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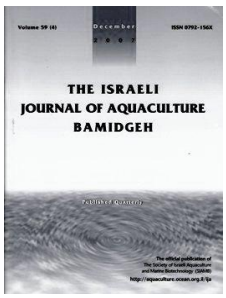

Hematology of Diploid and Triploid Rainbow Trout (*Oncorhynchus mykiss*), Black Sea Trout (*Salmo labrax* Pallas, 1814), and their F_1 Hybrids

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Key words: hematological parameters, *Salmo labrax*, *Oncorhynchus mykiss*, interspecific hybridization, diploidy, triploidy

Abstract

Erythrocyte sizes and counts, leukocyte counts, hemoglobin concentrations, and hematocrit values were determined for diploid and triploid rainbow trout (*Oncorhynchus mykiss*), Black Sea trout (*Salmo labrax*), and their hybrids. Comparison of the diploid and triploid hybrids with the parental species revealed low erythrocyte counts and high mean corpuscular hemoglobin values for the triploids, elevated hemoglobin concentrations for the diploids, and similar hematocrits for all fishes.

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Introduction

The Black Sea trout (*Salmo labrax* Pallas 1814) is distributed in rivers draining into the Black Sea, including those on the eastern Black Sea coast of Turkey (Kottelat and Freyhof, 2007). Recently, the Black Sea trout has attracted interest for aquaculture in the Black Sea Region of Turkey (Başçınar et al., 2007). Due to its high economic value, the Black Sea trout is farmed on small private trout farms in the eastern Black Sea region of Turkey despite its slow growth rate (Canyurt and Akhan, 2009).

In aquaculture, inter-specific hybridization is widely used to increase the growth rate, manipulate the sex ratio, produce sterile fish, improve flesh quality, increase disease resistance, and improve environmental tolerance (Bartley et al., 2001). Inter-specific hybridization in salmonids has been studied for a long time. Although viability is often poor in diploid hybrid salmonids (Blanc and Chevassus, 1982; Blanc and Maunas, 2005), it can be improved through artificial triploidization (Chevassus et al., 1983; Scheerer and Thorgaard, 1983; Gray et al., 1993; Blanc and Maunas, 2005).

Triploid fish differ from diploids in three fundamental ways: they are generally more heterozygous, they have larger but fewer cells in a variety of tissues, and their gonad development is somewhat disrupted (Benfey, 1999). Triploid fish have larger erythrocytes than diploids but the number of erythrocytes is reduced to maintain the hematocrit at the diploid level (Barker et al., 1983; Benfey and Sutterlin, 1984; Ueno, 1984; Graham et al., 1985; Small and Randall, 1989; Aliah et al., 1991; Sezaki et al., 1991; Parsons, 1993; Yamamoto and Iida, 1994). Because the ratio of surface area to volume is reduced as the size of the cell increases, the erythrocytic surface area available for gas exchange may limit the aerobic capacity in triploids (Benfey, 1999).

Fish hematology is the basic index for monitoring fish welfare and health status. In this study, key hematological indices in triploid and diploid hybrids of rainbow trout × Black Sea trout were investigated to provide baseline data and evaluate the feasibility of intensive hybrid production and culture.

Materials and Methods

Fish. Twelve >3-year-old female (3960±141 g) and eight >2-year-old male (1814±172 g) rainbow trout (*Oncorhynchus mykiss*) plus fifteen >2-year-old female (904±76 g) and ten >2-year-old male (716±74 g) Black Sea trout (*Salmo labrax*) were obtained from a private trout farm in Rize, Turkey. Eggs were stripped from ovulating females and approximately 24,000 eggs from each species were fertilized with milt of donor males. Fertilized eggs were transferred to the Fish Breeding Research Unit at the Rize University Fisheries Faculty and used to create six groups of intra-specific and inter-specific crosses: O♀ × O♂ diploids and triploids, O♀ × S♂ diploids and triploids, S♀ × S♂ diploids and triploids. Triploids were produced by a 26.5°C thermal shock lasting 20 min, initiated 25 min after fertilization (Blanc and Maunas, 2005). Fertilized eggs were incubated in 10-11°C spring water prior to and after shock. Alevins were fed according to standard protocols (Willoughby, 1995). Forty-five juveniles from each group (3.31±0.37-11.74±1.44 g) were placed in 100-l circular tanks with open flow spring water, kept under natural conditions, and fed commercial trout pellets twice daily. Water temperature was 14°C and oxygen was 8.7 mg O_2/l when blood was taken. The ploidy level in the diploids and induced triploids was verified according to the size of the erythrocyte nuclei (Piferrer et al., 2000). Spontaneous allotriploid hybrids were eliminated from the diploid hybrid group, and only seven diploid hybrids were used for data analysis.

