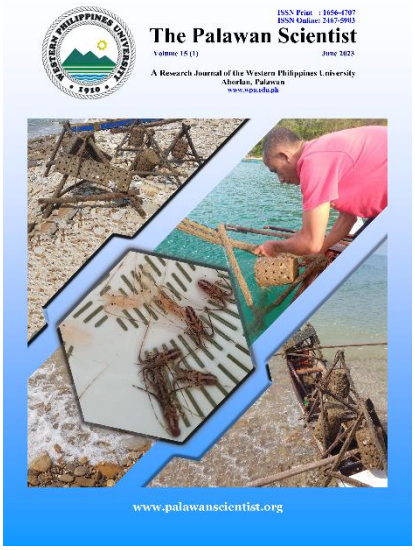


# Histopathology of the gills of Lake Van Fish *Alburnus tarichi* (Güldenstädt, 1814) infected with *Dactylogyrus* spp. parasites.

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## ABSTRACT

The Lake Van fish is an anadromous carp endemic to the Lake Van basin. In this study, the histopathological effects of *Dactylogyrus* spp. parasites, which naturally infected fish, on the gills were determined during reproductive migration. Fish gills were stained with hematoxylin and eosin after routine histological procedures and apoptotic cells in the gills were determined immunohistochemically. *Dactylogyrus* spp. parasites were found in 8 of the 60 fish caught in the study. No parasites were found in the fish sampled from the lake. It was observed that the parasites caused hypertrophy, hyperplasia, edema, epithelial desquamation, hemorrhage, fusion of secondary lamellae, and necrosis in the gills. Immunohistochemically, no increase in the number of apoptotic cells was observed in the gills of the parasite-infected fish when compared with the non-infected fish gills. As a result, it can be inferred that the lake water has a restrictive effect in fish against parasites. Histopathologic lesions caused by the observed parasites in fish caught in fresh water could significantly affect gill functions.

**Keywords:** apoptosis, gill, histopathology, Lake Van

## INTRODUCTION

Fish is one of the most important components in the human diet due to its high nutritional quality and content. Although inland waters constitute 0.01% of the world's waters, fishing in these parts provides approximately 40% support to world fish production (Lynch et al. 2016). The Lake Van fish (*Alburnus tarichi* Güldenstädt, 1814) provides approximately 1/3 of Türkiye's domestic fish production. Approximately 10,000 t fish is caught per year, constitutes a great source of protein for the local people (Oğuz and Ünal 2011). The Lake Van fish is a carp species endemic to Türkiye's Lake Van, one of the largest soda lakes in the world. It is the only vertebrate species that has adapted to the extreme conditions of Lake Van such as high pH (9.8), alkalinity (155 mEq/L), and salinity (22‰) (Danulat and Kempe 1992). They migrate in fish schools to the streams pouring into the lake to spawn between April and July every year.

Despite its great importance for the people of the region, the Lake Van fish was included in the category of declining species in 2014 (Freyhof 2014). The decrease in its numbers was due to illegal hunting during spawning, habitat destruction and loss, river sand mining, and waste water pollution have also been shown to decrease egg success in fish as the causes of population decline. The effects of parasites on the decrease in numbers were ignored.

Fish parasites are an integral part of aquatic ecosystems and are common in natural fish populations. Parasites can directly cause the death of fish, as well as having many negative effects such as preventing growth, causing behavioral disorders, reducing resistance to stress factors, and causing histological disorders (Kumaraguru et al. 1995; Feist and Longshaw 2008). Parasitic diseases are among the factors limiting the development of aquaculture (Scholz 1999).

Although many biochemical, physiological, and histological studies have been conducted on Lake Van fish, studies on parasites and their effects on fish are very limited (Oğuz 2015; Oğuz and Kaval Oğuz 2020). The pathogenic effects of parasitic organisms on fish, especially those that cause lesions in tissues, have been studied primarily in fish of economic importance. Monitoring of parasites in cultured fish is of great importance to prevent the spread of pathogens. Histopathological examination of fish tissues is an important method used to determine the health status of fish individually and as a population (Takashima and Hibiya 1995; Genten et al. 2009). The most common gill lesions in fish infected with parasites are hypertrophy, edema, necrosis, epithelial desquamation, hyperplasia, fusion of secondary lamellae, and telangiectasia. However, it is not possible to see the same symptoms in all fish species (Abdelmonem et al. 2010). It has been stated that as a result of gill infection by parasites, fish may die due to a decrease in body weight and condition factor, respiratory disorders, and serious changes in osmoregulation (Raissy and Ansari 2011).

