Int. J. Aquat. Biol. (2025) 13(6): 115-136
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Impact of acute salinity exposure on physiological indices and survival of Caspian roach (*Rutilus caspicus*)

Naeemeh Kasalkheh[1], Solmaz Shirali[*1], Mohammad Ali Salari Aliabadi[1], Seyed AliakbarHedayati[2], Esmaeil Paghe[3]

[1]Department of Marine Biology, Faculty of Marine Sciences, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran.
[2]Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[3]Agricultural Research, Education and Extension Organization (AREO), Iranian Fisheries Science Research, Gorgan, Iran.

Abstract: Increased salinity, a major stressor in aquatic environments, can significantly affect the health and survival of fish. The Caspian roach (*Rutilus caspicus*) is an economically and ecologically valuable species of the Caspian Sea; the decline in its stocks in recent years has heightened the need to study its adaptation to adverse environmental conditions. This research was conducted to investigate the effect of different salinities on hematological, biochemical, and histological indices in Caspian roach. In this study, 480 fish with an average weight of 15±2 grams were maintained under laboratory conditions at salinities of 0 (Control), 6, 12, and 15 ppt. Blood and tissue samples were collected at 36, 72, and 108 hours after placement in the specified salinity concentrations. Hematological and biochemical indices, as well as the pathology of liver, gill, and kidney tissues, were investigated. The results showed that as salinity increased, the numbers of red blood cells (RBCs), hemoglobin, and hematocrit increased. In addition, glucose and the enzymes ALT, AST, and ALP increased significantly (P<0.05). The activity of Na+-K+-ATPase in the gills initially increased and then decreased at high salinities, indicating the fish's attempt to maintain osmotic regulation. Histological studies showed that increasing salinity induced necrosis, hyperplasia, and severe damage in the gill, liver, and kidney tissues. In total, this research showed that high salinity negatively affects the health and osmotic regulation of Caspian Roach and increases mortality in this species.

Article history:
Received 17 October 2025
Accepted 8 December 2025
Available online 25 December 2025

Keywords:
Salinity
Caspian roach
Biochemistry
Histology

Introduction

Environmental stressors, including salinity, can significantly impact the physiology of aquatic organisms, leading to changes in hematological and biochemical indices and disruptions to tissue and organ function (Rashamol et al., 2020; Shahjahan et al., 2022a). In conditions of water scarcity and drought, salinity stress is recognized as a major stressor for aquatic animals, particularly fish species. The Caspian roach (*Rutilus caspicus*) is a commercially valuable species that inhabits the fresh and brackish waters of the Caspian Sea basin (Hasanpour et al., 2015, 2016; Eagderi eta al., 2022; Salauat et al., 2024). Due to its economic and ecological importance (Rasekhi et al., 2023), this species is directly exploited by people and plays a vital role in the life cycle of other valuable fish, such as sturgeons (Eremkina and Yarushina, 2022; Rasekhi et al., 2023).

The stocks of this fish have declined in recent decades due to overfishing, destruction of spawning habitats in rivers and wetlands, and pollution from agricultural, domestic, and industrial wastes (Salauat et al., 2024). Despite the annual release of millions of juveniles, produced at considerable expense by breeding and rearing centers, into the rivers flowing into the southern Caspian Sea basin (Kashiri et al., 2019; Jamebozorgi et al., 2023), this action appears to have had little impact on the recovery of the species' stocks. Observations and reports indicate that a significant proportion of the released juveniles die before entering the sea, which may be due to improper release management, environmental problems, and insufficient ability to adapt to environmental

[*]Correspondence: Solmaz Shirali
E-mail: Solmazshirali_awz@yahoo.com
DOI: https://doi.org/10.22034/ijab.v13i6.2689

conditions, particularly issues with ionic regulation in this species (Kashiri et al., 2019; Jamebozorgi et al., 2023).

Changes in salinity pose a significant challenge to fish in various aquatic environments. Adaptation to these changes and maintenance of osmotic balance depend on the function of multiple organs, including the gills, kidneys, intestines, and skin (Ruiz-Jarabo et al., 2019; Takvam et al., 2021). These organs, under complex physiological and hormonal control, maintain the balance of ions and water in the fish's body. While numerous studies have examined salinity tolerance in various fish species, investigating salinity tolerance and its physiological effects in Caspian Roach juveniles is particularly important. Tian et al. (2020) showed that the growth of *Nibea albiflora* is limited to a salinity of 42 ppt, and lower salinities (6 to 30 ppt) do not significantly affect growth, blood ion concentration, and the activity of Na^+-K^+-ATPase and antioxidant enzymes. Other studies have also demonstrated that salinity stress can induce changes in blood parameters, hormones, and gene expression related to osmotic regulation, as well as tissue damage in various fish organs (Mohamed et al., 2021; Winarti et al., 2022). Nevertheless, there are few studies on the effects of short-term salinity changes and on the survival of Caspian Roach after acute salinity exposure. Therefore, this study investigates the impact of acute salinity exposure on the physiological indices and survival of Caspian Roach. This research aims to better understand the mechanisms by which this species adapts to salinity changes and to develop strategies to improve the survival of individuals released into rivers flowing into the Caspian Sea.

Materials and Methods

Experimental design and treatment: A total of 480 Caspian Roach with an average weight of 15±2 grams were transported to the Gharesu Aquaculture Research Station, Bandar Turkaman region, Iran. They were acclimated to the new conditions for 1 week. During this period, the fish were fed daily at 3% of their body weight, divided into three meals, using a commercial starter feed (Zargari et al., 2024). Throughout the acclimation period, water physicochemical parameters were monitored daily, and the fish's feeding behavior was observed.

At the beginning of the study, a preliminary test was conducted to determine the appropriate salinity range and exposure time. In this test, one treatment group (with three replicates) was subjected to a gradual increase in salinity from 0 to 40 ppt (with a daily increment of 3 ppt). The results of this preliminary test indicated that a concentration of 15 ppt for 108 hours resulted in over 50% mortality. Subsequently, the fish were randomly divided into four treatment groups (each with three replicates): a control group at 0 ppt salinity (Control) and three experimental groups at 6 ppt (T1), 12 ppt (T2), and 15 ppt (T3). Salinity in the experimental groups was gradually increased, and samples were collected at 36, 72, and 108 hours after the onset of salinity stress for each concentration. Daily, 50% of the water in the tanks was replaced with fresh water (with adjusted salinity).

Mortality rate: To calculate the mortality rate for each sampling period (36, 72, and 108 hours) for each treatment, the standard method was used, based on the number of deaths in each period using the formula of Mortality Rate (%) = (Number of Dead Fish) / (Total Number of Fish Initially) × 100. Briefly, to calculate the mortality percentage for each treatment, 60 fish were used. In calculating the mortality rate in the second and third sampling periods (72 and 108 hours), the difference in mortality from the previous periods was also considered (Zargari et al., 2024).

Sampling*:* Fish were fasted for 24 hours before sampling. At the end of the experimental period, after anesthetizing the fish with a 100 ppm Eugenol solution, blood and tissue samples were collected from each treatment group (Zargari et al., 2024). Blood samples were taken from the caudal vein using heparinized syringes. Each sample was then divided into two parts for hematological studies and plasma separation. For sampling the gill, liver, and kidney tissues, samples were taken after complete anesthesia.

Hematological studies: Hemoglobin (Hb), hematocrit (Hct), white blood cell (WBC) count, red

blood cell (RBC) count, and WBC differential count were determined using standard methods (Zargari et al., 2024). RBC and WBC counts were performed after dilution with a dye solution using a Neubauer hemocytometer. For the WBC differential count, blood smears were prepared according to standard procedures and stained with Giemsa (Zargari et al., 2024). Hemoglobin (Hb) concentration was measured using the cyanmethemoglobin method with a commercial kit (ZiestChem Diagnostics, Tehran, Iran). Hematocrit (Hct) percentage was determined using hematocrit tubes and a microcentrifuge (Zargari et al., 2024).

Plasma biochemical studies: To separate plasma, heparinized blood samples were centrifuged at 3000 RPM for 7 minutes at 4°C. The plasma was then collected and stored at -18°C until the assays were performed. Plasma biochemical parameters, including glucose, total protein, and albumin, were measured using commercial kits (ZiestChem Diagnostics, Tehran, Iran) according to the manufacturer's instructions. Total immunoglobulin levels were determined using a standard method: the difference between pre- and post-sedimentation levels was calculated after sedimentation with 12% polyethylene glycol, and total protein was measured with a total protein assay kit (Zargari et al., 2023). The activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured spectrophotometrically using commercial kits (Pars Azmoon, Tehran, Iran). The activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were measured colorimetrically using an ELISA reader, following the manufacturer's instructions for the ZellBio commercial kits (Germany). Na^+-K^+-ATPase enzyme activity was measured following a modified version of the methods described by McCormick (1993, 1995).

Histopathological studies: Fish gill, kidney, and liver tissues were initially fixed in 10% buffered formalin, and the fixative was replaced after 12 hours for long-term preservation (Zargari et al., 2023). Five-micrometer tissue sections were prepared by dehydration, clearing, paraffin embedding, sectioning, and staining with hematoxylin and eosin (H&E), then mounted on slides. Finally, the prepared tissue sections were examined under a light microscope at 100x to 400x magnification.

Statistical analyses: In this study, data normality was first assessed using the Kolmogorov-Smirnov test. Subsequently, SPSS 26 software was used for statistical analyses. Data were analyzed using one-way analysis of variance (ANOVA), and Duncan's multiple-range test was used to determine significant differences between means at $P<0.05$. Graphs were designed using Microsoft Excel. The final results are presented as mean ± standard deviation.

Results

Hematology studies: Examining the results (Table 1) showed that, with increasing salinity concentration and exposure time, blood indices, such as red blood cell count, hemoglobin, and hematocrit, increased significantly ($P<0.05$). The lowest levels of these indices were observed at 15 ppt salinity. However, no significant differences in the blood indices MCV, MCH, and MCHC were observed between treatments ($P>0.05$). The results also indicated that changes in salinity concentration did not alter the percentages of leukocyte types (lymphocytes, monocytes, and neutrophils) in Caspian roach ($P>0.05$).

Mortality rate: According to Table 2, mortality increased with increasing salinity and exposure time. The highest mortality rate was observed in fish under salinity stress at 15 ppt. After 72 hours of exposure to 15 ppt, 20 out of 60 fish had died; with exposure time extended to 108 hours, 34 out of 39 fish had died, corresponding to 33.3% and 87.2%, respectively. Also, at the end of the period, 54 of 60 fish in this group died, i.e., the total mortality percentage was 90%.

Biochemical indicators

***Transaminases (AST and ALT) and alkaline phosphatase (ALP)*:** The activity level of ALT enzyme at 12 and 15 ppt significantly increased compared to the control group and treatment 1 ($P<0.05$) (Fig. 1). No significant difference was

Table 1. Changes in red blood cell count, white blood cell count, hematocrit percentage, hemoglobin concentration, blood indices MCV, MCH and MCHC and white blood cell differential count in Caspian roach under salinity stress at different concentrations across three sampling periods.

