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# Variations in the lipid peroxidation and antioxidant biomarkers in some tissues of anadromous cyprinid fish during migration

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**Abstract:** *Alburnus tarichi* is an endemic fish species inhabiting the Lake Van basin of Turkey. This anadromous cyprinid fish migrates for spawning to the freshwater inlets pouring into Lake Van, which has highly alkaline and brackish water. During the migration, the fish moves to a new habitat from a different habitat and encounters many challenges. The objective of the present study is to investigate whether antioxidant responses occur in the tissues of *A. tarichi* during its anadromous migration. To this end, fish were sampled at three different points and in two different periods from the migration route, including in Lake Van at prespawning, at the entrance of a freshwater stream (Karasu) at spawning and in the upstream of the freshwater stream at spawning. Malondialdehyde (MDA) content and antioxidant defenses including activities of the enzymes superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and catalase and the level of glutathione were assayed in liver, gill, white muscle, trunk kidney and anterior intestine tissues. Our results showed increased MDA levels in liver, white muscle and anterior intestine and tissue-specific antioxidant responses in the freshwater environment. This study reports that alterations occurred in the antioxidant defense system indicators in the tissues of anadromous cyprinid fish during migration and that the antioxidant defenses might reflect an important role in spawning migration that ultimately leads to accomplishment of reproductive activity.

***Key words:*** *Alburnus tarichi*; Antioxidant responses; Lipid peroxidation; Migration.

## Introduction

Reactive oxygen species (ROS) are generated constantly during normal aerobic cellular metabolism of animals through the mitochondrial electron transport chain (1). Antioxidant defenses have evolved in cells to protect biological structures against the harmful and damaging effects of ROS such as superoxide anion, singlet oxygen, hydroxyl radical, peroxyl radical, and hydrogen peroxide (2). Enzymatic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase) and nonenzymatic (e.g., vitamins C and E, glutathione) members of antioxidant defense systems also provide a balance between prooxidants and antioxidants. However, a variety of environmental stressors can lead to overproduction of ROS and shift the balance in favor of prooxidants that result in oxidative stress. When oxidative stress occurs in cells, ROS attack cellular macromolecules including nucleic acids, proteins, lipids and carbohydrates, ultimately causing cellular damage (3, 4).

Studies from birds and mammals show a linkage between oxidative stress and life history traits such as migration, which has high-energy demand and results in elevated metabolism (5, 6, 7). In fish, a myriad of extrinsic factors such as temperature, oxygen availability, salinity, pollution and anthropogenic activities can directly affect ROS levels and cause increased oxidative stress (1, 3). Reproduction, immune response, diet and food deprivation, physical activity and aging and senescence are life history traits that affect the prooxidant/antioxidant balance and are ecological inducers of oxidative stress (1, 7). Reproduction is an energetically demanding activity in which basal and field rates increase for long periods and resources such as antioxidants and nutrients are allocated to reproductive activity (8). Recently, studies conducted in fish with different reproductive strategies reported that oxidative stress and antioxidant capacity changes occur during spawning migration. The semelparous Pacific salmons are challenged by various physical and biological factors during spawning migration. Wilson *et al.* (9) observed increased oxidative stress in the tissues of the Pacific salmon (*Oncorhyncus gorbuscha*) sampled from the spawning grounds compared with that in the fish sampled from freshwater entry, which is related with reproductive strategy. In another study, oxidative stress played a role during migration in migrants of semianadromous brown trout (*Salmo trutta*) (5).

*Alburnus tarichi* (Güldenstädt, 1814) (Cyprinidae) is an endemic species living in the Lake Van basin located in the Eastern Anatolia Region of Turkey. Lake Van is the largest soda lake on Earth that possesses highly alkaline and highly brackish water (Table 1). *A. tarichi* is the only fish species that inhabits Lake Van, which demonstrates the extreme living environment, in addition to the absence of higher plants in its littoral. In general, living conditions are lethal for fishes and inver-

