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Comparison of Growth Performance and Smoltification of Triploid and Diploid Black Sea Trout (*Salmo labrax*) in Freshwater and Seawater

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Abstract

This study aimed to evaluate the growth performance and smoltification process of triploid Black Sea trout, produced using the pressure shock method. Length, weight, survival rate (SR), specific growth rate (SGR), condition factor (CF), feed conversion rate (FCR), haematological parameters (WBC, LYM, MID, GRAN, RBC, HGB, HCT, MCV, MCH, MCH, MCHC, PLT, MPV, PDW), stress responses (cortisol and glucose values), osmoregulatory capacity indicators, Ca, Cl, Na, and K levels in blood serum were examined. The triploid groups showed higher length, weight and specific growth rate in both freshwater and seawater with a salinity of 18‰ ($P<0.05$). After a 90-day growth period, the best growth performance was seen in triploid freshwater group with the mean initial lenght of 13.60±0.06 cm and weight of 26.60±0.45 g. The final length, weight and specific growth rate of this group were 22.10±0.21 cm, 139.32±3.35 g and 1.84±0.13, respectively. Temporal changes in blood Ca, Cl, Na, and K ion levels in blood, cortisol and glucose levels showed that the adaptation ability of triploid fish to seawater might be different from that of diploids. The cortisol values of triploid groups were lower than that of diploid groups in freshwater but higher in seawater, while glucose values showed fluctuations between groups during the whole study period ($P<0.05$). Ca reached the highest values in triploid freshwater group on day 17, Cl in diploid and triploid seawater groups on day 24 and Na in triploid seawater group on day 17 ($P<0.05$). K value showed no statistical difference between the groups throughout the study. Understanding the physiological and biological responses of fish to environmental changes during seawater transition and adaptation is expected to enhance the survival and growth performance of diploid and triploid Black Sea trout.

Introduction

Aquaculture has emerged as an important tool to provide sustainable fish production in the world. To ensure sustainability in fish farming and increase productivity, various biotechnological approaches are applied (Çakmak et al., 2022; Song et al., 2023). Triploid fish production is one of the most widely used biotechnological applications in aquaculture. Triploid fish are preferred due to their infertility, increased biomass, and better fillet quality as they divert metabolic energy to somatic growth rather than gamete formation. In addition, this application is recommended by international organisations such as NASCO, FAO, ICES as infertile triploid fish are unlikely to disrupt natural stocks if they escape into the wild (Yılmaz et al., 2017; Sonay et al. 2021). Despite its disadvantages, triploidization is commonly used in trout farming to support sustaniable aquaculture (Poontawee et al., 2007; Aydin, 2021; Crouse et al., 2021; Crouse et al., 2023; Sonay et al., 2024).

Triploidy is induced by applying physiological shocks (low or high temperature, hydrostatic pressure and chemical shock) to freshly fertilised eggs.

Temperature shock is the most cost-effective and simple method, whereas pressure shock is the most effective and successful. Triploidy can be achieved by blocking the second meiosis and retaining the second polar cell after fertilisation (Felip et al., 1997; Başçınar & Sonay, 2009; Piferrer et al., 2009; Akhan et al., 2011a; Preston et al., 2013; Madaro et al., 2022). Studies on the growth performance of triploid and diploid fish in freshwater and seawater have produced conflicting results, with some indicating superior performance by triploids (Thorgaard & Gall, 1979; Taylor et al., 2011; Schafhauser-Smith & Benfey, 2001; Sonay et al., 2024) and others favoring diploids (Galbreath et al., 1994; Chiasson et al., 2009; Sacobie et al., 2015). In some cases, growth performances of triploids and diploids were found as comparable (McGearhy et al., 1996; Wagner et al., 2006).

Trout experience significant stress when transitioning from freshwater to seawater environments. During the initial period of seawater transfer, trout undergo morphological changes (e.g., streamlined body, darker fin margins, loss of body spots, silver coloration) and physiological adjustments to mitigate salinity effects and maintain homeostasis (Taylor et al., 2007; Ge et al., 2021; Xiang et al, 2022). Each species has its own salinity tolerance limits to maintain normal physiological activities. Optimum salinity levels vary depending on the life stage (growth, reproduction and juvenile) and species (Sonay & Başçınar, 2017). Both diploids and triploids exhibit tolerance to salinity changes, although seawater can significantly impact their growth (Bœuf & Payan, 2001; Fraser et al., 2022). Previous studies on triploid salmonids have examined seawater adaptation, survival and growth rates, and responses to acute and physiological stressors (Biron & Benfey, 1994; McGeachy et al., 1994; McGeachy et al., 1996; Benfey & Biron, 2000; Sadler et al., 2000; Cotter et al., 2002).

In this context, the Black Sea trout (*Salmo labrax*) is a promising anadromous species for its use of triploid technology. Given its ecological and economic significance, the effects of triploid trout production on this species requires thoroughout investigation. Black Sea trout are naturally distributed in the Eastern Black Sea rivers such as Fırtına, Çağlayan, Kapistre, Solaklı, Baltacı and İyidere. The species spawns in freshwater and upon reaching smolt size (11.5 cm and over length), it moves downstream to the river estuary and then migrates to the sea after finishing the adaptation process to seawater salinity (Tabak et al., 2001; Çakmak et al., 2008; Çakmak et al., 2018). Black Sea trout is cultured in many trout farms in the Eastern Black Sea Region in Türkiye,

This study aimed to compare the growth performance and smoltification process of triploid Black Sea trout fry, induced via pressure shock, with their diploid counterparts in both freshwater and seawater.