		WBC (mm^3)	RBC×10^6 (mm^3)	Hct (%)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
36 hours	Control	14333.33±416.33^{bA}	1.43±0.05^{bB}	31.33±1.15^{bA}	5.92±0.7^{cA}	219.4±14.12^{aA}	41.36±3.6^{abA}	18.98±2.9^{aA}	87±1^{aA}	6.67±1.53^{aA}	6.3±0.58^{aA}
	6 ppt	15131.33±305.51^{bA}	1.49±0.09^{bAB}	33.67±1.53^{aA}	6.15±0.22^{bcA}	226.98±17.7^{aA}	41.46±3.51^{abA}	18.3±1.42^{aA}	88±1^{aA}	6±1^{aA}	6±1.73^{aA}
	12 ppt	17333.33±503.32^{aA}	1.67±0.06^{aA}	34±1^{aA}	6.81±0.2^{abB}	203.51±13.31^{aA}	40.75±2.76^{bA}	20.02±0.09^{aA}	87.3±1.53^{aA}	6±1$_{aA}$	6.67±1.52^{aA}
	15 ppt	17133.33±832.67^{aA}	1.55±0.09^{abA}	35±1^{aA}	7.3±0.35^{aA}	226.26±13.55^{aA}	47.12±1.56^{aA}	20.85±0.68^{aA}	87.6±1.15^{aA}	5.67±1.15^{aA}	6.67±1.15^{aA}
72 hours	Control	12533.33±611.01^{cB}	1.54±0.08^{aA}	34.67±1.15^{aA}	6.96±0.35^{aA}	225.09±16.93^{aA}	45.18±4.13^{aA}	20.09±1.44^{aA}	87±1^{aA}	8±1^{aA}	5±1^{bA}
	6 ppt	13933.33±503.32^{bB}	1.6±0.06^{aA}	34±1^{aA}	7.13±0.44^{aA}	212.63±11.27^{aA}	44.58±3.55^{aA}	20.95±0.7^{aA}	86.3±2.08^{aA}	8.3±1.15^{aA}	5.3±1.15^{abA}
	12 ppt	16000±346.41^{aA}	1.4±0.04^{bB}	29±1^{bB}	5.56±0.57^{bB}	207.6±1328^{aA}	39.78±4.67^{abA}	19.16±1.88^{aA}	85.67±0.58^{aA}	7.67±1.53^{aA}	6.67±2.08^{abA}
	15 ppt	16666.67±305.51^{aA}	1.22±0.07^{cB}	28.67±1.53^{bB}	4.27±0.39^{cB}	236.21±17.04^{aA}	35.23±4.44^{bA}	14.88±0.79^{bA}	86±1^{aA}	6±1^{aA}	8±1^{aA}
108 hours	Control	16800±529.15^{aA}	1.55±0.05^{aA}	34.33±1.15^{aA}	6.6±0.38^{aB}	221.7±8.55^{bA}	42.57±1.09^{bA}	19.23±1.11^{aA}	86.3±0.58^{aA}	8±1^{aA}	5.67±0.58^{aA}
	6 ppt	16066.67±305.51^{aA}	1.52±0.03^{aB}	35±1^{aA}	6.98±0.25^{aB}	230.28±6.03^{bA}	45.94±2.3^{aA}	19.96±1.23^{aA}	87±1^{aA}	7.3±0.58^{aA}	5.67±0.58^{aA}
	12 ppt	14933.33±611.01^{aAB}	1.32±0.06^{bC}	30.33±1.53^{bAB}	4.99±0.39^{bC}	230.47±4.43^{bA}	37.9±1.56^{cA}	16.46±0.87^{bA}	87±1^{aA}	7.3±1.53^{aA}	5.67±1.15$_{aA}$
	15 ppt	12600±529.15^{cB}	1.03±0.06^{cC}	26.33±1.53^{cC}	4±0.41^{cB}	256.37±10.81^{aA}	38.84±1.72^{cA}	15.17±0.95^{bA}	87.3±1.53^{aA}	6.67±1.15^{aA}	6±1^{aA}

Dissimilar lowercase letters within each column, corresponding to each sampling period, indicate a significant difference at the $p<0.05$ level. Dissimilar uppercase letters within each column, corresponding to the comparison of each fish group across the three sampling periods, indicate a significant difference at the $P<0.05$ level. Data are presented as mean ± standard deviation.

observed in the enzyme activity level between treatments 2 and 3, which were exposed to 12 and 15 ppt salinity, respectively ($P>0.05$). Also, no significant difference in mean ALT levels was found across groups at different sampling times ($P>0.05$).

Figure 2 shows AST enzyme activity levels in Caspian Roach under different salinity concentrations across three distinct time periods. According to the results, increasing salinity to 12 and 15 ppt significantly increased AST enzyme levels compared

Table 2. Percentage of mortality calculated separately for each sampling period and the percentage of total mortality calculated for each salinity concentration at the end of the sampling period.

	Control	6 ppt	12 ppt	15 ppt
36 Hours	1.7	0	0	0
72 Hours	1.7	1.7	1.7	33.3
108 Hours	0	1.7	6.8	87.2
Total Mortality Rate	3.3	3.3	8.3	90

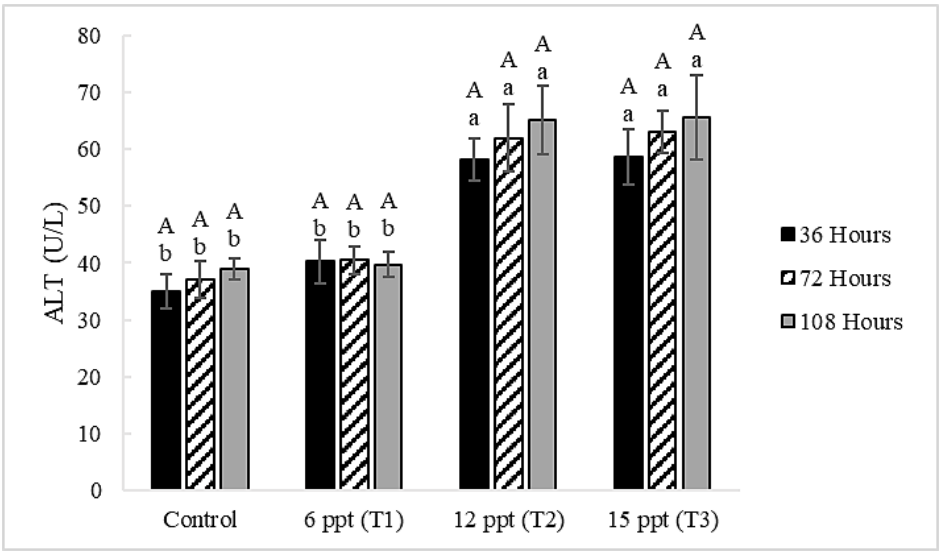

Figure 1. ALT enzyme activity levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

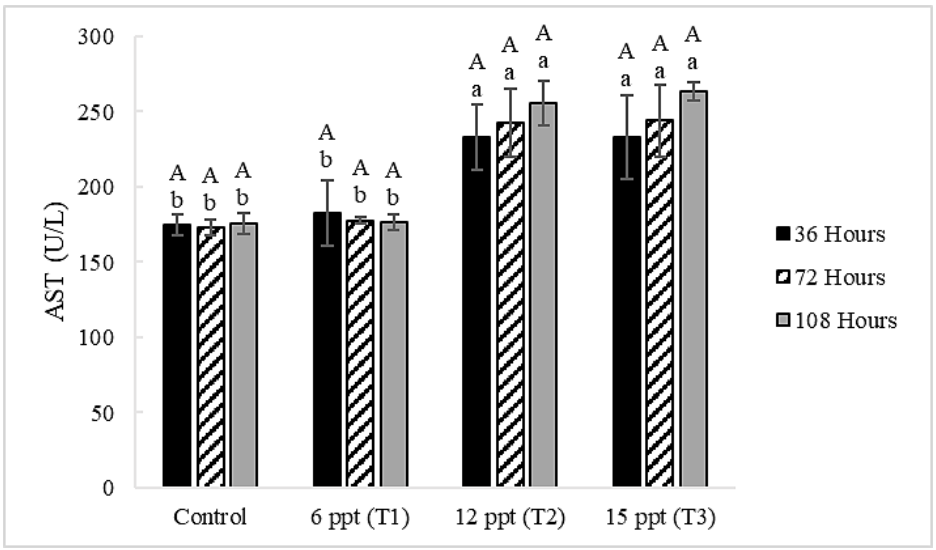

Figure 2. AST enzyme activity levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

with the control group and treatment 1 (P<0.05). However, no significant difference in AST enzyme activity was observed between treatments 2 and 3 (P>0.05). Furthermore, the levels measured in treatment 1 did not differ significantly from those in the control group (P>0.05). Also, comparing mean AST levels across the different sampling times revealed no significant difference between treatments

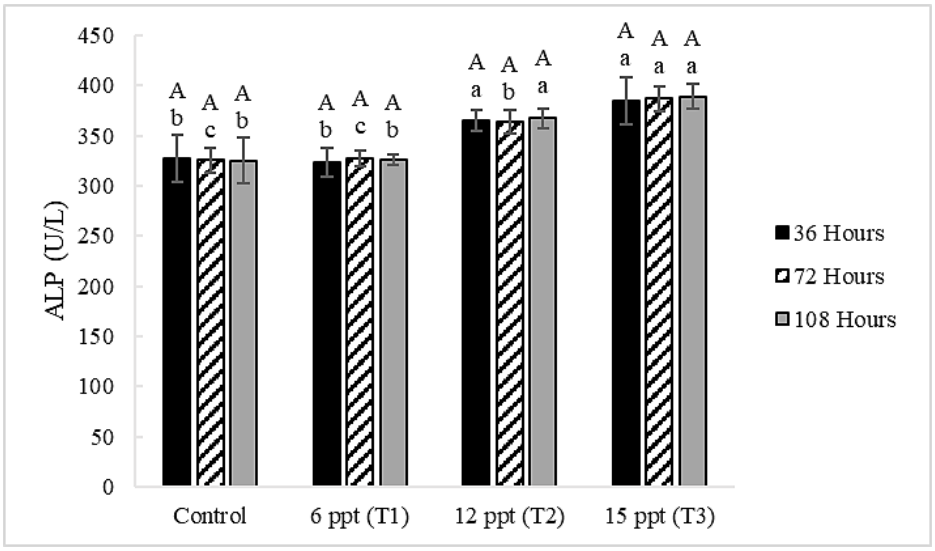

Figure 3. Glucose levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

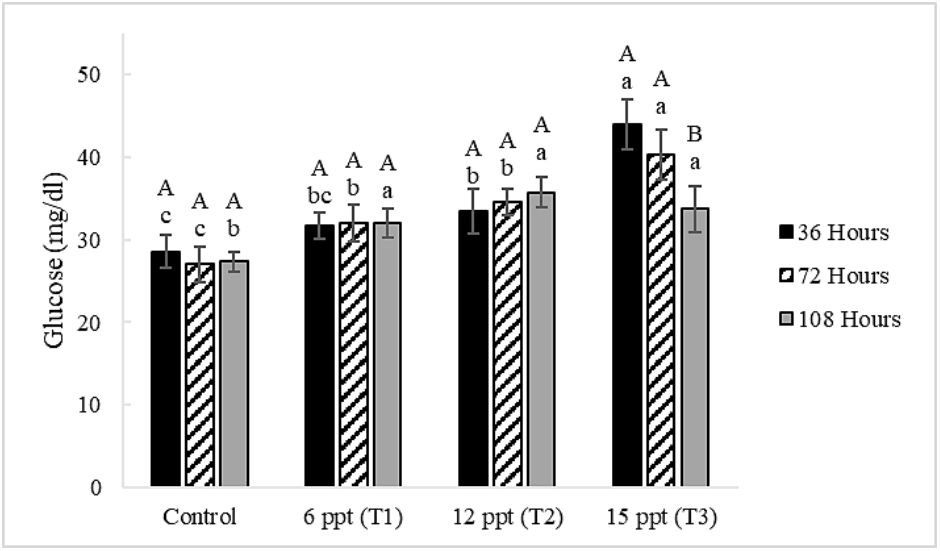

Figure 4. Glucose levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

(P>0.05).

The lowest significant level of ALP enzyme activity was observed in the second sampling period for the control group and treatment 1 (P<0.05). The highest level of significance for this enzyme activity was observed at a salinity of 15 ppt across all three sampling periods (P<0.05). In comparing the mean ALP levels across the three different sampling periods, no significant difference was observed between treatments (P>0.05) (Fig. 3).

