SHORT COMMUNICATION

CHANGES IN BLOOD ION LEVELS AND MORTALITY RATES IN DIFFERENT SIZED RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* FOLLOWING DIRECT TRANSFER TO SEA WATER

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Abstract

Plasma ion values and mortality rates were compared for 450 rainbow trout *(Oncorhynchus mykiss)* of three sizes following direct transfer from fresh water to Black Sea water of about 18 ppt. In fish of 14.29±0.30 g, plasma Na^+, Cl^- and K^+ levels significantly (p<0.05) rose above initial values five days after the transfer and peaked at 178.6±5.66, 153.9±0.14 and 1.14±0.04 mM/l, respectively. In 20.45±0.48 g fish, these values also rose significantly, reaching 172.4±4.24, 151.8±6.65 and 0.98±0.04 mM/l by day 5. In fish of 29.91±0.99 g, however, plasma Na^+ and Cl^- concentrations peaked 19 days after transfer, reaching only 165.5±6.43 and 142.9±8.34 mM/l, while plasma K^+ reached its highest concentration of 1.02±0.06 mM/l on day 12, All three concentrations dropped to near initial values on day 26. In all groups, the plasma Ca^{2+} level rose significantly (p<0.05) above the initial value five days after transfer and then declined while the plasma P^{5+} concentration dropped on day 5, reaching a minimum on day 12 and recovering the initial level on day 26. The lowest mortality (8.0±1.89%) was recorded in the 30 g group, followed by 19.3±0.94% and 24.7±0.94% in the 20 g and 14 g groups. The failure of the smallest fish to adapt after direct transfer to sea water was likely due to excessively high plasma Na^+ and Cl^- concentrations and tissue dehydration, indicating that fish of 30 g best adapt to a seawater environment of 18 ppt.

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Introduction

Rainbow trout *(Oncorhynchus mykiss)* has been commercially cultured in freshwater ponds in Turkey since the 1970s and culture in sea cages in the Black Sea has grown steadily since 1990. Although this species is cultured in sea water, there are no physiological studies of their ability to adapt during early life stages to Black Sea conditions. Teskeredzic et al. (1989) and Yigit and Aral (1999) demonstrated that young rainbow trout grow better and have better food conversion in seawater than in freshwater conditions. Since early transfer to sea water may reduce the period required to reach market size, there is great interest in knowing the most effective and least expensive method for adapting and growing rainbow trout in cages in the Black Sea. Achieving seawater tolerance at an early age can reduce production costs and improve investment returns.

Seawater adaptation in rainbow trout is related to the development of branchial ionoregulatory mechanisms. When fish are transferred from fresh water to sea water, they lose water and dehydrate. Ionic differences cause sodium (Na^+) and chloride (Cl^-) ions to enter the body through diffusion or drinking. Elevated plasma Na^+ and Cl^- levels stimulate the ion extrusion mechanism and induce an increase in (Na^+,K^+)-adenosine triphosphatase (ATPase) activity. Increased (Na^+,K^+)-ATPase activity is accompanied by lowered serum Na^+ and Cl^- levels and adaptation to saline conditions. Adaptation to sea water is size dependent, where larger trout adapt better than small trout (Jackson, 1981; Johnston and Cheverie, 1985; Gorie, 1993; Wedemeyer, 1996).

Since various factors such as salinity, temperature, fish size, handling, and stress affect seawater tolerance in salmonids, results of earlier studies differ. Landless (1976) demonstrated that 55 g rainbow trout were not affected by direct transfer from fresh water to 25 ppt sea water, but that a rise from 25 to 33 ppt resulted in several deaths. Jackson (1981) reported that rainbow trout smaller than 30 g had difficulty maintaining osmotic control when transferred to salinities above 28 ppt.

The objective of the current study was to evaluate the development of osmotic and ionic regulation in rainbow trout of different sizes when transferred directly into sea water and determine the minimum size at which fish are suitable for transfer.

