



Growth Performance and Digestive Physiology of Black Sea Salmon (*Salmo labrax*) Following Seawater Transfer

Osman Tolga Özel¹ · Eyüp Çakmak¹ · İsa Coşkun² · Selin Ertürk Gürkan³ · Recayi Çimagil¹

Received: 11 February 2026 / Revised: 7 July 2026 / Accepted: 16 July 2026 / Published online: 29 July 2026
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2026

Abstract

The present work was performed to determine the changes in digestive physiology during parr-smolt transformation of Black Sea salmon (*Salmo labrax*), reached smoltification size, fed in freshwater (RAS) and seawater conditions. The fish was randomly placed in experimental tanks. The experimental was performed as triplicate, and lasted for 90 days. The initial weights of fish were 18.53 ± 0.07 g in freshwater and 18.57 ± 0.02 g in seawater. The fish were fed by hand three times a day up to apparent satiation. The test diet contained 46.20% of crude protein and 14.97% of crude lipid. After transfer to seawater, the WG, SGR, FI and RGR in the first 30 days was in favor of fresh water, but no difference was observed between the groups in the following days. FW, FCR and SR were found to be similar on all trial days. Whereas thermal growth coefficient was higher in seawater. The villi length was higher in seawater, but muscularis thickness in freshwater. In additional, goblet cell count and VL/WV were not differences in freshwater and seawater. The activity of pepsin was higher in freshwater. While lipase enzyme was in favor of freshwater in the first 30 days transfer later, amylase enzyme was in favor of freshwater on the 90th day. No difference was observed between the groups on the other days. While trypsin enzyme was higher in freshwater on the 30th and 60th days, no difference on the 90th day. We can say that after transfer to seawater would cause significant changes in intestinal histomorphology and some digestive enzyme activity of Black Sea salmon.

Keywords Aquaculture · Growth · Enzyme · Smoltification · Villus

Introduction

Black Sea salmon (*Salmo labrax*) is an endemic species of the Black Sea basin. Like other salmonids, it exhibits an anadromous life cycle, with its natural distribution covering the Black Sea and the rivers draining into it, as well as the Sea of Azov basin (Çakmak et al. 2025). The species comprises three ecotypes: stream, sea, and lake forms. Among these, the sea ecotype inhabits marine environments

until reaching sexual maturity and subsequently migrates to freshwater for reproduction, after which it returns to the sea (Tabak et al. 2002; Çakmak et al. 2022). Sea ecotype individuals exhibit two main migration patterns occurring in spring and autumn. In spring, parr initiate smoltification near river mouths and migrate toward the sea, while adults migrate upstream for spawning. In autumn, a predominantly unidirectional migration toward the sea occurs, including post-spawning adults returning to marine habitats and smolt individuals migrating to the sea for the first time (Tabak et al. 2002). This migration period may continue until mid-December for broodstock fish.

Due to its high economic value, attractive appearance, and desirable taste, Black Sea salmon has gained increasing importance in aquaculture in Türkiye (Tabak et al. 2002; Çakmak et al. 2022). The species is cultured using three main production systems: stream-based, dam lake, and marine systems. These systems involve different stages of production ranging from egg to smolt (≈ 12 cm), portion size (200–300 g), or larger market sizes (> 600 g to > 3000 g),

✉ Osman Tolga Özel
osmantolgaozel@gmail.com

¹ Department of Aquaculture, Central Fisheries Research Institute, General Directorate of Agricultural Research and Policies, Trabzon, Türkiye

² Department of Animal Science, Faculty of Agriculture, Ahi Evran University, Kırşehir, Türkiye

³ Department of Biology, Faculty of Science, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

depending on the production strategy (Özel 2022). However, seawater conditions in the Black Sea are suitable for cage culture only for approximately seven months per year, from November (<18–20 °C) to June (>18–20 °C).

In the commercial production of anadromous species such as Black Sea salmon, understanding the smoltification stage and associated physiological changes is critical for successful adaptation and growth performance. Premature transfer to seawater prior to complete smolt development may result in increased mortality and impaired growth due to insufficient physiological adaptation.

In the present study, experimental conditions were determined based on the available rearing systems of the research infrastructure. Freshwater fish were maintained in a recirculating aquaculture system (RAS) under stable temperature conditions, whereas seawater groups were reared in open-flow seawater tank systems where temperature naturally fluctuates. Therefore, temperature differences between groups were not experimentally imposed but rather reflected the inherent characteristics of the rearing systems used. Accordingly, this study was conducted to monitor the physiological changes during the smoltification process of Black Sea salmon individuals, whose commercial interest is increasing day by day.

Materials and Methods

Fish and Rearing Conditions

This study was used seventh filial generation (F7) of Black Sea salmon (sea ecotype) as latest culture line produced by Central Fisheries Research Institute in 2018–2019 breeding season. This study was conducted in two different aquaculture conditions: The first, freshwater recirculating aquaculture system (RAS) and the second, seawater open flow system which has supplied from Black Sea had a salinity 18‰. Fish were grown at RAS from hatchery to smolt size. Subsequently, Fish were randomly placed in 400 L (100×100 cm square with depths 40 cm) fiberglass tanks. Experiment was carried out in triplicate. The number of fish is adjusted to 60 in each replicate, for a total of 360 fish. Fish had initial weights of 18.53 ± 0.07 g (Body length: 11.41 ± 0.01 cm) and 18.57 ± 0.02 g (Body length: 11.46 ± 0.01 cm) in freshwater and seawater, respectively. The study lasted for 90 days. The fish were fed by hand three times a day up to apparent satiation. Feed size was 4 mm during the trial period. Water temperature, pH (7.67 ± 0.17) and oxygen (8.76 ± 0.21 mg/L) were recorded daily in RAS. Ammonia (0.09 ± 0.07 mg/L) was measured weekly. Water temperature was 14.62 ± 0.97 °C in freshwater and 11.48 ± 0.93 °C in seawater. Water change in trial

tanks was 24 times in a day. Trial tanks were cleaned by siphoning daily. Trial diet was presented in Table 1.