**Table 1.** Physicochemical characteristics of water from Lake Van and Karasu Stream.

| Characteristic | Lake Van | Karasu Stream |
|---|---|---|
| pH | 9.81[a,b]; 9.78[e]; 9.65[f]; 9.77–9.80[g]; | 8.63–8.65[c]; 7.40[d]; 8.28[e]; 8.34[g]; 8.40[h] (May data) |
| Salinity | 22.7 (‰)[a]; 22.7 (ppt)[b]; 15.0 (‰)[e]; 16.90 (ppt)[f]; 16.5–16.8 (ppt)[g] | 0.3 (ppt)[g] |
| Conductivity | 25.5–26.5 (mS × $cm^2$)[a]; 29.76 (mS/cm)[f]; 27.88–28.65 (mS/cm)[g] | 572 (µS/cm)[g]; 582.2 (µS/cm)[h] (May data) |
| Dissolved oxygen | 10.2 (mg/ml)[f]; 10.4–9.8 (mg/ml)[g] | 8.7 (mg/ml)[g]; 8.2–8.6 (May data) (mg/ml)[c]; 8.8 (mg/ml)[h] |
| Saturation | 107 (%)[f]; 105–108 (%)[g] | 100 (%)[g]; 105 (%)[h] |
| *Ion concentrations* | | |
| $Na^+$ | 336.9 (meq/l)[d]; 337.9 (mmol/l)[b] | 1.32 (meq/l)[d]; 15–25 (mg/l)[c] |
| $K^+$ | 13.0 (meq/l) [d]; 10.90 (mmol/l)[b] | 0.103 (meq/l)[d]; 2.5–4.0 (mg/l)[c] |
| $Mg^{2+}$ | 7.80 (meq/l) [d]; 4.42 (mmol/l)[b] | 2.01 (meq/l)[d]; 64.41–70.47 (mg/l)[c]; 83.3 (mg/l)[h] (May data) |
| $Ca^{2+}$ | 0.23 (meq/l)[d]; 0.11 (mmol/l)[b] | 1.01 (meq/l)[d]; 84.21–93.0 (mg/l)[c]; 98.4 (mg/l)[h] (May data) |
| $Cl^-$ | 153.70 (meq/l)[d]; 160.60 (mmol/l)[b] | 0.37 (meq/l)[d]; 17.80–21.30 (mg/l)[c]; (27.7 mg/l)[h] |
| Total cations | 358.17 (meq/l)[d] | 4.44 (meq/l)[d] |
| Total anions | 354.94 (meq/l)[d] | 6.03 (meq/l)[d] |

a: (10); b: (11); c: (13); d: According to Kempe *et al*. (14) and Tugrul *et al.* (15) as cited by Arabaci *et al.* (16); e: (16); f: (17); g: (18); h: (19).

tebrates. Thus, *A. tarichi* has physiological abilities to fit Lake Van conditions. Another biologically important feature of *A. tarichi* is that is an anadromous species and performs annual spawning migration toward the freshwater streams pouring into Lake Van for reproduction. The reproduction period of the fish extends from mid-April to mid-July but most fish pass to freshwater inlets from the highly alkaline and brackish waters of the lake during May (10). During the reproductive migration, the fish moves from one habitat (lake) to a different habitat (freshwater) and faces a multitude of challenges and stressors. The fish travel long distances to upstream in freshwater inlets to locate suitable spawning grounds and must overcome stream currents during this journey, demonstrating physiological and biochemical adaptations to cope with osmotic stress (10, 11) and struggles with predators (e.g., illegal fishing during spawning migration (12) and wild predators such as gulls). Reproduction is also an energetically demanding activity and elevates metabolic rates (8). Thus, *A. tarichi* must overwhelm all extrinsic and intrinsic factors during migration for successful reproduction, which provides us an excellent model to learn the role of oxidative stress during migration. The primary objective of the current study was to determine whether antioxidant responses occurred during anadromous migration in different tissues of *A. tarichi* using the lipid peroxidation and antioxidant defense system indicators such as, the level of glutathione and the activities of superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and catalase. For this purpose, *A. tarichi* were caught from the lake at prespawning season in April and were captured from the entry and upstream in a freshwater inlet (Karasu Stream) (Table 1) during spawning migration in May. Then, samples from five tissues including liver, gills, trunk kidney, white muscle and anterior intestine were evaluated in terms of the antioxidant defense system indicators.


**Figure 1.** Map of the study area on Lake Van or Karasu Stream (Van, Turkey). Sampling points of *A. tarichi* are indicated by numbers (1: Lake Van, 2: entrance of Karasu Stream, 3: approximately 6.5 km upstream of the entrance of Karasu Stream) (Sources: Google Earth, Google Maps).