Materials and Methods

The study was conducted in November, when seawater culture of rainbow trout commonly begins in Turkey. A total of 450 rainbow trout of three size groups (14, 20 and 30 g) was transported from a commercial fish farm (Akbalik Co., Bafra, Samsun, Turkey) to our facilities at the Faculty of Fisheries in the University of Ondokuz Mayis, Sinop, Turkey. The transfer from the freshwater site to the seawater site required one hour. The fish were transferred directly into six identical 50-l rectangular polypropylene tanks supplied with running sea water of 1000 ml/min (salinity 18 ppt). Each treatment consisted of 150 fish (75 fish per tank with two replicate tanks per size group). The fish were cultured under a natural photoperiod regime and the temperature was seawater ambient, ranging 10.8-11.4ºC. Fish were fed to satiation twice a day with a commercial diet containing 45% crude protein, 23% crude lipid, 21% nitrogen free extracts (NFE), gross energy of 23.5 kJ/g diet and a P/E ratio of 19.15 mg protein/kJ. The fish were weighed four days before the transfer to allow them to recover from any stress caused by the weighing.

During the adaptation period, fish from each size group were randomly sampled for plasma Na^+, Cl^-, K^+, Ca^{2+} and P^{5+} levels. Three, four and five fish were sampled from the large, medium and small size groups, respectively. The sampled fish were sacrificed, the caudal peduncle was severed and blood was collected (1 ml per fish) in lithium-heparinized blood collecting tubes. The blood was immediately placed in an ice bath. The plasma was separated by centrifugation at 5000 rpm for 5 min and used in ion determinations. Chloride was determined using a Buchler Chloridometer, while Na^+, K^+, Ca^{2+} and P^{5+} levels were determined using a Nova 1 Ion Analyzer.

Results are expressed as means±SD. Statistical comparisons between size groups were conducted using SPSS 10.0 for Windows. One-way ANOVA was used and significant ANOVA were followed by a post-hoc multiple comparison test (Duncan's new multiple range test; General Linear Model - Univariate procedure). Differences were considered significant at $p<0.05$. Data expressed in percent were arcsine-transformed prior to analysis by ANOVA and the post-hoc multi comparison test.

Results

The Na^+, Cl^-, K^+ and Ca^{2+} concentrations of the 14 g and 20 g fish had a similar pattern (Figs. 1-4). The initial Na^+, Cl^- and K^+ concentrations of the 14 g fish were 145.3±0.00 mM/l, 121.4±1.56 mM/l and 0.50±0.00 mM/l, respectively, while those of the 20 g fish were 147.7±3.25 mM/l, 122.3±2.12 mM/l and 0.40±0.14 mM/l. The Na^+, Cl^- and K^+ levels in the 14 g and 20 g fish rose significantly ($p<0.05$) and peaked at five days at 178.6±5.66 mM/l, 153.9±0.14 mM/l and 1.14±0.04 mM/l for the 14 g fish, and 172.4±4.24 mM/l, 151.8±6.65 mM/l and 0.98±0.04 mM/l for the 20 g fish. Afterward, these values gradually decreased and returned to initial concentrations by the end of the experiment.

In the 30 g fish, the initial Na^+, Cl^-, K^+ concentrations were 143.0±3.54 mM/l, 120.6±1.56 mM/l and 0.54±0.19 mM/l. However, the Na^+ and Cl^- concentrations peaked 19 days after transfer at 165.5±6.43 mM/l and 142.9±8.34 mM/l, while K^+ reached the highest concentration (1.02±0.06 mM/l) on day 12.

All three ion levels in all three groups returned to initial values by day 26.

The initial plasma Ca^{2+} concentrations were 9.0±0.28 mg/dl, 8.9±0.78 mg/dl and 7.9±0.12 mg/dl for the 14, 20 and 30 g size groups, respectively. Ca^{2+} peaked in all groups at five days at 13.4±0.20 mg/dl, 12.7±0.25 mg/dl and 12.9±0.21 mg/dl, respectively, and then dropped. It slightly increased