Fish Performance

For performance evaluation, fish were measured monthly. For this, fish were starved for 24 h, slightly anesthetized with benzocaine (50 ppm). Length measurement was performed using Von-Bayer scale and weight was measured using digital scales with an accuracy of 0.01 g (Holden and Reitt 1974). Fish performance was determined using equations shown below.

$$\text{Weight gain (WG)} = (\text{final weight} - \text{initial weight})$$

$$\text{Relative growth rate (RGR)} : 100 \times [(\text{final weight} - \text{initial weight}) \div \text{weight}]$$

Table 1 Formulation and proximate composition of experimental diets (%)

Ingredients	%
Soybean meal	20
Fish meal ¹	31
Wheat gluten	6
Pea protein	12
Sunflower seed meal	7
Wheat flour	12.5
Fish oil ²	11
Vitamin mix ³	0.22
Mineral mix ⁴	0.16
Vit C	0.12
Proximate composition	
Dry matter	93.86
Crude protein	46.20
Crude lipid	14.97
Ash	9.38
NFE	17.71
ME (Mj kg ⁻¹)	18.15
Gross Energy (Mj kg ⁻¹)	21.07

¹Fish meal was a blends of Atlantic herring (*Clupea harengus*) and European sprat (*Sprattus sprattus*) meals containing 10.7% of crude lipid and 65.37% of crude protein

²Fish oil was European anchovy (*Engraulis encrasicolus*) oil

³Supplied the following: retinol (Vit A) 0.6 mg, thiamine (Vit B1) 20 mg, riboflavin (Vit B2) 30 mg, niacin (Vit B3) 6 mg, calcium pantothenate (Vit B5) 50 mg, pyridoxine (Vit B6) 20 mg, biotin (Vit B7) 200 mg, folic acid (Vit B9) 0.5 mg, cobalamin (Vit B12) 0.05 mg, cholecalciferol (Vit D3) 0.05 mg, tocopherol (Vit E) 200 mg, menadione (Vit K3) 12 mg, inositol 300 mg

⁴Supplied the following: calcium iodate (Ca2IO6) 0.8 mg, cobalt carbonate hexahydrate (CoCO3·6H2O) 0.15 mg, copper sulfate pentahydrate (CuO4S·5H2O) 10 mg, ferric sulfate heptahydrate (FeSO4·7H2O) 50 mg, manganese (II) oxide (MnO) 50 mg, sodium selenite (Na2SeO3) 0.15 mg, zinc oxide (ZnO) 50 mg

$$\text{Specific growth rate (SGR)} = 100 \times [(\ln \text{ final weight} - \ln \text{ initial weight}) \div \text{days}]$$

$$\text{Thermal growth coefficient (TGC)} = 100 \times \left(\text{final weight}^{\frac{1}{3}} - \text{initial weight}^{\frac{1}{3}} \right) \div (\text{day} \times \text{temperature})$$

$$\text{Feed conversion ratio (FCR)} = (\text{feed intake} \div \text{weight gain})$$

$$\text{Survival rate (SR)} = 100 \times [(\text{final number of fish} \div \text{initial number of fish})]$$

Enzyme Analyses

The gastrointestinal tract tissue samples were taken in 45th minutes post feeding and fixed using liquid nitrogen, homogenized in porcelain mortars, and centrifuged at 10,000 rpm for 1 h at (+4 °C). The resulting supernatants were utilized for subsequent analyses. To evaluate α -amylase enzyme activity, the analytical method established by Metais and Bieth (1968) was employed, using soluble starch as the substrate. Measurements were conducted at a wavelength of 540 nm for 5 min. For the assessment of trypsin enzyme activity, the methodology utilized by Tseng et al. (1982) was adopted, employing Na-Benzoyl-DL-arginine-p-nitroanilide as the substrate. Spectrophotometric measurements were performed at a wavelength of 253 nm over a 5-minute period. Pepsin enzyme activity was assessed using a modified version of the analytical method established by Worthington (1982), as adapted by Infante and Cahu (1994). Bovine hemoglobin served as the substrate, and samples were measured at a wavelength of 280 nm for a duration of 5 min. Lipase enzyme activity was determined following the protocol outlined by Versaw et al. (1989), with spectrophotometric readings taken at 490 nm for 10 min. To interpret the results, the enzyme activity values were normalized to the protein concentration in the homogenate, which was determined using the Bradford (1976) method.

Intestinal Histomorphology

Intestine tissue samples from middle intestine at the beginning part near to pyloric caeca cut as 5 cm placed into 10% formaldehyde solution for tissue fixation processing. After fixation proses, tissue samples were cut at 5 mm thickness and inserted into tissue cassettes for dehydration. Dehydration was made with graded ethanol with 70% to 100%. After dehydration, tissue samples were kept in xylene for 15 min

and polished. After polishing, tissue samples were embedded in paraffin blocks in 3 stages for 3 h at 70 degrees Celsius. Afterwards, the paraffin blocks were cut at a thickness of 5 microns with the help of a microtome and the tissue samples were glued onto the slide. The paraffin was removed from the tissues adhered on the slide with the help of xylene and xylene was removed with the help of ethanol. Algian blue PAS staining procedure was applied for staining the tissues in according to manufacturer instructions. ZEISS Primostar HD Light microscope attached with 5 mp axio cam ERC 5s digital microscope camera was used to illustrate tissue samples and e measurements of the tissue samples.

Statistical Analyses

The statistical analyses were carried out SPSS 21.0 software. Prior to analysis, data were tested for normality and for homogeneity of variances. The effects of group, day, and their interaction were analyzed using two-way ANOVA. When significant differences were detected, Tukey's HSD post hoc test was used. Data are presented as mean $\pm$ SE, and differences were considered significant at $p < 0.05$.