Materials and Methods

Supply of Triploid and Diploid Black Sea Trout

The broodstocks for producing triploid and diploid fish were sourced from the 7th-generation marine eco-type Black Sea trout broodstock cultured at the Central Fisheries Research Institute (SUMAE) Trabzon, Türkiye. Fish eggs were fertilized using the dry milking method, and triploidization was induced at 40, 45, and 50 minutes through shock application at a pressure of 9,500 psi for 5 minutes at 10°C. Triploidization studies on Atlantic salmon, rainbow trout and other brown trout species by pressure shock method were generally accepted as reference for triploidization (Chourrout, 1984; Yesaki et al., 1996; Couture et al., 2007; Loopstra and Hansen, 2008; Taylor et al., 2012; Preston et al., 2013; Lahnsteiner and Kletzl, 2018). A Triploid High Pressure Machine (İleri Otonom Sistemler ve Savunma Teknolojileri Ltd. Com., Ankara, Türkiye) was used for pressure shock applications. Following shock application, the eggs were incubated at 10 °C in vertical flow incubation cabinets. To determine ploidy, blood erythrocyte and nucleus measurements were conducted on fry averaging 5 g in weight. In order to determine ploidy from erythrocyte diameter and nucleus measurements, preparations were prepared in three replicates from each fish and the major axis, minor axis, nucleus major axis and nucleus minor axis measurements (mean ± SD) of 50 erythrocyte cells from each preparation were made (Akhan et al., 2011a; Akhan et al., 2011b; Dorafshan et al., 2008). Triploid ratios were determined based on the measurement results and the groups with 100% triploid ratio were used for a smoltification follow-up experiment (50 minutes after fertilisation via shock application at a pressure of 9.500 psi for 5 minutes at 10 °C).

Growth Performance

In order to determine the growth performance and smoltification changes of triploid and diploid individuals in freshwater and seawater, four groups with three replicates were formed. These were DF (diploid freshwater), TF (triploid freshwater), DS (diploid sea water) and TS (triploid sea water) groups. Two different recirculating aquaculture systems (RAS) were used. In the sea water RAS the main water parameters included an average salinity of 18 ‰, water temperature of 14±1°C, pH of 7.5±0.5, and dissolved oxygen levels of 7±1 mg/L. In the freshwater RAS ll water parameters (water temperature, pH, and oxygen levels) matched those of the seawater system, except that salinity was 0 ‰. In both RAS, ammonia was measured below 0.02 mg/l and nitrite was measured below 0.01 mg/l. Considering the stocking density, a total of 12 fibreglass tanks of 100x100 cm were used during the study, 6 tanks in the seawater unit and 6 tanks in the freshwater unit.

A total of 2400 fish were used to determine growth performance. Half of the fish were transferred to sea water. Each tank was stocked with 200 fish. Length and weight measurements of 30 fish randomly from each tank were made in 15-day periods. (Table 1). Clove oil (30 mg/l) (Metin et al., 2015) was used as a mild anaesthetic before the length and weight measurements of the fish. Fish were fed with commercial trout feed (SÜRSAN Su Ürünleri San. ve Tic. A.Ş.) (crude protein: 45%, crude fat: 20%, crude cellulose: 2%, ash: 6%) at the rate of 2% of biomass for two meals a day (09:00-17:00). The study was performed for 90 days. Growth performance and feed conversion values were determined using the following equations (Cho and Bureau 1998; Korkut et al., 2007; Akhan et al., 2010):

Specific growth rate:

$$SGR=\left[\frac{\ln(W_f)-\ln(W_i)}{t}\right]x100 \quad (1)$$

Condition factor:

$$K=\frac{100xW}{TL^3} \quad (2)$$

Feed conversion rate:

$$FCR=\frac{\text{Feed eaten by fish}}{\text{Weight gained by fish}} \quad (3)$$

Survival rate:

$$SR=\frac{\text{Final number of fish}}{\text{İnitial number of fish}}x100 \quad (4)$$

Where; Wf: final weight, Wi: initial weight, t: time (days), TL is total length of a fish.

Smoltification Tracking

Smoltification was monitored alongside the growth performance study. Hemogram parameters included WBC (white blood cells, 10^3/µL), LYM (lymphocytes, 10^3/µL), MID (monocytes, 10^3/µL), GRAN (granulocytes, 10^3/µL), RBC (red blood cells, 10^6/µL), HGB (hemoglobin, g/dL), HCT (hematocrit, %), MCV (mean cell volume, fL), MCH (mean cell hemoglobin, pg), MCHC (mean cell hemoglobin concentration, g/dL), PLT (platelet count, 10^3/µL), MPV (mean platelet volume, fL), and PDW (platelet distribution width, %). Biochemical parameters analyzed included potassium (K, mmol/L), sodium (Na, mmol/L), chloride (Cl, mmol/L), calcium (Ca, mmol/L), glucose (mmol/L), and cortisol (ng/mL). For hemogram analysis, blood samples were taken on 0, 3, 7, 12, 17, 31, 38, 52 days from two fish of each group. Their blood was collected in purple capped etda tubesand and measurements were made with fulotomatic hematology analyzer (PROKAN PE-6800 VET, China) using a cold chain system in the fish health laboratory of Recep Tayyip Erdoğan University, Faculty of Fisheries.