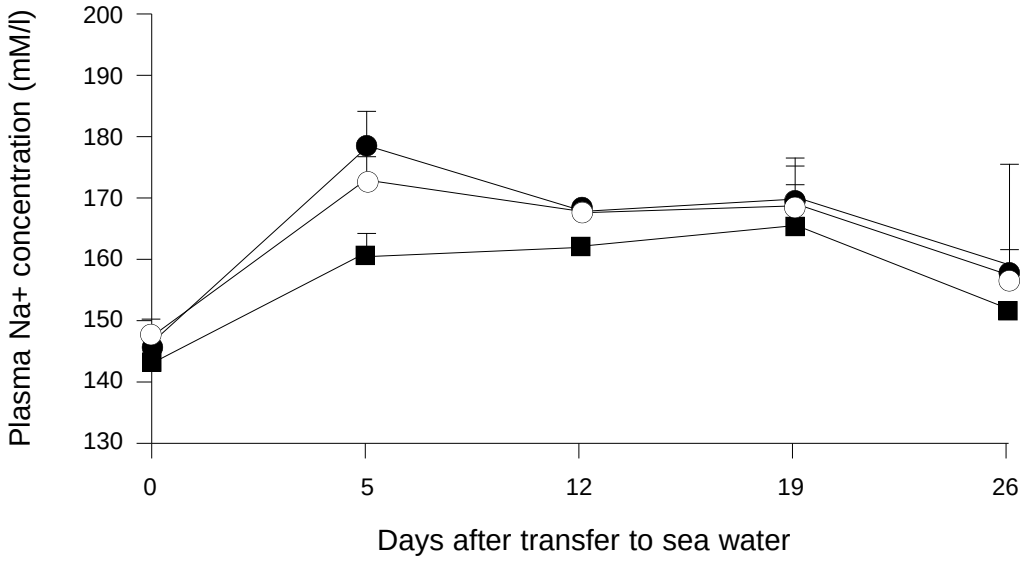

Fig. 1. Plasma Na^+ concentrations of 14 (●), 20 (○) and 30 (■) g rainbow trout after transfer to sea water. Values are means±SD.

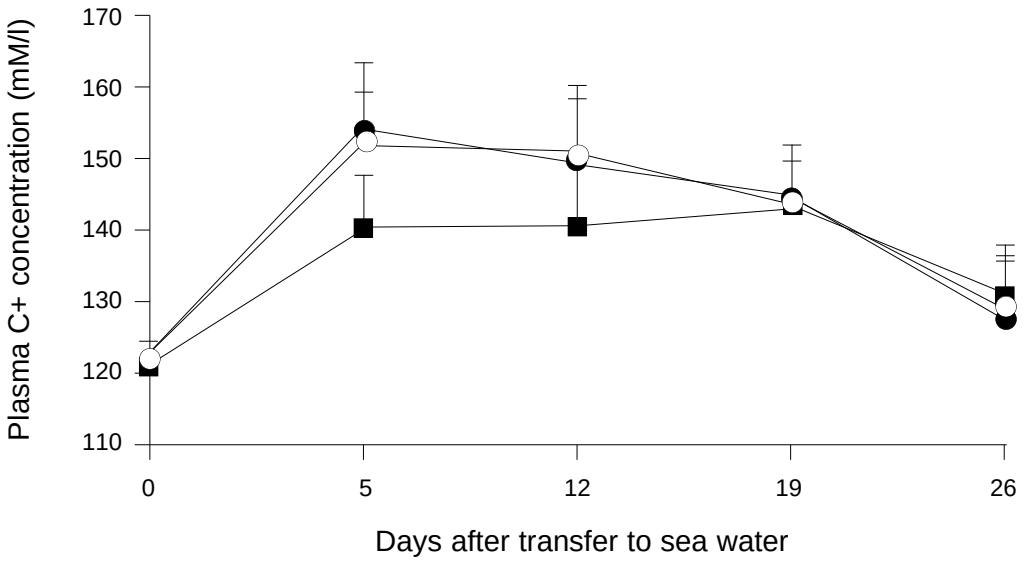

Fig. 2. Plasma Cl^- concentrations of 14 (●), 20 (○) and 30 (■) g rainbow trout after transfer to sea water. Values are means±SD.