Total protein, total immunoglobulin, albumin, and glucose: The highest total protein level was observed in the control and treatment 1 groups (P>0.05). With increasing salinity concentration up to 15 ppt, the level of total blood protein significantly decreased after 72 and 108 hours (P<0.05). The lowest total blood protein level was observed after 108 hours in treatment 3 at 15 ppt salinity (P<0.05), but no significant difference was observed during the same period between the control group and treatments 1 and

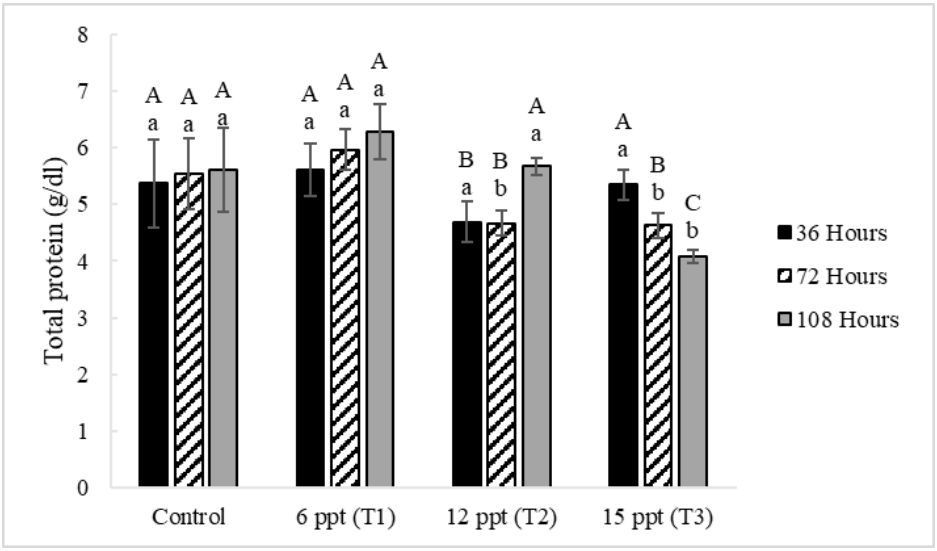

Figure 5. Total protein levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

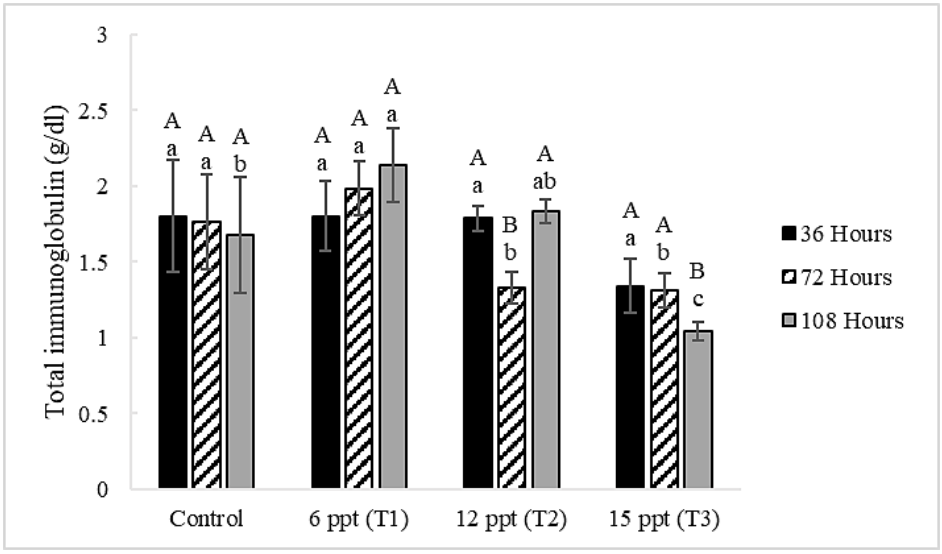

Figure 6. Total immunoglobulin levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

2 (P>0.05). Compared with the three sampling periods, a significant difference in mean total blood protein levels was observed in the groups at 12 and 15 ppt salinity (P<0.05) (Fig. 5).

According to the results of the current work, the total immunoglobulin levels showed no significant difference between the control group and treatment 1 after 36 and 72 hours (P>0.05) (Fig. 6). Increasing salinity from 6 to 15 ppt for 36 hours did not lead to a significant difference between the treatments compared to the control group (P>0.05). The lowest significant level of total immunoglobulin was observed after 72 hours in treatments 2 and 3 (P<0.05), and after 108 hours, the level of total immunoglobulin at 15 ppt salinity decreased significantly (P<0.05).

The highest serum albumin levels were observed at 6 and 12 ppt ambient salinity (P<0.05). No significant difference was observed at 36, 72, and 108 hours of exposure to 6 and 12 ppt salinity (P>0.05). After 36,

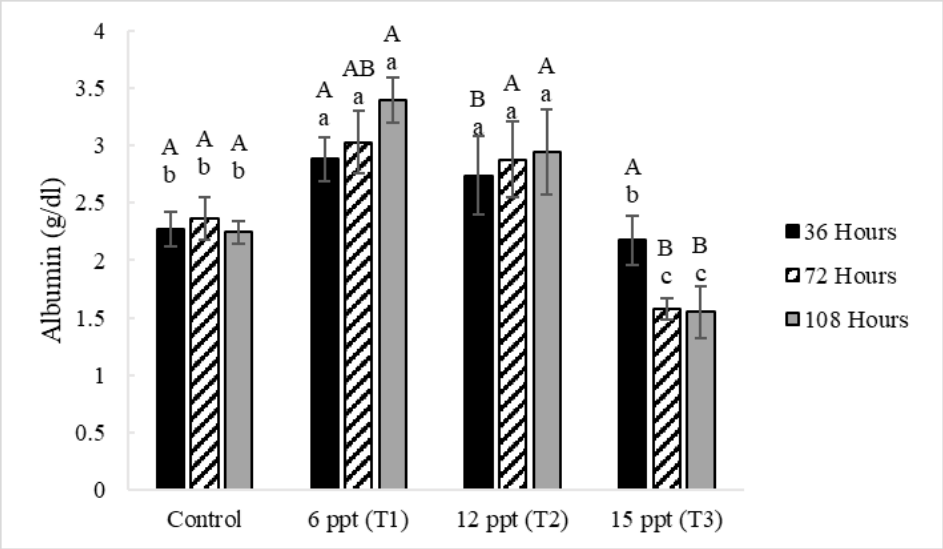

Figure 7. Albumin levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

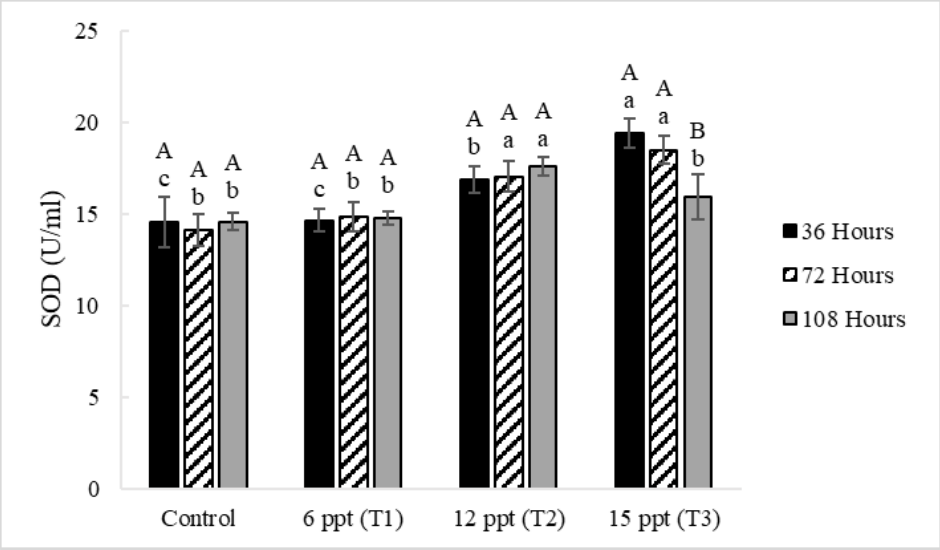

Figure 8. SOD enzyme activity levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

72, and 108 hours, the lowest level was observed in the group with salinity below 15 ppt (P<0.05). Exposure to high salinity concentrations leads to a significant decrease in blood albumin levels over time (P<0.05) (Fig. 7).

Glucose levels in Caspian roach significantly increased with increasing salinity concentration up to 15 ppt (P<0.05) (Fig. 4). Comparing the mean blood glucose levels across the three different sampling periods showed that exposing the Caspian roach to 15 ppt salinity for 36 and 72 hours leads to a significant increase in blood glucose levels and after 96 hours, glucose levels significantly decrease (P<0.05).

Antioxidant enzymes: Exposure of the fish to 12 and 15 ppt salinity for 36 and 72 hours, respectively, increases SOD enzyme activity (P<0.05). At similar time points, no significant difference was observed between treatment 1 and the control group (P>0.05). The effect of exposure to 15 ppt salinity shows that, despite the absence of a significant change in SOD

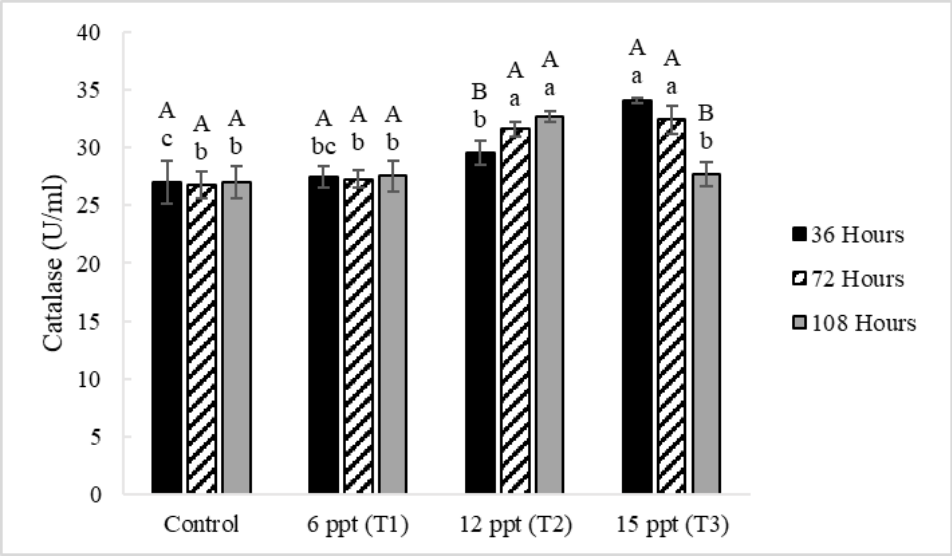

Figure 9. Catalase enzyme activity levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference in the mean data for each treatment across salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference in the mean data across sampling periods. Data are presented as mean ± standard deviation.

enzyme activity at 36 and 72 hours (P>0.05), a significant difference was observed at 108 hours (P<0.05) (Fig. 8).

Changes in catalase enzyme activity levels with increasing salinity concentration showed a significant increase (P<0.05) (Fig. 9). Examining the effect of 12 ppt salinity concentration revealed that increasing the duration of exposure to salinity from 36 hours to 72 and 108 hours also shows a significant increase in catalase enzyme activity (P<0.05). According to the results, exposing Caspian roach to 15 ppt ambient salinity for 108 hours resulted in a significant decrease in catalase activity (P<0.05).

