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TEMPORAL VARIATIONS IN HEMATOLOGICAL, IMMUNOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) ACCLIMATED TO HIGH-SALINE WATER IN THE NORTHERN AEGEAN SEA*

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Abstract

The present study evaluated the health status of rainbow trout (30.24±5.34 g) acclimated to high-saline water (28‰). Among adaptation procedures, gradually-acclimated (for 48 h) fish showed best performance, which were then introduced to the offshore cage-farm for further monitoring of fish health in marine environment over a long-term period of 100 days, until harvest weight of 319.9±48.51 g. Fish health and welfare was evaluated by means of biochemical parameters (viscerasomatic index, hepatosomatic index, mesenteric fat index, and spleen somatic index), hematological parameters (serum glucose, triglyceride, cholesterol, total protein, albumin, globulin), and immune parameters (lysozyme, myeloperoxidase, respiratory burst and potential killing values). Gradual acclimation to high-saline water did not show any adverse effects on health parameters. No significant differences were found in biometric measures (P>0.05). However, a time-dependent increase was recorded in hematology markers (P<0.05) after seawater transfer, and the health status improved over long term from March 7 to June 14, 2018. All serum biochemical markers, except the total protein showed significant alterations (P<0.05) in long term but without detrimental influence by day 100. Therefore, it seems possible to expand trout farms from brackish water sites to higher saline environments up to 28‰ salinity, with no detrimental impacts on fish health, that in turn may significantly contribute to the extension of potential aquaculture sites to wider areas.

Key words: rainbow trout, biochemical parameters, hematological profile, health status, high saline water

Capture fisheries are no longer capable to cover the increasing demand of the world population, estimated to reach 9 billion in 2050 (UN-WPP, 2019). The aquaculture industry may provide sustainable solutions for the increasing consumer demands (Subasinghe et al., 2009; Golden et al., 2017; López-Mas et al., 2021) with high quality protein for human beings (Stankus, 2021).

Limitations in near-shore sites as a result of the rapid expansion of the sector, as well as increasing public concern regarding environmental impacts of cage farms, shifts new investments to extreme offshore marine areas (Halwart et al., 2007; Bostock et al., 2010). Owing to their osmoregulatory features, the transfer of rainbow trout from freshwater to marine sites with brackish water characteristics after a certain growth, is a common practice in trout farming (Yigit et al., 2004). In the early 1990s, higher growth performance has been reported for rainbow trout in the estuaries of the Adriatic Sea (Edwards, 1984), Norwegian fjords (Dill, 1990), and the Black Sea (Yigit and Aral, 1999) with brackish water conditions, compared to those cultured in freshwater farms. Since then, trout farms in brackish marine sites with low-saline water conditions dominated over freshwater farms (Deniz, 2022). However, strict control mechanisms of local governments for environmental impact assessment, brought new challenges for trout farms. The increasing production yields coupled with reduced carrying capacities of PASs forced farmers to seek new potential marine sites for trout aquaculture. Therefore, research regarding impacts of higher saline conditions on the health status of rainbow trout is still open and needs assessment, that in turn may support local governments and decision makers in designating new PASs for the expansion of trout production in cage systems.

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In recent years, blood parameters have been used as a qualified technique for assessing the health status and welfare in fish (Fazio, 2019), and changes in blood parameters reflected physiological responses in fish (Silkin and Silkina, 2005; Uiuiu et al., 2021). Most of reports primarily focused on osmoregulation ability of rainbow trout and stress recovery when fish is exposed to seawater adaptation (Morro et al., 2019; Ge et al., 2021). Physiological variations in fish during seawater acclimation, as well as stress-related downsides, are well documented by these investigations. However, reports on metabolic changes in fish blood after seawater acclimation is quite limited. Therefore, this study carried out from March to June, representing the periods of smoltification in wild individuals of sea trout (Rutkovska et al., 2019), aimed to provide information on variations of blood parameters in rainbow trout exposed to high saline water conditions (28 ‰). The assessment of the health-status of trout in high-saline water conditions may support local governments and decision makers in planning and allocation of new PASs for a sustainable expansion of trout aquaculture from brackish to seawater sites in Europe with reliable information based on real cage farm conditions.