Results

The growth performance is shown in Table 2. In the 30 days of feeding, WG, SGR, FI, RGR and TGC in fish fed in seawater had higher than those in freshwater. However, no differences in terms of FW and FCR. In the 60 days of feeding, no differences were observed in growth performance between freshwater and seawater except for FI and TGC. The end of experimental, growth parameters including FW, WG, FCR, SGR, RGR and FI were not differences among groups. The TGC in fish fed in seawater was higher than those of freshwater.

In the 30 and 60 days of the experiment, trypsin activity in fish fed in freshwater had higher than those in seawater, but not differences in 90 days. In all days of experimental, pepsin activity in the fish fed in freshwater had higher than those in seawater. There was no difference in terms of amylase activity among groups in the 30 and 60 days of the experiment, whereas the higher amylase activity in freshwater in the 90 days of the experimental was determined. In the 30 days of the experiment, lipase activity in fish fed in freshwater had higher than those in seawater, but not differences in 60 and 90 day (Table 3).

Although there was no difference in intestinal villi length among groups in the 30 days of the experiment, it was the higher in seawater in the 60 and 90 days of the experimental. In all days of experimental, the number of goblet cells in freshwater and seawater was found to be similar to each

Table 2 Growth performance of fish kept in freshwater and seawater

Groups (G)	FW	WG	FCR	SGR	SR	FI	RGR	TGC
Freshwater	49.31±17.79	30.78±17.78	1.07±0.08	1.57±0.05 ^a	96.11±1.67 ^a	29.39±16.28 ^a	166.16±95.86	0.11±0.01 ^b
Seawater	47.85±16.24	28.97±16.24	1.10±0.12	1.48±0.09 ^b	93.89±1.18 ^b	27.77±16.58 ^b	153.36±86.04	0.13±0.02 ^a
Sig.	NS	NS	NS	*	**	*	NS	***
Days (D)								
Day 30	29.33±0.67 ^c	10.62±0.82 ^c	1.02±0.08 ^b	1.50±0.11	96.11±2.28	10.23±0.93 ^c	56.80±4.49 ^c	0.11±0.01 ^b
Day 60	48.16±2.07 ^b	29.45±2.03 ^b	1.05±0.06 ^b	1.58±0.07	94.44±1.36	27.45±1.40 ^b	157.44±10.77 ^b	0.13±0.02 ^a
Day 90	68.26±3.93 ^a	49.56±4.02 ^a	1.19±0.07 ^a	1.50±0.07	94.44±1.36	48.05±1.74 ^a	265.03±23.02 ^a	0.13±0.01 ^a
Sig.	***	***	***	NS	NS	***	***	**
Interaction								
GXD	NS	NS	NS	NS	NS	NS	NS	**

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; $\bar{X}$: mean; $S_{\bar{x}}$: standard error; NS: not statistically significant ($p > 0.05$); Means with different superscript letters in a column are significantly different at $p \leq 0.05$, values were given as means with standard errors. *FW* (g) Final weight, *FI* (g) Feed intake, *FCR* Feed conversion rate, *WG* (g) Weight gain, *SGR* (%) Specific growth rate, *RGR* (%) Relative growth rate, *TGC* (%) Thermal growth coefficient, *SR* (%) Survival rate. All fish were measured from each replicate

Table 3 Digestive enzyme activities of fish kept in freshwater and seawater

Groups (G)	Pepsin	Trypsin	Amylase	Lipase
Freshwater	39.17±15.51 ^b	28.23±6.34	2.93±1.81	0.04±0.02
Seawater	54.25±15.95 ^a	29.94±4.62	3.39±1.44	0.04±0.01
Sig.	***	NS	NS	NS
Days (D)				
Day 30	42.35±7.49 ^b	27.52±4.12 ^b	2.38±1.07 ^b	0.03±0.01
Day 60	45.44±29.52 ^b	27.83±7.62 ^b	3.08±2.14 ^{ab}	0.03±0.02
Day 90	52.33±2.54 ^a	31.91±3.41 ^a	4.03±1.16 ^a	0.04±0.01
Sig.	***	*	**	NS
Interaction				
GXD	***	***	***	**

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; $\bar{X}$: mean; $S_{\bar{x}}$: standard error; NS: not statistically significant ($p > 0.05$); Means with different superscript letters in a column are significantly different at $p < 0.05$, values were given as means with standard errors. 6 fish were analyzed from each replicate

Table 4 Intestinal histomorphology of fish kept in freshwater and seawater

Groups (G)	VL	VW	ML	VL/VW	GC
Freshwater	285.78±69.68 ^b	69.90±18.65	57.32±21.78 ^a	8.58±2.62	44.88±16.98 ^a
Seawater	356.76±103.36 ^a	76.87±14.08	40.51±15.32 ^b	9.32±2.46	35.71±15.74 ^b
Sig.	***	NS	***	NS	*
Days (D)					
Day 30	257.21±64.22 ^c	66.00±13.30	30.81±9.11 ^c	8.03±2.32 ^b	46.25±15.67 ^a
Day 60	305.80±84.37 ^b	73.07±13.96	49.97±17.88 ^b	8.63±2.80 ^b	43.19±17.37 ^{ab}
Day 90	404.37±65.80 ^a	81.57±19.66	69.97±17.88 ^a	10.26±2.04 ^a	31.44±14.54 ^b
Sig.	***	**	***	**	*
Interaction					
GXD	***	*	***	NS	NS

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; $\bar{X}$: mean; $S_{\bar{x}}$: standard error; NS: not statistically significant ($p > 0.05$); Means with different superscript letters in a column are significantly different at $p < 0.05$, values were given as means with standard errors. *VL* Villi length, *VW* Villi width, *ML* Muscularis layer, *GC* Goblet cells. 6 fish were examined from each replicate

other. Intestinal villi width was not found difference in the 30 and 60 days of the experiment, but higher in seawater in the 90 days of the experimental. In additionally, muscularis layer was thicker in freshwater compared to seawater (Table 4) (Fig. 1).

