Apoptosis and cell proliferation in the epithelia of the esophagus and intestine of *Alburnus tarichi* Güldenstädt, 1814 (Cyprinidae) during migration from highly alkaline and brackish water to fresh water

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Abstract

In this study, cellular turnover in the epithelia of the esophagus and intestine of the anadromous Lake Van fish (*Alburnus tarichi*) that migrated from the highly alkaline and brackish water of Lake Van to fresh water were investigated by apoptosis and cell proliferation. Toward this aim, fish were collected from or near the entrance to Lake Van, and upstream in a freshwater stream (Karasu). The tissues were subjected to terminal deoxynucleotidyl transferase nick end labeling and proliferating cell nuclear antigen stainings for the detection of apoptosis or cell proliferation, and were then quantified. In the esophagus epithelium, the apoptotic index (AI) displayed no differences between fish from the lake and those from the freshwater stream. The AI increased significantly at the anterior or mid-intestine epithelia and throughout the intestine epithelium, whereas it remained unchanged in the posterior intestine epithelium of fish from both upstream and at the entrance of the freshwater stream. In the esophagus epithelium, the proliferation index (PI) values decreased gradually in fish from the freshwater stream. The PI increased significantly in the anterior intestine epithelium or throughout the intestinal epithelium of fish collected from the entrance of the stream; however, it did not change along the intestinal epithelium of fish collected upstream in the freshwater stream. It was determined that cellular changes occurred in the esophageal and intestinal epithelium of Lake Van fish during freshwater acclimation.

Keywords: *Alburnus tarichi*, apoptosis, cell proliferation, esophagus, intestine

Introduction

Past studies have found that the digestive tract of marine and euryhaline teleost fish plays an important role in osmoregulation. Teleost fish inhabiting seawater have to drink it to compensate for an osmotic loss of body fluids. The ingested seawater is desalted by the esophagus, which is permeable to sodium and chloride ions or impermeable to water, and the subsequent absorption of water by the intestine occurs along with the active uptake of monovalent ions (Yamamoto & Hirano 1978; Kirsch & Meister 1982; Kirsch et al. 1984; Ciccotti et al. 1993; Buddington & Kuz’mina 2000). During acclimation to a hyperosmotic environment, changes occur morphologically in the esophageal epithelium of eel (*Anguilla anguilla*) as a consequence of ion/water permeability from a stratified epithelium containing abundant mucous cells, and is replaced by a simple columnar epithelium without mucous cells in freshwater-acclimated eels (Yamamoto & Hirano 1978). In relation to osmoregulatory processes, tiny regions of a simple columnar epithelium were observed in the esophageal epithelium with increases in water salinity in juvenile *Oreochromis niloticus* (Cataldi et al. 1988b). A recent study reported that apoptosis occurs in the epithelium of the anterior intestine, which is comparable to that in the esophagus of a euryhaline mudskipper (*Periophthalmus modestus*) during seawater acclimation, while cell proliferation increases during freshwater acclimation, indicating the role of cellular turnover in the regulation of permeability or transport capacity in the alimentary tract (Takahashi et al. 2006a).
The Lake Van fish (Alburnus tarichi Güldenstädt, 1814) (Cyprinidae) is a species that is endemic to Turkey's Eastern Anatolian Lake Van basin, which is the Earth's largest soda lake and possesses highly alkaline and highly brackish water (Table I). Lake Van represents an extreme living environment and, in general, living conditions are lethal for fish and invertebrates. Thus, the Lake Van fish has physiological abilities to fit Lake Van's conditions. Another biologically important feature of Lake Van fish is that it is an anadromous species, and it performs an annual spawning migration toward the freshwater streams that pour into the lake for reproduction. The reproduction period of the fish begins in the middle of April and extends to the middle of July, but most of the fish pass to freshwater inlets from the highly alkaline and brackish waters of the lake during May (Danulat & Selcuk 1992). During the reproductive migration, the fish move from one habitat (the lake) to a different habitat (fresh water) and face a multitude of challenges and stressors. They travel a long distance upstream in freshwater inlets to locate suitable spawning grounds and must overcome stream currents during this journey (Danulat & Selcuk 1992), demonstrating physiological and biochemical adaptations to cope with osmotic stress (Danulat 1995) via their high osmoregulatory capacity during freshwater acclimation (Arabaci et al. 2001). Cyprinids do not possess a true stomach, instead having an expansion at the anterior part of the intestine (Strüsmann & Takashima 1995). The digestive tract of the Lake Van fish is composed of a buccal cavity, pharynx, esophagus, post-esophageal swelling, and intestine segments. In this study, we aimed to examine the cellular turnover using apoptosis and cell proliferation in the epithelia of the esophagus and intestine of these fish throughout their migration from the lake's highly alkaline and brackish water to a freshwater stream (Karasu) (Table I). For this purpose, tissues were obtained from the fish during their prespawning period in the lake and during the spawning period at two different points in Karasu Stream (at the entrance and upstream). Next, the tissues were subjected to terminal deoxynucleotidyl transferase nick end labeling (TUNEL) and proliferating cell nuclear antigen (PCNA) staining for the detection of apoptosis or cell proliferation, and were then quantified.