Changes in GPx enzyme activity were calculated in the same way as those for SOD and catalase. According to the results, no significant difference in exposure duration at 6 ppt salinity was observed compared to similar times in the control group (P>0.05). However, at 12 ppt, a significant increase in GPx enzyme activity levels was observed after 72 and 108 hours compared to 36 hours of salinity tolerance (P<0.05). At 15 ppt, GPx enzyme activity showed an inverse relationship with salinity tolerance time. As the duration of salinity tolerance increased, GPx enzyme activity levels significantly decreased, and the lowest level was observed in fish that had tolerated 108 hours of salinity (P<0.05) (Fig. 10).

Gill Na^+-K^+-ATPase activity: Measurements of Na+-K+-ATPase activity in the gills of Caspian Roach show that exposure to ambient salinity significantly decreases Na+-K+-ATPase activity (P<0.05). The lowest level of gill Na^+-K^+-ATPase activity was observed in treatment 2 after 36, 72, and 108 hours (P<0.05). No significant difference in Na^+-K^+-ATPase activity was observed between treatment 1 and the control group (P>0.05). 15 ppt salinity led to a significant increase in Na^+-K^+-ATPase activity at 36 and 72 hours of exposure compared with all groups (P<0.05). However, tolerance to 15 ppt salinity after 108 hours was associated with a significant decrease (P<0.05) in Na+-K+-ATPase activity compared with 36 and 72 hours (Fig. 11).

Gill tissue histology: Examination of gill tissue in Caspian roach revealed that the degree of tissue damage increased with increasing salinity (Fig. 12). Table 3 shows the extent of the complications observed in the gill tissue for each group. The major alterations observed in histopathological examinations include necrosis, hyperplasia at the base of the secondary lamellae, epithelial detachment, aneurysm, and edema (Fig. 12).

Liver tissue histology: Based on the results, exposure to high salinity in Caspian Roach causes tissue damage in the liver. With increasing salinity, liver

Table 3. Determination of the extent of gill tissue complications in Caspian Roach under salinity stress at different concentrations.

	Control	6 ppt	12 ppt	15 ppt
Hyperplasia	-	+	++	++++
Epithelial detachment	-	-	+	+++
Aneurysm	+	+	+	+++
Edema	-	-	-	-

No observed complication (-), Observation of 1 to 3 complications (+), Observation of 3 to 5 complications (++), Observation of 5 to 7 complications (+++), Observation of more than 7 complications (++++).

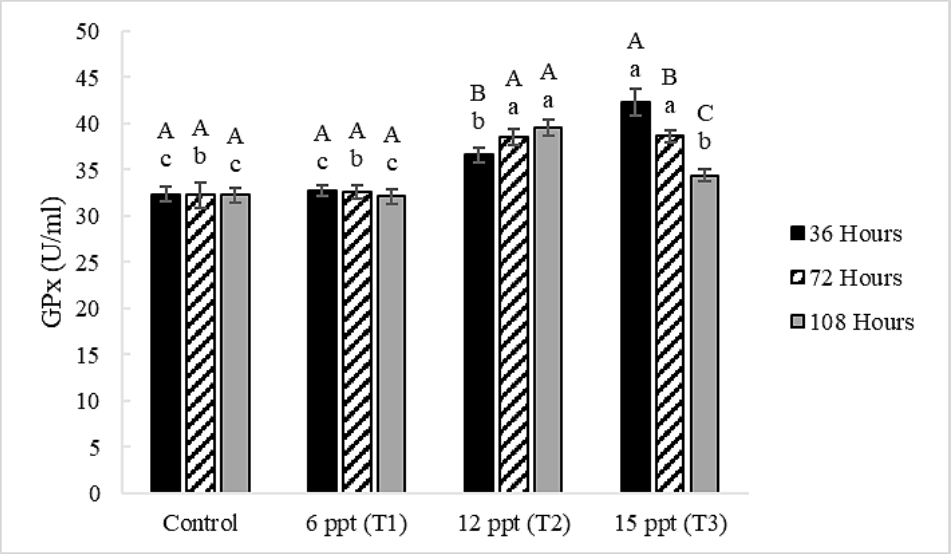

Figure 10. GPx enzyme activity levels in Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

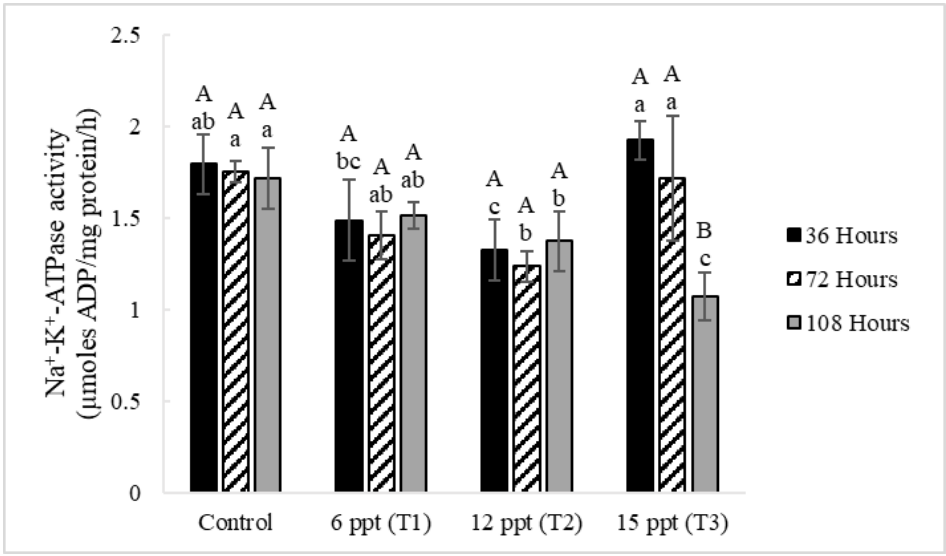

Figure 11. Na^+-K^+-ATPase enzyme activity levels in the gills of Caspian roach under different salinity concentrations across three different time periods. Different lowercase letters indicate a significant difference between the mean data for each treatment at different salinity concentrations compared to the control group. Different uppercase letters indicate a significant difference between the mean data at each sampling period. Data are presented as mean ± standard deviation.

tissue damage increased (Fig. 13). The major damage in liver tissue under salinity stress was vacuolation of hepatocytes, and, in severe cases, degeneration was observed (Table 4).

Table 4. Determination of the extent of liver tissue complications in Caspian Roach under salinity stress at different concentrations.

	Control	6 ppt	12 ppt	15 ppt
Degeneration	-	-	+	+++
Vacuolation	-	-	++++	++++
Hemorrhage	-	-	-	-
Karyolysis	-	-	-	-
Enlargement of the cell nucleus	-	-	-	-

No observed complication (-), Observation of 1 to 3 complications (+), Observation of 3 to 5 complications (++), Observation of 5 to 7 complications (+++), Observation of more than 7 complications (++++).

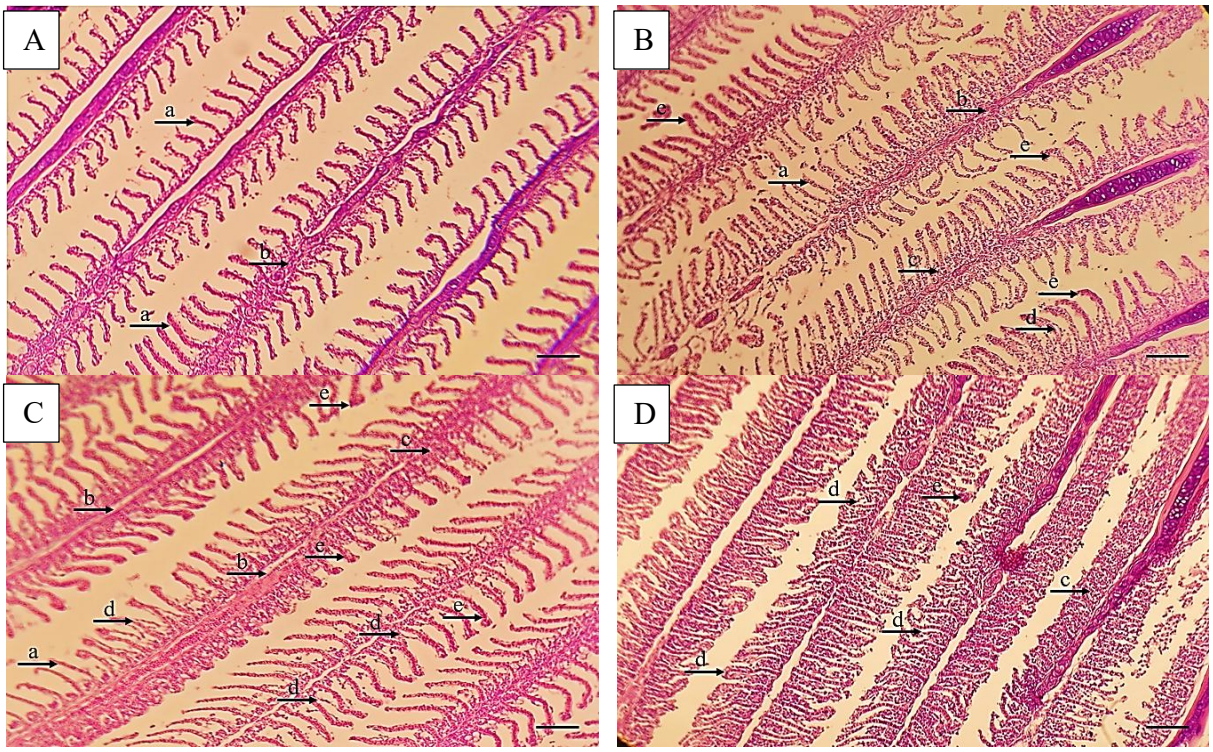

Figure 12. Gill tissue sections in Caspian roach under salinity stress at different concentrations and fish captured from their natural habitat. Magnification 100-400x. (A) Gill tissue in control group fish; normal secondary lamellae (a), primary lamellae (b). (B) Gill tissue in fish under 6 ppt salinity; normal secondary lamellae (a), primary lamellae (b), hyperplasia at the base of the secondary lamellae (c), epithelial detachment in the secondary lamellae (d), mild aneurysm or clubbing at the end of the secondary lamella (e). (C) Gill tissue in fish under 12 ppt salinity; normal secondary lamellae (a), primary lamellae (b), hyperplasia at the base of the secondary lamellae (c), epithelial detachment in the secondary lamellae (d), aneurysm or clubbing at the end of the secondary lamella (e). (D) Gill tissue in fish under 15 ppt salinity; hyperplasia at the base of the secondary lamellae (c), epithelial detachment in the secondary lamellae (d), aneurysm or clubbing at the end of the secondary lamella (e).

Kidney tissue histology: Histopathological examinations of kidney tissue showed that increasing ambient salinity can damage the tissue (Fig. 14), leading to destruction of interstitial tissue, dilation of the glomerular space, and accumulation of exudate in kidney tubules, disrupting homeostasis. The type and severity of tissue damage in each group of fish are presented in Table 5.

Discussions

Increased salinity from evaporation and reduced freshwater inflow led to habitat destruction, biodiversity loss, and lower fish stocks. Drought, by reducing water volume and altering chemical conditions, makes living conditions more difficult for aquatic organisms. Restoring fish stocks is particularly important, and aquaculture can play a significant role in this.

Hematology studies: In the current study, increasing salinity and exposure time significantly increased hematological indices, including red blood cell count, hemoglobin, and hematocrit, in Caspian roach. The lowest levels of these indices were observed at 15 ppt salinity. In addition, no significant differences in the

Table 5. Determination of the extent of complications in the kidney tissue of Caspian Roach under salinity stress at different concentrations.

	Control	6 ppt	12 ppt	15 ppt
Renal tubule degeneration	-	-	-	+
Glomerular space dilation	-	-	+	++
Exudate accumulation	+	+	+	++
Melanomacrophage centers accumulation	-	-	-	-
Destruction and degeneration of interstitial cells	-	-	-	++

No observed complication (-), Observation of 1 to 3 complications (+), Observation of 3 to 5 complications (++), Observation of 5 to 7 complications (+++), Observation of more than 7 complications (++++).