For smoltification follow-up, blood samples of three fish from each group were taken into yellow-capped gel tubes on days 0, 3, 7, 12, 17, 24, 24, 31, 38, 45 and 52 of the study. The serum was removed from blood samples by centrifugation at 5.000 rpm for 10 minutes. Plasma ions (sodium (Na), chloride (Cl), potassium (K), and calcium (Ca), glucose and cortisol values serum portions of the samples were analysed by a private laboratory (Teknik Ortopedi Kimya San. Tic. Ltd. Şti. Konya, Türkiye). Sodium, chlorine and potassium were analyzed by ion-selective electrolyte (ISE) method in electrolyte device, calcium and glucose were analyzed by the spectrophotometric method in biochemistry autoanalyzer and cortisol was analyzed by immunoassay method in hormone analyzer.

Statistical Analysis

Sigmaplot 11 (SYSTAT Software, Inc., Chicago, IL, USA) and Microsoft Office Excel 2016 Pro. softwares were used for statistical analyses. The normal distribution of all data was tested using the Shapiro-Wilk test. The comparison of diploid and triploid erythrocyte measurements were analysed by t-test or by the Mann-Whitney test when the data did not have a normal frequency distribution, growth performance and smoltification groups were compared by two-way ANOVA and differences between groups were determined by Duncan's Multiple Range test ($P<0.05$).

Table 1. Mean values (± SEM) of length (cm), weight (g), specific growth rate (SGR), condition factor (CF), feed conversion rate (FCR) and survival rate (SR; %) for triploid and diploid of Black Sea trout (*Salmo labrax*) in freshwater and seawater.

	Diploid		Triploid	
Parameter	**Freshwater**	**Seawater**	**Freshwater**	**Seawater**
L_i (cm)	13.25±0.06	13.22±0.06	13.60±0.06	13.60±0.05
L_f (cm)	18.99±0.19^a	18.40±0.11^a	22.10±0.21^c	21.09±0.17^b
W_i (g)	25.21±0.51	24.63±0.49	26.60±0.45	27.01±0.32
W_f (g)	91.89±1.69^b	78.11±1.42^a	139.32±3.35^d	102.08±1.79^c
SGR (% day^{-1})	1.44±0.05^a	1.28±0.05^a	1.84±0.13^b	1.48±0.04^a
CF (g/cm^3)	1.34±0.03^b	1.25±0.01^b	1.29±0.05^b	1.09±0.03^a
FCR	1.00±0.02	1.06±0.01	0.99±0.03	0.99±0.02
SR (%)	95.33±4.67	90.68±2.44	100±0.00	96.33±1.89

The superscript letters in the same row represent significant differences between the groups ($P<0.05$). W_i; Initial weight, W_f; Final weight, L_i; Initial length, L_f; final length, SGR: Specific growth rate, CF: Condition factor, FCR: Feed conversion ratio, SR: Survival rate.

Results

Determination of Triploidy

The mean length and width of erythrocytes were 15.39±0.52 µm and 8.66±0.16 µm in diploids, 20.31±0.38 µm and 11.77±0.39 µm in triploids, respectively. Nuclei length and width were 7.19±0.27 µm and 3.98±0.42 µm in diploids, 9.23±0.25 µm and 4.69±0.37 µm in triploids.

Growth Performance

Analysis revealed significant differences in the growth parameters of diploid and triploid Black Sea trout across freshwater and seawater conditions ($P<0.05$). The highest length (22.10±0.21 cm), weight (139.32±3.35 g), and specific growth rate (1.84±0.13) were determined in triploid freshwater group (Table 1; Figure 1). The lowest CF value was observed in the triploid seawater group ($P<0.05$), while FCR values did not differ significantly among the groups (Table 1; Figure 1). Although FCR values did not differ significantly, triploids exhibited lower values compared to diploids. The highest survival rate (100±0.00%) was recorded in the triploid freshwater group. In addition, triploid groups showed greater length and weight gain in both water environments compared to diploid groups. When diploid and triploid groups were evaluated in freshwater and seawater, both groups showed a superior growth performance in freshwater.

Blood Parameters and Smoltification Tracking

Results of blood analyses (WBC, LYM, MID, GRAN, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, MPV and PDW) are presented in Tables 2 and 3. The results of blood analyses, MID and HGB on day 0, MID, GRAN, HGB, HCT, MCV and PDW on day 3, WBC, LYM, MID, GRAN, RBC, HGB, HCT, MCH, MCHC, PLT, MPV and PDW on day 7, PDW on day 12, PDW on day 17. day, WBC, LYM, MID, HGB, HCT, MCV, MCH, MCHC and PLT, MCV, PLT, MPV and PDW on the 31st day, and MCH, MCHC and PLT on the 52nd day reached the highest value ($P<0.05$) (Table 2, 3). Minimum and maximum values of these parameters were not determined for trout. However, when compared within groups and between environments, it is possible to have an idea about the general health status of fish (Fazio, 2019).