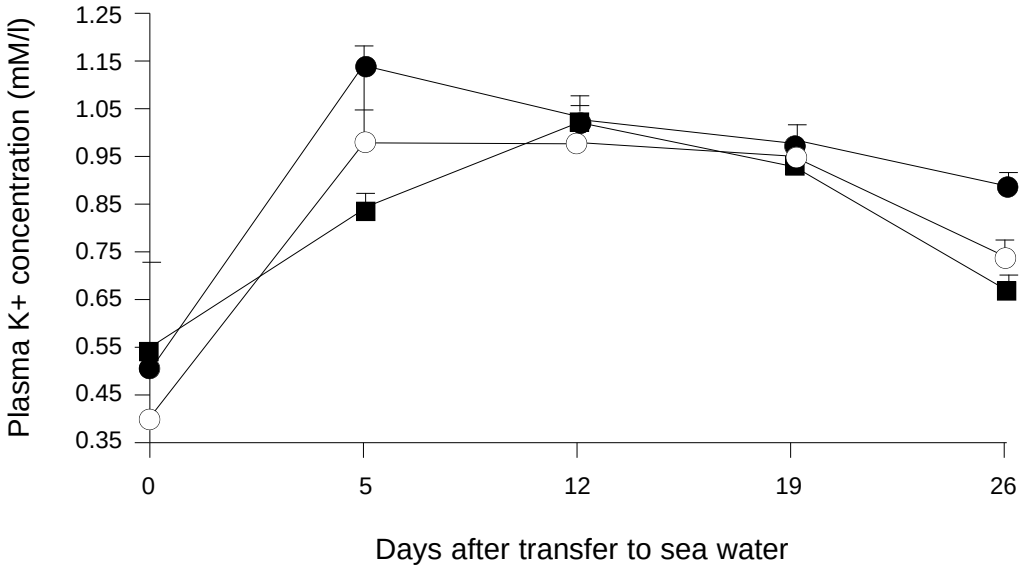

Fig. 3. Plasma K^+ concentrations of 14 (●), 20 (○) and 30 (■) g rainbow trout after transfer to sea water. Values are means±SD.

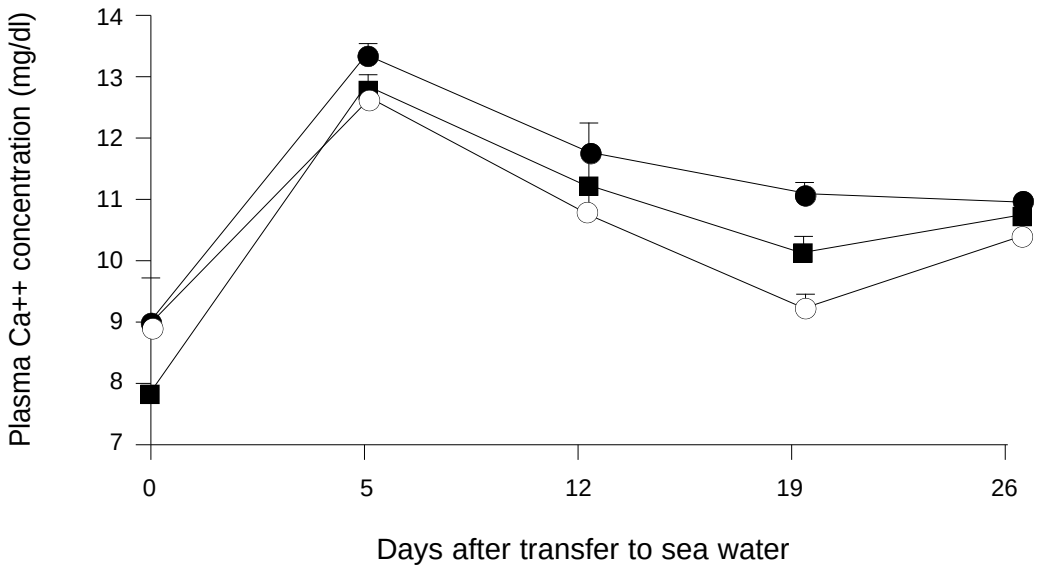

Fig. 4. Plasma Ca^{2+} concentrations of 14 (●), 20 (○) and 30 (■) g rainbow trout after transfer to sea water. Values are means±SD.

between days 19 and 26 in the two larger groups. The plasma P^{5+} concentrations (Fig. 5) in all groups decreased and had similar patterns, i.e., the levels dropped to about 10 mg/dl by day 12 and rose to the initial value by the end of the experiment.

Cumulative mortality (Fig. 6) was highest in the 14 g group, followed by the 20 g group. Mortality in the 30 g group was significantly lower ($p<0.05$) than in the 14 g and 20 g groups.

