.16	7.40	8.10
	3	8	7.83 \pm 0.17	7.50	8.11
			7.72 \pm 0.11	6.07	8.10
MCV (μm^3)	7	5	133.78 \pm 1.41	130.10	139.40
	6	6	149.99 \pm 11.54	128.40	205.78
	4	7	154.10 \pm 5.20	145.60	168.60
	3	8	164.18 \pm 20.33	131.60	201.54
	20	-	147.27 \pm 4.93	128.40	205.78
MCH (pg)	7	5	33.68 \pm 2.94	23.40	45.10
	6	6	41.93 \pm 5.98	23.10	60.21
	4	7	41.82 \pm 7.24	26.40	60.21
	3	8	41.60 \pm 6.13	29.70	50.10
	20	--	38.98 \pm 2.61	23.10	60.21
MCHC (%)	7	5	26.57 \pm 0.99	23.00	30.00
	6	6	24.39 \pm 1.47	21.00	30.00
	4	7	25.75 \pm 1.88	21.00	30.00
	3	8	31.36 \pm 1.78	28.00	34.10
	20	-	26.47 \pm 0.84	21.00	34.10
PCV (%)	7	5	28.28 \pm 0.60	26.00	31.00 ^a
	6	6	28.16 \pm 1.07	26.00	33.00 ^a
	4	7	29.75 \pm 1.93	26.00	34.00 ^{ab}
	3	8	34.33 \pm 2.33	30.00	38.00 ^b
	20	-	29.45 \pm 0.75	26.00	38.00
				*	
Total Weight (g*)	7	5	2714.28 \pm 43.64	2400.00	2900.00 ^a
	6	6	2883.33 \pm 66.96	2500.00	3300.00 ^a
	4	7	3050.00 \pm 86.60	2700.00	3300.00 ^a
	3	8	3833.33 \pm 90.37	3400.00	4200.00 ^b
	20	-	3120.33 \pm 90.37	2400.00	4200.00

Total Length (cm)	7	5	66.85 \pm 1.50	60.00	71.00 ^a
	6	6	67.83 \pm 0.74	66.00	71.00 ^a
	4	7	70.25 \pm 2.46	63.00	74.00 ^a
	3	8	79.33 \pm 0.66	78.00	80.00 ^b
	20	-	69.73 \pm 4.92	60.00	80.00
				**	

*: P<0.05, **: P<0.01, ***: P<0.001 RBC: Red Blood Cell, WBC: White Blood Cell, Hb: Hemoglobin, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PVC: Packed Cell Volume,

Table 3. The correlation between spermatological and hematological properties of *B. grypus* in the spawning season (n=20)

PARAMETERS	VOL	MOT	MOT DUR	pH	SPZ CON	PCV	Hb	WBC	RBC	MCV	MHC	MCHC
VOL	-	0.543*	-0.167	0.068	-0.120	-0.408	-0.381	0.321	-0.318	0.209	0.263	0.020
MOT	0.543*	-	0.160	0.055	-0.107	-0.428	-0.364	0.266	-0.452*	0.287	0.328	0.057
MOT DUR	-0.167	0.160	-	-0.005	-0.049	-0.205	-0.095	-0.099	0.321	-0.434	-0.382	0.133
pH	0.068	0.055	-0.005	-	0.472*	0.048	-0.149	0.112	0.131	-0.27	-0.326	-0.134
SPZ CON	-0.120	-0.107	-0.049	0.472*	-	0.126	-0.132	-0.015	-0.105	0.105	0.017	-0.168
PVC	-0.408	-0.428	-0.205	0.048	0.126	-	0.011	-0.224	0.170	0.205	-0.233	-0.705**
Hb	-0.381	-0.364	-0.095	-0.149	-0.132	0.011	-	-0.755**	0.265	-0.206	0.189	0.682**
WBC	0.321	0.266	-0.099	0.112	-0.015	-0.224	-0.755**	-	-0.056	-0.069	-0.275	-0.443
RBC	-0.318	-0.452*	0.321	0.131	-0.105	0.170	0.265	-0.056	-	0.876**	0.837**	0.044
MCV	0.209	0.287	-0.434	-0.270	0.105	0.205	-0.206	-0.069	0.876**	-	0.822**	-0.262
MHC	0.263	0.328	-0.382	-0.326	0.017	-0.233	0.189	-0.275	0.837**	0.822**	-	0.324
MCHC	0.020	0.057	0.133	-0.134	-0.168	-0.705**	0.682**	-0.443	0.044	-0.262	0.324	-