Cortisol, which is one of the most important indicators of stress, glucose, Ca, Cl, Na and K values in blood serum are given in Figure 2. Cortisol levels ranged from 52 to 61 ng/ml in all groups on day 0. After the 7th day, levels were lower in freshwater groups compared to seawater groups. The change between seawater and freshwater continued until the end of the study (Figure 2). The highest glucose level was recorded in the diploid seawater group (9.64 mmol/L) on day 7, while the lowest was observed in the triploid seawater group (3.61 mmol/L) on day 38. On day 7, the diploid marine group, on days 17 and 38 diploid and triploid seawater and freshwater groups and on day 45 diploid seawater group showed statistical differences (Figure 2). While Ca

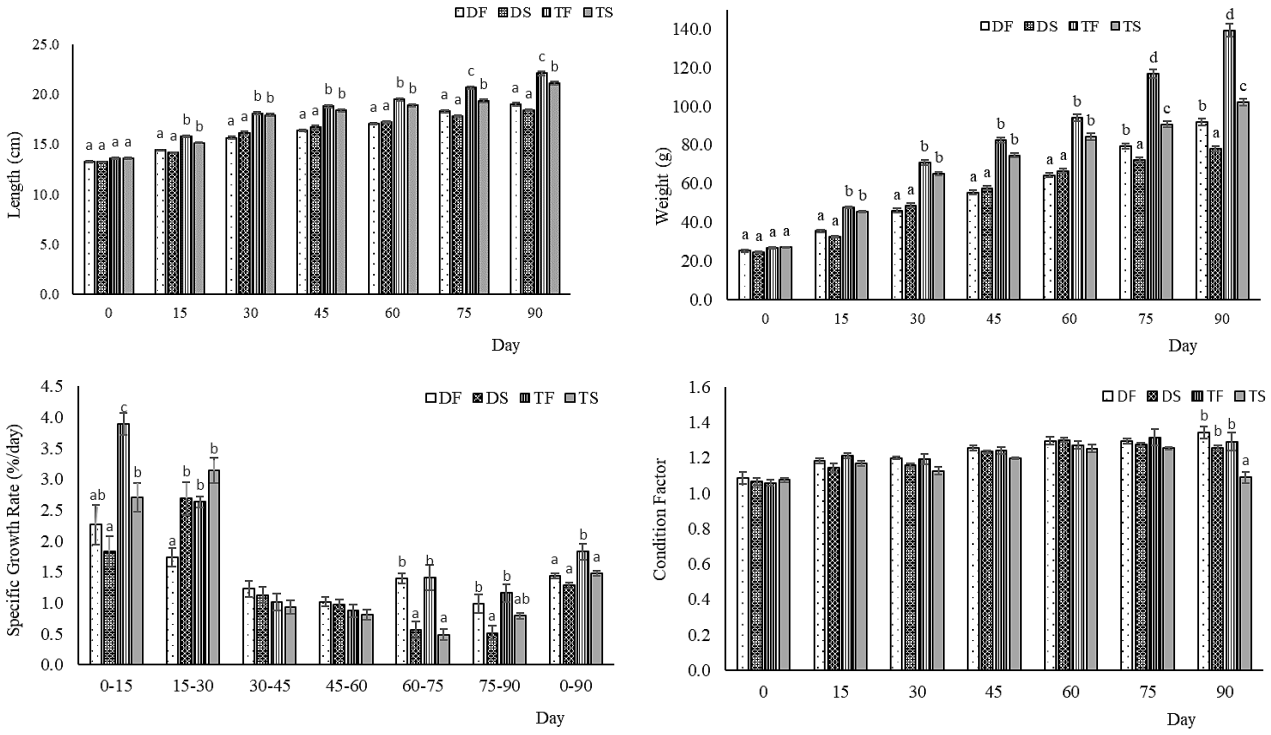

Figure 1. Mean values (±SEM) of weight, total length, specific growth rate (SGR), and condition factor (CF) of triploid and diploid Black sea trout (*Salmo labrax*) in freshwater and seawater (DF: Diploid freshwater, TF: Triploid freshwater, DS: Diploid sea water, TS: Triploid sea water groups). Letters on the bars indicate that the difference between the groups is significant ($P<0.05$).

values showed fluctuations during the study period, Cl values showed higher values in seawater groups than freshwater groups except for days 0 and 38. As expected, Na values, one of the important indicators of smoltification, were higher in seawater groups between days 3 and 24 ($P<0.05$). The highest Na value in the diploid seawater group was 166.67 mmol/L on the 7th day of the study, while the highest sodium value in the triploid seawater group was 171.33 mmol/L on the 17th day. K values were similar between all groups except day 3 (Figure 2).

Discussion

The superior growth of triploid fish and the interest of aquaculture producers have increased the production of triploid fish in the world. The effects of triploidy may vary for different species (Fast et al., 1995). This difference in growth can be explained by the fact that triploids have more genes, larger nuclei, cell structure and function, and different stages of gonadal development than diploids (Maxime, 2008; Preston, 2014; Sonay et al., 2024). Growth performance in triploid fish varies not only across species but also among individuals within the same species. While Galbreath et al. (1994) found that triploid Atlantic salmon (*Salmo salar*) exhibited faster growth during early stages, McGeachy et al. (1995) observed slower growth in the same species. Studies across various species and life stages have shown that diploids often exhibit similar or superior growth compared to triploids (Gervai et al., 1980; Cassani and Caton, 1986; Fast et al., 1995; McGeachy et al., 1996; Sacobie et al., 2015; Koedprang and Na-Nakorn, 2000; Teuscher et al., 2003; Aydın et al., 2021; Fraser et al., 2021; Meng et al., 2022). In general, the results of the studies (Wolters et al., 1982; Benfey, 1999; Maxime, 2008; Kızak et al., 2013; Sonay et al., 2024) show that triploid fish have better growth and better feed conversion rates compared to diploids. Triploid fish reach higher weights, SGR and CF values, indicating that they have a more efficient feed conversion ratio and better energy efficiency (Schafhauser-Smith and Benfey, 2001; Glover et al., 2013; Weber et al., 2014; Aydın et al., 2021; Chapinduka et al., 2022; Meng et al., 2022). Triploid groups showed higher length, weight and specific growth in both freshwater and seawater. In addition, when diploid and triploid groups were evaluated within themselves, the

Table 2. Changes in some blood parameters (WBC, LYM, MID, GRAN, RBC, HGB, HCT) for triploid and diploid of Black Sea trout (*Salmo labrax*) in freshwater and seawater (Mean ± SEM). (DF: Diploid freshwater, TF: Triploid freshwater, DS: Diploid sea water, TS: Triploid sea water groups).