Discussion

Growth in fish is affected by various factors such as time, temperature, photoperiod, body size, environmental conditions, management practices, nutrition, and disease (Aunsmo et al.

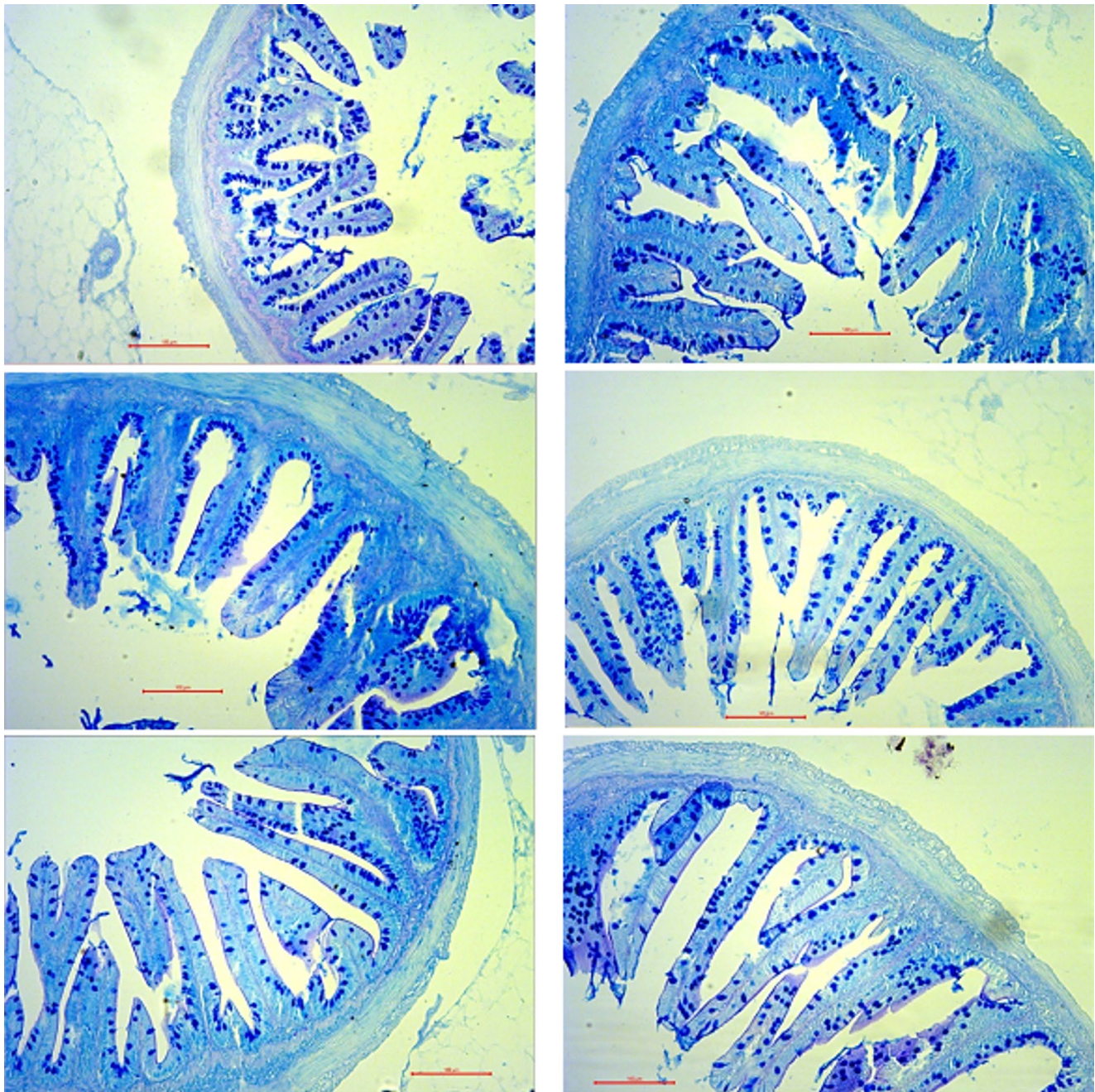


Fig. 1 The intestinal histology of fish kept in freshwater and seawater, (4x, H&E). **A:** Freshwater 30. day, **B:** Seawater 30. day, **C:** Freshwater 60. day **D:** Seawater 60. day, **E:** Freshwater 90. Day, **F:** Seawater 90. Day. 6 fish were measured from each replicate

2014). Growth performance is commonly evaluated using indices such as specific growth rate (SGR) and the thermal growth coefficient (TGC). SGR generally decreases as fish increase in size and is strongly influenced by water temperature. Growth rate typically increases with temperature up to the species-specific optimum, after which it declines (Thorarensen and Farrell 2011). TGC was developed to facilitate growth comparisons across different thermal conditions by incorporating cumulative thermal exposure and body size

into the calculation (Thorarensen and Farrell 2011; Crouse et al. 2022). However, TGC is based on assumptions regarding isometric growth and the relationship between temperature and growth, and these assumptions may not be fully satisfied when environmental factors such as temperature and salinity vary simultaneously. Therefore, its interpretation should be made with caution. The discrepancy between TGC and SGR observed in the present study likely reflects the mathematical properties of these indices rather than

a true biological advantage. Because TGC incorporates cumulative thermal exposure, fish achieving similar weight gain under lower temperature conditions may exhibit higher TGC values without necessarily demonstrating superior growth efficiency. Accordingly, the higher TGC observed in the seawater group should not be interpreted in isolation as evidence of superior growth performance but rather considered together with SGR, final body weight, FCR, and the other measured growth parameters.