Material and methods

Study location, fish sampling and tissue processing

Turkey's Eastern Anatolian Lake Van is the country's biggest lake. 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 the anadromous Lake Van fish

| Table I. Physicochemical characteristics of water from Lake Van and Karasu Stream. |
|-----------------|-----------------|-----------------|
| Characteristics | Lake Van         | Karasu Stream   |
| pH              | 9.81<sup>a</sup>; 9.78<sup>b</sup>; 9.65<sup>c</sup>; 9.77–9.80<sup>e</sup> | 8.63–8.65<sup>a</sup>; 7.40<sup>e</sup>; 8.28<sup>e</sup>; 8.34<sup>e</sup>; 8.40<sup>b</sup> (May data) |
| Salinity        | 22.7 (%)<sup>a</sup>; 22.7 (ppt)<sup>a</sup>; 15.0 (%)<sup>e</sup>; 16.90 (ppt)<sup>e</sup>; 16.5–16.8 (ppt)<sup>e</sup> | 0.3 (ppt)<sup>e</sup> |
| Conductivity    | 25.5–26.5 (mS × cm)<sup>a</sup>; 29.76 (mS/cm)<sup>a</sup>; 27.88–28.65 (mS/cm)<sup>a</sup> | 572 (µS/cm)<sup>a</sup>; 582.2 (µS/cm)<sup>b</sup> (May data) |
| Dissolved oxygen| 10.2 (mg/mL)<sup>a</sup>; 10.4–9.8 (mg/mL)<sup>e</sup> | 8.7 (mg/mL)<sup>a</sup>; 8.2–8.6 (May data) (mg/mL)<sup>a</sup>; 8.8 (mg/mL)<sup>b</sup> |
| Saturation      | 107 (%)<sup>a</sup>; 105–108 (%)<sup>e</sup> | 100 (%)<sup>e</sup>; 105 (%)<sup>b</sup> |
| Ion concentrations | Na<sup>+</sup> 336.9 (meq/L)<sup>a</sup>; 337.9 (mmol/L)<sup>b</sup> | 1.32 (meq/L)<sup>a</sup>; 15–25 (mg/L)<sup>b</sup> |
|                | K<sup>+</sup> 13.0 (meq/L)<sup>d</sup>; 10.90 (mmol/L)<sup>b</sup> | 0.103 (meq/L)<sup>a</sup>; 2.5–4.0 (mg/L)<sup>b</sup> |
|                | Mg<sup>2+</sup> 7.80 (meq/L)<sup>d</sup>; 4.42 (mmol/L)<sup>b</sup> | 2.01 (meq/L)<sup>a</sup>; 64.41–70.47 (mg/L)<sup>b</sup>; 83.3 (mg/L)<sup>b</sup> (May data) |
|                | Ca<sup>2+</sup> 0.23 (meq/L)<sup>d</sup>; 0.11 (mmol/L)<sup>b</sup> | 1.01 (meq/L)<sup>a</sup>; 84.21–93.0 (mg/L)<sup>d</sup>; 98.4 (mg/L)<sup>d</sup> (May data) |
| Cl<sup>−</sup> | 153.70 (meq/L)<sup>d</sup>; 160.60 (mmol/L)<sup>b</sup> | 0.37 (meq/L)<sup>d</sup>; 17.80–21.30 (mg/L)<sup>d</sup>; 27.7 (mg/L)<sup>d</sup> |
| Total cations   | 358.17 (meq/L)<sup>d</sup> | 4.44 (meq/L)<sup>d</sup> |
| Total anions    | 354.94 (meq/L)<sup>d</sup> | 6.03 (meq/L)<sup>d</sup> |