## Materials and Methods

### Study location and fish sampling and processing

Lake Van is the largest lake in Turkey. The lake is located in the east of the Eastern Anatolia Region of Turkey. Karasu Stream is located in the Lake Van basin and pours into Lake Van from its eastern side. This stream is one of the freshwater inlets in which anadromous *A. tarichi* engages in spawning activity. The fish migrate from Lake Van through the Karasu mouth (Çitören reeds) and travel upstream in the Karasu Stream for spawning commonly in May (Fig. 1). A total of 24 sexually mature and healthy female *A. tarichi* (fork length: 18–22 cm and total weight: 80–110 g) were used in the study. Sampling time was selected according to the reproductive period of *A. tarichi* described by the studies of Ünal *et al.* (20) and Kaptaner and Kankaya (21). Because of possible changes depending on sex difference, only females were examined in the study. *A. tarichi* were captured at three sites throughout the migration route and at two differ-

ent times (prespawning and spawning periods). The first sampling was performed at Lake Van on April 1, 2018 (prespawning), the second one at the entrance of Karasu Stream on May 5, 2018 (spawning), and the third one at approximately 6.5 km upstream of the entrance of Karasu Stream on May 12, 2018 (spawning) (Fig. 1). Eight fish were caught from each site, and pH and temperature were measured in surface water samples taken from the sampling area. After samplings, the fish were anesthetized by using 2-phenoxyethanol. Then, the tissues including liver, gill, trunk kidney, white muscle and anterior intestine were carefully obtained from fish. The tissues were immediately flash frozen in liquid nitrogen and stored at −80 °C until biochemical analyses.

**Biochemical analyses**

Before the analyses, the tissues were thawed and homogenized for 5 min in 50 mM ice-cold $KH_2PO_4$ solution (1:10 w/v) using a glass-porcelain ultrasonic homogenizer (Jencons Scientific Co., UK). The homogenates were centrifuged at 10,000 rpm for 30 min. All processes were conducted at 4 °C. Supernatant fractions were removed and used to determine the total protein content, lipid peroxidation and antioxidant defenses.

Lipid peroxidation (LPO) was determined by measuring the malondialdehyde (MDA) content, a product of lipid peroxidation, in the samples. The MDA concentration was measured spectrophotometrically at 532 nm, using the method described by Jain *et al.* (22), based on thiobarbituric acid reactivity. The results are expressed as nanomole per gram of protein.

Superoxide dismutase (SOD) activity was measured using a commercial kit (Ransod, Randox Lab., UK) at 505 nm and 37 °C according to the manufacturer's instructions. SOD activity is expressed as unit per gram of protein.

Catalase (CAT) activity was measured spectrophotometrically according to Aebi (23). The activity of CAT was determined by assaying the decrease in absorbance (hydrogen peroxide consumption) at 240 nm. CAT activity is expressed as nanomole of $H_2O_2$ consumed per minute per gram of protein.

Glutathione peroxidase (GPx) activity was measured using a commercial kit (Ransel, Randox Lab., UK) at 340 nm and 37 °C according to the manufacturer's instructions based on the method of Paglia and Valentine (24). The GPx activity is expressed as unit per gram of protein.

Glutathione (GSH) content was measured spectrophotometrically at 412 nm using the method described by Beutler *et al.* (25). The GSH levels were obtained using a standard curve derived from external GSH standards. The results are expressed as micromoles GSH per milligram of protein.

Glutathione-*S*-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate (26). GST activity is expressed as nanomoles of CDNB-glutathione conjugate per minute per milligram of protein.

The total protein content in the supernatant fractions was assayed spectrophotometrically using the method of Bradford (27), with bovine serum albumin as the standard.

**Statistical analyses**

Statistical analyses were performed using the statistical software package for the Social Sciences, version 16.0. Differences among the data from each sampling site were analyzed using a one-way analysis of variance with a post hoc Duncan's multiple comparison test. The results are expressed as the mean ± standard deviation (SD). Values with $P < 0.05$ were considered statistically significant.

**Results**

Temperature and pH in the surface water samples taken from the lake and the freshwater were 8.5 °C / 9.67 and 16-18 °C /8.30-8.40, respectively.