Growth in fish is affected by various factors such as time, temperature, light, body size, environment, management, nutrition and disease (Aunsmo et al. 2014), and be estimated using two important indexes such as SGR and TGC. Of these, SGR tend to decrease as the fish gains weight, and affected significantly by water temperature. SGR increases until the temperature reaches the optimum requirement for growth, beyond which it decreases (Thorarensen and Farrell 2011). The second is TGC, which is more useful and flexible than SGR. It expresses growth rate in fish independently of temperature and size (Thorarensen and Farrell 2011), and can be eliminated the effect on growth rate of water temperature (Crouse et al. 2022). However, TGC is based on assumptions regarding body growth and the relationship between temperature and growth, and therefore its interpretation should be made cautiously when environmental variables such as temperature and salinity differ simultaneously. Furthermore, (Thorarensen and Farrell 2011) noted that although TGC can be a model for predicting growth performance in the long term, it can be variable in the short term and may fluctuate seasonally. The difference between TGC and SGR observed in the present study likely reflects the mathematical properties of these indices. Because TGC incorporates cumulative thermal exposure, fish achieving similar weight gain under lower temperature conditions may exhibit higher TGC values without necessarily demonstrating superior biological growth performance. Therefore, TGC should be interpreted together with conventional growth indices rather than in isolation. Our study found that although the fish were reared in seawater at sub-optimum temperatures, growth performance was similar to that of those reared in freshwater conditions under optimum temperatures. Although TGC was significantly higher in seawater, other growth indicators including final weight, SGR, FCR and weight gain did not differ between treatments. Therefore, the higher TGC should be interpreted as a temperature-adjusted growth response under the experimental conditions rather than direct evidence of superior growth performance in seawater. Because freshwater and seawater groups were maintained under different temperature regimes, the observed TGC difference likely reflects the combined influence of salinity and temperature rather than salinity alone. Indeed, Crouse et al. (2022) reported

that the general opinion is that growth in fish will increase in parallel with the increase in water temperature. Araújo et al. (2023) reported that growth in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) was better at temperatures of 16 and 20 °C than at lower temperatures. Crouse et al. (2022) found that FW, FI, FCR and SR of Atlantic salmon (*Salmo salar*) at freshwater RAS were similar at 12 and 14 °C treatments, but TGC had higher value in the low temperature (12 °C). Our finding obtained on TGC was similar to that of Crouse et al. (2022), stated that high TGC in the low temperature provided a more biologically balanced environment. Unlike our study, besides, Sonay and Başçınar (2017) found that in the Brook trout (*Salvelinus fontinalis*) weighing approximately 1 g transferred to seawater in monoculture environment, TGC was statistically similar to freshwater at the end of 45 days. This may differ depending on factors such as season, temperature, adaptation, time and fish size etc. Indeed, Kantserova et al. (2017) reported that parr individuals resident at freshwater experience independent but coordinated morphological, behavioral, physiological, and biochemical transformations for survival and growth in the seawater during smoltification or parr-smolt transformation.

Black Sea salmon to be adapted to the Black Sea salinity must be at least 12 cm in length and 15 g in weight. The fact that the fish to be transferred to the sea water are larger than the smolt size facilitates the adaptation (SUMAE 2010). Moreover, Black Sea trout bigger sized than 11.5 cm length can be successfully adapted to Black Sea salinity (Kurtoğlu and Çelikkale 2016). Brown trout has genetically a high growth potential in seawater that can be maintained up to 3 kg (Bonnet et al. 1999). This may be due largely to the food chain, in addition to salinity. In previous study, van Rihn et al. (2021) reported that poor growth performance can be seen in commercial salmon culture in the early stages of the seawater growth phase. Indeed, Jonsson et al. (2001) reported that the limiting of nutrients on fish growth is less in seawater than in freshwater. Growth performance in fish may differ in marine cages or seawater-fed tank systems or in freshwater RAS or freshwater-fed ponds. Kurtoğlu and Çelikkale (2016) reported that SGR obtained in Black Sea trout grown in freshwater and seawater was found to be high in favor of the freshwater. We found that SGR were found to be similar in both aquatic environments at the end of the experiment. This may be due to temperature, trial period or physical and chemical parameters of water resources. In our study, during the first 60 days after transfer to seawater, a temporary loss of appetite associated with early adaptation stress was observed. This resulted in a temporary decrease in feed intake. However, the return of feed intake to normal on day 90 indicates successful adaptation to seawater. The unchanged growth and FCR suggest that the negative

impact of the transfer process was minimal. In the 90th days, furthermore, the gradual decrease in feed consumption and its statistical equalization is also an indication that the fish has completed its thermal adaptation. Indeed, Walde et al. (2022) reported that at sea condition, the appetite and growth of Atlantic salmon are greatly affected by temperature as well as weight; growth decreases during winter and appetite increases as day length increases, and growth increases with increasing temperatures up to about 12–19 °C and then decreases as temperature increases. Moreover, Kurtoğlu and Çelikkale (2016) found that specific growth rate in the first generation Black Sea trout grown in freshwater and seawater was 1.03–1.47 and 1.06–1.08, respectively. In our study, it varied between 1.55 and 1.58 in freshwater and 1.43–1.55 in seawater. The fact that our results are higher reveals that the applied selectivity breeding programs make a positive contribution to the improvement of the culture characteristics of the species. Thodesen et al. (2001) reported that growth in Atlantic salmon may be improved by selective breeding.