Blood samples. Blood samples were taken from 20 randomly chosen fish from each group by cutting dorsoventrally through the caudal peduncle. After microscopy, only diploid fish were used for diploid groups and triploid fish for triploid groups. Blood (0.5-1 ml) was placed in a K_3-EDTA coated blood collection tube or heparinized capillary microhematocrit tube at the end of the caudal vessel. Blood samples were refrigerated (4ºC) for later hemoglobin (Hb) and hematocrit (Hct) determination.

Hematological measurements. Red blood cells (RBC) and white blood cells (WBC) were counted by standard hematological techniques (Dacie and Lewis, 2006). Micro hematocrit tubes (75 mm length, 1.1-1.2 mm internal diameter) were used to determine

hematocrit: duplicate tubes for each sample were filled with blood, one end of the tubes was covered with glass putty and plaster, the tubes were centrifuged at 2500 rpm for 5 min, and the contents were measured with a hematocrit ruler. Total blood hemoglobin concentration was measured by the spectrophotometric cyanmethemaglobin test at 540 nm with a spectrophotometer (Shimadzu UV-1800, Japan) and computed as Hb (g/dl) = [(OD test)/(OD std)] × 15. The mean cell volume was calculated as MCV (fl) = Hct (%)/RBC (10^6/µl), mean cellular hemoglobin content as MCH (pg) = [Hb x 10]/RBC (10^6/µl), and mean cell hemoglobin concentration as MCHC (g/l) = [Hb x 10]/Hct (Dacie and Lewis, 2006).

Blood smears were prepared from each fish by the conventional method, fixed in methyl alcohol, and stained with May**-**Grünwald-Giemsa (Fig. 1). The surface area (S) of erythrocytes and nuclei was calculated as S = (π/4)ab, where a and b are the major and minor semiaxes, respectively, of the cell or nucleus. The volume (V) of erythrocytes and nuclei was calculated as V = 4/3πab^2, where a and b are the major and minor semiaxes, respectively, of the cell or nucleus. Dimensions of red blood cells and nuclei were measured at 1,000× magnification with a calibrated ocular micrometer and light microscope (Olympus BX51, Japan). Twenty arbitrarily-chosen erythrocytes were analyzed from each sample, thus 400 erythrocytes were examined for each ploidy, hybrid, or species group.

Data analysis. Data were analyzed by one-way analysis of variance (ANOVA). The significance of differences between means was determined by Duncan's multiple range test (p<0.05) using SPSS for Windows, version 13.0. Values are expressed as means±SE. Differences were accepted as significant when p<0.05.

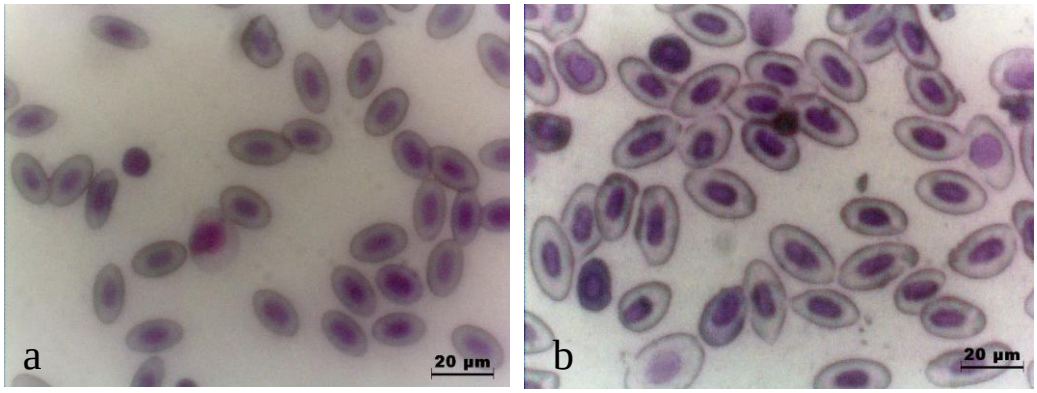

Fig. 1. Erythrocytes in (a) diploid and (b) triploid F_1 hybrids of rainbow trout (*Oncorhynchus mykiss*) × Black Sea trout (*Salmo labrax* Pallas, 1814).