Discussion

The mortality rates of the 14 and 20 g fish indicate that small rainbow trout are unable to adapt to 18 ppt salinity when transferred directly from fresh water to sea water. Earlier studies have shown that salinity of 20 ppt is the upper threshold for survival of 10-30 g rainbow trout (Landless, 1976; Eddy and Bath, 1979; Jackson, 1981; Johnsson and Clarke, 1988). Landless (1976) reported that rainbow trout of 15 g have routinely been put directly into sea water at a salinity of 22 ppt with low mortality of 1-8%. However, in the present study, when transferred directly to 18 ppt, mortality (8%) was significantly lower in the 30 g fish than in the smaller fish of 14 and 20 g (25% and 19%, respectively). Death was possibly caused by an inability to regulate high plasma Na^+ and Cl^- concentrations and maintain cell and plasma water. In contrast, Gorie (1993) reported high mortality and high plasma Na^+ concentration in 50 g rainbow trout, showing that the fish had not acquired seawater adaptability. Gorie (1993) found that rainbow trout larger than 150 g are of sufficient size to develop in seawater culture and can be transferred directly into seawater without acclimation. The higher salinity (31.6 ppt) in Gorie's study than in the present study (18 ppt) may account for the difference in results.

The Na^+ and Cl^- values prior to seawater transfer in the present study are in agreement with those reported by Johnston and Cheverie (1985), Finstad et al. (1988), Morgan and

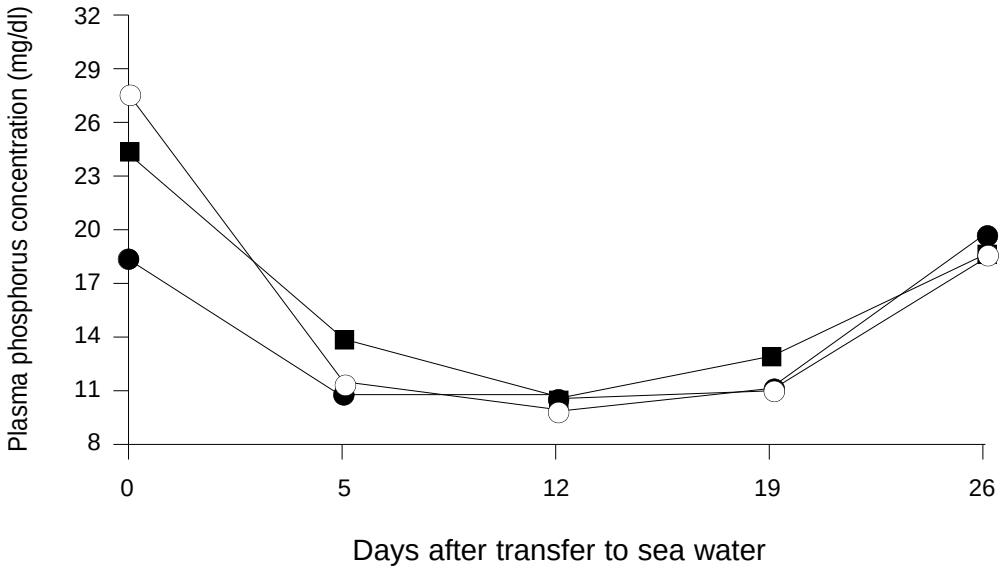

Fig. 5. Plasma P^{5+} concentrations of 14 (●), 20 (○) and 30 (■) g rainbow trout after transfer to sea water. Values are means±SD.