Fish adopt various strategies to regulate their intrabody fluids depending on water salinity, which may affect the activity of digestive enzymes (Ciavoni et al. 2024a). The enzyme activities in the gastrointestinal tract of fish varies according to the different feeding habits of the species (Mardones et al. 2018). For instance, the feed intake and stomach fullness affect the secretion rate of trypsin, while nutritional status of fish affects its activity rate (Sunde et al. 2001). In our study, similarly, trypsin activity decreased in parallel with the decreased feed consumption on the 30 and 60 days after Black Sea salmon were transferred to sea water. Besides, neither changed on the 90 day. Between 1+Atlantic salmon smolt adapted to seawater and freshwater-adapted fish, no significant differences were found in proteolytic enzyme activity in the midgut (Usher et al. 1990) and the specific growth rate (Usher et al. 1991). Similar to our study, Ciavoni et al. (2024b) reported that pepsin activity in the gastric decreased in Atlantic salmon smolts after transfer to seawater. Decreased pepsin activity may occur as a result of accumulated salt in fish body after exposure to salinity due to low osmoregulation capacity of the gastric (Pujante et al. 2018). It can also occur as a result of a decrease in the amount of acid and pepsinogen secreted from the gastric glands. However, since the decrease in the pepsin enzyme does not affect growth, it can be considered to be more of a physiological rearrangement than a functional deficiency. Differences observed in digestive enzyme activities during the adaptation process of salmonids to salinity may vary depending on fish species, environmental conditions, adaptation period, intestinal sections, and physiological readiness of the fish before adaptation. In previous study, Liu et al. (2017) found that the activities of pepsin

and amylase in the whole gut of juvenile American shad (*Alosa sapidissima*) were not affected by salinity treatment while lipase decreased significantly. In an additional study, Ming et al. (2020) reported that the activities of amylase, trypsin and lipase in intestine of rainbow trout subjected to 8 weeks of salinity adaptation decreased, while their activities in the stomach followed a stable course. An additional consideration is that digestive processes are strongly influenced by water temperature. Lower temperatures generally slow gastric emptying, intestinal transit, and the postprandial secretion and activation of digestive enzymes. Because the seawater group was maintained at a lower water temperature than the freshwater group, identical post-feeding sampling times may not have represented equivalent physiological stages in the two groups. Consequently, the interpretation of digestive enzyme activities, particularly where no significant differences were detected, should be made with caution, as the postprandial peak of enzyme activity may have occurred later in the seawater group. Future studies incorporating temperature-adjusted sampling schedules or more comprehensive postprandial time-course analyses are needed to determine whether digestive enzyme responses differ between freshwater and seawater under comparable physiological conditions.

The first part of the midgut section of teleost intestine is the main place where nutrients are absorbed, and the folds in this part increase the absorption area together with the microvilli of the epithelial cells (Lokka et al. 2013). Intestinal epithelium has osmoregulatory functions as well as nutrient absorption (Khojasteh 2012). The intestine also has important function for maintenance of water and ion balance. The rates of water drinking or ion and water absorption in intestine of fish in seawater are high (Sundell et al. 2003). In Atlantic salmon, the anterior and posterior sections of intestine exhibit various histomorphological transformations during smoltification and seawater adaptation, and have different functions in this process (Duarte et al. 2023). In our study, villus length increased on the 60 and 90th day after transferring to sea water, and villus width increased on the 90th day, with no change on the other days statistically. This shows that intestinal villi development the resulting increased surface area in Black Sea salmon were completely in favor of sea water on the 90th day after transfer to seawater. In seawater conditions, the weakening of the muscularis layer is thought to occur as a result of decreased intestinal peristaltic movements, while the increase in villi length and width is thought to occur as a result of the prolonged residence time of food in the intestine due to decreased peristaltic movements. Besides, we found that there was also no change in the number of goblet cells. The fact that the number of goblet cells remains unchanged despite the increased villus length and width during transfer

to seawater may indicate that intestinal mucosal barrier was preserved, mucus secretion remains balanced, that this does not lead to pathological stress, and that a gradual and functional physiological adaptation occurs when considered together with villus development. Unlike our study, Bi et al. (2021) reported that applying different levels of seawater to juvenile golden trout (*Oncorhynchus mykiss*) weighing an average of 60 g for 7 days, the number of goblet cells in the middle of the intestinal villi increased. This contrast may be due to size, species and residence time in seawater of fish or villi development. Additionally, as smoltification progresses and size increases in Atlantic salmon, changes in intestinal histomorphology occur to gain tolerance to seawater (Duarte et al. 2023). In our study, intestinal villi length and muscularis thickness increased as fish weight increased in both freshwater and seawater conditions.

A limitation of our study is that freshwater and seawater groups were exposed to different temperature regimes in addition to salinity differences. Therefore, the observed physiological responses cannot be attributed exclusively to salinity, as temperature may also have contributed to the observed effects. However, this reflects the inherent characteristics of the rearing systems employed in the study and should be considered when interpreting the results. Another limitation of the present study is that fish were fed to apparent satiation throughout the experimental period. It may have allowed fish to compensate for differences in appetite or digestive efficiency through voluntary adjustments in feed intake.

Conclusion

The present results demonstrate that transfer of Black Sea salmon to seawater after reaching smoltification size induces marked changes in digestive physiology without adversely affecting overall growth performance. Although freshwater conditions were characterized by more stable and near-optimal temperatures compared with seawater, growth performance was generally comparable between freshwater and seawater groups. Although TGC was higher in seawater, this result should be interpreted as a temperature-adjusted growth response under the environmental conditions of this study rather than definitive evidence of superior growth performance in seawater. Reductions in trypsin and lipase activities observed during the early phase of seawater transfer likely reflect an acute adaptation response. Similarly, decreased pepsin activity after transfer may be associated with combined effects of salinity and temperature changes. Enhanced intestinal villus development following seawater adaptation indicates an increased absorptive surface area and improved digestive capacity. Because salinity

and temperature differed between freshwater and seawater systems, the physiological responses observed in this study should be interpreted as the combined effects of these environmental factors rather than salinity alone. Overall, the results indicate an early adaptation phase (0–30 days), a transitional phase (30–60 days), and a stable adaptation phase (60–90 days) following seawater transfer. These findings suggest that Black Sea salmon can successfully adapt to seawater conditions after reaching appropriate smolt size. However, future studies using factorial experimental designs that independently assess the effects of salinity and temperature, as well as different smolt sizes, are required to further elucidate the mechanisms underlying smoltification and seawater adaptation.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s41208-026-01198-x>.