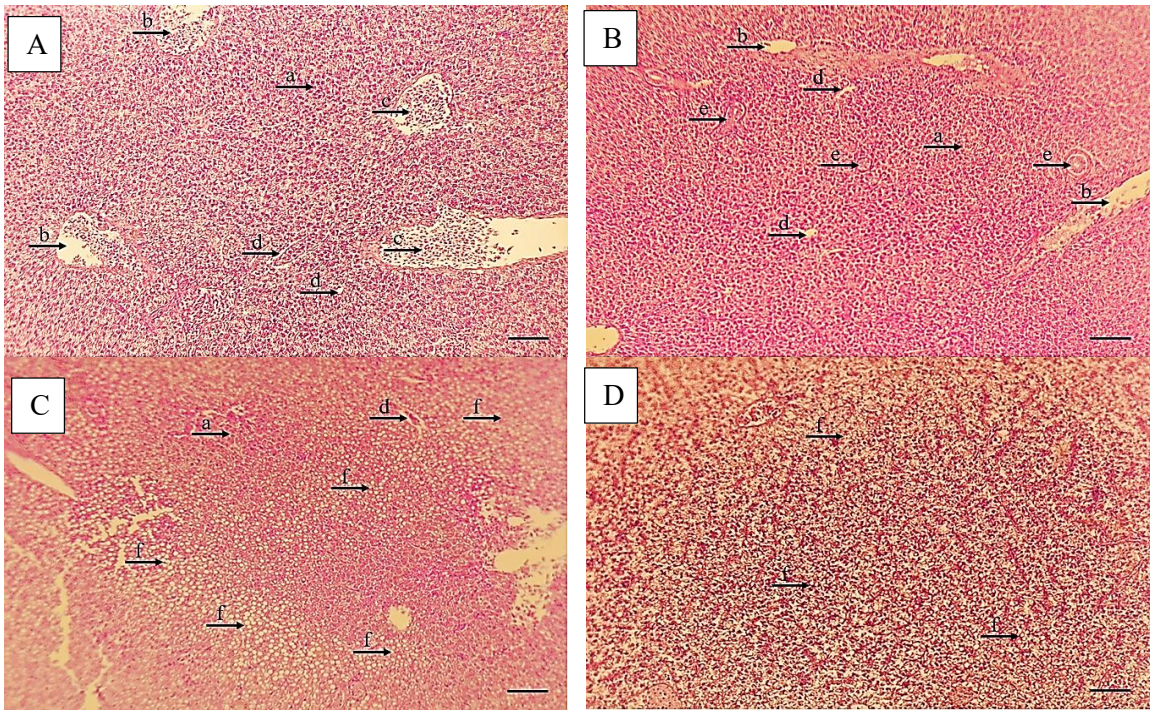

Figure 13. Liver tissue sections in Caspian roach under salinity stress at different concentrations and fish captured from their natural habitat. Magnification 100-400x. (A) Liver tissue in control group fish; liver hepatocytes (a), vein (b), red blood cells (c), sinusoidal space (d). (B) Liver tissue in fish under 6 ppt salinity; liver hepatocytes (a), vein (b), red blood cells (c), sinusoidal space (d), bile ducts (e). (C) Liver tissue in fish under 12 ppt salinity; liver hepatocytes (a), sinusoidal space (d), vacuolation of hepatocytes (f). (D) Liver tissue in fish under 15 ppt salinity; vacuolation of hepatocytes (f).

blood indices MCV, MCH, and MCHC were observed between treatments, and changes in salinity concentration did not alter the percentages of leukocyte types. Hematological changes are important indicators of fish health under stressors such as salinity. Salinity stress can affect the fish's oxygen-carrying capacity and immune system by altering red and white blood cell counts, hematocrit, and hemoglobin (Khanzadeh et al., 2024, 2025). Also, the number of white blood cells, especially neutrophils, increases in response to stress (Khanzadeh et al., 2024, 2025). Changes in hematocrit indicate hydration status and oxygen-carrying capacity. Decreased blood indices such as MCH and MCHC can be caused by anemia, oxidative stress, or impaired nutrient absorption, and reduce oxygen-carrying capacity and tissue function, which was not observed in the current work.

Research on the effects of water salinity on fish blood parameters shows differences across species. Goda et al. (2019) reported that increasing water salinity to 15% increased the numbers of red blood cells (RBC) and white blood cells (WBC) in European seabass (*Dicentrarchus labrax*). Ali et al. (2024) reported that the number of red and white blood cells in Nile tilapia (*Oreochromis niloticus*) was significantly affected by salinity, and the number of red and white blood cells decreased significantly with increasing salinity concentration up to 20 ppt, and increased significantly with increasing salinity to 25

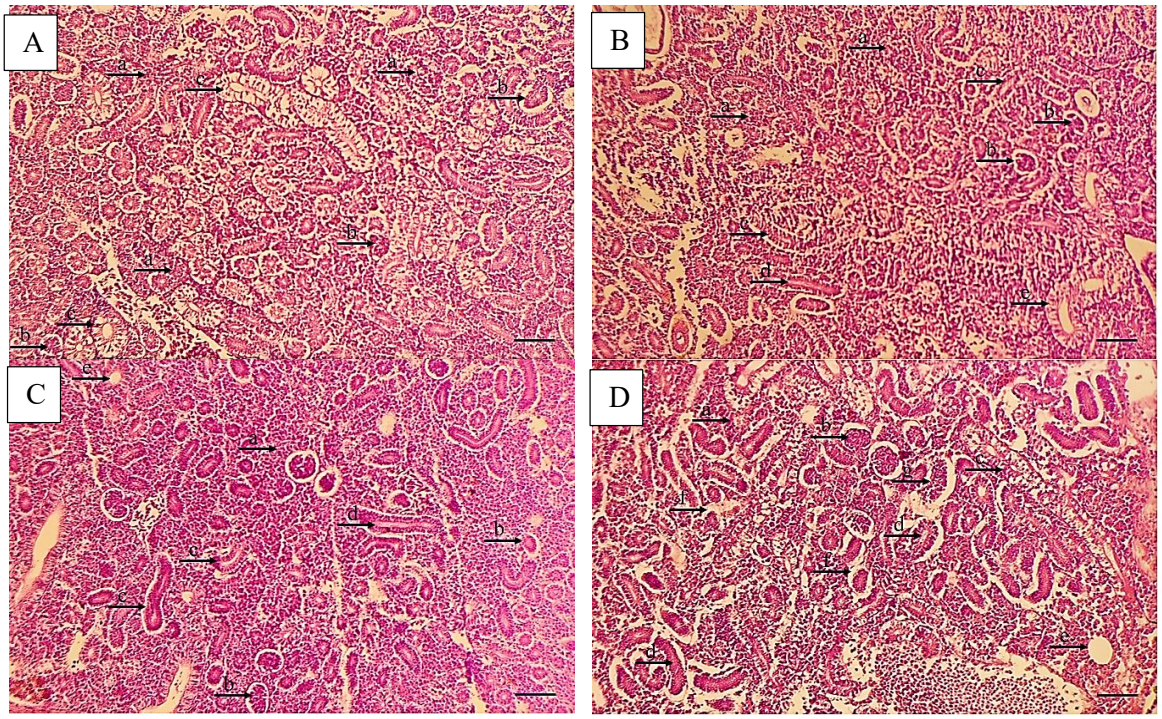

Figure 14. Kidney tissue sections in Caspian Roach under salinity stress at different concentrations and fish captured from their natural habitat. Magnification 100-400x. (A) Kidney tissue in control group fish; interstitial cells (a), renal glomeruli (b), renal tubules (c). (B) Kidney tissue in fish under 6 ppt salinity; interstitial cells (a), renal glomeruli (b), renal tubules (c), presence of exudate fluids in the renal tubules (d), collecting ducts (e). (C) Kidney tissue in fish under 12 ppt salinity; interstitial cells (a), renal glomeruli (b), renal tubules (c), presence of exudate fluids in the renal tubules (d), collecting ducts (e). (D) Kidney tissue in fish under 15 ppt salinity; interstitial cells (a), renal glomeruli (b), renal tubules (c), presence of exudate fluids in the renal tubules (d), collecting ducts (e), dilation of the glomerular space (f), degeneration of renal tubules (g).

ppt. They also reported that salinity stress can cause histo-morphological changes in the hematopoietic tissue of Nile tilapia (Ali et al., 2024). In common carp (*Cyprinus Carpio*), a decrease in the level of blood indices such as red blood cell count (RBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hematocrit (HCT) and platelet count (PLT) was observed when the salt concentration gradually increased from zero to 8 ppt (Owais et al., 2024). On the other hand, Handayani et al. (2020) reported that the levels of hematocrit and hemoglobin and the number of red and white blood cells did not differ significantly between all salinities in *O. niloticus*. Overall, these studies show that water salinity significantly affects the blood parameters of aquatic organisms and may affect their growth, stress levels, and overall health. The decrease in blood indices in Caspian roach under salinity stress indicates impaired hematopoietic function and reduced blood oxygen-carrying capacity at higher salinity. These changes can reduce fish health and survival.

Mortality rate: In the control group, fish movements were normal, and no stress-related abnormal behavior was observed. However, with increasing salinity, especially at 15 ppt, after about 3 days, symptoms such as lethargy, slow swimming, and decreased response to environmental stimuli began to appear. Recording the number of deaths after 108 hours of salinity stress at 15 ppt showed a mortality rate of approximately 86±4 percent. This is consistent with the results of other researchers, including Murmu et al. (2020), Jensen et al. (2015), Kızak et al. (2013), and Altinok et al. (1998). These results seemed to contradict those of Malakpour Kolbadinezhad et al. (2012), who reported no casualties during the gradual transfer of Caspian roach fry release. The differences between these findings may be attributed to differences in laboratory conditions across species, fish size, genetic diversity, water quality parameters (including temperature, brine composition, and duration of salinity stress), and the ability of fish to adapt to

changing environmental conditions.

Each fish species has an ideal salinity tolerance range that depends on its physiological conditions and results from multifaceted interactions among the nervous system, metabolism, and other physiological processes (Khanzadeh et al., 2024, 2025). Any change in salinity can cause osmotic and oxidative stress in aquatic animals by disrupting physiological homeostasis and biological processes, thereby affecting the survival, metabolism, and distribution of fish species. Therefore, decreased survival with increasing salinity may be due to the organism's increased need to provide the energy required for osmoregulation. Also, the results showed that the choice of transfer method (sudden or gradual) directly affects survival and the time required for ionic and osmotic regulation in fish.

Biochemical indicators

***Transaminases (AST and ALT) and alkaline phosphatase (ALP)*:** In the present study, transaminase (AST and ALT) and ALP levels increased when Caspian roach were exposed to salinity stress. There are numerous reports on changes in transaminase (AST and ALT) and ALP levels, which sometimes show contradictory results. In this regard, studies by Mozanzadeh et al. (2021), Shukry et al. (2021), Abdel-Rahim et al. (2020), Al-Khshali and Hilali (2019), and AlKatrani et al. (2018) show that ALT and AST levels increase with increasing salinity. In contrast, Goda et al. (2019) reported that as water salinity increased, ALT and AST levels decreased. The studies by Wang et al. (2023) and Huang et al. (2021) showed that water salinity has no significant effect on ALT and AST levels.