The MDA content and antioxidant defenses including SOD, CAT, GPx, GST and GSH in the tissues of fish sampled from the lake and river environments are presented in Table 2. MDA levels were significantly higher in the liver, white muscle and anterior intestine of fish from the stream entry and in the upstream than the levels in those tissues of fish from the lake environment. MDA levels remained unchanged in the gill and trunk kidney of fish from the stream environment. SOD activity increased significantly in the liver and anterior intestine of fish from the upstream of Karasu Stream; whereas SOD activity increased significantly in the trunk kidney of fish sampled from both the entry and in the upstream of Karasu Stream. SOD activity remained unchanged in the gill and white muscle of fish from the stream. The liver, gill, trunk kidney and anterior intestine of fish from both the entry and in the upstream of Karasu Stream had significantly increased CAT activity, whereas no change in enzyme activity was observed in white muscle. GPx activity decreased significantly in the liver of fish from the stream environment, whereas significant increases in GPx activity were observed in gill, white muscle and anterior intestine of fish from both the entry and in the upstream of Karasu Stream. A significant decrease was even observed in anterior intestine GPx activity of the fish from the upstream of Karasu Stream compared with that in fish from the stream entry, although this decrease remained significantly higher than the activity level in the fish from the lake. GPx activity in the trunk kidney was elevated only in fish from the upstream of Karasu. GST activity in the liver, gill and white muscle of fish from the entry and in the upstream of Karasu decreased significantly compared with that in fish from the lake environment. Although GST activity recovered in the gill and liver of fish from the upstream of Karasu Stream compared with that in fish from the stream entry, this activity remained significantly lower than that in fish from the lake. The levels of GSH in the liver, gill and trunk kidney were significantly elevated in fish from the stream environment compared with those in fish from the lake environment, whereas no significant changes were detected in white muscle and anterior intestine.

**Discussion**

*A. tarichi* is an endemic cyprinid fish that inhabits the Lake Van basin. This anadromous fish performs annual migration activity from Lake Van, which is the largest soda lake on Earth and possesses highly alkaline

**Table 2.** Antioxidant defense system indicators in liver, gill, trunk kidney, white muscle and anterior intestine of anadromous *Alburnus tarichi* captured in Lake Van at prespawning or at the entrance and in the upstream of the Karasu Stream at spawning.

| Tissue | Indicators | Lake Van | Karasu Stream (entrance) | Karasu Stream (upstream) |
|---|---|---|---|---|
| Liver | MDA | 13.08 ± 3.67[a] | 30.20 ± 10.61[b] | 30.65 ± 17.20[b] |
| | SOD | 166.18 ± 25.86[a] | 184.23 ± 65.80[a] | 320.35 74.74[b] |
| | CAT | 42.03 ± 21.17[a] | 58.33 ± 18.50[ab] | 69.94 20.31[b] |
| | GPx | 228.94 ± 179.72[a] | 24.78 ± 27.06[b] | 65.22 61.69[b] |
| | GST | 7.66 ± 2.92[a] | 4.49 ± 2.57[b] | 5.68 ± 2.12[ab] |
| | GSH | 6.00 ± 0.97[a] | 7.73 ± 1.73[b] | 10.02 ± 1.75[c] |
| Gill | MDA | 36.50 ± 13.12[a] | 26.82 ± 7.77[a] | 33.78 9.48[a] |
| | SOD | 26.50 ± 2.26[a] | 22.81 ± 8.88[a] | 24.83 9.09[a] |
| | CAT | 0.11 ± 0.05[a] | 0.23 ± 0.11[ab] | 0.34 ± 0.23[b] |
| | GPx | 2.14 ± 0.89[a] | 2.92 ± 1.40[ab] | 4.00 ± 1.57[b] |
| | GST | 5.34 ± 1.53[a] | 3.82 ± 0.99[b] | 4.95 ± 0.87[ab] |
| | GSH | 3.02 ± 1.48[a] | 2.99 ± 1.60[a] | 6.01 ± 2.11[b] |
| Trunk kidney | MDA | 63.08 ± 12.37[a] | 73.15 ± 16.64[a] | 72.96 24.50[a] |
| | SOD | 49.30 ± 12.76[a] | 62.53 ± 14.06[b] | 69.86 ± 10.46[b] |
| | CAT | 0.63 ± 0.31[a] | 0.95 ± 0.42[ab] | 1.19 ± 0.35[b] |
| | GPx | 1.07 ± 0.82[a] | 1.17 ± 0.49[a] | 1.91 ± 0.67[b] |
| | GST | 2.88 ± 1.05[a] | 2.54 ± 0.91[a] | 4.34 ± 1.93[b] |
| | GSH | 4.95 ± 1.14[a] | 6.99 ± 1.68[b] | 7.55 ± 1.49[b] |
| White muscle | MDA | 2.49 ± 1.06[a] | 6.21 ± 1.53[b] | 7.03 ± 3.53[b] |
| | SOD | 43.31 ± 8.27[a] | 51.66 ± 12.68[a] | 54.67 11.02[a] |
| | CAT | 0.05 ± 0.03[a] | 0.04 ± 0.03[a] | 0.05 ± 0.03[a] |
| | GPx | 1.04 ± 0.37[a] | 1.41 ± 0.37[b] | 1.88 ± 0.31[c] |
| | GST | 2.72 ± 0.96[a] | 1.65 ± 0.75[b] | 1.74 ± 0.84[b] |
| | GSH | 3.83 ± 1.44[a] | 3.91 ± 1.29[a] | 4.91 ± 1.22[a] |
| Anterior intestine | MDA | 36.18 ± 24.24[a] | 77.59 ± 42.39[b] | 69.67 ± 23.29[b] |
| | SOD | 139.07 ± 25.53[a] | 142.02 ± 46.30[a] | 235.41 86.39[b] |
| | CAT | 0.12 ± 0.10[a] | 0.25 ± 0.10[b] | 0.53 ± 0.16[c] |
| | GPx | 1.12 ± 0.40[a] | 6.47 ± 7.36[b] | 3.91 ± 2.61[ab] |
| | GST | 0.59 ± 0.57[a] | 3.19 ± 2.37[b] | 2.32 ± 1.95[ab] |
| | GSH | 2.40 ± 1.50[a] | 2.93 ± 0.72[a] | 3.25 ± 1.09[a] |

MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; GPx: glutathione peroxidase; GST: glutathione-*S*-transferase. Values are the mean ± SD. Different letters indicate significant differences between fish groups using Duncan's multiple range test. Significance was at $P \leq 0.05$. The SOD activity is expressed as unit/g protein; CAT activity is expressed as nmoles of $H_2O_2$ consumed/min/g protein; GPx activity is expressed as unit/g protein; GST activity is expressed as nmoles of CDNB-glutathione conjugate/min/mg protein; MDA content is expressed as nmoles/g protein; GSH content is expressed as μmoles/mg protein.

and brackish water, to freshwater streams for spawning. During the migration, *A. tarichi* encounters a myriad of external and internal factors such as acclimation to freshwater, swimming activity moving upstream in freshwater against currents to identify suitable spawning grounds, increasing water temperature, avoiding predators and reproductive costs. In a previous study, we observed increased erythrocyte osmotic fragility causally related with physicochemical changes during the *A. tarichi* migration to freshwater that indicates a physiological stress in the freshwater environment during spawning (28). In this study, we examined antioxidant defense system indicators in *A. tarichi* during its challenging migration travel and sampled fish and collected tissues at the prespawning period when the fish was in the lake (April) and in a stream (Karasu) pouring into the lake that fish migrated to for spawning (May). Fish were sampled at two points in the stream, one at the entry and one approximately 6.5 km upstream of the stream entrance. Our results clearly indicated changes in the lipid peroxidation and antioxidant defenses in the tissues of this cyprinid fish during its anadromous migration.

Migration is a highly energy demanding activity requiring that energy reserves are allocated to growth, reproduction, and survival of the organism (7). The role of oxidative stress during the expression of life history traits is extensively studied in birds and mammals (1); however, few studies and less information regarding the linkage between oxidative stress and life history traits are available for fish. Therefore, *A. tarichi* is an excellent model to study oxidative stress mechanisms during migration and to elucidate its migration biology. In the present study, MDA content, a product of lipid peroxidation, increased significantly in liver, white muscle and anterior intestine of fish from the entry and in the upstream in Karasu Stream compared with that in Lake Van samples, indicating oxidative stress. Similar to our results, lipid peroxidation increases significantly in the liver of migrating Chinook salmon smolts with concom-