Day	Groups	Blood Parameters						
		WBC (10^3/µL)	LYM (10^3/µL)	MID (10^3/µL)	GRAN (10^3/µL)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)
0.	DF	31.22±1.22	28.93±0.96	1.20±0.15^{a}	0.92±0.16	0.96±0.06	8.87±0.43^{a}	16.62±0.57
	TF	32.37±0.91	29.75±0.80	1.53±0.09^{b}	1.08±0.06	0.96±0.10	8.67±0.35^{a}	16.62±1.16
	DS	33.48±1.97	31.30±1.77	1.30±0.15^{a}	0.88±0.11	1.16±0.12	10.08±0.78^{b}	18.88±1.54
	TS	33.73±0.67	31.30±0.58	1.73±0.12^{b}	0.85±0.07	1.06±0.03	9.38±0.19^{b}	18.38±0.50
3.	DF	30.10±2.56	28.17±2.27	1.13±0.23^{a}	0.80±0.09^{a}	1.00±0.06	8.77±0.41^{a}	15.37±0.94^{a}
	TF	29.53±1.63	27.77±1.49	1.10±0.15^{a}	0.67±0.05^{a}	1.01±0.05	8.97±0.33^{a}	16.73±0.67^{b}
	DS	32.70±0.61	30.52±0.56	1.38±0.10^{a}	0.80±0.04^{a}	1.01±0.03	9.70±0.13^{b}	17.60±0.19^{b}
	TS	35.25±0.74	32.42±0.69	1.83±0.15^{b}	1.00±0.12^{b}	1.10±0.04	10.03±0.10^{b}	18.30±0.66^{b}
7.	DF	24.12±1.38^{a}	23.38±1.12^{a}	0.80±0.11^{ab}	0.82±0.06^{bc}	0.85±0.03^{a}	8.20±0.11^{a}	15.52±0.59^{a}
	TF	21.97±0.64^{a}	20.57±0.92^{a}	0.50±0.04^{a}	0.43±0.03^{a}	0.88±0.03^{a}	7.88±0.28^{a}	15.53±0.41^{a}
	DS	35.90±2.73^{b}	33.30±2.14^{b}	1.47±0.20^{c}	1.12±0.22^{c}	1.39±0.15^{b}	12.02±1.41^{b}	24.35±2.54^{b}
	TS	29.33±0.70^{b}	28.20±0.62^{b}	0.97±0.07^{b}	0.58±0.05^{ab}	1.07±0.03^{a}	9.53±0.30^{b}	18.68±0.41^{a}
12.	DF	30.57±1.47	28.83±1.41	1.13±0.07	0.60±0.03	0.95±0.03	9.03±0.31	17.18±0.71
	TF	29.53±1.18	27.78±1.04	1.12±0.11	0.63±0.07	0.96±0.04	8.63±0.27	17.10±0.41
	DS	28.68±0.99	27.22±0.86	0.92±0.08	0.55±0.06	0.94±0.03	8.77±0.40	16.82±0.64
	TS	30.37±1.15	28.57±1.02	1.18±0.10	0.62±0.04	1.07±0.06	9.80±0.45	18.72±0.70
17.	DF	30.55±1.26^{ab}	28.75±1.08^{ab}	1.10±0.13^{a}	0.68±0.08	1.00±0.03	9.50±0.28^{a}	16.68±0.72^{a}
	TF	33.92±1.18^{b}	31.72±1.08^{b}	1.48±0.07^{b}	0.72±0.05	1.12±0.06	11.02±0.65^{b}	19.70±0.87^{b}
	DS	28.58±1.46^{a}	27.08±1.40^{a}	0.97±0.06^{a}	0.53±0.02	0.94±0.05	9.28±0.42^{a}	17.10±0.67^{a}
	TS	28.28±1.52^{a}	26.72±1.35^{a}	0.98±0.11^{a}	0.58±0.06	1.02±0.04	9.17±0.41^{a}	17.52±0.73^{a}
31.	DF	33.63±1.59	31.63±1.23	1.30±0.25	0.70±0.12	1.13±0.04	10.63±0.35	18.70±0.85
	TF	32.50±1.59	30.47±1.42	1.37±0.15	0.67±0.03	1.09±0.04	10.10±0.35	19.50±0.64
	DS	30.37±2.46	28.53±2.17	1.17±0.22	0.67±0.12	1.09±0.11	9.57±0.75	17.97±0.96
	TS	28.33±2.28	26.87±2.05	1.00±0.15	0.47±0.09	0.99±0.03	9.50±0.97	18.07±1.08
52.	DF	31.17±1.45	29.40±1.19	1.13±0.17	0.63±0.12	1.08±0.06	10.63±0.64	17.87±1.14
	TF	33.33±0.88	31.27±0.69	1.40±0.20	0.67±0.07	1.19±0.02	11.20±0.91	20.70±1.21
	DS	31.00±0.81	29.27±0.75	1.17±0.09	0.57±0.09	1.13±0.05	10.70±0.30	19.43±0.79
	TS	28.17±2.84	26.77±2.53	0.97±0.23	0.43±0.09	1.07±0.12	9.13±1.01	18.57±1.89

Different letters on numbers in the same column indicate that the difference between groups is significant ($P<0.05$). WBC: White blood cell, LYM: Lymphocyte, MID: Monocyte, GRAN: Granulocyte, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit.

differences in growth performance in freshwater and seawater could be due to the smoltification process.