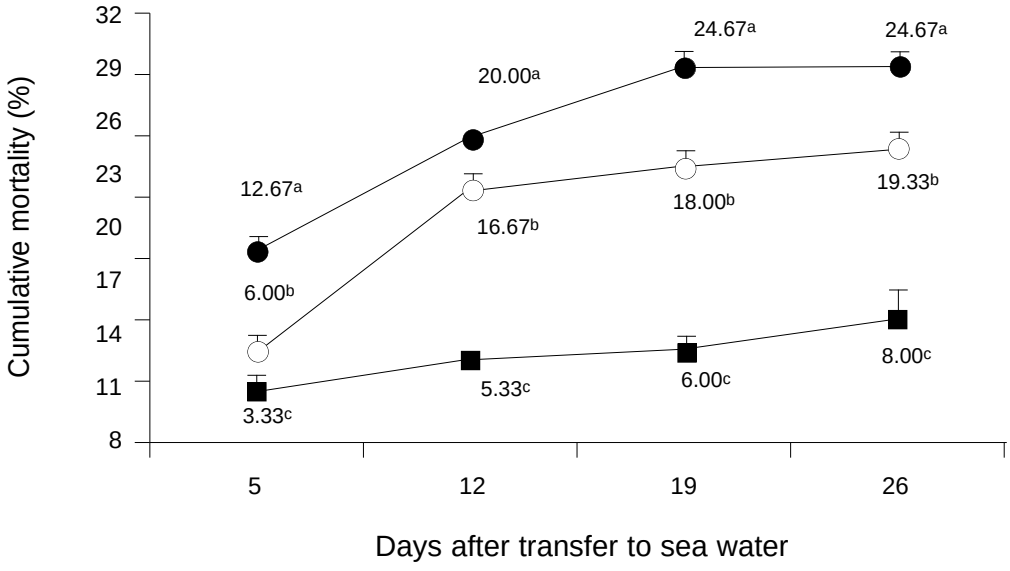

Fig. 6. Cumulative mortality of 14 (●), 20 (○) and 30 (■) g rainbow trout after transfer to sea water. Values are means±SD. Values within a time interval with different superscripts differ significantly at the 5% level.

Iwama (1991) and Gorie (1992, 1993) for rainbow trout, by Johnston et al. (1987) for Atlantic salmon (*Salmo salar*) and by Gorie (1996) for Japanese char (*Salvelinus pluvius*).

The plasma Na^+ concentrations of the dead and moribund fish were much higher (267±31 mM/l) than of freshwater-cultured rainbow trout (135±10 mM/l). Thus, most of the fish likely died as a result of inadaptability to sea water (Gorie, 1992). In another study, Gorie (1993) reported that plasma Na^+ concentrations of fish in the 50 and 100 g weight classes rapidly increased to around 200 mM/l six hours after transfer to sea water (31.6 ppt) and that by the end of the experiment, Na^+ levels were still at 190 mM/l, while Na^+ levels in larger fish were stable at around 160-170 mM/l throughout the experiment. Almendras (1996) reported that plasma Cl^- concentrations of fingerling and subadult sea bass (*Lates calcarifer*) rapidly rose to around 190 mM/l three hours after transfer to 32 ppt salinity. In the present study, all the trout were affected by the transfer but the Na^+ and Cl^- concentrations in the 30 g group did not exceed 165.5 mM/l and 142.9 mM/l. The failure of the small fish (14-20 g) to adapt was due to excessively high plasma Na^+ and Cl^- concentrations and tissue dehydration.

Johnsson and Clarke (1988) reported that salinity tolerance in rainbow trout was highest at 11ºC, lowest at 17ºC and intermediate at 5ºC. Seawater temperature during the course of the present study was 11ºC. Besides the low salinity level in the present study, the difference in water temperature may have affected the plasma ion concentrations and might explain the lower levels in this study when compared to other reports. Jürss et al. (1984) reported that 60 g fish had poorer growth and lower gill ATP-ase activity at 16ºC than at 8ºC. Hogstrand and Haux (1985) obtained 100% mortality in smolted sea trout (*Salmo trutta*) transferred to 20ºC seawater (25 ppt) while mortality was 0 at 15ºC. The interacting effects of temperature and salinity on seawater adaptation should be considered in marine farming of salmonids.

Johnsson and Clarke (1988) found that small (7-15 g) rainbow tout grew well at 14 ppt and 19 ppt salinity, while the growth rate was lower at 24 ppt. Speshilov (1978) found 10-22% higher weight gains in underyearling and yearling rainbow trout at salinities of 4-15 ppt than in fresh water. Teskeredzic et al. (1989) reported that rainbow trout (84-301 g) growth was 3-4 times greater in brackish water than in fresh. Likewise, Yigit and Aral (1999) reported that rainbow trout of about 88 g grew 1.5 times better in brackish than in fresh water. In contrast, negative effects of salinity on the growth of rainbow trout and fall chinook salmon (*Oncorhynchus tshawytscha*) were reported by Morgan and Iwama (1991).

Generally, the plasma phosphorus content is negatively correlated with the plasma calcium content (Ersoy et al., 1979). This inverse relationship was observed in the present study.

In summary,14 g rainbow trout had the highest mortality and plasma ion levels while 30 g fish had the lowest, indicating that 30 g fish best adapted to the seawater environment of 18 ppt.

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