Material and methods

Experimental fish and seawater acclimatization procedures

A total of 8400 rainbow trout (*Oncorhynchus mykiss* W., 1792) with mean weight of 30.00±10.0 g were obtained from a commercial freshwater farm, and transferred to the Marine Research Station of Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology (Dardanos-Turkey, 40°04'37"N – 26°21'39"E). Transport time from freshwater farm to the marine research facility was 2.5 hours in total. When fish reached the marine research facility, two different seawater adaptation procedures were applied, namely (a) direct transfer without adaptation, and (b) gradual adaptation with replacement of freshwater by seawater over a period of 48 hours. For each of the seawater adaptation treatments, six circular-shaped fiberglass tanks with 750 L volume were used and seawater acclimation experiments were carried out in six replicates. Prior to the transport fish in the freshwater facility was withheld from feeding for two days and no feeding was performed throughout seawater acclimation period. When mortalities stopped and fish condition stabilized, experimental fish were accepted as acclimated to the new high-saline water condition. This was also a sign of appetite, when fish started voluntary feeding.

Cage farm conditions and fish monitoring after seawater adaptation

The offshore cage farm used for health status monitoring in rainbow trout, consisted of a two-bay grid system moored with 12 identical 500 kg deadweight anchors in the Strait of Canakkale (Northern Aegean Sea, Turkish coast), deployed 0.6 nautical miles off the coast of Guzelyali town (40°03'42" N – 26°20'36" E, 40°03'51" N – 26°20'45" E, 40°03'45" N – 26°20'55" E, 40°03'36" N – 26°20'48" E). The seafloor slope ranged between 35 and 55 m with a mean water depth of 45 m. Gravity-type cage, with 6 m diameter and 150 m^3 volume was used in this study.

After seawater acclimation in the marine research facility at waterfront area of Dardanos campus (40°04'37"N – 26°21'39"E), 1,000 fish were randomly withdrawn out of the batch with highest survival rate, which was the gradual-adaptation group in this study, and transferred to the cage farm site of Canakkale University for monitoring of the health status based on variations in biometric indices, hematological, serum biochemical, and immunological parameters of seawater-acclimated trout over a long term of 100 days. Feed was offered to fish twice daily (09:00 and 17:00) by hand. A commercial trout diet with optimum protein and lipid levels (P/E ratio) was used. Nutritional compositions of commercial diet used during the 100 days of monitoring study are given in Table 1.

Table 1. Nutritional content of the feed used in the grow-out experiment. Information based on feed chart of commercial trout diet

	Proximate composition (g/100 g air dry basis)
Moisture	10
Crude protein	44
Crude lipid	18
Crude ash	11
Crude fiber	2
Vitamin D_3 (IU)	2500
Vitamin E (mg)	200
Vitamin C (mg)	200
Gross energy (kcal/kg diet)*	4800
Digestible energy (kcal/kg diet)	4080
DP/DE (mg/kJ)	22.4
PE:GE	0.51

*Based on energy fuels of 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, 17 kJ g^{-1} nitrogen free extract; the energy unit "joule" was converted to "kcal" using the conversion factor of 4.182.

Measurements of the water quality parameters

Water quality parameters such as temperature, salinity, dissolved oxygen, and pH at the cage farm location were measured biweekly at 2 m depth from March to June 2018, using a YSI 600 QS Multiprobe System (Yellow Springs Instruments).

Collection of blood samples

Monthly samples of 12 fish were collected by divers for blood collection throughout the study period of 100 days. Prior to blood sampling, fish were sedated using clove oil (50 mg L^{-1}) as earlier reported by Iversen et al. (2003). Blood was taken from the caudal vein through

a 2.5 mL plastic syringe. For the analyses of hematological (RBC, Hgb and Hct) and innate immune parameters (respiratory burst and potential killing activities), a part of the blood samples was transferred into tubes containing K_3EDTA. After the rest of blood was taken into serum tubes, the serum was gained through 5000 *g* speed centrifugation for 10 min. Serum samples were stored at –80°C for further analysis. Thereafter, the fish were euthanized with high dose (250 mg L^{-1}) of anesthesia and fish samples were transferred to the laboratory in laboratory-class ice box, for further dissection for biometric indexes of VSI (viscerasomatic index), HSI (hepatosomatic index), MFI (mesenteric fat index), SSI (spleen somatic index). Inside temperature of samples was kept at 4 ± 0.1°C during the transfer.