The significant weight gain and SGR observed in the triploid freshwater group are likely influenced by the smoltification process upon exposure to seawater. After diploid and triploid fish were transferred to seawater, growth and survival rates were different in seawater due to incomplete smoltification (Taylor et al., 2011, 2012). These findings suggest that triploid fish are better adapted to living conditions and have higher survival rates (Piferrer et al., 2009; Fraser et al., 2021). In different studies, triploid fish transferred to the sea showed more weight loss and mortality than diploids. This might be due to adaptation to seawater, feeding during the fry period, deformity, genetic, etc. (Fraser et al., 2013; Madaro et al., 2022).

Blood parameters may reflect physiological and metabolic differences associated with the nutritional and health status of fish (Tan et al., 2018; Köse, 2024). Fluctuations in blood parameters serve as key indicators for assessing infections, fish welfare, nutritional disorders, and environmental stressors (Sandnes et al., 1988; Ivanc et al., 2005). In particular, haematological blood analyses include red blood cell parameters, white blood cell parameters and platelet count per unit of blood volume (Ivanc et al., 2005). In this study, the variations in haematological blood parameters and fluctuations in different periods might be due to the variable internal environment of the fish and changes in environmental factors. Due to the factors affecting blood values in fish, the results obtained were evaluated by comparing with the control group. Triploid fish have fewer but larger erythrocytes which are nucleated and contain oxygen-binding hemoglobin. Blood haemoglobin concentration is expected to be low in triploid fish (Sadler et al., 2000; Jamalzadeh et al., 2008). However, in this study, although haemoglobin values showed differences in some measurements, there was no significant difference between diploids and triploids in the whole study ($P>0.05$). These results were similar to those of triploid brook trout (Benfey, 1999), triploid Atlantic salmon (Sadler et al., 2000) and rainbow trout and brook trout (Benfey and Biron, 2000) in that haematocrit and haemoglobin levels were not different between triploids and diploids despite the decrease in blood cell count in triploids.

Smoltification, a crucial adaptation process for salmonids transitioning from freshwater to seawater,

Table 3. Changes in some blood parameters (MCV, MCH, MCHC, PLT, MPV, PDW) for triploid and diploid of Black Sea trout (*Salmo labrax*) in freshwater and seawater (Mean ± SEM). (DF: Diploid freshwater, TF: Triploid freshwater, DS: Diploid sea water, TS: Triploid sea water groups).

Day	Groups	Blood Parameters					
		MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/µL)	MPV (fL)	PDW (%)
0.	DF	176.18±5.45	93.08±1.24	53.23±1.21	48.17±3.89	10.52±0.17	13.57±0.68
	TF	176.87±9.04	93.92±8.32	52.88±2.33	62.17±13.28	10.65±0.12	13.48±0.76
	DS	165.00±5.34	88.33±4.12	53.55±1.34	65.33±9.19	10.85±0.20	13.52±0.51
	TS	173.18±2.05	88.33±1.59	51.08±0.77	47.67±3.78	10.62±0.10	12.80±0.41
3.	DF	154.40±1.14^{a}	88.07±1.20	57.25±0.87	54.67±6.03	10.57±0.08	13.68±0.73^{a}
	TF	167.38±4.51^{b}	90.87±3.04	54.45±0.99	46.67±2.78	10.82±0.17	13.53±0.68^{a}
	DS	174.67±4.09^{b}	94.20±2.12	54.08±0.82	37.50±2.64	10.95±0.10	13.27±0.63^{a}
	TS	166.90±1.47^{b}	91.62±2.69	55.07±1.69	46.17±5.20	11.00±0.27	16.83±1.26^{b}
7.	DF	181.18±0.87	97.17±0.91^{b}	54.68±0.66^{b}	33.83±2.90^{a}	10.58±0.11^{ab}	11.50±0.71^{a}
	TF	177.38±0.98	91.53±0.54^{a}	51.65±0.91^{a}	30.17±1.45^{a}	10.98±0.06^{ac}	13.90±0.07^{ab}
	DS	178.75±2.08	89.85±1.08^{a}	50.83±0.77^{a}	50.17±2.04^{b}	11.32±0.19^{c}	13.47±1.86^{ab}
	TS	175.87±2.54	88.30±2.04^{a}	50.43±0.57^{a}	46.50±2.26^{b}	10.75±0.19^{ab}	14.38±0.60^{b}
12.	DF	180.42±3.41	94.72±1.93	52.62±0.76	35.83±2.24	10.50±0.19	12.08±0.61^{a}
	TF	179.47±3.99	90.27±2.63	50.40±0.62	29.50±5.98	11.13±0.25	14.25±1.30^{b}
	DS	179.00±3.24	92.80±2.34	52.10±1.41	27.17±4.38	11.13±0.13	15.18±1.16^{b}
	TS	176.17±4.28	92.22±1.78	52.55±1.05	35.17±5.56	10.82±0.16	14.43±1.10^{b}
17.	DF	167.68±3.29^{a}	95.37±1.54^{b}	57.07±0.87^{b}	35.00±5.55^{b}	10.83±0.14	14.03±0.68
	TF	175.43±3.94^{ab}	98.02±2.09^{b}	56.08±1.09^{b}	25.33±1.69^{a}	11.10±0.14	14.52±0.82
	DS	182.83±3.34^{b}	98.77±2.58^{b}	54.22±1.26^{b}	39.83±8.11^{b}	10.67±0.13	13.90±0.70
	TS	172.38±2.35^{s}	89.80±1.27^{a}	52.27±0.74^{a}	51.17±5.46^{b}	11.02±0.19	15.85±1.29
31.	DF	164.93±3.93^{a}	94.07±0.72	56.90±1.27	67.00±7.61^{b}	10.60±0.12^{a}	14.50±0.56^{b}
	TF	179.57±1.99^{b}	92.33±1.14	51.73±0.30	60.33±5.15^{b}	10.27±0.20^{a}	11.77±0.99^{a}
	DS	168.03±3.09^{a}	92.47±2.57	53.07±1.54	45.67±10.11^{a}	11.03±0.18^{b}	14.70±0.60^{b}
	TS	190.63±4.72^{b}	95.90±7.17	52.30±2.16	21.67±1.76^{a}	11.33±0.17^{b}	16.23±1.09^{b}
52.	DF	165.80±3.30	98.37±1.81^{b}	59.57±1.98^{b}	37.67±3.33^{b}	10.73±0.18	14.83±0.80
	TF	173.60±8.01	93.63±6.55^{b}	53.93±1.97^{b}	34.00±5.51^{b}	11.30±0.25	13.50±0.96
	DS	173.23±2.15	95.13±3.50^{b}	55.17±2.27^{b}	42.33±6.01^{b}	11.07±0.09	16.77±1.43
	TS	174.77±2.04	85.60±0.31^{a}	49.07±0.75^{a}	32.00±3.06^{a}	10.90±0.15	16.63±0.82