*Dactylogyrus* is a parasite genus that is common in all fish species, especially cyprinid fish. It is known that especially *Dactylogyrus vastator*, *Dactylogyrus anchoratus*, and *Dactylogyrus extensus* from the family Dactylogyridae infect carp (Trujillo-González et al. 2018). They reproduce with eggs, attach to the host with two hooks at their posterior end (Kennedy 2007), and settle in the fish gill filaments. In large numbers, they spread throughout the fish body. Different levels of tissue damage and necrotic and degenerative pathological changes were observed in infected fish (Abdelmonem et al. 2010).

The present study describesthe effects of the parasite on the gills of *A. tarichi.*

## METHODS

### Sampling

Lake Van fish were caught from Lake Van and streams pouring into the lake between April and July of 2022, when breeding migration takes place (Figure 1A). Thirty fish each were sampled from the lake and in the River Karasu which flows into the lake. (Figure 1B). After the fish were caught, they were transported to the laboratory in oxygen-connected transport containers.

The total weight and fork length of the caught fish were determined. Age was determined from the operculum. The gills of the fish were removed under anesthesia (phenoxyethanol 320 µl/l), placed in fixatives, and kept in Bouin fixative and 4% paraformaldehyde solutions at +4°C for 24 h (Bancroft and Gamble 2002).

Fish aged between 3 and 5 years, weighing 94-118 g, and of fork length of 17.5-20 cm were used in the present study. The gills of 8 out of the 60 fish caught in the lake and fresh water during the breeding migration of Lake Van fish were infected with *Dactylogyrus* species. Parasitic infection was observed only in the fish adapted to fresh water, but not in those caught in the lake.

All of the animal experimental procedures were performed in accordance with the animal study protocols approved by the Animal Research Local Ethics Committee of Van Yüzüncü Yıl University (Protocol no: 2020/20).

### Water Parameter

At the time of fish sampling, water parameters from the lake and freshwater were monitored. The pH, water temperature, dissolved oxygen, and salinity were measured with a multiparameter device (Milwaukee MW805, São Paulo, Brazil). The physicochemical parameters were measured using a HACH spectrophotometer (HACH DR/2010, HACH Co., Loveland, CO, USA). Water samples were analyzed for nitrite (HACH method 8507), nitrate (HACH method 8171), and ammonia (HACH method 8155) according to the manufacturer's instructions. The water parameters in Lake Van and the River Karasu where the fish were sampled, are shown in Table 1. When the measured values were compared, it was observed that there was a significant difference in the parameters between the two sampling areas. The difference between lake and stream water parameter was analyzed using the t-test (IBM SPSS Statistics 22, USA). The difference between the groups was considered significant at $p < 0.05$.

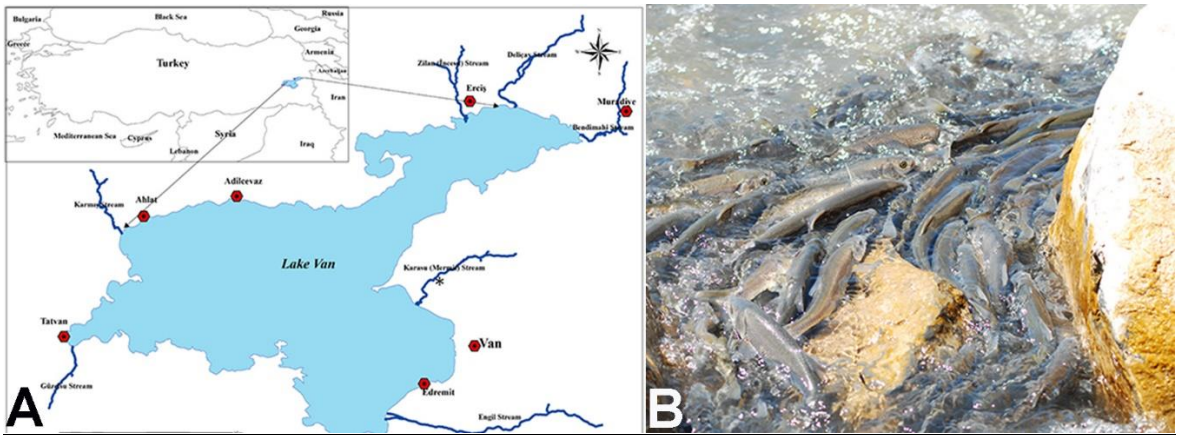


**Figure 1.** Location of the sampling area of Lake Van fish in Lake Van (A) and the fish school during reproductive migration of Lake Van fish (B).