Calculation of biometric indices

The samples were transferred to the laboratory and dissected by micro dissection tools after weighing. The viscera and separated parts were gravimetrically measured. Biometric indexes were calculated using following equations (Yigit et al., 2016):

Viscerasomatic index (VSI, %) = (viscera weight / fish body weight) × 100 (1)

Hepatosomatic index (HSI, %) = (liver weight / fish body weight) × 100 (2)

Mesenteric fat index (MFI, %) = (lipid weight around viscera (g) / body weight) × 100 (3)

Spleen somatic index (SSI, %) = (spleen weight / fish body weight) × 100 (4)

Hematological analysis

Red blood cell (RBC) count (×10^6 per mm^3), hematocrit (Hct, %), and hemoglobin (Hgb) concentrations (g/dL) were determined using the method of Blaxhall and Daisley (1973). The RBC count was obtained with a Thoma hemocytometer using Dacie's diluting fluid. The Hct value was determined using a capillary Hct tube. The Hgb concentration was determined by spectrophotometry (540 nm) via the cyanmethemoglobin method. Hematological parameters of mean corpuscular volume (MCV), mean corpuscular Hgb (MCH), and mean corpuscular Hgb concentration (MCHC) were calculated using the following equations (Zandecki et al., 2007):

MCV (fL) = [(Hct, %) × 10] / (RBC, ×10^6 per mm^3) (5)

MCH (pg) = [(Hgb, g/dL) × 10]/ (RBC, ×10^6 per mm^3) (6)

MCHC (g/dL) = [(Hgb, g/dL) × 100] / (Hct, %) (7)

Biochemical analysis

Serum biochemical indices, including glucose (GLU), triglyceride (TG), cholesterol (CHOL), total protein (TP), albumin (ALB) and globulin (GLO) were determined using bioanalytic test kits (Bioanalytic Diagnostic Industry Co.) and were measured with an Optizen POP QX spectrophotometer (Mecasys Co. Ltd., Daejeon, Korea).

Statistical analyses

Each value was expressed as mean ± SD for each of the measured variables. The statistical significance ($P<0.05$) of biometric indices, hematological and serum biochemical variables were tested using one-way ANOVA followed by a Tukey multi-comparison test with SPSS 17.0 packaged software.

Results

Survival rates of rainbow trout exposed to either direct or gradual seawater adaptation were used to assess best seawater acclimation group in order to begin health status monitoring study with healthy individuals. Out of the total 4,200 fish in the direct transfer group, only 756 individuals survived 6 days after seawater transfer, that accounted for only 18% survival. In the gradual-adaptation process, however, fish were acclimated to seawater by gently replacing freshwater with seawater over a 48-h course. This way of adaptation reduced mortality and the number of alive fish after 6 days, was 3,654, equal to a survival rate of 87% of the total fish introduced (4,200), which was then used for the long-term monitoring study. After cage transfer, fish behavior was monitored and it was observed that fish started voluntary feeding on day 4 after seawater transfer. Experimental conditions, survival rates, seawater adaptation procedures and fish growth over a 100-day period in high saline (28 ‰) water environment are shown in Table 2.

Table 2. Survival rates of rainbow trout exposed to direct and gradual seawater adaptation and growth performance and survival during the grow-out period in seawater cage farm conditions for 100 days

	Direct transfer	Gradual transfer
1	2	3
Fish body weight (g)	30 ± 10 g	30 ± 10 g
Fish transport from FW to SW station	27 February	
Fish transport time (hours)	2.5 h	2.5 h
Start of seawater adaptation	27 February	
Seawater acclimation period	0 h	48 h
End of seawater adaptation	27 February	1 March

Table 2 – contd.

1	2	3
Seawater salinity (‰)	28	28
Salinity increase from 0 to 28‰	direct	~0.6 ‰/h
Number of fish (initial)	4,200	4.200
Number of fish (final)	756	3,654
Monitoring of adaptation (days, 1-6 March, no appetite – no feeding)	6	6
Survival rate (%)	18	87
Cage transfer	NO (discarded)	YES (cage transferred)
Date of cage transfer	7 March	
Number of cage-transferred fish	–	1,000
Start of feeding (days after transfer)	–	4
Initial fish weight (g) (7 March)	–	30.24±5.34
Initial biomass (kg/m^3)	–	0.21
Final fish weight (g) (14 June)	–	319.9±48.51
Final biomass (kg/m^3)	–	2.27
Seawater monitoring (days)	–	100
Wet weight gain (g)	–	289.66
Relative growth rate (%)	–	957.87
Specific growth rate (%/day)	–	1.97
Survival after 100 days (%)	–	95

FW: freshwater, SW: seawater.
Relative growth rate, % = (final wet weight – initial wet weight/initial wet weight) × 100.
Specific growth rate,° % growth/day = ((ln(final weight) – ln(initial weight))/(days)) × 100.
Feed conversion ratio = feed intake (g)/weight gain (g).
Survival rate (%) = (number of fish at final/number of fish at initial) × 100.