Different letters on numbers in the same column indicate that the difference between groups is significant ($P<0.05$). MCV: Mean cell volume, MCH: Mean cell hemoglobin, MCHC: Mean cell hemoglobin concentration, PLT: Platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width.

significantly impacts their survival and growth rates (Langdon, 2019; Morera et al., 2021). In order to evaluate the performance of triploid fish in this adaptation process and to reveal important findings in terms of aquaculture, blood plasma, cortisol, glucose, Ca, Cl, Na and K changes were observed in diploid and triploid groups of fish in freshwater and seawater environments. In this study, the smolt length of brown trout was 11-23 cm (mean 13 cm) as reported in the literature (L'Ab´ee-Lund et al., 1989). Changes in cortisol hormone, which is an important biochemical indicator of stress; cortisol levels were lower in freshwater groups compared to seawater groups, suggesting that the freshwater environment causes less stress in fish and the effects of the adaptation process to seawater. However, cortisol levels of triploid groups were lower than diploid groups in freshwater but higher in seawater, suggesting that triploid fish respond differently to salinity-induced stress. This findings suggests that the adaptation capacity of triploid fishes to salinity changes might be different from diploids. Because, abiotic (temperature, salinity, photoperiod, etc.) and biotic (disease, parasites, etc.) factors can cause stress in fish and other aquatic organisms since fish are more vulnerable to stress than other vertebrates (Lemos et al., 2023).

One of the primary markers of stress in fish is glucose. Glycogenolysis produces it, which is then released into the circulation to meet the cells' increased energy needs (Köse, 2024). The relationship between glucose and cortisol is critical for fish to cope with stress. Blood glucose levels are changed by cortisol as a stress reaction (Barton and Iwama, 1991; Barton, 2002).

In addition, diploid and triploid groups showed similarities and differences according to plasma Ca, Cl, Na and K values. While Cl value reached the peak level