Based on our findings, ALT levels were significantly higher in higher salinities, but no significant difference in mean ALT levels was found across groups at different sampling times. ALT enzyme, due to its major presence in liver cytosol, is a specific indicator of liver health, and liver damage increases its levels. In the current study, increasing salinity to 12 and 15 ppt resulted in a significant rise in AST levels. AST is a non-specific indicator of tissue damage and is present in the liver, red blood cells, kidneys, pancreas, and cardiac muscle (Khanzadeh et al., 2024, 2025). In addition, various environmental and physiological factors such as age, water salinity, seasons, maturity status, sex, water temperature, and diet type affect the levels of these enzymes and their activity (Zargari et al., 2023).

In the present study, plasma transaminase activity increased with increasing water salinity. It is also observed that with increasing time of exposure to salinity, the activity of these enzymes increases, but not significantly. When a fish is exposed to salinity stress, its body activates various defense mechanisms to cope with it (Khanzadeh et al., 2024, 2025). These mechanisms can damage liver cells and other tissues, leading to increased release of these enzymes into the bloodstream. High salinity can damage liver cells and muscle tissue, leading to the release of AST and ALT into the blood (Zargari et al., 2023). Therefore, their increased levels in the blood indicate tissue damage. In addition to tissue damage, under stressful conditions, the secretion of glucagon and cortisol activates gluconeogenic enzymes (transaminases). In this process, to provide the energy the body needs to cope with salinity stress, non-carbohydrate precursors, such as amino acids, are used to produce glucose. Alanine and aspartate are among the most important amino acids for gluconeogenesis. Under stressful conditions, alanine and aspartate are released from muscle tissue and enter the liver, where they are converted to pyruvate and oxaloacetate, respectively, by ALT and AST enzymes, which then enter the gluconeogenesis pathway for glucose production. The ALP level was significantly higher at 15 ppt across all treatments in the current study. The increased ALP levels may indicate damage to the liver, bones, or intestines. In the case of fish under salinity stress, increased ALP is usually due to liver damage. ALP is a membrane-bound enzyme and is present in most tissues, and is one of the important enzymes in the process of osmotic regulation in aquatic animals. Also, in some studies, ALP has been recognized as a potential stress indicator in fish. Salinity fluctuations often trigger physiological stress responses that disrupt the balance of plasma hormones, energy

metabolism, and electrolytes in aquatic animals. The ALP enzyme plays an important role in fish adaptation to saltwater. Many researchers have reported that, when fish are transferred from freshwater to saltwater, ALP activity increases significantly, suggesting an important role for this enzyme in ion and hormone transport during osmotic regulation (Zargari et al., 2023).

Total protein, total immunoglobulin, albumin, and glucose: Based on the results of the current study, with increasing salinity concentrations up to 15 ppt, total blood protein levels decreased significantly at 72 and 108 hours. Changes in plasma proteins are important indicators of the health and stress status of aquatic animals (Khanzadeh et al., 2024, 2025). Plasma proteins play vital roles in functions such as nutrient transport, maintaining osmotic pressure, and immune response. A decrease in plasma protein under salinity stress can result from increased protein catabolism, decreased appetite, and impaired protein synthesis due to damage to the liver, kidneys, and gills. These changes indicate metabolic and physiological disorders and can negatively affect fish health and survival (Khanzadeh et al., 2024, 2025). In studies by Mozanzadeh et al. (2021), Goda et al. (2019), Abdel-Rahim et al. (2020), and Fagan et al. (2003), as water salinity increases, total plasma protein levels rise. In other studies, reviewed by Peyghan et al. (2014), Cho et al. (2013), Martinez-Alvarez et al. (2002), and Fast et al. (2002), no significant difference in plasma protein levels was observed with changes in water salinity. Other researchers, including Wang et al. (2023), Shukry et al. (2021), and Elarabany et al. (2017), found that as water salinity increases, plasma protein levels decrease. The difference in results may be due to differences in fish species, environmental conditions, and how the fish are exposed to salinity.

The results of the present study showed that as salinity increased, total immunoglobulin levels decreased significantly, consistent with the findings of Amin et al. (2022) on Caspian roach. Immunoglobulins (antibodies) are important proteins that play a key role in the immune response of animals, including fish (Zargari et al., 2023). These proteins bind to foreign antigens and mark them for destruction by the immune system (Khanzadeh et al., 2024, 2025). A decrease in blood immunoglobulin levels can indicate a weakened immune system and increased susceptibility to disease (Zargari et al., 2023). Salinity stress is one of the most important environmental stressors for fish. This stress can cause extensive physiological and biochemical changes in the fish's body, which in turn affect immune system function (Mozanzadeh et al., 2021). The exact mechanism by which salinity stress affects immunoglobulin levels in bony fish is not well understood, but possible mechanisms include increased energy consumption to adapt to the new environment or reduced energy available for synthesizing immune proteins such as immunoglobulins. Also, weakening of the immune system and reduced activity of immune cells, such as lymphocytes, can lead to decreased immunoglobulin production. The kidneys, which play an important role in the production and regulation of immunoglobulins, can be damaged, leading not only to their loss in the urine but also to reduced synthesis. In addition, salinity stress increases the production of stress hormones such as cortisol, which can have immunosuppressive effects and reduce immunoglobulin production. Mozanzadeh et al. (2021) and Cuesta et al. (2005) show that with increasing salinity, total immunoglobulin levels increase. Elarabany et al. (2017) reported that immunoglobulin levels did not change significantly with increasing salinity.

In the present study, increasing ambient salinity and exposure time to salinity stress can significantly decrease albumin levels in Caspian roach. This decrease may be due to damage to liver hepatocytes and disruption of the albumin synthesis process during salinity stress. Also, high salinity can affect the absorption of essential nutrients for protein synthesis, such as amino acids, and consequently reduce albumin synthesis. In addition, salinity stress can increase protein catabolism to provide the energy needed to deal with stress. The kidneys and gills play an important role in maintaining the balance of plasma proteins. Damage to the kidneys can lead to increased

excretion of albumin through the urine and leakage of protein from the blood to the environment (Batool et al., 2024). The decrease in albumin levels in fish under salinity stress indicates impaired liver and kidney function and, in general, is an indicator of stress and disease in these organisms. Decreased albumin levels can lead to edema (swelling), fluid accumulation in tissues, reduced transport of certain nutrients, and a weakened immune system.

Albumin, one of the most important water-soluble proteins in plasma, constitutes more than 50% of blood proteins and plays vital roles, including maintaining blood osmotic pressure, transporting nutrients and drugs, and maintaining blood pH. This protein is synthesized in the liver and is regenerated in the blood at 8% daily, with a half-life of 10-18 days. The main functions of albumin include regulating the colloidal osmotic pressure of the blood and assisting in the transport of drugs, fatty acids, hormones, and bilirubin. The increase in albumin levels during the transfer of fry to salt water can be attributed to the fish's response, which provides the energy needed to regulate osmotic pressure through albumin synthesis. The results of studies by Batool et al. (2024), Abdel-Rahim et al. (2020), and Peyghan et al. (2014) showed that albumin levels are affected by environmental salinity and that plasma salinity is directly positively related to salinity concentration. In contrast, Shukry et al. (2021) and Goda et al. (2019) reported that changes in plasma albumin levels are inversely related to increasing environmental salinity.

Glucose levels in Caspian Roach increased significantly with increasing salinity concentrations up to 36 and 72 hours, and then decreased. Glucose is one of the most reliable parameters for measuring stress, which is strongly influenced by management and environmental stresses (Shukry et al., 2021). Under stressful conditions, glucose is mainly used as an energy source, a process regulated by hormones. In stressful conditions, such as increased environmental salinity, catecholamines (adrenaline and noradrenaline) and cortisol, by affecting the liver, induce glycolysis and gluconeogenesis and consequently increase plasma glucose, thereby providing the energy needed to deal with stressful conditions (Shukry et al., 2021). Changes in blood glucose levels can indicate significant physiological responses to environmental stress. Salinity stress is one of the most important environmental stressors for fish, causing extensive changes in their metabolism and physiology.

Salinity stress disrupts the osmotic balance of a fish's body (Mozanzadeh et al., 2021). To deal with this disorder, fish expend more energy to excrete excess ions from their bodies and maintain osmotic balance. This increased energy consumption leads to an increased demand for glucose as an energy source. Salinity stress causes the release of stress hormones such as cortisol. These hormones activate gluconeogenesis enzymes in the liver (Shukry et al., 2021; Mozanzadeh et al., 2021). Gluconeogenesis is a process in which glucose is produced from amino acids, glycerol, and some fatty acids. Stress hormones also increase the breakdown of glycogen stored in the liver and muscles. Glycogen is a polysaccharide that acts as an energy reserve in the body. Under stress, some tissues, such as muscles, may reduce glucose use to rely on alternative energy sources, such as fatty acids, leading to an increase in blood glucose levels.

Despite reports of decreased glucose levels during stress, studies by Shukry et al. (2021), Mozanzadeh et al. (2021), Azodi et al. (2021), Goda et al. (2019), Elarabany et al. (2017), and Taylor et al. (2007) reported similar results regarding increased glucose levels in aquatic animals under stress, which are consistent with the results of the present study. Studies by Mian and Siddiqui (2020) showed that with increasing salinity, plasma glucose levels in *O. mossambicus* decreased. Martinez-Alvarez et al. (2002) reported that no significant change in glucose levels was observed during adaptation to salinity in Adriatic sturgeon. Increased blood glucose levels can lead to the production of reactive oxygen species that damage cells and cause oxidative stress. Oxidative stress itself can weaken the immune system and make fish susceptible to various infectious diseases.

Antioxidant enzymes: Based on the current study, with increasing stress time and salinity concentration

in Caspian roach, the activities of superoxide dismutase, catalase, and glutathione peroxidase increased significantly. Salinity stress can increase the production of reactive oxygen species in fish. This increase is due to impaired mitochondrial function, increased activity of enzymes producing reactive oxygen species, and decreased activity of the endogenous antioxidant defense system. In addition, this increase in their level may be due to increased expression of antioxidant enzyme genes and, consequently, increased synthesis of enzyme proteins. This trend may have been aimed at protecting cells and body tissues, reducing oxidative stress caused by disruption of the body's homeostatic balance, and improving physiological function.

Numerous studies have examined how the levels of antioxidant enzymes change, often yielding contradictory results. The effects of environmental salinity on antioxidant enzyme levels in fish vary by species and exposure conditions. In *Hypophthalmichthys molitrix*, exposure to water with 6% salinity increases the activity of SOD and CAT enzymes, while the activity of the GPX enzyme did not show any change (Jiang et al., 2022). However, in *Acanthopagrus latus*, a gradual increase in salinity increased GPX and SOD activity, whereas CAT activity decreased (Mozanzadeh et al., 2021). In *Lates calcarifer*, CAT and GPX activities decreased with increasing salinity from 6 to 24%, and then increased from 35 to 48%. SOD activity level increased with increasing salinity from 6 to 24% (Mozanzadeh et al., 2021). These studies show that the effects of salinity on antioxidant enzymes are species-specific and depend on exposure duration and environmental factors. In a study by Dawood et al. (2022), the activity of SOD, CAT, and GPx enzymes significantly decreases with increasing salinity, but the levels of malondialdehyde (MDA) increased in fish under 15 and 20 ppt salinity stress.

Naturally, reactive oxygen species (ROS) are produced during metabolic processes, but under stress conditions, their production in the body increases (Jiang et al., 2022). These species are highly reactive free radicals that can react with important biological molecules, such as lipids, proteins, and nucleic acids, in the body, leading to oxidative stress (Khanzadeh et al., 2024, 2025). All living organisms have protective systems against free radical reactions and oxidative stress (Mozanzadeh et al., 2021; Jiang et al., 2022). In fact, these protective systems can create a balance in normal conditions between the production and removal of reactive oxygen species. Imbalance in this process may disrupt the body's homeostasis and cause oxidative damage to various tissues in living organisms. Antioxidant defense systems include enzymatic and non-enzymatic systems. The most important antioxidant enzymes include catalase, superoxide dismutase, and glutathione peroxidase. Superoxide dismutase converts the superoxide radical to hydrogen peroxide (H_2O_2). Subsequently, catalase decomposes hydrogen peroxide into water and oxygen. Glutathione peroxidase reduces hydrogen peroxide and other peroxides using glutathione (Mozanzadeh et al., 2021; Jiang et al., 2022).