*:p<0.05, **:p<0.01 Vol: Milt Volume, Mot: The Percentage of Motile Spermatozoa, Mot Dur: Duration of Sperm Motility, pH: Sperm pH, Spz Con: Spermatozoa Concentration, PVC: Packed Cell Volume, Hb: Hemoglobin, WBC: White Blood Cell, RBC: Red Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration

In this study, the average spermatozoa motility of *B. grypus* sperm was determined as 71.25 ±4.56 % (Table1). Similarly, the mean spermatozoa motility at *B. aeneus* which is one of the different *Barbus* species was reported 65 ±8.95 % (Vlok and Van Vuren, 1988) and is similar to *Carassius gibelio* as 79 ±3.0 % (Taati et al., 2011). Verma et al. (2009) reported spermatozoa motility of some fish species like *Catla catla*, *Labeo rohita*, *Labeo calbasu*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* that belongs to *Cyprinidae* as 90 ±1.50 %, 90 ±2.30 %, 92 ±1.40 %, 88 ±30 %, 93 ±1.70 % and 89 ±3.20 % respectively. The spermatozoa motility of this study was found lower than the previous studies. The low spermatozoa motility in present study could be explained by differences among the species, environment, spawning season (Büyükhatipoğlu and Holtz, 1984). Because, spermatozoa motility can change among different fishes that belong to same species (Honeyfield and Krise, 2000). There were no significant differences observed in spermatozoa motility based on age.

In this study, mean duration of motility in *B. grypus* was found to be 115.10±6.19 s (Table1). Verma et al. (2009) reported mean duration of motility that some *Cyprinidae* species like *Catla catla*, *Labeo rohita*, *Hypophthalmichthys molitrix*

and *Ctenopharyngodon idella* as 80±4.50 s, 90±5.50 s, 75±3.50 s and 85±2.50 s, respectively. Also, Taati et al. (2011) found that mean duration of motility in *C. gibelio* was 33.63±4.03 s. Our results were found higher than those values. The high duration of motility in the present study could be explained by the differences between two species, dilution types, and the high rates of immature spermatozoa in milt (Zhukinskiy and Alekseenko, 1983). Besides, mean duration of motility of *B. grypus* was found similar with both *Labeo calbasu* (110 ±5.00 s) and *Cirrhinus mrigala* (115.10 ±6.19 s) (Verma et al., 2009). When the average duration of spermatozoa motility of the fish caught was analyzed, the duration of spermatozoa motility was not statistically significant despite the slight increase depends on age.

The concentration of spermatozoa was 10.61 ±1.67 x10⁹/mL in this study (Table 1). Alavi et al. (2008-10) reported that the spermatozoa concentration of *B. sharpeyi* and *B. barbus* were 9.8x10⁹/mL and 12.5x10⁹/mL, respectively. Policar et al. (2011) reported the spermatozoa concentration in *B. barbus* as 11.8 ±0.9 x10⁹/mL. Similarly, Vlok and Van Vuren (1988) reported the spermatozoa concentration of *B. aeneus* as 11.8 ±0.9 x10⁹/mL. Büyükhatipoğlu and Holtz (1984) stated that spermatozoa concentration could be decreased from the beginning to end of

the spawning season. In this study, statistically significant change was not observed on spermatozoa concentration value of the samples depends on the age obtained.

The semen pH (Table1) in *B. grypus* was found as 8.13 ± 0.45 in the present study. The semen pH values of *Catla catla*, *Labeo calbasu*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* were found as 7.8 ± 0.07 , 7.9 ± 0.05 , 8.1 ± 0.09 , 7.8 ± 0.03 and 7.9 ± 0.06 , respectively (Verma et al., 2009). Our results were similar to those values, but higher than *Labeo rohita* (7.3 ± 0.06) (Verma et al., 2009). These high values could be due to the physico-chemical structure of oligotrophic Atatürk Dam Lake that is slightly alkaline. This could be caused of the similarity of the sperm pH of freshwater fish and the pH of the water that fish live in (Suquet et al., 1993). Statistically significant change was not observed on spermatozoa pH value of the samples depends on the age obtained in this study.