itant reduced levels of vitamin E after they leave the hatchery origin and encounter the first dam of the hydropower system (29). Lipid peroxidation is a complex process in which polyunsaturated fatty acids (PUFAs) in biological membranes are exposed to changes by chain reactions and form lipid hydroperoxides, which decompose double bonds of unsaturated fatty acids and destroy membrane lipids (30). As a primary energy storage organ, fish liver plays a major role in lipid metabolism. Lipids and their constituent fatty acids are energy sources for metabolic activities such as growth, reproduction and migration (31). PUFA concentrations in liver increase during the reproductive season in teleost fish (32, 33). PUFAs are highly susceptible to ROS attack, which results in lipid peroxidation (34). The high production of ROS as a result of a stress condition during migration can cause increased lipid peroxidation in the liver (35). In the present study, MDA level also increased in the white muscle of fishes sampled from the stream. In Pacific salmon (*Oncorhynchus gorbuscha*), white muscle had increased concentrations of 8-OHdG indicating DNA damage during spawning migration (9). The physiological status in white muscle tissue can change in response to exercise, fasting, salinity transition and sexual maturity during migration (36). White muscle tissue is also rich in terms of PUFA (33) and mitochondrial content (37). During exercise, overproduction of ROS occurs in mitochondria as a result of contractile activity associated with increased oxygen consumption and other cellular processes such as energy metabolism (38, 39). An increase in lipid peroxidation was also observed in the anterior intestine in the current study. Due to the lack of studies related to increased lipid peroxidation in the intestine of fish, the reasons for our findings are difficult to evaluate. The gastrointestinal tract of fish plays an important role in osmoregulation in addition to digestion (40), and the intestine is very susceptible to external factors such as salinity changes (41), pollution (42), and internal factors such as hormones (e.g., cortisol) (43) which are capable of inducing apoptosis in the intestine with the cues from increased lipid peroxidation. However, studies performed in mussels (*Perna* sp.) show that increased levels of lipid peroxidation in digestive glands may be caused by increased oxidative stress as a result of increased ROS formation due to high metabolic rates during the breeding season (44, 45). Elevated lipid peroxidation in the tissues might also be the result of osmoregulatory processes (46) and increasing water temperature during migration, because water temperatures are implicated as contributing to increases in lipid peroxidation in fish (47).

Our results indicated that tissue-specific responses occurred in the antioxidant enzymes SOD, CAT, GPx and GST. Under oxidative stress conditions, antioxidant enzyme activities frequently increase because of overproduction of ROS (48). The SOD-CAT system functions as the primary defense mechanism against oxidative stress, and SOD is a crucial enzyme that accelerates the dismutation of superoxide radical to hydrogen peroxide and water, whereas the role of CAT is to degrade hydrogen peroxide, a precursor of the hydroxyl radical, to water (49, 50). The simultaneous increases in the activities of these two enzymes are usually observed in response to ROS (51). Increased SOD activities in fish from the stream (Karasu) compared with those sampled from the lake, suggested production of superoxide anions and cellular oxidative stress during the anadromous migration to freshwater. A relationship between increased CAT activity and lipid peroxidation has been reported due to the role of CAT in the Fenton reaction in which the enzyme scavenges hydrogen peroxide to produce highly reactive molecules such as the hydroxyl radical and weakens the lipid peroxidation rate (45, 52). Seasonal variations in SOD and CAT activity are related with reproductive cycles and physicochemical parameters of water in mussels (45). Increased concentrations of SOD and CAT in marine fish are correlated with oxygen consumption followed by temperature elevation, which result in an increase in ROS generation (53). Food restriction and starvation are another factor that induces new SOD isoforms (54) and increases in SOD/CAT activities in tissues (55, 56) of fishes. Arabacı *et al.* (16) observed hypokalemia in *A. tarichi* migrating to freshwater, and they speculated that it develops as a result of starvation during spawning migration in the fish. Regardless of the species, osmoregulation is an energetically expensive process. Hypo-osmotic stress results in high ROS production and energy expenditure in the Mediterranean green crab (*Carcinus aestuarii*) when undergoing exposure to diluted sea water, and an induction of SOD and CAT activities accompanied by an increase in mitochondrial density occurs in posterior gills to overwhelm the osmotic stress and salinity-induced oxidative stress (57). GPx is an important enzyme that converts hydrogen peroxide into hydrogen oxide using GSH as a substrate. The enzyme protects cells against damage induced by oxyradicals and reduces formation of lipid peroxides by termination of radical chain propagation (58). Increases in GPx activity can be due to a protective response to overproduction of hydrogen peroxide in the tissues under stressful conditions to prevent damage by lipid peroxidation. Data from marine fish reveal that GPx1 and GPx4 activities are correlated with membrane unsaturation, and marine fish likely defend against initial oxidative insults using primarily GPx1, in addition to increases in repair mechanisms by GPx4 to counter damage in PUFA-rich tissues (59). However, GPx activity decreased in some tissues of fish from the river environment in the present study. This finding is consistent with a previous study demonstrating high levels of GPx mRNA expression as salinity decreased (8.75−4 psu) and a low level of GPx expression at 0 psu, which are related with tissue damage, in olive flounder (*Paralichthys olivaceus*) (60). Thus, decreases in GPx activity might be a result of tissue damage. Similar to the present study, GPx activity was reduced in teleost fish (*Sparus aurata*) exposed to cadmium (61) and in a shrimp (*Litopenaeus vannamei*) undergoing acute salinity changes (62). GST is an enzyme that plays a role in the detoxification of xenobiotics by conjugating electrophilic metabolites into GSH and protecting the cells from oxidative stress (63). This enzyme is also a suitable and sensitive biomarker of environmentally induced oxidative stress in fish (64). Bebianno *et al.* (65) studied the influence of abiotic factors such as salinity, pH and temperature on GST activity in mussel (*Mytilus galloprovincialis*) and found that GST activity decreased with increases in salinity; however, a sig-

