Research Article

The effect of anesthetic (2-phenoxyethanol) application on some biochemical and hematological parameters in Russian sturgeon (*Acipenser gueldenstaedtii*) and Siberian sturgeon (*Acipenser baerii*) during transport

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Abstract: Experiments were carried out to determine the effect of 2-phenoxyethanol to reduce stress response during the transportation of Russian and Siberian sturgeons. In transportation experiment 1 (TE_1), Siberian sturgeons (687.2 ± 17.41 g) were stocked in tanks and transported for 90 min (stocking density = 65 ± 0.71 kg/m³). Before transport, 0.5 mg/L 2-phenoxyethanol was applied to the first group (TE_{1-A}, n = 285) but not to the second group (TE_{1-B}, n = 287). In transportation experiment 2 (TE_2), Russian sturgeons (1873 ± 159 g) were divided into two groups and stocked in tanks as they have a density of 60 ± 0.23 kg/m³ water. Before transport, 0.6 mg/L of 2-phenoxyethanol was applied to the first group (TE_{2-A}, n = 99), the second group (TE_{2-B}, n = 101) was left without application. During the 10 h of transport period (at the 2, 4, 6, and 8 h), 0.6 mg/L 2-phenoxyethanol was applied to the fish in the first group (TE_{2-A}) every 2 h. The results revealed that plasma glucose and cortisol reached their highest level in the anesthetic-free groups (TE_{1-B} and TE_{2-B}) during transportation in both experiments. There was no difference in the protein levels between anesthetic and anesthetic-free groups. In the TE_{1-B} and TE_{2-B}, red blood cells and hematocrit decreased, neutrophils and white blood cells increased. However, no changes were observed in eosinophils and mean corpuscular volume between groups in both experiments. Lymphocyte increased in TE_{1-B} in comparison to TE_{1-A} and TE_{1-BT} (before transport anesthetic-free fish in TE_1). In addition, there was no change in levels of monocyte, hemoglobin, mean hemoglobin concentration (MCH) and mean corpuscular hemoglobin concentration (MCHC) between TE_{1-BT}, TE_{1-A}, and TE_{1-B}. Monocyte, hemoglobin, MCH, and MCHC increased in TE_{2-B} compared with TE_{2-BT} (before transport anesthetic-free fish in TE_2) and TE_{2-A} but the lymphocyte count did not change. The results indicated that the use of anesthetic agent 2-phenoxyethanol during transfer reduced mortality rate (0% and 12.89% in TE_{1-A} and TE_{1-B}, respectively, and 2.02% and 15.84% in TE_{2-A} and TE_{2-B}, respectively) and physiological stress in sturgeons.

Key words: Acipenser sp., sturgeon, anesthesia, 2-phenoxyethanol, hematological parameters, biochemical parameters

1. Introduction

Wild populations of sturgeon have significantly decreased due to urbanization and human-made constructions such as dams preventing route lines of migration, overfishing, and water pollution, or a combination of these factors with others as well. As a result of the depletion of natural stocks, these sturgeon species have almost disappeared a long time ago. The high-value cultivation of sturgeon is important not only for economic returns but also for conservation of wild fish populations worldwide. Nowadays, a promising growth of sturgeon culture is drawing attention with an increase from 3100 t in 2000 to around 40,000 t in 2010, reaching about 100,000 t in 2017 (1). The Russian sturgeon (*Acipenser gueldenstaedtii*, also known as diamond or Danube sturgeon) and the Siberian sturgeon (*Acipenser baerii*) are among the important species in recent sturgeon aquaculture practices. Sedatives and anesthetics are very

useful for decreasing stress caused by aquaculture activities such as fish transport, artificial reproduction, handling, sorting, tagging, vaccination, or surgical procedures (2–4). Hematological parameters have a significant effect on the determination of conditions that can cause stress in fish (5). The determination of changes in blood parameters provides significantly important information regarding the physiological status of fish (6). Changes in some blood parameters are frequently used in the monitoring of the effect of physiological or sublethal stress caused by endogenous or exogenous changes in fish (7).