Overall mean values of some hematological parameters of *B. grypus* in the spawning season are presented in the Table 2. The WBC values of our study ($30.01 \pm 4.11 \times 10^3/\text{mm}^3$) were higher than the reported values of Örün and ErdemLi (2002) in *C. trutta* ($17.65 \pm 2.15 \times 10^3/\text{mm}^3$), Aydın et al. (1998) in *S. glanis* ($17.00 \pm 1.29 \times 10^3/\text{mm}^3$), Yavuzcan et al. (1997) in *Oreochromis niloticus* ($7.02 \pm 0.99 \times 10^3/\text{mm}^3$), Gbore et al. (2006) in *T. zilli* ($1.29 \pm 0.12 \times 10^3/\text{mm}^3$) and in *C. gariepinus* ($1.80 \pm 0.85 \times 10^3/\text{mm}^3$). Besides, The data of WBC of *B. grypus* were found lower than the reported values of Aydın et al. (1998) in *C. lazera* ($35.00 \pm 3.48 \times 10^3/\text{mm}^3$), Groff and Zinki (1999) in *C. carpio* ($37.8 \pm 2.88 \times 10^3/\text{mm}^3$) and *C. auratus* ($52.3 \pm 4.88 \times 10^3/\text{mm}^3$). These different values from previous studies may be as a response of the immune system against infectious agents. To learn the reason we need to more data about to this fish species. But, increase at the leukocyte count could be result of the increasing macrophages and other phagocytic cells which is a key element of the immune system (Misra et al., 2006).

The RBC values were found as $2.05 \pm 0.07 \times 10^6/\text{mm}^3$. On the contrary of our data, Talal et al. (2011) reported that the RBC values of *B. xanthopterus* and *B. sharpeyi* as 3.45 ± 0.77 and $3.55 \pm 0.52 \times 10^6/\text{mm}^3$, respectively. If the RBC values of different fish species that belongs to same family are examined, it is seen that Groff

and Zinkl (1999) reported the values of *C. carpio* ve *C. trutta* as $1.67 \pm 0.08 \times 10^6/\text{mm}^3$ and $1.61 \pm 0.81 \times 10^6/\text{mm}^3$, respectively. Similarly, former researchers stated that RBC values as $1.10 \pm 0.51 \times 10^6/\text{mm}^3$, $1.26 \pm 0.87 \times 10^6/\text{mm}^3$, $1.13 \pm 0.25 \times 10^6/\text{mm}^3$, $1.10 \pm 0.14 \times 10^6/\text{mm}^3$ and $0.73 \pm 0.02 \times 10^6/\text{mm}^3$ in *C. trutta*, *S. glanis*, *C. lazera*, *O. niloticus*, *T. zilli* and *C. gariepinus*, respectively (Örün and ErdemLi, 2002; Aydın et al., 1998; Yavuzcan et al., 1997; Gbore et al., 2006). Khadjeh et al. (2010) reported the RBC values in shabout as $1.41 \pm 0.04 \times 10^6/\text{mm}^3$. Our RBC values were found higher than these reported values. RBC value tends to increase with age of the fishes (Das 1965). The RBC values of male fish did not show increase based on age in our study. RBC values were significantly higher in 7- and 8-years-old Beluga (*Huso huso* L., 1758) than those in 4 and 6-years-old Akrami et al. (2013). The RBC values of Shabbout could change depending on environment, infection and physiological activity (Brenden and Huizinga, 1986). The RBC values were not statistically significant despite the increase depends on age. The elevated RBC counts are a response to the higher metabolic demand and have no impact on erythrocyte volume (Satheeshkumar et al., 2011). The increased number of RBC indicates oxygen demand in the tropical region to meet the higher oxygen requirement at higher metabolic rates (Engel and Davis 1964).