Acknowledgements This study was supported by General Directorate of Agricultural Research and Policies of Türkiye within “Investigation of possibilities of using some phytobiotic added feeds in feeding of Black Sea salmon” project which its number is TAGEM/HAY-SUD/2017/A11/P-01/3.

Author Contributions Methodology, feed formulation, tissue sampling, data analysis, manuscript writing and reviewing: Osman Tolga Özel; experiments, feeding studies and data collection: Osman Tolga Özel, Eyüp Çakmak, Recayi Çimagil; digestive enzymes: Selin Ertürk Gürkan, intestinal histomorphology: İsa Coşkun.

Funding The funding was provided by General Directorate of Agricultural Research and Policies (Türkiye).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Statement The experimental protocols were conducted in accordance with the approval of the experimental animal’s ethics committee of the Trabzon Central Fisheries Research Institute (protocol No.: ETİK-2017/1).

Competing interests The authors declare no competing interests.

References

- Araújo BC, Miller MR, Walker SP, Symonds JE (2023) The influence of temperature on performance, biological indices, composition, and nutrient retention of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) reared in freshwater. *Comp Biochem Physiol A Mol Integr Physiol* 280:11412 <https://doi.org/10.1016/j.cbpa.2023.111412>
- Aunsmo A, Krontveit R, Valle PS, Bohlin J (2014) Field validation of growth models used in Atlantic salmon farming. *Aquaculture* 428–429:249–257. <https://doi.org/10.1016/j.aquaculture.2014.03.007>
- Bi B, Gao Y, Jia D, Kong L, Su Y, Rong H, Wu X, Wang X, Hu Z, Hu Q (2021) Growth influence of juvenile golden trout (*Oncorhynchus*