Table 3. Biweekly temporal variations of seawater quality parameters in the experiment site

Parameters	Temperature (°C)	Salinity (‰)	pH	DO (mg/L)
Freshwater facility				
February, 27	09.00	0.00	8.00	8.10
Transport tank	12.00	freshwater	8.00	8.90
Seawater facility				
March 1	11.00	28.00	8.25	8.10
March 7	11.00	28.20	8.30	8.00
March 21	11.20	27.30	8.20	8.70
April 4	12.30	27.50	8.10	9.00
April 18	12.50	28.40	8.32	9.20
May 2	15.20	26.50	8.25	10.60
May 16	16.30	26.50	8.25	9.20
May 30	16.50	26.20	8.25	8.50
June 6	22.00	24.00	8.20	7.70

Water quality parameters

At the freshwater farm where the experimental fish were obtained, the water quality parameters of temperature, pH and dissolved oxygen were recorded as 9°C, 8.0, and 8.9 mg L^{-1}, respectively. When the transport tank reached the marine research facility after a drive for 2.5 h, the temperature, pH and dissolved oxygen in the transport water were measured as 12°C, 8.0, and 8.9 mg L^{-1}, respectively. Fish transferred from the freshwater facility to marine station were acclimated to a saline water of 28‰, which was also the salinity of seawater when fish were caged after adaptation process in indoor tanks. At cage farm site, seawater temperature, salinity, pH and dissolved oxygen (DO) ranged between 11 and 22°C, 23.70 and 28.40‰, 8.22 and 8.32, and 7.70 and 10.60 mg L^{-1}, respectively, throughout the study period (Table 3).

Biometric indices

Several body indices of seawater-acclimated rainbow trout after 100 days of seawater growth have been given in Table 4. Body indexes of VSI, MFI, HSIs of rainbow trout did not alter significantly ($P<0.05$) by the end of 100-day period.

Hematological variables

Hematological variables of rainbow trout in freshwater prior to seawater transfer, and for the following 100 days of monitoring thereafter, have been given in Table 5. The RBC count, Hgb and Hct values increased significantly ($P<0.05$) when fish were acclimated to seawater. The increased levels of RBC count, Hgb and Hct in seawater-acclimated trout remained almost stable until the end of day 100 ($P>0.05$). In contrast, however, the MCV, MCH, and MCHC of rainbow trout showed a significant decline ($P<0.05$), when fish was gradually transferred from freshwater to seawater conditions.

Biochemical parameters

Biochemical variables in seawater-acclimated rainbow trout by the end of day 100 are given in Table 6. An increasing trend in serum TP levels was noted in seawater-acclimated fish over the freshwater samples, however, this increase was not significant ($P>0.05$). However, the increase of ALB, GLO, GLU, TRIG and CHOL in seawater-acclimated trout compared to the freshwater samples was significant ($P<0.05$). Further increase in ALB was noted until day 60 ($P<0.05$), and then stabilized. All other parameters also stabilized, despite the slight increase until day 100, but without significance ($P>0.05$).

Table 4. Initial and final biometric indices of seawater-acclimated rainbow trout in cage conditions

Parameters	Freshwater Initial February 27	Seawater Final June 14
VSI	10.14±0.66	10.53±0.48
HSI	1.4±0.08	1.39±0.07
MFI	0.89±0.19	1.11±0.18
SSI	0.08±0.02	0.08±0.01

VSI = Viscerosomatic index, HIS = Hepatosomatic index, MFI = Mesenteric fat index, SSI = Spleen somatic index. The data presented means ± SD, n=12.