Results

Erythrocyte measurements. Erythrocytes were significantly larger in all triploid groups than in diploids (Table 1). Erythrocytes were more ellipsoidal in triploid hybrids and triploid rainbow trout than in their corresponding diploids. The opposite was true for Black Sea trout where erythrocytes in triploids were more spherical than in diploids. The erythrocyte surface and volume were larger in triploids than in diploids. Likewise, the nuclear surface and volume were greater in triploids than in diploids.

Hematological characteristics. Hematological data are presented in Fig. 2. Triploidization significantly lowered the number of erythrocytes in rainbow trout, hybrids, and Black Sea trout. The number of WBC was also lower in triploids than in diploids, but the differences were not statistically significant. The mean WBC/RBC ratio was higher in triploids than in diploids in Black Sea trout and hybrids, but lower in triploids than in diploids for rainbow trout.

Mean hematocrit values were 35.46% for diploid and 35.61% for triploid rainbow trout, 34.61% for diploid and 33.59% for triploid hybrids, 37.53% for diploid and 36.08%

Table 1. Erythrocyte cell and nucleus dimensions (means±SEM) in diploid (D) and triploid (T) rainbow trout (*Oncorhynchus mykiss*), Black Sea trout (*Salmo labrax*), and their F_1 hybrids (*O. mykiss* × *S. labrax*).

	O. mykiss × *O. mykiss*			*O. mykiss* × *S. labrax*			*S. labrax* × *S. labrax*		
	Diploids (n = 20)	*Triploids (n = 19)*	*Ratio (T/D)*	*Diploids (n = 7)*	*Triploids (n = 20)*	*Ratio (T/D)*	*Diploids (n = 20)*	*Triploids (n = 17)*	*Ratio (T/D)*
Cell minor axis (µm)	8.79±0.227^{b}	10.47±0.197^{c}	1.19	9.12±0.296^{b}	10.78±0.155^{c}	1.18	7.85±0.091^{a}	10.27±0.084^{c}	1.30
Cell major axis (µm)	13.92±0.356^{a}	16.90±0.445^{b}	1.21	14.73±0.482^{a}	18.79±0.261^{c}	1.27	14.29±0.118^{a}	17.22±0.329^{b}	1.20
Cell surface (µm^2)	96.25±4.078^{ab}	139.53±6.021^{c}	1.45	105.68±5.455^{b}	159.29±3.852^{d}	1.50	88.16±1.584^{a}	138.83±2.741^{c}	1.57
Cell volume (fl)	567.84±36.18^{ab}	981.56±60.98^{c}	1.72	647.54±50.49^{b}	1151.19±44.68^{d}	1.77	462.22±13.45^{a}	950.73±22.25^{c}	2.05
Nucleus minor axis (µm)	4.51±0.108^{b}	5.41±0.148^{c}	1.19	4.49±0.070^{b}	5.29±0.11^{c}	1.17	4.10±0.050^{a}	5.21±0.111^{c}	1.27
Nucleus major axis (µm)	7.13±0.171^{a}	8.62±0.219^{c}	1.20	7.79±0.255^{b}	9.79±0.179^{d}	1.25	6.76±0.038^{a}	8.83±0.254^{c}	1.30
Nucleus surface (µm^2)	25.32±0.883^{ab}	36.79±1.615^{c}	1.45	27.46±0.657^{b}	40.91±1.667^{c}	1.48	21.76±0.291^{a}	36.29±1.675^{c}	1.66
Nucleus volume (fl)	76.60±4.121^{a}	134.24±9.320^{b}	1.75	82.32±1.845^{a}	146.51±9.981^{b}	1.78	59.57±1.483^{a}	127.17±8.274^{b}	2.13

Means in a row with different superscripts differ at $p<0.05$.

for triploid Black Sea trout. The hematocrit level did not significantly differ between diploids and triploids in rainbow trout, the hybrid, or Black Sea trout.