Even though transport of live fish provides a better quality and better market price, plasma cortisol levels in fish shows remarkable variations. Transport causes a significant increase in plasma cortisol levels in fish (8,9). According to Ross and Ross (10) one of the biggest stressors is mechanical abrasion caused by the contact

with each other while the fish are carried in high stocking densities. Robertson et al. (11) reported that medium (30 min) or long-term (5.5 h) fish transport creates severe stress on fish. If the fish encounters stress, it undergoes some biochemical changes to survive and maintains homeostasis (12,13). Cortisol is one of the hormones related to stress reactions in sturgeons and cortisol concentration in the serum rapidly increases in the event of acute stress (14). Metabolites are small molecules that are intermediate products of metabolic reactions catalyzed by various enzymes found naturally in cells. It was reported that metabolites such as glucose, glycogen concentration, and lactate increased during stress in fish (15). Generally, when physical and chemical difficulties are encountered, a stress response that includes physiological processes is formed. These physiological changes are the first response involving hormonal changes. The second response includes metabolites, blood ions, and hematological changes, and the third response includes the general condition of the fish (16,17). All organisms (such as bacteria, yeast, animals, humans) respond to stress by increasing the synthesis of cellular stress proteins against physical and chemical stress (18,19). Plasma cortisol, glucose, protein, and lactate are biochemical parameters used to monitor stress. Stress-forming factors include handling, capture, egg maturity control for fertilization, transportation and spawning (16,20).

Stress can delay or reduce some of the mechanisms in the defense system, especially in this case the defense against the pathogen may be compromised and the immune system of the stressed animal is suppressed (21). Anesthetic agent decreased the activity and stress in Atlantic salmon (*Salmo salar*) during transportation (22). Anesthetic to be used in the practice should be safe for fish and human health, it should be cheap and easy to find. In aquaculture applications, anesthetics can be used to minimize stress (23).

The primary objective of this study is to evaluate the effect of anesthetic agent 2-phenoxyethanol on hematologic (erythrocyte, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean hemoglobin concentration (MHC), mean corpuscular hemoglobin concentration (MCHC), leukocyte, monocyte, lymphocyte, neutrophil, eosinophil) and biochemical (cortisol, glucose, protein) parameters of Siberian sturgeon (*Acipenser baerii)* and Russian sturgeon (*Acipenser gueldenstaedtii*) fish during transportation, which may be a stress factor.

2. Materials and methods

Experiments were carried out during fish transport operations of a commercial farm involved in sturgeon breeding in Adana, Turkey. A total of 12 identical transportation tanks (1 m^3 volume each, 6 tanks in the TE_1, and 6 tanks in the TE_2) supported with dissolved oxygen were used in the experiments. Dissolved oxygen, pH, and water temperature levels in the transport tanks were controlled using a YSI 6600 CTD multiparameter measuring device. The active substance of 2-phenoxyethanol is ethylene glycol monophenyl ether. Its brief formula is $C_8H_{10}O_2$, the molar weight is 138.17 g L^{-1}, the density is 1.107–1.108, the peroxide content is less than 0.005%, and the boiling temperature is 245 °C (24).

2.1. Transportation experiment 1 (TE_1)

Siberian sturgeon (*Acipenser baerii* Brandt, 1869) with an average weight of 687.2 ± 17.41 g and mean length of 63.6 ± 8.11 cm were divided into two groups (TE_{1-A}, n = 285 and T_{1-B}, n = 287), tagged and stocked in tanks (tank-1, tank-2, and tank-3 anesthetic group as TE_{1-A}, and also tank-4, tank-5, and tank-6 anesthetic-free group as T_{1-B}) with a stocking density of 65 ± 0.71 kg/m^3 freshwater. Prior to transport, the first group (TE_{1-A}) was subjected to anesthesia treatment of 0.5 µL/L 2-phenoxyethanol (77699 Sigma-Aldrich), while no anesthesia treatment was applied in the second experimental group (TE_{1-B}). The total time of transportation lasted 90 min (starting point 37°18′08″N, 35°42′09″ E; end point 37°02′15″N, 35° 19′22″ E, Turkey).