<sup>a</sup>Danulat and Selcuk (1992).
<sup>b</sup>Danulat (1995).
<sup>c</sup>Cetinkaya et al. (1994).
<sup>d</sup>According to Kempe et al. (1978) and Tugrul et al. (1984) as cited by Arabaci et al. (2001).
<sup>e</sup>Arabaci et al. (2001).
<sup>f</sup>Kaptaner (2015).
<sup>g</sup>Kaptaner et al. (2016).
<sup>h</sup>Atıcı et al. (2018).
engages in spawning activity. The fish migrate from Lake Van through the Karasu mouth (Çitören reeds) and travel upstream into the Karasu Stream for spawning, usually in May. Eighteen mature female Lake Van fish (fork length: 18–22 cm and total weight: 80–110 g) were used in this study. The sampling period was chosen in alignment with the Lake Van fish reproductive period as described by Ünal et al. (1999) and Kaptaner and Kankaya (2013). The fish were captured at three sites along their migration route and at two different times (prespawning and spawning periods). The first sampling was performed at Lake Van on 1 May 2018 (prespawning), the second was at the entrance of Karasu Stream on 5 May 2018 (spawning), and the third was at approximately 6.5 km upstream of the entrance of Karasu Stream on 12 May 2018 (spawning) (Figure 1). Six fish were caught from each site using gill and cast nets, and the pH and temperature were measured in surface water samples taken from the sampling area. After the samplings, the fish were anesthetized using 2-phenoxyethanol (320 µL/L) and sacrificed. The tissues, including the esophagus and anterior, mid and posterior segments of the intestine, were carefully obtained from the fish using fine scissors, forceps and scalpels. For the histological procedures, a fixation period of 24 h was used for the tissue samples in a 10% neutral buffered formalin solution. Phosphate-buffered saline, pH 7.4, was then used to wash the tissues, after which a gradual ethanol series was used for dehydration, followed by paraffin embedding.

**TUNEL staining**

A commercial terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit (In situ cell death detection kit, cat no: ab206386, Abcam, Cambridge, UK) was used to label the apoptotic cells. Sections measuring 5 µm thick were selected from the middles of the tissues and placed on adhesive-coated slides (Marienfeld GmbH, Lauda-Königshofen, Germany). Following deparaffinization and dehydration, the sections were washed with tris-buffered saline (TBS), and then tissue permeabilization was achieved through incubation at room temperature with proteinase K for 20 min. Next, the sections were covered with 3% H$_2$O$_2$ for 5 min to deactivate the endogenous peroxidase. After washing with TBS, the slides were
immunohistochemically detect the PCNA, sections 5 µm thick were selected from the middles of the tissues and placed on adhesive-coated slides (Marienfeld GmbH, Lauda-Königshofen, Germany). Following deparaffinization and dehydration, the sections were incubated at room temperature for 5 min in 3% H2O2 to inhibit the endogenous peroxidase activity in the tissues. Following TBS (pH: 7.4) washing, a target retrieval solution (Dako, Glostrup, Denmark) was used for 30 min to achieve antigen retrieval. After washing the sections with TBS, incubation was performed at room temperature for 20 min using horse serum for the prevention of nonspecific bindings, followed by overnight diluted primary antibody (1:200; monoclonal mouse anti-proliferating cell nuclear clone PC10, Dako, ref. number: M0879) incubation. A commercial immunohistochemistry kit (EXPOSE mouse and rabbit specific HRP/DAB detection IHC kit, cat no: ab80436, Abcam, Cambridge, UK) was used to immunohistochemically visualize the PCNA staining, and the remaining staining steps were carried out by following the instructions provided by the manufacturer. To visualize the peroxidase activity, incubation of the sections in a solution of substrate-chromogen containing DAB was performed until the appearance of a brown color (1–3 min) and the sections were then rinsed in deionized water. Counterstaining was performed using Mayer’s hematoxylin stain. Gradual concentrations of ethanol, 95% and 100%, were used to treat the slides, after which cover slips were placed on them and Canada balsam (Merck, Germany) was used to seal them. When preparing the negative control slides, either horse serum or TBS was used rather than the primary antibody. Examination of the slides was carried out using a microscope (Leica DMI 6000 B, Leica Microsystems CMS GmbH, Wetzlar, Germany) and photographs were taken.