Hemoglobin was higher in diploids than in triploids for rainbow trout, hybrids, and Black Sea trout, although the differences were not significant. The mean cellular hemoglobin was 59.87 for diploid and 74.78 for triploid rainbow trout, 60.94 for diploid and 80.31 for triploid hybrids, and 67.96 for diploid and 74.51 for triploid Black Sea trout. Mean cellular hemoglobin concentrations were slightly, but not significantly, higher in diploids, i.e., 22.9 for diploid and 20.71 for triploid rainbow trout, 22.73 for diploid and 21.43 for triploid hybrids, 21.63 for diploid and 20.77 for triploid Black Sea trout.

Discussion

Triploid hybrids had a lower erythrocyte count than diploid hybrids or their parental species. Similarly, triploid rainbow and Black Sea trout had few erythrocytes than diploid rainbow and Black Sea trout. A reduction in number of blood cells and an increase in cell size have been reported for triploid salmonids and other fishes (Purdom, 1972; Barker et al., 1983; Benfey and Sutterlin, 1984; Ueno, 1984; Graham et al., 1985; Aliah et al., 1991; Sezaki et al., 1991; Parsons, 1993; Yamamoto and Iida, 1994; Benfey, 1999; Ballarin et al., 2004; Peruzzi et al., 2005). This fact is so well accepted that erythrocyte size is frequently used as the sole criterion for determining ploidy in fish. The number of erythrocytes is reduced in triploids in proportion to the increase in erythrocyte size so that the total blood volume (in percentage) is the same as in diploids (Benfey, 1999). The increase in cell size in triploids is not identical to the increase in cellular or nuclear length or width. The erythrocytes and nuclei were more elliptical in the triploids than in the diploids, similar to results in triploid Caspian salmon (Dorafshan et al., 2008) and turbot (Cal et al., 2005).

The hematocrit levels did not significantly differ between diploids and triploids because the increase in cellular size was offset by a decrease in cell number, similar to other fish species (Benfey, 1999; Peruzzi et al., 2005). The serum hemoglobin level was higher, though not significantly, in diploids than in triploids. The effects of triploidy on total blood hemoglobin concentrations are equivocal. Sometimes levels in triploids are lower than in diploids (Dorafshan et al., 2008) and sometimes they are similar (Barker et al., 1983; Aliah et al., 1991; Ballarin et al., 2004; Cal et al., 2005; Peruzzi et al., 2005).

The average volume of red blood cells (MCV) was greater in triploids than in diploids, so that the mean cellular hemoglobin content (MCH) of triploid erythrocytes was also