2.2. Transportation experiment 2 (TE_2)

Russian sturgeon (*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833) with a mean weight of 1873 ± 159 g and length of 71.6 ± 4.1 cm were divided into two groups, tagged and stocked in tanks (tank-1, tank-2, and tank-3 anesthetic group as TE_{1-A}, also tank-4, tank-5, and tank-6 anesthetic-free group as T_{1-B}) at a stocking rate of 60 ± 0.23 kg/m^3 freshwater. The first group of fish (TE_{2-A}, n = 99) received anesthesia treatment with 0.6 µL/L 2-phenoxyethanol (77699 Sigma-Aldrich) at 2-h intervals (at 2, 4, 6, and 8 h) after the start of transportation from the sturgeon farm in Antalya to Adana (starting point 36°58′24″ N, 31°03′50″ E; end point 37°18′08″ N, 35° 42′09 ″E, Turkey) for a total of 10-h transport, while no anesthesia treatment was applied for the second experimental group (TE_{2-B}, n = 101).

2.3. Blood samples

Blood samples were taken before transport from anesthetic-free fish (TE_{1-BT}, TE_{2-BT}), after transport from anesthetized fish (TE_{1-A}, TE_{2-A}), and after transport in anesthetic-free group (TE_{1-B}, TE_{2-B}) in both experiments (TE_1, TE_2). Before and after the transport period, at each sampling hour, 15 fish were randomly selected from each tank in the TE_1 and 10 fish were randomly selected in the TE_2. Four milliliters of blood was drawn from the caudal vein of each labelled fish using a syringe (caudal vein washed with sterile water prior to collecting blood in order to avoid any contamination), the blood was immediately transferred into tubes with Ethylenediamine tetraacetic acid – EDTA (25,26). Taking blood sample procedures were carried out within 1 min to minimize stress. Two milliliters of blood samples for biochemical analysis and 2 mL for hematological analysis were transferred to separate

tubes. The samples were stocked in ice and transported to the laboratory. The blood was centrifuged at 3250 × *g* for 5 min and plasma was removed and stored at 4 °C for biochemical analysis (22). The samples were analyzed the same day.

2.4. Hematological and biochemical analyses

Red (RBC) and white blood cells (WBC) were counted using Natt–Herrick solution and Thoma microslide (27). In the determination of hemoglobin (Hb) and hematocrit (Hct), cyan-methemoglobin and microhematocrit methods were used (27,28). Leukocyte cell types (lymphocyte, monocyte, neutrophil, and eosinophil) were identified in blood smears from fishes. Peripheral blood smears were stained with a mixture of May-Grünwald and Giemsa. The percentages of leukocyte cell types were identified using these blood smears by a light microscope at 100× magnification. A total of 100 leukocyte cells were counted by thoroughly scanning each blood smear and the percentages of monocyte, lymphocyte, neutrophil, and eosinophil were determined (29,30). Erythrocyte indexes were calculated using the following equations as provided earlier (27,31):

MCV (μ³) = Hct (%) ÷ RBC (10⁶/mm³) × 10
MCH (pg) = Hb (g/100 mL) ÷ RBC (10⁶/mm³) × 10
MCHC (%) = Hb (g/100 mL) ÷ Hct (%) × 100

Cortisol level was conducted with competitive inhibition enzyme immunoassay technique (Fish Cortisol ELISA Kit - Catalogue Number CSB-E08487f) (32). Total protein and plasma glucose levels were measured with enzymatic techniques using a commercial enzyme kit (Sigma-Aldrich QuantiProä BCA Assay Kit- QPBCA, Sigma-Aldrich Glucose, and Sucrose Assay Kit-MAK013) (33,34).

2.5. Statistical analysis

The results acquired for the hematological and biochemical parameters were compared using one-way analysis of variance (ANOVA). Duncan's multiple comparison test of the one-way ANOVA was used to compare the mean differences. The differences were assumed to be significant at P ≤ 0.05 (35).