nificant relation between GST and other factors was not observed. On the other hand, increased and decreased GST mRNA expression was observed in the liver of olive flounder (*Paralichthys olivaceus*) during osmotic stress (60). Additionally, GST activity is under climatic stress and reacts differently in winter and spring (64, 66). Aras *et al.* (67) reported a decline in GST activity in spring and summer in fishes. Increased GST activities observed in the present study might result from ROS-generated oxidative stress, depending on its detoxification role. However, in this study, GST activity decreased with a corresponding increase in lipid peroxidation in some tissues. Similar relations between GST and lipid peroxidation are also reported in goldfish (*Carassius auratus*) after exposure to hypoxia (68) and iron ions (52), suggesting inactivation of the enzyme during detoxification of lipid peroxide products. GSH is a tripeptide that consists of *g*-glutamine, cysteine, and glycine and is responsible for maintaining the redox status of cells. GSH can react directly with ROS or electrophilic compounds, protects cells against oxidants and simultaneously is a co-factor for the antioxidant enzymes GPx and GST (69). Increases in GSH content were observed depending on the tissue in this study. Similar changes in GSH level are also reported in the blood of migrants of brown trout (*Salmo trutta*) performing partial migration in which migrants have increases in antioxidant capacity to cope with oxidative stress induced by a sustained increase in metabolic rate during migration (5). Wilson *et al.* (9) also reported tissue-dependent increases in antioxidant capacity (GSH, α-tocopherol, ascorbic acid) in Pacific salmon (*Oncorhyncus gorbuscha*) from spawning grounds in which oxidative stress occurred. A close relation between reproductive cycle and increased GSH levels is reported in the green-lipped mussel (*Perna viridis*) (45). Thus, our results for *A. tarichi* suggest that the increased tissue levels of GSH might be due to an adaptive and protective role of this biomolecule against oxidative stress as a result of prooxidant challenge during migration to freshwater (70).

This study reports changes in the antioxidant defense system indicators in the tissues of anadromous *A. tarichi* during its migration from Lake Van, which possesses highly alkaline and brackish water, to freshwater. Those alterations are likely associated with physicochemical alterations in the freshwater environment, avoiding predators, physical activity, reproductive activity and osmoregulation. However, further studies should be conducted with these fish to learn the exact reasons. The study provides a baseline data for further studies in *A. tarichi* to understand the migration biology. The results also illustrate the importance and role of the antioxidant system in the acclimation of organisms to different environments, particularly during migration.

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**Compliance with ethical standards**
All procedures were performed in agreement with national and institutional regulations for the protection of animal welfare during the study. This work was approved by the Animal Experiments Ethics Committee of Van Yuzuncu Yil University for the ethical concerns (YUHADYEK-2018/09).

**Conflict of interest**
The authors declare that they have no conflict of interest.

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