Table 5. Hematological parameters of seawater-acclimated rainbow trout in cage conditions

Parameters	Freshwater	Seawater		
	Day 0 February 27 Initial	Day 30 April 5	Day 60 May 5	Day 100 June 14 Final
RBC ($\times 10^6$ mm^{-3})	1.86±0.04 a	2.46±0.08 b	2.52±0.11 b	2.59±0.09 b
Hb (g dL^{-1})	9.58±0.16 a	9.88±0.21 b	10.18±0.40 b	10.73±0.53 b
Hct (%)	26.81±0.64 a	30.67±0.84 b	32.77±0.89 c	32.92±1.11 c
MCV (fL)	127.33±0.82 c	121.13±0.86 b	122.44±0.97 b	105.61±1.02 a
MCH (pg)	46.68±1.13 b	42.53±1.63 a	42.01±1.88 a	41.09±1.15 a
MCHC (g dL^{-1})	43.13±1.04 b	34.11±1.23 a	34.02±1.67 a	32.17±1.71 a

The data presented means ± SD, n=12.

Table 6. Serum biochemical parameters of seawater-acclimated rainbow trout in cage conditions

Parameters	Freshwater	Seawater		
	Day 0 February 27 Initial	Day 30 April 5	Day 60 May 5	Day 100 June 14 Final
Total protein (g/dL)	3.20±0.18 a	3.35±0.21 a	3.42±0.24 a	3.50±0.17 a
Albumin (g/dL)	0.23±0.02 a	0.29±0.02 b	0.38±0.04 c	0.37±0.03 c
Globulin (g/dL)	2.71±0.19 a	2.93±0.17 ab	3.16±0.21 b	3.21±0.16 b
Glucose (mg/dL)	55.64±6.15 a	66.19±4.37 b	71.16±8.33 b	72.95±7.07 b
Triglyceride (mg/dL)	79.08±4.31 b	83.85±5.19 a	96.02±6.36 b	97.08±5.88 b
Cholesterol (mg/dL)	102.18±3.15 a	112.75±5.47 b	112.61±9.78 ab	114.02±5.01 b

The data presented means ± SD, n=12.

Table 7. Immunological parameters of seawater-acclimated rainbow trout in cage conditions

Parameters	Freshwater	Seawater		
	Day 0 February 27 Initial	Day 30 April 5	Day 60 May 5	Day 100 June 14 Final
Lysozyme (µg/mL)	24.31±1.08 b	21.19±1.48 a	19.72±1.19 a	19.65±1.87 a
Myeloperoxidase (U/L)	246.16±12.83	228.74±14.88	220.15±16.13	217.5±17.01
Respiratory burst (OD630)	0.14±0.02 a	0.15±0.02 ab	0.16±0.01 ab	0.19±0.02 b
Potential killing (OD630)	0.15±0.02 a	0.17±0.01 a	0.21±0.01 b	0.27±0.02 b

The data presented means ± SD, n=12.

Immunological parameters

Immunological parameters are given in Table 7. Seawater-acclimated trout presented significantly lower ($P<0.05$) lysozyme activity compared to the freshwater counterparts. Even though a smoothly declining trend was noted over the 100 days following seawater transfer, the difference was not significant ($P>0.05$). The same trend was recorded for the myeloperoxidase parameters, with no significance between seawater-acclimated trout and their freshwater representatives ($P>0.05$). Respiratory burst and potential killing parameters increased significantly ($P<0.05$) after seawater transfer and reached the highest value in June by the end of the 100-day experimentation.

Discussion

The sales price per kg rainbow trout grown in marine systems was 220 percent higher than of those produced in freshwater in the year 2017, according to statistics provided by the United Nations Food and Agriculture Organization (FAO, 2019). The remarkable increase in market price with the increase of harvest size is an advantage for trout farming in seawater. In the 1990s, researchers reported better growth for rainbow trout in brackish waters of the Adriatic Sea (Edwards, 1984), Norwegian fjords (Dill, 1990), and the Black Sea (Yigit and Aral, 1999), the latter being the first report from the Turkish coast of Black Sea providing evidence for improved growth compared to their freshwater counterparts. Yigit and Aral (1999) underlined less daily growth for rainbow trout in freshwater (2.61%), compared to their representatives (3.37%) in brackish waters of the Black Sea with around 15–17‰ salinity. Further, it was reported that rainbow trout with 88 g initial weight reached around 295 g in 90 days at 8.4°C freshwater conditions, while their representatives in seawater with 85 g initial weight reached around 388 g by day 90, even though mean water temperature in seawater (6.9°C) was lower than that of the freshwater (8.4°C) (Yigit and Aral, 1999).