3. Results

Among the TE_1 groups, RBC and Hct values were significantly lower in the TE_{1-B} compared to those obtained from the measurement results in TE_{1-A} and TE_{1-BT} (P < 0.05). Lymphocyte and neutrophil percentages in TE_{1-B} significantly increased compared to the values obtained for the other groups (P < 0.05). WBC number in the TE_{1-B} showed an increase compared to the other experimental groups (TE_{1-BT} and TE_{1-A}). MCH level in TE_{1-A} decreased and were below the values obtained for the TE_{1-BT} and TE_{1-B} groups (P < 0.05). There was no change in the levels of Hb, MCV, MCHC, monocyte, and eosinophil between the TE_{1-BT} group and the experimental groups of TE_{1-A} and TE_{1-B} (P < 0.05, Table 1).

Cortisol and glucose values decreased in the TE_{1-BT} compared to the TE_{1-A} group, whereas increased levels of cortisol and glucose were found in the TE_{1-B} group compared to the TE_{1-BT} and TE_{1-A} groups. It was observed that

Table 1. Results of hematological analyses in the transportation experiment 1 (TE1)

	Before transport TE_{1-BT} (anesthetic-free, n = 15)	After transport TE_{1-A} (anesthetic, n = 15)	After transport TE_{1-B} (anesthetic-free, n = 15)
RBC (×10⁶/mm³)	0.93 ± 0.24[a]	1.03 ± 0.29[a]	0.79 ± 0.16[b]
Hct (%)	26.2 ± 0.81[a]	28.5 ± 0.63[a]	21.3 ± 0.55[b]
Hb (g/dL)	8.9 ± 0.30[a]	8.7 ± 0.23[a]	7.4 ± 0.52[a]
MCV (μ³)	281.72 ± 13.49[a]	276.70 ± 18.11[a]	269.62 ± 14.31[a]
MCH (pg)	95.70 ± 4.09[a]	84.46 ± 3.87[b]	93.67 ± 5.25[a]
MCHC (%)	33.97 ± 2.83[a]	30.53 ± 1.75[a]	34.74 ± 1.91[a]
WBC (×10³/mm³)	11.43 ± 0.52[a]	10.27 ± 0.33[a]	14.91 ± 0.64[b]
Lymphocyte (%)	51.14 ± 2.18[a]	49.82 ± 1.95[a]	57.31 ± 3.26[b]
Monocyte (%)	5.53 ± 1.17[a]	7.62 ± 2.43[a]	6.96 ± 1.36[a]
Neutrophil (%)	33.47 ± 2.81[a]	32.25 ± 1.32[a]	39.74 ± 1.56[b]
Eosinophil (%)	1.4 ± 0.15[a]	1.5 ± 0.11[a]	1.1 ± 0.19[a]

Data are represented as mean ± SE. The values in the same line with different superscripts are significantly different (P < 0.05).
Red blood cell (RBC), hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean hemoglobin concentration (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC).

the protein level did not change in the TE_{1-B} with regard to the other groups of TE_{1-BT} and TE_{1-A} (P < 0.05, Table 2).

In the TE_2 group, RBC and Hct values decreased in TE_{2-B} compared to TE_{2-A} and TE_{2-BT}. The levels of Hb, MCH, MCHC, WBC, monocyte, and neutrophil for the TE_{2-B} increased compared to the values recorded for the TE_{2-A} and TE_{2-BT} groups. No change in MCV, lymphocyte, and eosinophil levels was observed between the experimental groups (P > 0.05, Table 3).

Regarding the protein values, no difference was detected among the experimental groups of TE_{2-A}, TE_{2-B}, and TE_{2-BT}. A significant increase was recorded for cortisol and glucose levels in TE_{2-A} and TE_{2-B} compared to the TE_{2-BT}, with the highest level in the TE_{2-B} group (P < 0.05, Table 4).

Considering mortality rates in the TE_1-trial, no fish loss was observed in the TE_{1-A}, whereas a 12.89% of mortality occurred in the TE_{1-B} (13 fish in tank-4, 13 fish in tank-5, and 11 fish in tank-6. Mean with standard error 12.33

± 0.67) group exposed to stress of transportation. After transportation, fish losses were observed in the TE_2-trial with 2.02% for the TE_{2-A} (1 fish in tank-1, 0 fish in tank-2, and 1 fish in tank-3. Mean with standard error 0.67 ± 0.33) and 15.84% for the TE_{2-B} (5 fish in tank-4, 6 fish in tank-5, and 5 fish in tank-6. Mean with standard error 5.33 ± 0.33) test groups. In the experiments, the average oxygen (O_2) concentration, temperature and pH values in the tanks were presented in Table 5.