**Immuno-histochemical PCNA labeling**

To immunohistochemically detect the PCNA, sections 5 µm thick were selected from the middles of the tissues and placed on adhesive-coated slides (Marienfeld GmbH, Lauda-Königshofen, Germany). Following deparaffinization and dehydration, the sections were incubated at room temperature for 5 min in 3% H2O2 to inhibit the endogenous peroxidase activity in the tissues. Following TBS (pH: 7.4) washing, a target retrieval solution (Dako, Glostrup, Denmark) was used for 30 min to achieve antigen retrieval. After washing the sections with TBS, incubation was performed at room temperature for 20 min using horse serum for the prevention of nonspecific bindings, followed by overnight diluted primary antibody (1:200; monoclonal mouse anti-proliferating cell nuclear clone PC10, Dako, ref. number: M0879) incubation. A commercial immunohistochemistry kit (EXPOSE mouse and rabbit specific HRP/DAB detection IHC kit, cat no: ab80436, Abcam, Cambridge, UK) was used to immunohistochemically visualize the PCNA staining, and the remaining staining steps were carried out by following the instructions provided by the manufacturer. To visualize the peroxidase activity, incubation of the sections in a solution of substrate-chromogen containing DAB was performed until the appearance of a brown color (1–3 min) and the sections were then rinsed in deionized water. Counterstaining was performed using Mayer’s hematoxylin stain. Gradual concentrations of ethanol, 95% and 100%, were used to treat the slides, after which cover slips were placed on them and Canada balsam (Merck, Germany) was used to seal them. When preparing the negative control slides, either horse serum or TBS was used rather than the primary antibody. Examination of the slides was carried out using a microscope (Leica DMI 6000 B, Leica Microsystems CMS GmbH, Wetzlar, Germany) and photographs were taken.

**Cell counting**

Five randomly chosen optical areas of the epithelial layer with no intersection points were captured using a Leica Digital DFC490 camera (Leica Microsystems CMS GmbH, Wetzlar, Germany) connected to a Leica DMI 6000B model microscope at 20× magnification for each TUNEL- and PCNA-stained section. Processing of the captured images was done using ImageJ software (National Institutes of Health, USA, [http://rsbweb.nih.gov/ij/](http://rsbweb.nih.gov/ij/)) and the cells were counted as TUNEL/PCNA-positive or -negative using cell counter plugin software ([https://imagej.nih.gov/ij/plugins/cell-counter.html](https://imagej.nih.gov/ij/plugins/cell-counter.html)). At least 1000 esophageal epithelium cells for each section and 2000 cells from each intestinal segment belonging to each fish were counted. The apoptotic and proliferative cell indices for each fish were calculated in the esophagus and each segment of the intestine, and throughout the intestine. The apoptotic and proliferation indices were calculated as the TUNEL/PCNA-positive cell ratio from five different visual areas to the total number of cells (positive + negative) and multiplication of the ratios by 100.

**Statistical analyses**

The Statistical Software Package for the Social Sciences, version 16.0, was used to statistically evaluate data from the apoptotic index (AI) and proliferation index (PI) calculations. Differences among the data were analyzed using a one-way analysis of variance with a post hoc Duncan’s multiple comparison test. The results are presented as the mean ± standard deviation. Statistical significance was considered as P < 0.05.

**Results**

The temperature and pH of the surface water sample taken from the lake were 12.5°C and 9.64, respectively. The equivalent values were 17.3°C
and 8.31 at the entrance of the freshwater stream, and 18.6°C and 8.38 upstream in the fresh water.