Based on the great profitability afforded by marine production in trout culture, many studies have been carried out on physiological, behavioral, and biochemical aspects encountered during seawater adaptation of diadromous steelhead trout (*O. mykiss*) (Liebert and Schreck, 2016). Alterations in blood ion concentrations during seawater transfer of smolts have been investigated in order to understand the osmotic pressure induced by the change of salinity conditions for the assessment of minimum fish size performing best seawater adaptation (Yigit et al., 2004). Recent investigations demonstrated that monitoring blood parameters provide reliable data sets related to fish health (Abdelhamid et al., 2019; Fazio, 2019). Biometric indicators of MFI, VSI, and HSI have been widely used for the evaluation of fish conditions influenced by means of factors such as diet availability or adequacy (Jobling et al., 1998; Demirci et al., 2021). Also, HSI is often used in field research in order to identify possible liver diseases in fish (van der Oost et al., 2003). As poikilothermic animals, the metabolic rate of fish is influenced by a variety of factors, but mainly by temperature (Mitranescu et al., 2010). Due to fattening around the abdomen, adverse environmental conditions may directly influence MFI and VSI values. The HSI is associated with liver energetic reserves and metabolic activities (Pyle et al., 2005). Especially, ambient temperature directly affects nutritional metabolism, that in turn may severely influence liver functions with possible fluctuations in HSIs (Adams et al., 1996; Amirkolaie et al., 2013). In addition, HSI is a parameter of overall fish health and is frequently used to assess fish stress (Lohner et al., 2001). In the present study, measured biometric indicators did not show significant differences ($P>0.05$) between freshwater and seawater representatives, that could be explained by the availability of sufficient food and nutritional conditions of adequate and non-stress environments.

Blood ion concentrations were used in earlier studies for the assessment of osmoregulatory skills of smolts from different size groups during seawater adaptation of rainbow trout (Gamperl et al., 1994; Yigit et al., 2004). Today, a wide range of scientific knowledge has been documented with endocrinological changes in trout during smoltification (Björnsson et al., 2011; Stefansson et al., 2020).

Fish blood parameters are important indicators of fish welfare and health status (Acar et al., 2019; Fazio et al., 2020), which are widely used when fish is exposed to stress condition (Yılmaz et al., 2015), nutritional altera-

tion (Kesbiç et al., 2016; Yigit et al., 2018), and diseases (Baba et al., 2017; Yılmaz et al., 2022).

Hematological parameters are directly related to energy metabolism in fish, and especially Hgb and RBC are directly linked to O_2 transport (Fazio, 2019). Earlier studies on seawater adaptation of trout, underlined increased energy requirements during the adaption process. Namely, the increase in ATPase activity is an indication of the increasing energy demand (Sangiao-Alvarellos et al., 2005). Electron transfer chain (ETC) reactions allow the synthesis of ATP, the cellular energy source. The oxygen demand of the metabolism increases with the increase of these processes (Bagkos et al., 2014). In case of high energy requirement, increases in hematological parameters are predicted during and after the adaption phase (Taylor et al., 2007). Based on the findings in the present study, hematological parameters such as RBC, Hgb and Hct increased significantly ($P<0.05$) in fish exposed to seawater transfer. This increase continued but slightly, during the seawater phase and remained at significantly higher levels ($P<0.05$) compared to their freshwater representatives.

Reference values of hematological responses found for *O. mykiss* in both freshwater and seawater conditions were comparable with those reported earlier (RBC: 0.74–4.45 × 10^6 mm^{-3}, Hgb: 6.2–12.24 g dL^{-1}, and Hct: 22.2–45%) by Field et al. (1943), Zorriehzahra et al. (2010), Fazio et al. (2016), and Nabi et al. (2022). However, once the fish were transferred to seawater, an acclimation was likely noted, because the RBC and Hgb first increased when fish were transferred from freshwater to seawater, but thereafter RBC and Hgb values slightly increased without significance ($P>0.05$) throughout the 100 days of monitoring. Macrocytic anemia, characterized by an increased MCV, is typically induced by hypoxia, toxins, parasites, bacteria and deficiency in iron (Clauss et al., 2008). This suggests that the highest Hct value in June can be explained by extreme water quality conditions, since lowest oxygen level and highest temperature in the present study were measured in June.