4. Discussion

In recent years, live fish transport in aquaculture has become an important procedure. Therefore, it is of paramount importance to develop strategies to minimize physiological changes during transport. Earlier investigations on fish stressors reported that fish transportation appears to be one of the prominent stress factors affecting fish health status in aquaculture conditions (36). Gülen (37) reported

Table 2. Results of biochemical analyses in the transportation experiment 1 (TE_1)

	Before transport TE_{1-BT} (anesthetic-free, n = 15)	After transport TE_{1-A} (anesthetic, n = 15)	After transport TE_{1-B} (anesthetic-free, n = 15)
Cortisol (ng/ml)	6.33 ± 0.7[a]	31.7 ± 5.3[b]	53.2 ± 9.6[c]
Glucose (mg/dL)	48.7 ± 2.9[a]	96.4 ± 8.5[b]	150.1 ± 10.7[c]
Protein (g/dL)	1.77 ± 0.3[a]	1.91 ± 0.2[a]	2.3 ± 0.5[a]

Data are represented as mean ± SE. The values in the same line with different superscripts are significantly different (P < 0.05).

Table 3. Results of hematological analyses in the transportation experiment 2 (TE_2)

	Before transport TE_{2-BT} (anesthetic-free, n = 10)	After transport TE_{2-A} (anesthetic, n = 10)	After transport TE_{2-B} (anesthetic-free, n = 10)
RBC (×10^6/mm³)	0.84 ± 0.16[a]	0.87 ± 0.37[a]	0.72 ± 0.32[b]
Hct (%)	23.4 ± 0.59[a]	25.1 ± 0.94[a]	18.9 ± 0.37[b]
Hb (g/dL)	7.9 ± 0.80[a]	8.3 ± 0.51[a]	9.7 ± 0.36[b]
MCV (μ³)	278.57 ± 11.93[a]	288.52 ± 13.18[a]	262.50 ± 14.7[a]
MCH (pg)	94.05 ± 5.81[a]	95.41 ± 4.69[a]	134.72 ± 6.47[b]
MCHC (%)	33.76 ± 2.29[a]	33.07 ± 3.41[a]	51.32 ± 5.75[b]
WBC (×10³/mm³)	10.14 ± 0.46[a]	9.11 ± 0.62[a]	13.87 ± 0.53[b]
Lymphocyte (%)	58.64 ± 3.72[a]	59.78 ± 1.48[a]	62.25 ± 2.43[a]
Monocyte (%)	6.98 ± 1.57[a]	6.35 ± 1.74[a]	9.58 ± 2.11[b]
Neutrophil (%)	40.17 ± 2.26[a]	38.50 ± 2.43[a]	48.65 ± 3.14[b]
Eosinophil (%)	1.9 ± 0.23[a]	2.1 ± 0.14[a]	2.3 ± 0.28[a]

Data are represented as mean ± SE. The values in the same line with different superscripts are significantly different (P < 0.05) .
Red blood cell (RBC), hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean hemoglobin concentration (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC).

Table 4. Results of biochemical analyses in the transportation experiment 2 (TE_2).

	Before transport TE_{2-BT} (anesthetic-free, n = 10)	After transport TE_{2-A} (anesthetic, n = 10)	After transport TE_{2-B} (anesthetic-free, n = 10)
Cortisol (ng/mL)	7.25 ± 1.2^a	42.6 ± 3.9^b	71.4 ± 8.3^c
Glucose (mg/dL)	45.3 ± 2.2^a	112.9 ± 9.7^b	182.1 ± 12.6^c
Protein (g/dL)	1.52 ± 0.7^a	1.89 ± 1.1^a	2.8 ± 0.8^a

Data are represented as mean ± SE. The values in the same line with different superscripts are significantly different (P < 0.05).