Apoptotic cells with fragmented DNA in their nuclei were randomly scattered from the basal layer to the superficial layer of the esophageal epithelia containing mucous cells, in fish from both the lake and the fresh water (Figure 2). The AI values did not show a significant difference in the esophageal epithelium between fish from the lake and those from fresh water (Table II). Apoptotic cells were randomly and scarcely distributed throughout the entire epithelium from the base to the apex of the intestinal villi in fish from the lake, whereas these cells increased throughout the entire epithelium and, in some cases, they were condensed in the epithelial layer covering the apexes (tips) of the villi (Figure 3). In fish from the entrance to and upstream in the freshwater stream, the AI increased significantly in the epithelia of the anterior and mid-intestine or throughout the intestinal epithelium (P < 0.05), while it remained unchanged in the posterior intestinal epithelium (Table II).

Proliferating cells were randomly scattered throughout the esophageal epithelium of fish from the lake and the stream. The distribution of those cells was more intense in the esophageal epithelium in fish from the lake than in those from the stream (Figure 4). The PI was seen to gradually decrease in the freshwater fish and these decreases were statistically significant when compared to those from the lake (P < 0.05) (Table III). In the intestine, the proliferating cells were observed to be located mainly in the epithelium covering the basal regions and troughs of the intestinal folds in fish from both the lake and the fresh water. However, it was determined that the distribution of those cells increased from the base to the apex of the villi in fish from the entrance of the freshwater stream (Figure 5). The PI was significantly elevated in the epithelial layer of the anterior intestine or throughout the intestine (P < 0.05), while it remained unchanged in the epithelia of the mid- and posterior intestine segments of fish from the freshwater stream entry (Table III). There was no statistically significant alteration in the PI values in any of the epithelia of the intestinal segments or throughout the intestinal epithelium of fish upstream in the freshwater stream.

**Discussion**

The esophagus epithelium undergoes morphological alterations in relation to permeability during seawater and freshwater acclimation. In seawater-acclimated eel (*Anguilla anguilla*), the epithelium is composed of a simple and columnar cellular layer containing numerous mucous cells, while in freshwater-acclimated eel, the esophageal epithelium is stratified.

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**Table II.** Apoptotic index values in the epithelia of esophagus and intestine (anterior, mid, and posterior or throughout) during migration from the highly alkaline and brackish water of Lake Van to freshwater (Karasu Stream).

| Sampling site               | Esophagus | Anterior intestine | Mid intestine | Posterior intestine | Throughout the intestine |
|-----------------------------|-----------|--------------------|---------------|---------------------|--------------------------|
| Lake Van                    | 15.65 ± 9.98<sup>a</sup> | 7.08 ± 1.30<sup>a</sup> | 7.82 ± 3.36<sup>a</sup> | 12.05 ± 3.62<sup>a</sup> | 8.98 ± 3.57<sup>a</sup> |
| Entrance of Karasu Stream   | 16.95 ± 9.04<sup>a</sup> | 13.56 ± 7.37<sup>b</sup> | 12.83 ± 4.25<sup>ab</sup> | 12.98 ± 4.94<sup>a</sup> | 13.12 ± 5.34<sup>b</sup> |
| Upstream of Karasu Stream   | 15.20 ± 3.96<sup>a</sup> | 13.00 ± 4.24<sup>ab</sup> | 16.41 ± 9.77<sup>a</sup> | 11.48 ± 3.46<sup>a</sup> | 13.63 ± 6.43<sup>b</sup> |

Different letters represent significant differences between the groups of fish, determined using the Duncan multiple range test. Values are expressed as the mean ± standard deviation (P < 0.05 is considered significant; column comparison).
without mucous cells (Yamamoto & Hirano 1978). The esophagus is also associated with osmoregulation in tilapia (Oreochromis niloticus), where a stratified epithelium containing rich mucous cells is replaced by a highly vascularized monolayered epithelium with microvilli during saltwater acclimation (Cataldi et al. 1988a), and regions of single columnar epithelium appear with increased salinity concentrations in

Table III. Proliferation index values in the epithelia of esophagus and intestine (anterior, mid, and posterior or throughout) during migration from the highly alkaline and brackish water of Lake Van to freshwater (Karasu Stream).