**Table 1.** Water quality at the locations where fish were sampled.

| Parameter | Lake Van | Karasu River |
|---|---|---|
| Dissolved oxygen (mg/l) | 9.100 | 9.680 |
| pH | 9.660 | 8.960 |
| Temperature (ºC) | 12.100 | 19.90 |
| Salinty (ppt) | 16.970 | 0.200 |
| Conductivity (mS/m) | 24.200 | 0.498 |
| Nitrite (mg/l) | 0.001 | 0.009 |
| Nitrate (mg/l) | 0.270 | 1.900 |
| Ammonia-N (mg/l) | 0.420 | 0.050 |

**Histology**

The gills in Bouin fixative were then stored in 70% alcohol at +4°C until paraffin embedding. Tissues passed through graded alcohol series (70%, 80%, 90%, and 100%) and xylol were embedded in paraffin blocks. After 5-µm sections were taken from the paraffin blocks with the help of a microtome (HM 325 manual microtome, MICROM International GmbH, Waldorf, Germany), they were stained with hematoxylin and eosin to determine the general histological structure (Bancroft and Gamble 2002). The stained preparations were covered with Entellan and examined under a light microscope (Leica DMI 6000B microscope, Germany) and photographed using a Leica DFC 490 digital camera (Leica Microsystems, Germany).

**TUNEL Assay**

A TUNEL Assay Kit HRP-DAB (ab206386, Abcam, UK) was used for detecting cell death in gill sections of the Lake Van fish according to the manufacturer's instructions. Briefly, the tissue sections were deparaffinized and rehydrated at room temperature. Sections on coated glass slides were incubated in Proteinase K solution for 20 min at room temperature and then rinsed with Tris-buffered saline (1X TBS: 20 mM Tris pH 7.6- and 140-mM sodium chloride) for 5 min. To inactivate the endogenous peroxidases, the dried slides were incubated in 100 µl of 3% $H_2O_2$ at room temperature for 5 min, washed with 1X TBS, and left to dry. Then the specimen were covered with 100 µl of terminal deoxynucleotidyl transferase (TdT) equilibration buffer for 30 min and with 40 µl of TdT labeling mixture solution for 90 min at room temperature. The slides were incubated in a stop buffer at room temperature for 5 min to terminate the labeling reaction, followed by washing with 1X TBS. Then the sections were covered with 100 µl of blocking buffer at room temperature for 5 min, with the conjugate solution for 30 min and with diaminobenzidine (DAB) for 15 min consecutively. Finally, the sections were treated with methyl green counterstain, dehydrated with ethanol, and mounted with DPX. The slides were examined under a light microscope (Leica DMI 6000B microscope, Germany) and photographed using a Leica DFC 490 digital camera (Leica Microsystems, Germany).

**RESULTS**

When the gills of the uninfected fish were examined histologically, it was observed that the gills were composed of primary lamellae and secondary filaments, and pavement cells, mucus cells, and chloride cells were concentrated in these parts (Figure

2A). When the fish gills of infected fish were examined, it was observed that each gill was infected by parasites at different intensities and the damage varied depending on the parasite density. Tissue loss was observed in the parts where the parasites attached to the gills (Figure 2B). Fish gill epithelial cells infected with *Dactylogyrus* spp. showed hyperplasia, resulting in lamellar fusion (Figure 2B, C). Hemorrhage increased with the intensity of infection in fish (Figure 2D, E). Epithelial separation was observed in the gills of both the healthy and the infected fish, but it was more severe in the gills of the infected fish (Figure 2F).

When the healthy group was compared with the infected group, there was no difference in apoptotic cell density (Figure 3).