Table 5. Oxygen (O_2), temperature and pH values in the tanks.

	Before transport		After transport		Before transport		After transport	
	TE_{1-A}	TE_{1-B}	TE_{1-A}	TE_{1-B}	TE_{2-A}	TE_{2-B}	TE_{2-A}	TE_{2-B}
Oxygen (mg/L)	7.9 ± 0.1	7.8 ± 0.1	7.8 ± 0.2	7.5 ± 0.1	7.2 ± 0.2	7.0 ± 0.2	7.1 ± 0.2	6.9 ± 0.2
Temperature (°C)	17 ± 0.2	17.1 ± 0.1	16.9 ± 0.1	17 ± 0.1	14.8 ± 0.1	14.8 ± 0.2	15.1 ± 0.1	15 ± 0.2
pH	8.2 ± 0.1	8.2 ± 0.1	8 ± 0.2	8.1 ± 0.1	7.8 ± 0.1	7.7 ± 0.1	7.9 ± 0.1	7.8 ± 0.1

Data are represented as mean ± SE.

that hematology provides important information for diagnosis and treatment in Siberian sturgeon (*Acipencer baerii*). Therefore, it has been emphasized that hematology can be combined with other routine diagnostic means to determine the health status of fish and it may be a very useful health indicator in evaluating stress conditions (38). In the aquaculture sector, anesthetic agents are used for reducing the mortality rate during live fish transport and to prevent the deterioration of the general health condition of fish. Fish transport from hatchery to the grow-out farm or from a grow-out farm to the market location is important since fish encounter serious stress conditions during the transport, including noise and vibration from vehicle or road structures that might result in high mortality. The fish that survived the transportation and reached the new facility might need a certain time to recover from the stressful conditions. Kayali et al. (36) reported that European seabass (*Dicentrarchus labrax*) exposed to transport and handling stress for 2 h needed around 24 h for full-recovery and start of active feeding based on physiological excretion patterns of fish. In the experiments (TE_1, TE_2) of the present study, analysis and monitoring of stress indicators revealed that transport causes stress in Siberian and Russian sturgeons, which is in agreement with the earlier report on European Seabass by Kayali et al. (36). Transport procedures in fish may cause changes in physiological stress indicators such as cortisol and glucose (16). Iversen et al. (22) transported Atlantic salmon (*Salmo salar* L.) smolts for different durations (0.5, 2.5, and 4.5 h), and measured the plasma cortisol levels

of the fish before the transport (control) and 1 h after the transport. As a result of the measurements carried out after the transport, it was determined that the cortisol values increased significantly in all transport applications compared to the control group. The researchers have stated that this increase can be caused by the stress related to the transportation. In the present study, cortisol levels were significantly higher than those of the control groups in the transport applications (TE_1, TE_2). However, compared to baseline measurements (control), the increase in cortisol values in the 2-phenoxyethanol-treated groups was lower than those of the groups with no 2-phenoxyethanol application. This was associated with the effect of the anesthetic agent to reduce the transportation stress. Ross and Ross (10), observed beneficial effects of anesthetics during transport of live fish. Sedation provided advantages to minimize physical damage or stress caused by crowding, capture, and transport. In our study, cortisol level increased as an indication of stress condition during both short- and long-distance live transport (TE_1, TE_2) in all experimental groups (TE_{1-A}, TE_{1-B}, TE_{2-A}, and TE_{2-B}). Lower cortisol levels were detected in the anesthetically treated groups (TE_{1-A} and TE_{2-A}) compared to the TE_{1-B} and TE_{2-B} groups (anesthetic-free). According to the results obtained in the present study, our findings are supported by the previous research results mentioned above.