| Sampling site          | Esophagus   | Anterior intestine | Mid intestine | Posterior intestine | Throughout the intestine |
|------------------------|-------------|--------------------|---------------|---------------------|-------------------------|
| Lake Van               | 56.18 ± 10.49<sup>a</sup> | 17.84 ± 4.57<sup>a</sup> | 14.65 ± 1.68<sup>a</sup> | 14.65 ± 4.97<sup>a</sup> | 16.37 ± 4.79<sup>a</sup> |
| Entrance of Karasu Stream | 37.09 ± 8.34<sup>b</sup> | 33.36 ± 8.08<sup>b</sup> | 15.55 ± 2.64<sup>a</sup> | 17.43 ± 9.03<sup>a</sup> | 23.23 ± 10.23<sup>b</sup> |
| Upstream of Karasu Stream | 20.42 ± 8.45<sup>c</sup> | 16.30 ± 6.55<sup>a</sup> | 12.92 ± 1.81<sup>a</sup> | 10.63 ± 2.87<sup>a</sup> | 12.50 ± 5.25<sup>a</sup> |

Different letters represent significant differences between the groups of fish, determined using the Duncan multiple range test. Values are expressed as the mean ± standard deviation (P < 0.05 is considered significant; column comparison).
15-day-old tilapia (*Oreochromis niloticus*) within the normal pluristratified epithelium associated with an ion transport role (Cataldi et al. 1988b). In contrast to the above mentioned reports, a morphological change was not observed in Lake Van fish sampled from the highly alkaline and brackish Lake Van when compared to fish from fresh water (Karasu Stream).

On the other hand, the indices of the proliferating cells gradually decreased in the freshwater environment in the present study. The esophagus of Lake Van fish is a short tube (approximately 0.5 cm in length) connecting the pharynx with post-esophageal swelling (data not shown), and the mucosa of the esophagus is lined with a stratified epithelium containing mucous-secreting cells in fish from both the lake and the fresh water. It seems that the esophagus of the Lake Van fish was less affected by the freshwater environment and, as in many teleost fish, the major function of the esophagus is food transportation and lubrication (Awaad et al. 2014) rather than osmoregulatory processes. Our observations of the epithelium of the esophagus remain unclear for now and it should be investigated in more detailed studies in the future.

Apoptotic cells were scattered throughout the epithelium of the intestine in fish from the lake in this study, while those cells increased in the entire epithelium and were localized in the epithelium lining the apexes of the intestinal villi of the fish from fresh water. Similar distributions of apoptotic cells in the intestinal epithelia were also reported in studies conducted on different fish species (Takahashi et al. 2006a, 2014; Sirri et al. 2014). Proliferating cells were observed to be mainly localized in the epithelium lining the troughs and basal regions of the intestinal folds. This finding is consistent with studies of other fish with the detection of proliferating cells in the intestinal epithelium that covered the troughs and basal intestinal fold regions (Sirri et al. 2014; Takahashi et al. 2014). In the present study, the AI values increased and remained at high levels in the anterior and mid segments of the intestine or throughout the intestine of fish from the entrance to and upstream in the freshwater stream, whereas the PI increased in the anterior intestine and throughout the intestine. Modifications in apoptosis and cell proliferation are associated with the differentiation and permeability of the esophageal epithelium of euryhaline species, and it is generally reported that an elevation in cell proliferation occurs throughout the esophageal epithelium of freshwater-acclimated fish (Takahashi et al. 2006a, 2007). This report is consistent with our work, where an increase in cell proliferation might have occurred as a consequence of low permeability during...
freshwater acclimation (Takahashi et al. 2006b).