Gill Na^+-K^+-ATPase activity: The present study showed that exposing the Caspian roach to 15 ppt salinity after 108 hours increased gill Na+-K+-ATPase activity. The reason for the increase in the activity of the Na^+-K^+-ATPase enzyme in these fish may be due to the increase in the concentration of extracellular sodium and its effect on the expression level of the gene encoding the alpha subunit of the sodium-potassium pump, which subsequently leads to an increase in the synthesis of this subunit and, as a result, an increase in enzyme activity.

Fish require highly efficient ionic and osmoregulatory mechanisms to maintain their body homeostasis, which is essential for the normal functioning of all biochemical and physiological processes. The Na^+-K^+-ATPase enzyme plays an important role in maintaining the osmotic balance of fish and is specifically active along the basolateral membranes (Ding et al., 2023). By actively transporting sodium ions out of the cell and potassium ions into the cell, this enzyme maintains the concentration gradient of these ions and, as a result, helps regulate cell volume and maintain osmotic pressure (Li et al., 2022; Ding et al., 2023). Under

salinity stress, the activity of this enzyme, located on the basolateral side of mitochondrion-rich cells (MRC) in the gills, increases significantly, helping the fish cope with changes in environmental salinity (Ding et al., 2023). The osmoregulatory capacity of young fish is one of the most important physiological factors in the success of restoration during release and during transportation. When a fish is exposed to salt water, the sodium concentration in the gill cells increases. This increase in concentration activates the Na^+-K^+-ATPase enzyme. This enzyme in mitochondrion-rich cells of the gills (the sodium-potassium pump) uses ATP to pump sodium ions out of the cell and potassium ions into the cell (Li et al., 2022). The sodium-potassium pump creates a concentration gradient for potassium ions, which leads to water leaving the cell (Li et al., 2022).

How changes in the activity of the gill Na^+-K^+-ATPase enzyme after transfer to different environmental salinities depend on the species. Studies shown that changes in the activity of the gill Na^+-K^+-ATPase enzyme after transfer from a hypoosmotic environment to a hyperosmotic environment in *Fundulus heteroclitus* (Mancera et al., 2000), in other anadromous and euryhaline species such as *Oncorhynchus gorbuscha* (Nemova et al., 2021), *D. labrax* (Kır et al., 2019; Islam et al., 2021), *Salmo salar* (Bystriansky and Schulte, 2011; van Rijn et al., 2020), and *Lates calcarifer* (Ding et al., 2023) have been reported. There are reports of the direct effect of environmental salinity on the activity of the gill Na^+-K^+-ATPase enzyme in *Scophthalmus maximus* (Cui et al., 2020), *O. mossambicus* (Angadi et al., 2021), and *Oncorhynchus keta* (Li et al., 2022). While some other researchers reported a negative correlation between water salinity and the activity of the gill Na^+-K^+-ATPase enzyme in *O. mossambicus* (Handayani et al., 2020), in some reports, no significant correlation was found between water salinity and the activity of the gill Na^+-K^+-ATPase enzyme in *Fundulus heteroclitus* (Giacomin et al., 2020).

Histopathology: Based on the results of the current study, increasing salinity caused visible damage to the gill, liver, and kidney tissues. The major damage in the liver tissue of Caspian roach under salinity stress in our work was vacuolation of hepatocytes. In the kidney, interstitial tissue, dilation of the glomerular space, and accumulation of exudate in the tubules, which disrupt homeostasis, were major histopathological signs. Epithelial detachment, epithelial necrosis, gill degeneration, and hyperplasia were found in the current study. Ali et al. (2024), in evaluating the effects of environmental salinity stress on European seabass (*D. labrax*), reported various abnormalities in the gills and kidneys. In this study, consistent with our findings, although hypertrophy, epithelial detachment, and epithelial necrosis were observed in gill degeneration, hyperplasia was common across almost all salinity levels (Ali et al., 2024). Furthermore, glomerular necrosis, nuclear pyknosis, hyaline droplet degeneration, and glomerular shrinkage were observed in the kidney (Ali et al., 2024). Unusual behavioral responses with various pathological symptoms in the gills of *Channa punctate* fish were reported by Khatun et al. (2020). The results of their research showed that the intrusion of salt water into fresh water has destructive effects on gill morphology; therefore, to prevent losses, the intrusion of salt water should be controlled (Khatun et al., 2020). In addition, gill, liver, and kidney tissue damage have been reported in studies by other researchers in the presence of environmental pollutants (Shahjahan et al., 2022b; Vineetha et al., 2024).

Conclusion

Salinity stress in aquatic environments adversely affects the physiology of Caspian roach. Increased activity of antioxidant enzymes and Na^+-K^+-ATPase indicates the fish's attempt to deal with oxidative stress and maintain electrolyte balance. However, these mechanisms lose their effectiveness at high salinity. Damage to vital tissues and increased mortality indicate the harmful effects of salinity on fish health and survival. Decreased blood plasma immune factors also indicate a weakened immune system. Increased salinity can lead to physiological disorders and death

of the Caspian roach. Hence, the results showed that the choice of transfer method (sudden or gradual) directly affects survival and the time required for ionic and osmotic regulation in fish. Proper management of freshwater resources and reducing salt pollution is essential to preserving native fish populations and biodiversity.

References

Abdel-Rahim M.M., Lotfy A.M., Toutou M.M., Aly H.A., Sallam G.R., Abdelaty B.S., Helal A.M. (2020). Effects of salinity level on the survival, growth, feed utilization, carcass composition, haematological and serum biochemical changes of juvenile Meagre (*Argyrosomus regius*)(Asso, 1801) grown in ground saltwater. Aquaculture Research, 51(3): 1038-1050.

Ali A., Azom M.G., Sarker B.S., Rani H., Alam M.S., Islam M.S. (2024). Repercussion of salinity on hematological parameters and tissue morphology of gill and kidney at early life of tilapia. Aquaculture and Fisheries, 9(2): 256-264.

AlKatrani L.M., Jaafer F.M., Aldoghachi M.A. (2018). Effect of sudden and gradual transfer of *Oreochromis aureus* to different water salinities on the activity of AST and ALT serum enzymes. Journal of King Abdulaziz University, 28(2): 81-88.

Al-Khshali M.S., Al Hilali H.A. (2019). Some physiological changes (ALP, AST and ALT) of common carp (*Cyprinus carpio*) caused by high salinity. Biochemical and Cellular Archives, 19(2).

Altinok I., Galli S.M., Chapman F.A. (1998). Ionic and osmotic regulation capabilities of juvenile Gulf of Mexico sturgeon, *Acipenser oxyrinchus* de sotoi. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 120(4): 609-616.

Amin N., Shirangi S.A., Kashiri H., Jafaryan H., Adineh H. (2022). Effects of abrupt and gradual transfer methods to the salinity of the Caspian Sea on ion regulation, some of immunity responses and stress indices in Caspian Roach (*Rutilus caspicus*, Yakovlev 1870). Fisheries Science and Technology, 11(1): 42-54.

Angadi P., Das M., Roy R. (2021). Effect of high salinity acclimation on glucose homeostasis in Mozambique tilapia (*Oreochromis mossambicus*). Fish Physiology and Biochemistry, 47(6): 2055-2065.

Azodi M., Bahabadi M.N., Ghasemi A., Morshedi V., Mozanzadeh M.T., Shahraki R., Khademzadeh O., Hamedi S., Avizhgan S. (2021). Effects of salinity on gills' chloride cells, stress indices, and gene expression of Asian seabass (*Lates calcarifer*, Bloch, 1790). Fish Physiology and Biochemistry, 47: 2027-2039.

Batool M., Naz S., Abbas G., Chatha A.M.M., Mahmood M., Aziz A., Yasmin F. (2024). Hematological and biochemical studies in commercially important fish *Labeo rohita* exposed to cadmium chloride. Sarhad Journal of Agriculture, 40(1): 58-170.

Bystriansky J.S., Schulte P.M. (2011). Changes in gill H+-ATPase and Na+/K+-ATPase expression and activity during freshwater acclimation of Atlantic salmon (*Salmo salar*). Journal of Experimental Biology, 214(14): 2435-2442.

Choi K., Cope W.G., Harms C.A., Law J.M. (2013). Rapid decreases in salinity, but not increases, lead to immune dysregulation in Nile tilapia, *Oreochromis niloticus* (L.). Journal of Fish Diseases, 36(4): 389-399.

Cuesta A., Laiz-Carrión R., Del Río M.M., Meseguer J., Mancera J.M., Esteban M.Á. (2005). Salinity influences the humoral immune parameters of gilthead seabream (*Sparus aurata* L.). Fish and Shellfish Immunology, 18(3): 255-261.

Cui W., Ma A., Wang X., Huang Z. (2020). Myo-inositol enhances the low-salinity tolerance of turbot (*Scophthalmus maximus*) by modulating cortisol synthesis. Biochemical and Biophysical Research Communications, 526(4): 913-919.

Dawood M.A., Alkafafy M., Sewilam H. (2022). The antioxidant responses of gills, intestines and livers and blood immunity of common carp (*Cyprinus carpio*) exposed to salinity and temperature stressors. Fish physiology and biochemistry, 48(2): 397-408.

Ding Y.C., Lee S.S., Peng S.K., Yang W.K., Lee T.H. (2023). Salinity-dependent changes in branchial morphometry and Na+, K+-ATPase responses of euryhaline Asian sea bass, *Lates calcarifer*. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 339(5): 451-463.

Eagderi S., Mouludi-Saleh A., Esmaeli H.R., Sayyadzadeh G., Nasri M. (2022). Freshwater lamprey and fishes of Iran; a revised and updated annotated checklist-2022.Turkish Journal of Zoology, 46(6): 500-522.

Elarabany N., Bahnasawy M., Edrees G., Alkazagli R. (2017). Effects of salinity on some haematological and biochemical parameters in Nile tilapia, *Oreochromus niloticus*. Agriculture, Forestry and Fisheries, 6(6): 200-205.

Eremkina T.V., Yarushina M.I. (2022). Ural River Basin. In: Rivers of Europe. Elsevier. pp: 883-899.

Fagan M.S., O'Byrne-Ring N., Ryan R., Cotter D., Whelan K., Mac Evilly U. (2003). A biochemical study of mucus lysozyme, proteins and plasma thyroxine of Atlantic salmon (*Salmo salar*) during smoltification. Aquaculture, 222(1-4): 287-300.

Fast M.D., Sims D.E., Burka J.F., Mustafa A., Ross N.W. (2002). Skin morphology and humoral non-specific defence parameters of mucus and plasma in rainbow trout, coho and Atlantic salmon. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 132(3): 645-657.

Giacomin M., Onukwufor J.O., Schulte P.M., Wood C.M. (2020). Ionoregulatory aspects of the hypoxia-induced osmorespiratory compromise in the euryhaline Atlantic killifish (*Fundulus heteroclitus*): the effects of salinity. Journal of Experimental Biology, 223(13): jeb216309.

Goda A.M., Srour T.M., Mansour A.T., Baromh M.Z., Sallam G.R., Baromh A.Z. (2019). Assessment of stressful ambient water salinity on growth, feed utilization and hematological indices of European sea bass, *Dicentrarchus labrax*, juveniles. Aquaculture, Aquarium, Conservation and Legislation, 12(2): 553-563.

Handayani K.S., Irawan B., Soegianto A. (2020). Short-term mercury exposure in tilapia (*Oreochromis niloticus*) at different salinities: impact on serum osmoregulation, hematological parameters and Na+/K+-ATPase level. Heliyon, 6(7).