The increase of plasma glucose levels as an energy source, is accepted as an important secondary response that allows fish to combat stressful conditions (39). Barton (17) reported a significant increase in plasma glucose level

after 2 h of transport, reaching a maximum level at 3 h of transport in rainbow trout. According to the results of the glucose levels of the fish before and after transportation, the researcher reported that the stress caused by the transport increased the level of glucose in fish. Similarly, according to the results of the present study, transportation caused stress in the fish resulting in an increase in the level of glucose. In addition, according to our results, glucose levels were found to be higher in groups with no anesthetic agent applications during transport compared to those with the anesthetic application. This is an indicator of the stress-reducing effect of 2-phenoxyethanol in fish. In the study of Barcellos et al. (40), South American catfish (*Rhamdia quelen*) were exposed to acute and chronic stress by usual aquaculture practices. Acute stress was created by harvesting and then transferring them from one tank to another. Chronic stress was created by stocking the fish in concrete tanks in intensive stock ratio. In this experiment, the glucose values reached the highest level during acute stress. Pedron et al. (41) applied 2 and 6 mg L^{-1} benzocaine for 8-h transportation in juvenile cobia (*Rachycentron canadum*) samples. As a result, the glucose increased in both doses compared to that in the control group, but the most significant increase was in benzocaine in 6 mg L^{-1} dose. The researchers, in their study where no deaths were reported during transport for both doses, reported that the increase in glucose levels might be due to benzocaine-related stress. In our study, it was observed that in the group with no 2-phenoxyethanol application (TE_{2-B}) glucose levels increased 4-fold compared to the baseline measurements (TE_{2-BT}). Moreover, significant mortality rates (15.84%) were observed compared to the anesthesia application (TE_{2-A}) group. 2-phenoxyethanol (0.6 µl/L), which we applied during the 10-h transport, resulted in minimal mortality (2.02%) in fish, indicating that the anesthetic agent used could not be a source of stress in fish.

In a study by Hutchinson and Manning (42), stress was created by transportation in dab fish (*Limanda limanda*), subsequently, total serum protein concentrations were measured. It was reported that there was no significant change in protein values compared to the control group. Inoue et al. (43) applied clove oil (5 mg/L) as an anesthetic agent to reduce the stress during transport (4 h) in matrinxã (*Brycon cephalus*) fish. According to their results, no differences were found in the plasma protein levels of the groups with and without anesthesia. They noted that clove oil has alleviated the stress response as compared to the fish transported without anesthesia. Additionally, Shaluei et al. (44) examined the hematological and biochemical changes to beluga sturgeon (*Huso huso*) by administering 2-phenoxyethanol at different doses (0.3, 0.5, 0.7, and 0.9 mL/L). They reported that anesthesia in 0.7 and 0.9 mL/L doses had the lowest effect on hematological and serum biochemical indices in these fish and these doses can be

recommended as appropriate doses. They also informed that there was no significant difference in serum protein levels between the control and administration groups. Our results both in the TE_1 and TE_2 indicated that no difference was detected in serum protein values between the groups (TE_{1-A}, TE_{2-A}, TE_{1-B}, TE_{2-B}, TE_{1-BT}, and TE_{2-BT}). In terms of biochemical parameters (cortisol, glucose, and protein) the results obtained from our study showed similarity to the previous above-mentioned studies on the subject.

In a study on catfish (*Heterobranchus bidorsalis*), a decrease in RBC and Hct values were reported to be caused by transport stress. According to them, this reduction was a result of decreased activity of hematopoietic organs due to transport stress (45). Similarly, Akinrotimi et al. (46) examined the effects of transport stress on hematological parameters in Blackchin Tilapia (*Sarotherodon melanotheron*), and found that transport stressed caused a reduction in RBC and Hct levels. Additionally, Adeyemo et al. (47) reported a correlation between transport stress and hematological parameters of African catfish (*Clarias gariepinus*), with reduced levels of Hct and RBC in fish exposed to transportation compared to the control group (no transportation). In our research, similar results to those of the studies of Okafor and Achilefu (45), Akinrotimi et al. (46) and Adeyemo et al. (47) for RBC and Hct values were obtained. Red blood parameters are sensitive to environmental factors. Transportation stress in anesthetic-free groups had a destructive effect on hematopoietic organs in fish and decreases were observed in Hct and RBC levels. In the application groups, no suppression on fish due to the stress-reducing effect of the anesthesia reduced these values. In another study, duration of fish transport was a total of 12.5 h, of which the first 7 h was considered as the first stage and the rest 5.5 h was called the second stage. No differences were seen in Hb, MCH, MCHC, and lymphocyte levels in common carp (*Cyprinus carpio* L.) subjected to transport stress of various distances. Some differences were found in MCV, monocyte, and neutrophil levels (48). Eurasian perch (*Perca fluviatilis*) was subjected to stress by 4-h transport. Hemoglobin levels, MCH, and MCHC did not show any significant differences as a result of transport stress. However, MCV levels increased significantly (49). In an experiment with *Paralichthys olivaceus*, MCH and MCHC levels were reported to decrease during transport (180 min) stress (50). No difference was observed in MCV under stress conditions caused by fish transport (50,51). According to Houston et al. (52), different stress factors may have different effects on the erythropoiesis process in fish. In our study, in both trials, it was found that there was a decrease in RBC and Hct values in nonanesthetized groups after transportation. This result supported those reported in the previous studies. The decrease in RBC and Hct values affected the parameters including MCV,