On the other hand, few and randomly scattered
apoptotic cells were detected in the esophageal
epithelium of freshwater-acclimated mudskippers
(Periophthalmus modestus) (Takahashi et al.
2006a) and tilapia (Oreochromis niloticus)
(Takahashi et al. 2007). Throughout acclimation
to different salinities, the turnover of epithelial
cells falls under the control of endocrine regulation.
In teleost fish, prolactin is an essential hor-
mon that promotes freshwater acclimation,
performing this function by reducing ion and
water absorption by the esophagus and intestine
during freshwater acclimation (McCormick
2001; Takahashi et al. 2006b). Growth hormone plays
a role in seawater acclimation, and cortisol inter-
acts with both prolactin and growth hormone and
performs a dual function in osmoregulation.
Cortisol provides freshwater acclimation by main-
taining ion transporters and chloride cells. The
movement of water throughout the gut is con-
ducted by cortisol in freshwater eels, and there
are increased circulating levels of cortisol in eur-
hyaline and marine fish when transferred to hypo-
tonic environments, suggesting the role of this
hormone in ion uptake (McCormick 2001). Cell
proliferation is induced by prolactin during fresh-
water acclimation, while cell proliferation and
apoptosis are promoted by cortisol (Sakamoto
et al. 2005). In mudskippers (Periophthalmus mod-
estus), prolactin induces cell proliferation syner-
gistically with cortisol in the epithelium of the
anterior intestine, and the proliferating cells are
distributed throughout the epithelium in addition
to the troughs of the intestinal folds during fresh-
water acclimation. Apoptosis occurs at the tips of
epithelial folds, subsequently resulting in lower
permeability. Thus, even though the circulating
levels of prolactin and cortisol levels could not be
determined in the examined fish in this study, it
may be speculated that the increased cell prolif-
eration and apoptosis values observed in the
intestinal epithelia of Lake Van fish caught at
the entrance of Karasu Stream might have
occurred as the result of endocrine regulations
during freshwater acclimation. The cell PI
decreased, while the AI was found have remained
at high levels in the intestinal epithelia of fish
upstream in the freshwater stream. Cell prolifera-
tion, differentiation and apoptosis are continuous
homeostatic processes; however, a balance distur-
bance in cellular oxidants and pro-oxidants to the
advantage of the pro-oxidants causes differenti-
tiated growth, and arrested and apoptotic cells
(Aw 2003). Osmoregulatory responses are evolu-
tionarily conserved among different animal spe-
cies (Morritt & Richardson 1999; Xu et al.
2013). No matter the species, the process of osmoregulation is energetically costly, resulting
in hypo-osmotic stress that causes an increased
production of reactive oxygen species (ROS) and
a significant expenditure of energy in the
Mediterranean green crab (Carcinus aestuarii)
when undergoing exposure to diluted seawater
(Rivera-Ingraham et al. 2016). Studies performed
on mussels (Perna sp.) have shown that increased
levels of lipid peroxidation in the digestive glands
may have been caused by increased oxidative
stress as a result of increased ROS formation
due to high metabolic rates during the breeding
season (Wilhelm Filho et al. 2001; Verlecar et al.
2008). As a result of osmoregulatory processes,
elevated tissue lipid peroxidation or oxidative
stress and significant physiological alterations
have been reported in Acipenser naccarii
(Martinez-Alvarez et al. 2002). In our previous
studies conducted on Lake Van fish, we observed
increased erythrocyte osmotic fragility causally
related to physicochemical changes during migra-
tion to fresh water, that indicated physiological
stress (Arihan et al. 2017), and lipid peroxida-
tion, an indicator of oxidative stress, in the ante-
rior intestine of fish sampled from a freshwater
environment (Kaptaner & Dogan 2019). Apart
from the above-mentioned impact, the feeding
status and diet type also alter epithelial cell prolif-
eration and apoptosis in fish (Sirri et al. 2014;
Takahashi et al. 2014). Thus, increased apoptosis
in the intestinal epithelium might be associated
with the redox balance, which could be changed
by hypo-osmotic stress or feeding status. To
determine the exact mechanism underlying cell
proliferation or apoptosis in the intestinal epithe-
lia during freshwater acclimation, more detailed
studies must be conducted.

In conclusion, herein is reported the first investiga-
tion into cellular turnover using cell proliferation and apop-
tosis in the esophageal and intestinal epithelia of Lake
Van fish during migration from one habitat (lake) to
a different habitat (freshwater), and the alterations were
determined. Thus, this study provides new data regard-
ing the ecophysiology of the digestive tract of an ana-
dromous cyprinid fish; however, the reasons underlying
our observations remain unclear and more detailed
research is needed to define which factors, i.e. osmoregulatory-related impact, such as hormonal regulation and oxidative stress or feeding status, are effective.

Ethical statement

Approval for all of the procedures in this study was given by the Van Yuzuncu Yil University Animal Experiments Ethics Committee (Decision No.: YUHADYEK-2018/10).

Disclosure statement

No potential conflict of interest was reported by the authors.

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