In this study, the Hemoglobin (Hb) values of *B. grypus* sperm were 7.72 ± 0.11 g/dl. Similarly, Khadjeh et al. (2010) reported the Hb in *B. grypus* as 6.50 ± 0.10 g/dl. Talal et al. (2011) reported the Hb in *B. xanthopterus* and *B. sharpeyi* as 5.18 ± 0.22 g/dl and 5.32 ± 0.43 g/dl respectively. The similar Hb values were reported for different *Cyprinidae* species like *C. carpio* (8.20 ± 0.36 g/dl), *C. trutta* (7.90 ± 0.24 g/dl), *S. glanis* (9.02 ± 0.13 g/dl), *C. lazera* (9.35 ± 0.34 g/dl), *O. niloticus* (7.72 ± 0.21 g/dl) and *T. zilli* (6.60 ± 0.14 g/dl) (Örün and ErdemLi, 2002; Aydın et al. 1998; Yavuzcan et al. 1997; Gbore et al. 2006). Hemoglobin content of erythrocytes was associated with the volume and the development of RBCs. The effect of age on hemoglobin was determined insignificant. However, Das (1965) reported that Hb value tend to increase with the age of the fishes. Hrubec et al. (2001) reported that levels of hemoglobin increased with increasing age. Environmental factors and genetic factors could have affected the development of erythrocytes (Houston, 1990).

The MCV values of *B. grypus* caught in Atatürk Dam Lake were found as $147.27 \pm 4.93 \mu\text{m}^3$. We found similar results with *C. auratus* ($137 \pm 2.60 \mu\text{m}^3$) and *C. trutta* ($149.71 \pm 2.28 \mu\text{m}^3$). But, Khadjeh *et al.* (2010) found higher ($261 \pm 4.87 \mu\text{m}^3$) MCV values in same species. And also, higher MCV values were reported for different *Cyprinidae* species like *C. carpio* ($202 \pm 5.50 \mu\text{m}^3$), *S. glanis* ($249.60 \pm 10.10 \mu\text{m}^3$), *C. lazera* ($258.70 \pm 19.80 \mu\text{m}^3$), *O. niloticus* ($234.67 \pm 8.22 \mu\text{m}^3$) and *C. gariepinus* ($200.93 \pm 0.31 \mu\text{m}^3$) (Groff and Zinkl, 1999; Örün and ErdemLi, 2002; Aydın *et al.*, 1998; Yavuzcan *et al.*, 1997; Gbore *et al.*, 2006).

The MCH values of *B. grypus* were found as 38.98 ± 2.61 pg in this study. Similar values were reported in *B. grypus* (45.70 ± 0.88 pg), *C. trutta* (45.40 ± 1.80 pg), *C. carpio* (49.10 pg), *C. auratus* (42.00 ± 1.40 pg), *T. zilli* (46.48 ± 2.49 pg) and *C. gariepinus* (51.39 ± 0.04) (Khadjeh *et al.*, 2010; Örün and ErdemLi, 2002; Groff and Zinki, 1999; Gbore *et al.*, 2006). Aydın *et al.* (1998) in *S. glanis* (86.49 ± 5.01 pg) and in *C. lazera* (83.52 ± 2.85 pg) and also Yavuzcan *et al.* (1997) in *O. niloticus* (65.45 ± 2.10 pg) were found higher values than this study.

The MCHC values of *B. grypus* were calculated as 26.47 ± 0.84 % in this study. The MCHC values in *Cyprinidae* species like *C. trutta*, *S. glanis*, *C. lazera*, *O. niloticus* and *T. zilli* were reported as 30.32 ± 0.80 %, 30.66 ± 0.49 %, 31.20 ± 0.85 %, 31.00 ± 0.01 % and 33.14 ± 1.88 % respectively (Örün and ErdemLi, 2002; Aydın *et al.*, 1998; Yavuzcan *et al.*, 1997; Gbore *et al.*, 2006). But, both Khadjeh *et al.* (2010) (17.6 ± 0.27 %) and Gbore *et al.* (2006) in *C. gariepinus* (15.87 ± 0.03 %) were reported lower values than our results in *B. grypus*. The decreased MCV can be the sign for a defect in the maturation of erythrocytes (Kumar *et al.*, 2013). The fluctuation of MCH and MCHC could be due to the change of the hemoglobin concentration of RBCs in infected fishes (Wepener *et al.*, 1992).

The effect of the age on MCV, MCH and MCHC was observed insignificant at shabout fish.