Osmotic acclimation and subsequent growth phase in a saline environment are known to produce changes in plasma levels of metabolites, including glucose, proteins, and lipids, in fish (Giroux and Schlenk, 2021). As these metabolites can be used to understand the welfare of the acclimated diadromous fish, the assessment of these changes can provide information about metabolic changes in fish for osmotic acclimatization and saline environments (Giroux and Schlenk, 2021). In fish, total protein, albumin, and globulin play critical functions in the immune system (Wiegertjes et al., 1996). In addition, serum lipids are important indicators used in determining the effects of various environmental conditions on fish health (Tahir et al., 2021). The liver failure and/or renal dysfunction may cause a rise in serum albumin or triglyceride under toxic conditions (Banaee et al., 2013). However, there was no sign of toxic conditions, or changes in behavior such as loss of appetite, refusing to feed, swimming disorder, symptom of disease etc., until the end of the 100-day monitoring study. Except for the total protein in the blood, all of the serum biochemistry parameters assessed in the present study, slightly increased over the 100-day monitoring trial in seawater conditions. The alterations of serum biochemical parameters observed over the 100-day seawater phase, might be attributed to the fact that fish absorb more nutrients from the diet as the water temperature increases, and/or changes in water quality parameters during the course of the trial from March to June.

In this study, the hyperglycemic response showed that the fish produced more glucose to be used for energy needed in the fight against stress caused by seawater conditions. Increased levels of serum glucose were recorded after fish transfer to seawater condition however, the effect did not persist over time in seawater. Furthermore, while a rise in blood glucose levels is a secondary sign of stress, it is also a metric that varies based on factors including nutrition, appetite, and satiety status (Heath, 2019; Sahin et al., 2021). Hence, the significant increase of blood glucose in fish transferred to seawater from freshwater environment, was a clear indication of a stressful exposure. However, further monitoring of fish in seawater conditions over the 100-day period, showed that blood glucose levels did not increase significantly, an indication of well-adapted fish to higher salinity of 28‰, without serious stress encounters.

Respiratory burst and potential killing activities in blood, as well as serum lysozyme and myeloperoxidase activity, are the most common criteria used to assess the immunological status of fish (Das and Sahoo, 2014). In this study, the highest values for lysozyme activity and myeloperoxidase were seen in March before the seawater transfer, followed by a downward trend thereafter during the seawater phase, reaching the lowest level by day 100. In June, respiratory bursts and potential killing activities were at an all-time high level. There are limited studies available on the impacts of water temperature on immune response in rainbow trout. Increased respiratory burst activity in rainbow trout was found at higher acclimation temperatures (Nikoskelainen et al., 2004), which clearly support the findings in this study, where respiratory burst activity increased with the increase of water temperature from 11°C in March to 22°C in June. In contrast to the present study, however, Ndong et al. (2007) reported that respiratory burst activity decreased when Mozambique tilapia were transferred from 27°C to 19, 31 and 35°C, over 24–96 hours. Further, increasing temperatures from 10 to 19°C did not show any effect on respiratory burst activity in juvenile Atlantic cod (Pérez-Casanova et al., 2008).

Immunological memory is not found in the innate immune system in fish, as far as we know. Furthermore, the innate immune system of fish has a faster reaction time than the specialized immune system (Anderson et al., 1992). In addition, innate defenses are formed by the combination of many humoral and cellular components,

and they might have varied specificity in the presence of any immune-stimulating substances (Secombes, 1996). In this instance, each immune-related activity might achieve its maximum level, which then fades away due to time, biotic, or abiotic influences. The health and well-being parameters of rainbow trout revealed adequate development following seawater adaptation to 28‰ salinity, according to biochemical and immunological study through long-term monitoring of blood parameters in seawater acclimated rainbow trout.

In conclusion, this study provides evidence that gradual acclimation (48 h) of rainbow trout from freshwater to high-saline (28‰) sea conditions did not cause detrimental effects on fish health parameters. Based on health indicators and immunological factors related to blood values in sea-acclimated rainbow trout, it was observed that well-adapted fish remained in healthy status over the following 100 days after seawater transfer. According to the results in this study, extension of trout farms from brackish waters to more-saline marine environments with up to 28‰ salinity seems to be possible, without any detrimental effect on health status of fish over a long-term production course of 100 days. These findings may support decision makers for environmental management and planning of new potential aquaculture sites towards sustainable expansion of trout aquaculture industry in European waters.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

We acknowledge that all authors have contributed significantly, and confirm that all authors are in agreement with the content of the manuscript.

Ethical Statement

All practices during the study were carried out by European Union Directive no: 2010/63 and the approval of Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (Decision no: 2017/10-07).

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Conflict of interests

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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