MCH, and MCHC, and these results may be related with the negative effects of transportation stress on the erythropoiesis process. The lack of change in the values of anesthesia-applied groups is an indicator of the effect of 2-phenoxyethanol on stress.

Stress is one of the factors affecting the immune system, and poststress immunological suppression may occur. Pilinkovskij et al. (53) investigated the hematological effects of transport stress (3 h) on Atlantic sturgeon (*Acipenser oxyrinchus*) and noticed that WBC and Hb levels increased depending on the stress severity during transport. Rowley et al. (54) noticed that the monocytes and neutrophils play an important role in the nonspecific defense system in fish, which form the first line of cellular defense against any stress. The cellular defense is mediated by T lymphocytes that can specifically react with antigens. Some of the T lymphocytes activate phagocytes. Cellular defense involves the activation of phagocytes (monocytes/ macrophages and neutrophils), cytotoxic T cells and the release of cytokines and chemokines (55). In a study on African catfish (*Clarias gariepinus*), it was indicated that transport stress did not cause any change in eosinophil level (47). Similar to our research results, Parodi et al. (56) reported decreased mortality rates in South-American catfish (*Rhamdia quelen*) when using anesthetic during transportation. Dobšíková et al. (57) transported common carp (*Cyprinus carpio*) in two stages (7 h and 5 h) in a total 12 h. At the first stage (7 h) measurement, WBC (G L^{-1}) increased from 57.56 to 76.33 and to 79.94 at the second stage (5 h). Lymphocyte value increased from 51.02 to 65.32 in the first stage. Akinrotimi et al. (44) transported blackchin tilapia 50 km in two groups (with and without oxygen). The researchers examined the effects of transport

stress on hematological parameters and reported a higher increase in WBC, lymphocyte, monocyte, and neutrophil levels in nonoxygen treated groups compared to the other group as an indicator of defense. In our study, an increase in WBC level was observed in non-2-phenoxyethanol-applied sturgeons during the transportation, as a result of the defense mechanism against stress. Our results in terms of neutrophil values were consistent with those reported by Wendelaar Bonga (16), who reported that neutrophil values in blood increased during stress. The lack of an increase in these parameters in the anesthetic-applied groups during transportation is the best indication that 2-phenoxyethanol increases resistance and endurance to stress. The studies carried out on the transportation stress in different fish species showed that the blood cells in the immune response showed similar changes to those obtained in our study.

Most of the deaths that occur during live fish transportation are caused by incorrect transportation methods. Can and Sümer (58) reported that peppermint (*Mentha piperita*) and lavender (*Lavandula angustifolia*) essential oils can be safely used as anesthetic and sedative agent in tropical fish species. They recommended the essential oil concentration in the range of 10 to 30 µL L^{-1} for fish transport. Due to the sedative effect of anesthetics during transport, the general activity and metabolism in fish are being slowed down, and stress response is reduced (59). The result of the study indicated that the use of anesthetic agent 2-phenoxyethanol in Russian sturgeon (*Acipenser gueldenstaedtii*) and Siberian sturgeon (*Acipenser baerii*) during transport reduced mortality rate and physiological stress.

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