In this study, mean PCV values of *B. grypus* blood were 29.45 ± 0.75 %. These values of *B. grypus* were similar with Khadjeh *et al.* (2010) (36.9 ± 0.7 %). In addition, the obtained PCV results within the limits of the former researchers reported studies in different *Cyprinidae* species

like *C. carpio* (33.4 ± 1.51 %), *C. auratus* (22.3 ± 1.04 %), *C. trutta* (26.05 ± 2.38 %) *S. glanis* (29.75 ± 0.45 %), *C. lazera* (33.42 ± 1.27 %), *O. niloticus* (25.27 ± 0.67 %), *T. zilli* (20.07 ± 0.07 %) and *C. gariepinus* (20.78 ± 0.02 %) (Groff and Zinki, 1999; Örün and ErdemLi, 2002; Aydın *et al.*, 1998; Yavuzcan *et al.*, 1997; Gbore *et al.*, 2006). But, Talal (2011) in *B. xanthopterus* (36.9 ± 0.70) and in *B. sharpeyi* (40.56 ± 3.55 %) were reported higher values than our results. PCV concentration of infected fishes decreases according to destruction of RBC (Haney *et al.*, 1992). These differences between PCV values could be explained with the species differences and the environmental infectious factors.

PCV values of male fish at 8-years-old were observed significantly higher ($P < 0.05$). The increase in the PCV values may have resulted from the increase the weight of the fish. Similarly, PCV values were significantly higher in 7- and 8-years-old Beluga, than those in 4 and 6-years-old (Akrami *et al.*, 2013). Also, Preston (1960) concluded that an increase in the PCV values in flounder (*Plouronectes platessa* L., 1758) with the increase in weight. Besides, Hrubec *et al.* (2001) reported that levels of hematocrit increased with increasing age.

According to the study, we found positive correlation between milt volume and spermatozoa motility, and also sperm pH and spermatozoa concentration. Similarly, former researchers reported that there was a positive correlation between total spermatozoa motility and milt volume in *C. gariepinus* (Adewumi *et al.*, 2005).

The hematological characteristics of *B. grypus* caught in Atatürk Dam Lake, the highest correlation coefficient were found in PVC and MCHC ($r = -0.705$, $P < 0.01$); Hb and MCHC ($r = 0.682$, $P < 0.01$); Hb and WBC ($r = -0.755$, $P < 0.01$); RBC and MCV ($r = -0.876$, $P < 0.01$); RBC and MHC ($r = -0.837$, $P < 0.01$); MCV and MCH ($r = 0.822$, $P < 0.01$). Similarly, the negative correlation results of our study (RBC and MCV, $r = -0.876$ and RBC and MHC, $r = -0.837$), Akrami *et al.* (2013) reported same correlations in Beluga on RBC and MCV ($r = -0.489$; $P < 0.002$), RBC and MHC ($r = -0.465$; $P < 0.001$), MCV and MCH ($r = 0.373$, $P < 0.01$). These researchers reported that the significant negative correlations between RBC, MCV and MCH could be argumentative.

The relationship between milt characteristics and blood parameters analyzed, only a significant

correlation was found. There were negative correlation between spermatozoa motility and RBC values ($r=-0.452$, $P<0.05$). The factors that cause RBC to increase like high metabolic demand (Satheeshkumar et al., 2011), oxygen demand (Engel and Davis, 1964), environment, infection and physiological activity (Brenden and Huizinga, 1986) may have effect on spermatozoa motility.

The result of a previous study shown with increased a temperature RBC level increased. When temperature increased, activity of oxygen absorbing by RBC was reducing, thus body for compensation with the high number of RBC in blood (Bozorgnia et al., 2011). The spermatozoa motility of Siberian sturgeon (*A. baeri*), were significantly higher at 10°C (culture temperature) and the lowest at high temperature (17.5°C) (Williot et al., 2000).

Conclusion

In conclusion, this study is the first report for male *B. grypus* that include investigations of hematological parameters and milt characteristics in Atatürk Dam Lake. These results represent a valuable baseline dataset and provide background information in these species that has great aquaculture potential.

The sperm mixture of fish at different ages can be used as a simple procedure to achieve better results of fertilization capacity. The results of this study can be used in artificial breeding programs to produce suitable larvae for breeding and reproduction.

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