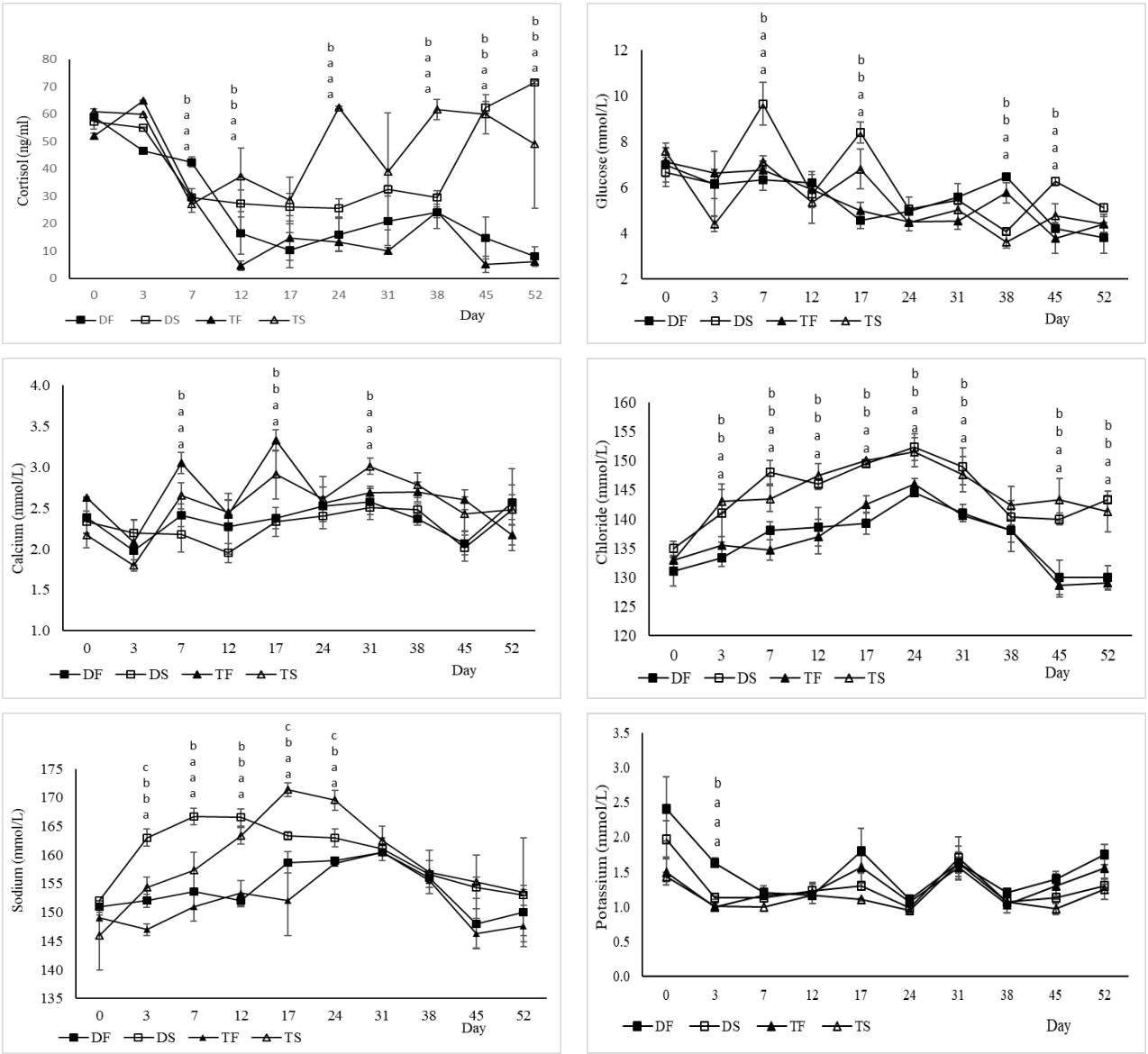

Figure 2. Means values (±SEM) of some plasma physiological and ionic parameters (cortisol (ng/ml), glucose (mmol/L), calcium (Ca) (mmol/L), chloride (Cl) (mmol/L), sodium (Na) (mmol/L) and potassium (K) (mmol/L)) of Black sea trout (*Salmo labrax*) in freshwater and seawater (DF: Diploid freshwater, TF: Triploid freshwater, DS: Diploid sea water, TS: Triploid sea water groups). Letters on the bars indicate that the difference between the groups is significant (P<0.05).

at day 24, Ca and Na values at day 17, only Na value showed a difference between diploids and triploids in seawater. Kurtoğlu and Çelikkale (2016) determined that during the smoltification process of Black Sea trout, the blood plasma sodium and chlorine ion levels of the fish increased suddenly in the first 3 days, the potassium ion level decreased until the 17th day and the physiological adaptation period of 11 cm fish lasted 17 days. Triploid and diploid groups formed similar curves in terms of chlorine values in freshwater and seawater, while no significant differences were observed between the groups. However, the higher chlorine levels in the seawater groups can be attributed to saltwater's high concentration of chlorine ions, which may also be related to the smoltification process. The chlorine levels, which increased until the 24th day of the study, decreased in the following days and remained at constant levels, indicating that the fish achieved ion balance in seawater and completed the smoltification process (Rutkovska et al., 2019; Mota et al., 2024). The fact that sodium values were higher in the seawater groups was an important indicator of smoltification as an expected finding. The decrease in sodium values, which increased until the 7th day in diploid marine groups, indicates that these fish responded faster to the smoltification process. However, in triploid seawater groups, the highest sodium value was reached on the 17th day and then the values decreased over time. This indicates that triploids follow a different adaptation pathway than diploids in the smoltification process and achieve sodium ion balance later. In both water environments, potassium values were similar in diploid and triploid groups.

Studies have shown that blood plasma changes throughout the smoltification process could vary depending on fish species, fish size, salinity, temperature, etc. (Fraser et al., 2021; Fraser et al., 2022). Although there was no statistical difference in the survival rate in triploid groups ($P>0.05$), when we evaluate it numerically, triploids had a higher survival rate than diploids. While this rate was similar to some previous studies (Fraser et al., 2022), it was different from some others (Galbreath et al., 1994; McGeachy et al., 1996).

Conclusion

In conclusion, in this study, the differences between the growth performances, stress responses during smoltification and mineral metabolism of triploid and non-shocked diploid Black Sea trout obtained by the use of locally produced triploid pressure device in freshwater and seawater environments were revealed. The findings indicate that the adaptation ability of triploid fish to seawater may be different from that of diploids. In terms of growth performance, triploid groups showed superior performance than diploids in both water environments. The results will provide important data to increase productivity in the trout farming sector and to better understand the effects of genetic interventions.

Ethical Statement

This study was carried out in accordance with the HADMEK guidelines. Experimental work was ethically reviewed, approved, and registered (January 28, 2022 - No.325.04.02-11) by the local representative of Animal Experiments Ethics Committee (SUMAE). All samplings and methods were performed as required by the Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees dated February 15, 2014.

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Author Contribution

Both authors contributed to manuscript preparation. First Author: conceiving the research, conducting laboratory experiments, data curation, interpretation, and writing; Second Author: supervision, conceptualization, data curation and interpretation, writing, review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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