- mykiss*) in different osmotic conditions: implications for tissue histology, biochemical indicators, and genes transcription involved in GH/IGF system. *Fish Physiol Biochem* 47:583–597. <https://doi.org/10.1007/s10695-021-00933-w>
- Bieth J, Metais P (1968) The simultaneous presence of trypsin and trypsin inhibitors in some pathological effusions. *Clin Chim Acta* 22:639–642
- Bonnet S, Haffray P, Blanc JM, Vallée F, Vauchez C, Fauré A, Fauconneau B (1999) Genetic variation in growth parameters until commercial size in diploid and triploid freshwater rainbow trout (*Oncorhynchus mykiss*) and seawater brown trout (*Salmo trutta*). *Aquaculture* 173(1–4):359–375. [https://doi.org/10.1016/S0044-8486\(98\)00460-8](https://doi.org/10.1016/S0044-8486(98)00460-8)
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Çakmak E, Özel OT, Batır E (2022) The Biggest Black Sea Salmon (*Salmo labrax* PALLAS, 1814) in Turkey. *MedFAR* 5(1):21–26
- Çakmak E, Özel OT, Düzgüneş ZD, Örnek V, Mısırs DS (2025) Türkiye’de Karadeniz Somonu (*Salmo labrax* Pallas, 1814) balıkçılığının Dönüşüm Seyri
- Ciavoni E, Schrama JW, Radhakrishnan G, Sæle Ø, Philip PJA (2024a) Salinity induced changes in the progression of water, ion and nutrient fluxes along the gastrointestinal tract of Atlantic salmon smolt (*Salmo salar*). *Aquaculture* 580:740331. <https://doi.org/10.1016/j.aquaculture.2023.740331>
- Ciavoni E, Schrama JW, Sæle Ø, Philip PJA (2024b) Dietary electrolyte imbalance alters drinking rate and gastrointestinal tract water fluxes of Atlantic salmon (*Salmo salar*) smolt in seawater. *Aquaculture* 585:740685. <https://doi.org/10.1016/j.aquaculture.2024.740685>
- Crouse C, Davidson J, Good C (2022) The effects of two water temperature regimes on Atlantic salmon (*Salmo salar*) growth performance and maturation in freshwater recirculating aquaculture systems. *Aquaculture* 553:738063. <https://doi.org/10.1016/j.aquaculture.2022.738063>
- Duarte V, Gaetano P, Striberny A, Hazlerigg D, Jørgensen EH, Fuentes J, Campinho MA (2023) Modulation of intestinal growth and differentiation by photoperiod and dietary treatment during smoltification in Atlantic salmon (*Salmo salar*, L). *Aquaculture* 566:739164. <https://doi.org/10.1016/j.aquaculture.2022.739164>
- Holden MJ, Reitt DFS (1974) Manual of Fisheries Science. Part 2. Methods of Resource Investigation and Their Application. FAO Fisheries Technical Paper No. 115. Food and Agriculture Organization, Quebec.
- Infante JZ, Cahu CL (1994) Influence of diet on pepsin and some pancreatic enzymes in sea bass (*Dicentrarchus labrax*) larvae. *Comp Biochem Physiol Part A: Physiol* 109:209–212
- Jonsson B, Jonsson N, Brodtkorb E, Ingebrigtsen PJ (2001) Life-history traits of Brown Trout vary with the size of small streams. 15(3):310–317 Published by: British Ecological Society
- Kantserova NP, Lysenko LA, Veselov AE, Nemova NN (2017) Protein degradation systems in the skeletal muscles of parr and smolt Atlantic salmon *Salmo salar* L. and brown trout *Salmo trutta* L. *Fish Physiol Biochem* 43:1187–1194. <https://doi.org/10.1007/s10695-017-0364-1>
- Khojasteh SMB (2012) The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. *Int J Aquat Sci* 3(2):71–88
- Kurtoğlu İZ, Çelikkale MS (2016) Culture and releasing potentials of Blacksea Sea trout (*Salmo trutta labrax* L.). *J Anatol Environ Anim Sci* 1(2):48–55
- Liu ZF, Gao XQ, Yu JX, Qian XM, Xue GP, Zhang QY, Liu BL, Hong L (2017) Effects of different salinities on growth performance, survival, digestive enzyme activity, immune response, and muscle fatty acid composition in juvenile American shad (*Alosa sapidissima*). *Fish Physiol Biochem* 43:761–773. <https://doi.org/10.1007/s10695-016-0330-3>
- Lokka G, Austbo L, Falk K, Bjerkaas I, Koppang EO (2013) Intestinal Morphology of the Wild Atlantic Salmon (*Salmo salar*). *J Morphol* 274:859–876. <https://doi.org/10.1002/jmor.20142>
- Mardones O, Devia E, Labbé BS, Oyarzún R, Vargas-Chacoff L, Muñoz JLP (2018) Effect of l-tryptophan and melatonin supplementation on the serotonin gastrointestinal content and digestive enzymatic activity for *Salmo salar* and *Oncorhynchus kisutch*. *Aquaculture* 482:203–210. <https://doi.org/10.1016/j.aquaculture.2017.10.003>
- Ming H, Yangen Z, Jian G, Thorleifur A, Li L, Qinfeng G, Shuanglin D (2020) Fatty acid composition and digestive enzyme activities of rainbow trout in response to dietary docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) during salinity acclimation. *J Ocean Univ China (Oceanic Coastal Sea Research)* 19:1430–1440. <https://doi.org/10.1007/s11802-020-4470-9>
- Özel OT (2022) The zootechnical performance of the seventh generation Black Sea salmon in recirculating aquaculture system. In: VI. International congress on domestic animal breeding genetics and husbandry. Samsun (Oral) 159–162
- Pujante IM, Moyano FJ, Martos-Sitche JA, Mancera JM, Martínez-Rodríguez G (2018) Effect of different salinities on gene expression and activity of digestive enzymes in the thick-lipped grey mullet (*Chelon labrosus*). *Fish Physiol Biochem* 44:349–373. <https://doi.org/10.1007/s10695-017-0440-6>
- Sonay DF, Başçınar N (2017) An investigation on the effects of juvenile rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) monoculture and duo-culture farming in freshwater and seawater on growth performance. *Iran J Fisheries Sci* 16(1):38–39
- SUMAE (2010) Karadeniz alabalığı (*Salmo trutta labrax*) yetiştiriciliği el kitabı. In: Çakmak E, Aksungur M (eds) Su Ürünleri Merkez Araştırma Enstitüsü Müdürlüğü, Yayın No: 2010–2 Trabzon, p 58 (in Turkish)
- Sunde J, Taranger GL, Rungruangsak-Torrissen K (2001) Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (*Salmo salar* L). *Fish Physiol Biochem* 25:335–345
- Sundell K, Jutfelt F, Ágústsson T, Olsen RE, Sandblom E, Hansen T, Björnsson BT (2003) Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr–smolt transformation of Atlantic salmon, *Salmo salar*. *Aquaculture* 222:1–4. [https://doi.org/10.1016/S0044-8486\(03\)00127-3](https://doi.org/10.1016/S0044-8486(03)00127-3)
- Tabak İ, Aksungur M, Zengin M, Yılmaz C, Aksungur N, Alkan A, Zengin B, Mısırs DS (2002) Project for the investigation of the cultural feasibility and the determination of the biotechnological properties of the Black Sea trout (*Salmo trutta labrax*, Pallas 1811). Final report. General Directorate of Agricultural Research and Policies. 98/12/01/007. p 173
- Thodesen J, Gjerde B, Grisdale-Helland B, Storebakken T (2001) Genetic variation in feed intake, growth and feed utilization in Atlantic salmon (*Salmo salar*). *Aquaculture* 194:273–281
- Thorarensen H, Farrell AP (2011) The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture* 312:1–14. <https://doi.org/10.1016/j.aquaculture.2010.11.043>
- Tseng HC, Grendell JH, Rothman SS (1982) Food, duodenal extracts, and enzyme secretion by the pancreas. *Am J Physiology-Gastrointestinal Liver Physiol* 243:304–312
- Usher ML, Talbot C, Eddy FB (1990) Effects of transfer to seawater on digestion and gut function in Atlantic salmon smolts (*Salmo salar* L). *Aquaculture* 90:85–96
- Usher ML, Talbot C, Eddy FB (1991) Effects of transfer to seawater on growth and feeding in Atlantic salmon smolts (*Salmo salar* L). *Aquaculture* 94:309–326

- van Rijn CA, Jones PL, Evans BS, Afonso LOB (2021) Physiological and growth responses of juvenile Atlantic salmon (*Salmo salar*) transferred to seawater during different stages of smolt development. *Aquaculture* 538:736527. <https://doi.org/10.1016/j.aquaculture.2021.736527>
- Versaw KW, Cuppet LS, Winters DD, Williams LE (1989) An improved colorimetric assay for bacterial lipase in nonfat dry milk. *J Food Sci* 54:1557–1558
- Walde CS, Stormoen M, Pettersen JM, Persson D, Røsæg MV, Jensen BB (2022) How delousing affects the short-term growth of Atlantic salmon (*Salmo salar*). *Aquaculture* 561:738720. <https://doi.org/10.1016/j.aquaculture.2022.738720>
- Worthington TM (1982) Pepsin enzymes and related biochemicals. Freehold, NJ: Worthington Diagnostic System Inc. Retrieved from <http://www.worthington-biochem.com/index/manual.html>
- Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.