Hasanpour S., Eagderi S., Mojazi Amiri B. (2015). Osteological development of the vertebral column, paired, dorsal and anal fins in *Rutilus caspicus*, Pravdin (1927)(Teleostei: Cyprinidae). Caspian Journal of Environmental Sciences, 13(3): 207-219.

Hasanpour S., Eagderi S., Mojazi-Amiri B., Moradi M. (2016). Skeletal development of the caudal complex in Caspian roach (*Rutilus caspicus*)(Yakovlev, 1927)(Teleostei: Cyprinidae). Biharean Biologist, 10(1): 16-19.

Huang M., Yang X., Zhou Y., Ge J., Davis D.A., Dong Y., Gao Q., Dong S. (2021). Growth, serum biochemical parameters, salinity tolerance and antioxidant enzyme activity of rainbow trout (*Oncorhynchus mykiss*) in response to dietary taurine levels. Marine Life Science and Technology, 1-14.

Islam M.J., Kunzmann A., Slater M.J. (2021). Extreme winter cold-induced osmoregulatory, metabolic and physiological responses in European seabass (*Dicentrarchus labrax*) acclimatized at different salinities. Science of the Total Environment, 771: 145202.

Jamebozorgi F.H., Abtahi B., Sharifpour I., Seyfabadi J., Rahmatabadi Z.T., Nazemroaya S., Lari E. (2023). The effects of the water-soluble fractions of crude oil on liver and kidney tissues of Caspian Kutum juveniles, *Rutilus frisii*. Marine Pollution Bulletin, 189: 114675.

Jensen L.F., Thomsen D.S., Madsen S.S., Ejbye-Ernst M., Poulsen S.B., Svendsen J.C. (2015). Development of salinity tolerance in the endangered anadromous North Sea houting *Coregonus oxyrinchus*: implications for conservation measures. Endangered Species Research, 28(2): 175-186.

Jiang Y., Yuan C., Qi M., Liu Q., Hu Z. (2022). The effect of salinity stress on enzyme activities, histology and transcriptome of silver carp (*Hypophthalmichthys molitrix*). Biology, 11(11): 1580.

Kashiri H., Shabani A., Gorgin S., Rezaei M., Jabale A. (2019). Temporal stability of genetic diversity revealed by microsatellite markers in restocked populations of Caspian roach, *Rutilus caspicus* (Yakovlev, 1870). Animal Biology, 69(3): 327-347.

Khanzadeh M., Hoseinifar S.H., Zargari A., Van Doan H. (2025). Effects of dietary supplements *Sargassum ilicifolium* and *Spirulina platensis* on growth parameters, immunity and gene expression in juvenile Asian seabass (Lates calcarifer). Journal of Agriculture and Food Research, 101689.

Khanzadeh M., Hoseinifar S.H., Zargari A., Tabibi H., Van Doan H., Rabetimarghezar N. (2024). Fucoidan derived from *Sargassum ilicifolium* affects growth and hemato-immunological parameters and antioxidant defense in Oscar (*Astronotus ocellatus*). Frontiers in Marine Science, 11: 1370871.

Khatun H., Mostakim G.M., Islam S. (2020). Acute responses of spotted snakehead (*Channa punctata*) to salinity stress: A study of a freshwater fish to salinity challenges during intrusion of saline water. Iranian Journal of Fisheries Sciences, 19(5): 2673-2687.

Kır M., Sunar M.C., Gök M.G. (2019). Acute ammonia toxicity and the interactive effects of ammonia and salinity on the standard metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture, 511: 734273.

Kızak V., Ozden O., Guner Y. (2013). Effects of seawater acclimatization on gill Na+-K+-ATPase activities and chloride cells in rainbow trout (*Oncorhynchus mykiss*)

and brown trout (*Salmo trutta forma fario*). Ege journal of fisheries and aquatic sciences, 50(1).

Li P., Liu W., Lu W., Wang J. (2022). Biochemical indices, gene expression and SNPs associated with salinity adaptation in juvenile chum salmon (*Oncorhynchus keta*) as determined by comparative transcriptome analysis. PeerJ, 10: e13585.

Malakpour Kolbadinezhad S., Hajimoradloo A., Ghorbani R., Joshaghani H., Wilson J.M. (2012). Effects of gradual salinity increase on osmoregulation in Caspian roach Rutilus caspicus. Journal of Fish Biology, 81(1): 125-134.

Mancera J.M., McCormick S.D. (2000). Rapid activation of gill Na+, K+-ATPase in the euryhaline teleost *Fundulus heteroclitus*. Journal of Experimental Zoology, 287(4): 263-274.

Martinez-Alvarez R.M., Hidalgo M.C., Domezain A., Morales A.E., García-Gallego M., Sanz A. (2002). Physiological changes of sturgeon *Acipenser naccarii* caused by increasing environmental salinity. Journal of experimental biology, 205(23): 3699-3706.

McCormick S.D. (1993). Methods for non-lethal gill biopsy and measurement of Na+/K+- ATPase activity. Canadian Journal of Fisheries and Aquatic Sciences, 50: 656-658.

McCormick S.D. (1995). Hormonal control of gill Na+/K+-ATPase and chloride cell function. In: C.M. Wood, T.J. Shuttlewoth, (Eds.), Fish Physiology, Vol. 14, San Diego, CA: Academic Press. pp: 285-315.

Mian J., Siddiqui P.Z.J.A. (2020). Effect of salinity and protein levels on haematological, and physiological changes and growth of hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*). Iranian Journal of Fisheries Sciences, 19(3): 1268-1279.

Mohamed N.A., Saad M.F., Shukry M., El-Keredy A.M., Nasif O., Van Doan H., Dawood M.A. (2021). Physiological and ion changes of Nile tilapia (*Oreochromis niloticus*) under the effect of salinity stress. Aquaculture Reports, 19: 100567.

Mozanzadeh M.T., Safari O., Oosooli R., Mehrjooyan S., Najafabadi M.Z., Hoseini S.J., Saghavi H., Monem, J. (2021). The effect of salinity on growth performance, digestive and antioxidant enzymes, humoral immunity and stress indices in two euryhaline fish species: Yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*). Aquaculture, 534: 736329.

Murmu K., Rasal K.D., Rasal A., Sahoo L., Nandanpawar P.C., Udit U.K., Patnaik M., Mahapatra K.D., Sundaray J.K. (2020). Effect of salinity on survival, hematological and histological changes in genetically improved rohu (Jayanti), *Labeo rohita* (Hamilton, 1822). Indian Journal of Animal Research, 54(6): 673-678.

Nemova N.N., Kaivarainen E.I., Rendakov N.L., Nikerova K.M., Efremov D.A. (2021). Cortisol content and Na+/K+-ATPase activity under adaptation of juvenile pink salmon *Oncorhynchus gorbuscha* (Salmonidae) to salinity changes. Journal of Ichthyology, 61: 771-778.

Owais M., Al Sulivany B., Abdulhalim B.A., Mehroz R. (2024). The pangas catfish *Pangasius pangasius*; growth efficiency and nutritional composition under variety of saltwater challenges. Egyptian Journal of Aquatic Biology and Fisheries, 28(6).

Peyghan R., Khadjeh G.H., Enayati A., (2014). Effect of water salinity on total protein and electrophoretic pattern of serum proteins of grass carp, *Ctenopharyngodon idella*. Veterinary Research Forum: An International Quarterly Journal, 5(3): 225.

Rasekhi S., Sharifian A., Shahraki M., Silvano R.A. (2023). Indigenous fishers' knowledge on fish behavior, fishing practices and climatic conditions in a conservation priority coastal ecosystem in the Caspian Sea. Reviews in Fish Biology and Fisheries, 33(3): 629-648.

Rashamol V.P., Sejian V., Bagath M., Krishnan G., Archana P.R., Bhatta, R. (2020). Physiological adaptability of livestock to heat stress: an updated review. Journal of Animal Behaviour and Biometeorology, 6(3): 62-71.

Ruiz-Jarabo I., Fuentes J., Mancera J.M. (2019). Osmoregulation. In: The biology of sole. CRC Press. pp: 354-374.

Salauat D., Shalgimbayeva S., Omarova Z., Jumakhanova G., Zhanysbay G. (2024). Current state of juvenile roach (*Rutilus rutilus caspicus*). In BIO Web of Conferences, 100: 04019.

Shahjahan M., Islam M.J., Hossain M.T., Mishu M.A., Hasan J., Brown C. (2022a). Blood biomarkers as diagnostic tools: An overview of climate-driven stress responses in fish. Science of the Total Environment, 843: 156910.

Shahjahan M., Taslima K., Rahman M.S., Al-Emran M., Alam S.I., Faggio C. (2022b). Effects of heavy metals on fish physiology–a review. Chemosphere, 300: 134519.

Shukry M., Abd El-Kader M.F., Hendam B.M., Dawood M.A., Farrag F.A., Aboelenin S.M., Soliman M.M.,

Abdel-Latif H.M. (2021). Dietary Aspergillus oryzae modulates serum biochemical indices, immune responses, oxidative stress and transcription of HSP70 and cytokine genes in Nile tilapia exposed to salinity stress. Animals, 11(6): 1621.

Takvam M., Wood C.M., Kryvi H., Nilsen T.O. (2021). Ion transporters and osmoregulation in the kidney of teleost fishes as a function of salinity. Frontiers in Physiology, 12: 664588.

Taylor J.F., Needham M.P., North B.P., Morgan A., Thompson K., Migaud H. (2007). The influence of ploidy on saltwater adaptation, acute stress response and immune function following seawater transfer in non-smolting rainbow trout. General and Comparative Endocrinology, 152(2-3): 314-325.

Tian L., Tan P., Yang L., Zhu W., Xu D. (2020). Effects of salinity on the growth, plasma ion concentrations, osmoregulation, non-specific immunity and intestinal microbiota of the yellow drum (*Nibea albiflora*). Aquaculture, 528L 735470.

van Rijn C.A., Jones P.L., Schultz A.G., Evans B.S., McCormick S.D., Afonso L.O. (2020). Atlantic salmon (*Salmo salar*) exposed to different preparatory photoperiods during smoltification show varying responses in gill Na+/K+-ATPase, salinity-specific mRNA transcription and ionocyte differentiation. Aquaculture, 529: 735744.

Vineetha V.P., Suresh K., Pillai D. (2024). Impact of sub-chronic polystyrene nanoplastics exposure on hematology, histology and endoplasmic reticulum stress-related protein expression in Nile tilapia (*Oreochromis niloticus*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 273: 110982.

Wang Y., Li H., Wei J., Hong K., Zhou Q., Liu X., Hong X., Li W., Liu C., Zhu X., Yu L. (2023). Multi-effects of acute salinity stress on osmoregulation, physiological metabolism, antioxidant capacity, immunity and apoptosis in *Macrobrachium rosenbergii*. Antioxidants, 12(10): 1836.

Winarti M., Sulmartiwi L., Lutfiyah L. (2022). July. The effect of salinity on the expression of heat shock protein 70 (hsp70) in the gills and kidneys of srikandi tilapia (*Oreochromis niloticus*). In IOP Conference Series: Earth and Environmental Science, 1036(1): 012092.

Zargari A., Mazandarani M., Safari R., Hoseinifar H., Hedayati A. (2024). Modulation of toxic effects of ammonia on growth, pathology of liver and kidney tissues and relative expression of GH and IGF-1 Genes by CoQ10 Supplementation in Oncorhynchus mykiss. Fish Physiology and Biochemistry, 1-19.

Zargari A., Nejatian M., Abbaszadeh S., Jahanbin K., Bagheri T., Hedayati A., Sheykhi M. (2023). Modulation of toxicity effects of CuSO4 by sulfated polysaccharides extracted from brown algae (*Sargassum tenerrimum*) in Danio rerio as a model. Scientific Reports, 13(1): 11429.