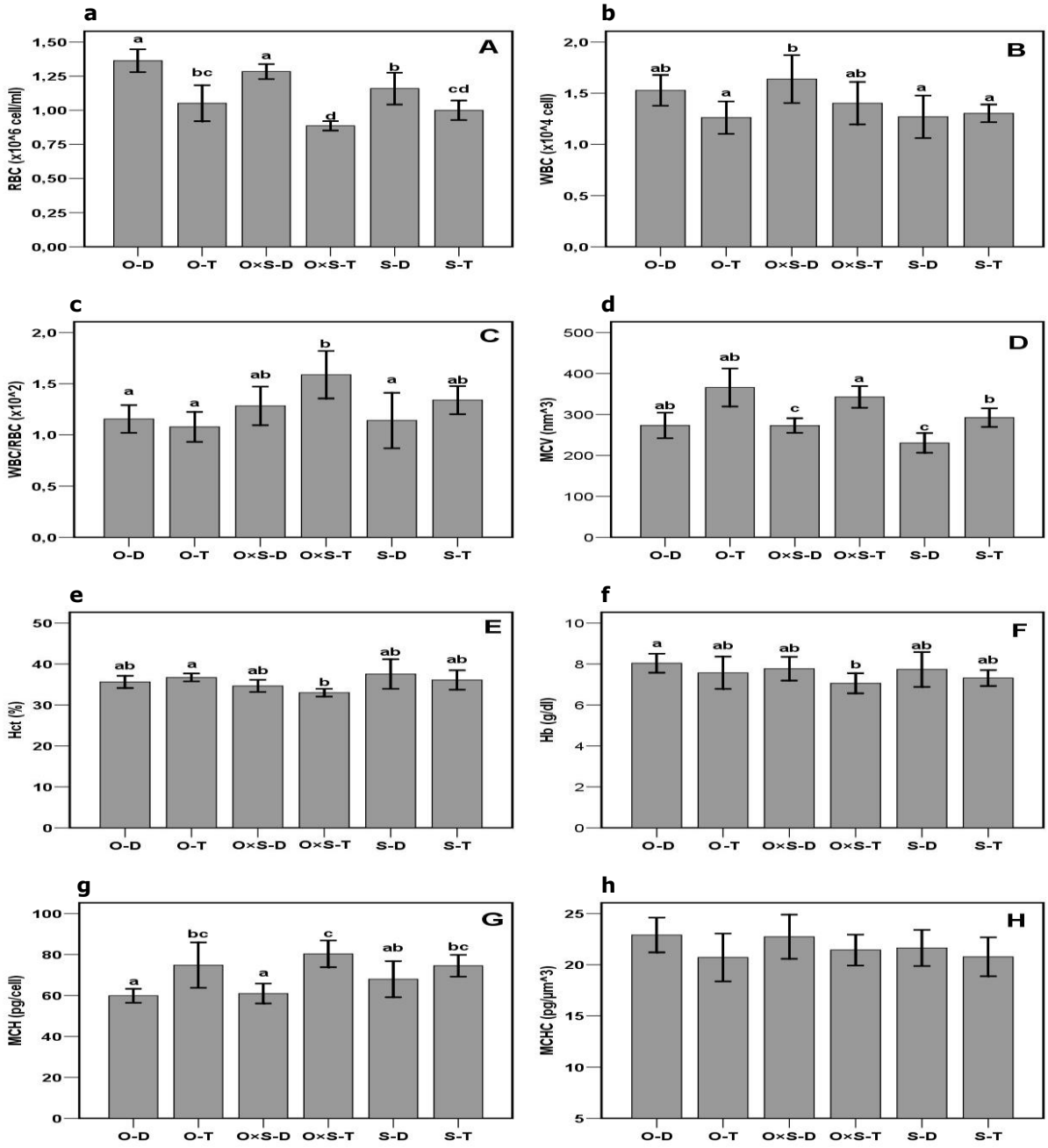

Fig. 2. Hematological parameters in diploid (D) and triploid (T) rainbow trout (O), hybrids (O×S), and Black Sea trout (S): (a) number of red blood cells (RBC), (b) number of white blood cells (WBC), (c) ratio of WBC to RBC, (d) mean corpuscular volume, (e) hematocrit, (f) hemoglobin, (g) mean cellular hemoglobin, and (h) mean cellular hemoglobin concentrations. Different letters above bars indicate significant differences (p<0.05).

significantly greater, similar to other fishes (Purdom, 1972; Barker et al., 1983; Benfy and Sutterlin, 1984; Graham et al., 1985; Aliah et al., 1991; Sezaki et al., 1991; Parsons, 1993; Yamamoto and Iida, 1994; Benfey, 1999; Ballarin et al., 2004; Peruzzi et al., 2005). The mean cellular hemoglobin concentrations (MCHC) were lower, but not significantly, in triploids than in diploids. Reported values for MCHC in diploid and triploid fish are inconsistent. Sometimes they are similar in triploids and diploids (Barker et al., 1983; Aliah et al., 1991; Cal et al., 2005) and sometimes they are lower in triploids (Benfey and Sutterlin, 1984; Graham et al., 1985; Small and Randall, 1989; Parsons, 1993; Yamamoto and Iida, 1994).

Our results show triploidy-associated changes in hematological parameters that might affect the physiology of rainbow trout × Black Sea trout hybrids. There are theoretical problems in carrying out biological processes in enlarged cells (Sadler et al., 2000). For example, tolerance to hypoxic conditions is lowered because of the low erythrocyte concentration and chronic stress can result from poor water quality, diet utilization, osmoregulation capacity, immunologic competence, or behavior (Benfey, 1999). Further study of husbandry-related experimental challenges should provide a better understanding of the comparative adaptation capacities of diploid and triploid Black Sea trout and its hybrids with rainbow trout.

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