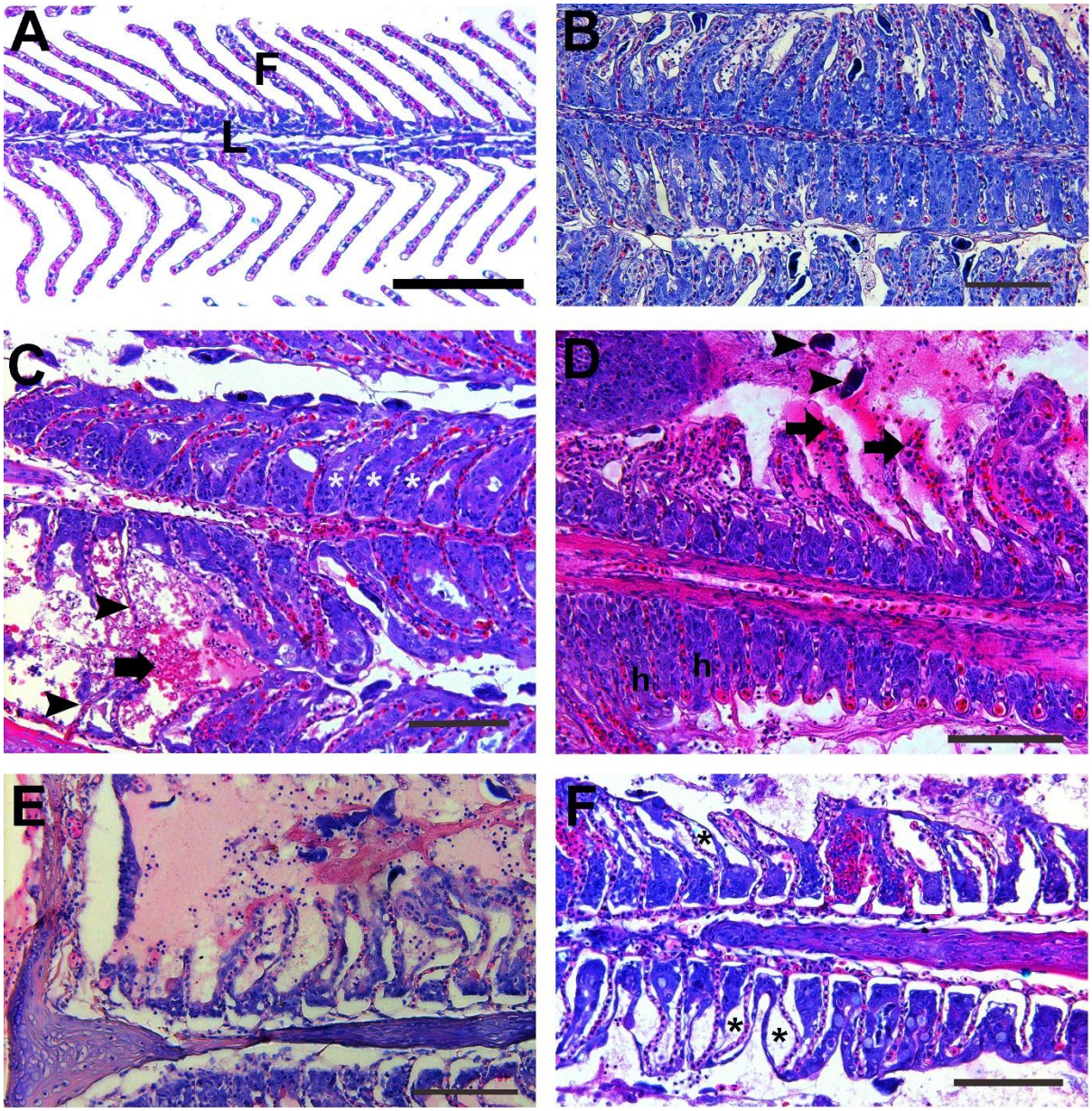


**Figure 2.** Histopathologic image of the gills of the Lake Van fish infected with *Dactylogyrus* spp. A) General view of normal fish gill histology (L: primary lamellae, F: secondary filament) B) Primary filament hyperplasia with secondary lamellar fusion (asterisk) C) Hemorrhages (arrows), lamellar fusion (asterisk), and necrosis of epithelial cells (arrowhead) D) Hemorrhages (arrows) and hypertrophy of cells (h). Arrowheads show parasites. E) Necrosis and desquamation of epithelial cells F) Epithelial lifting of varying severity (asterisks). Hematoxylin and eosin staining. Scale bars: 100 µm.

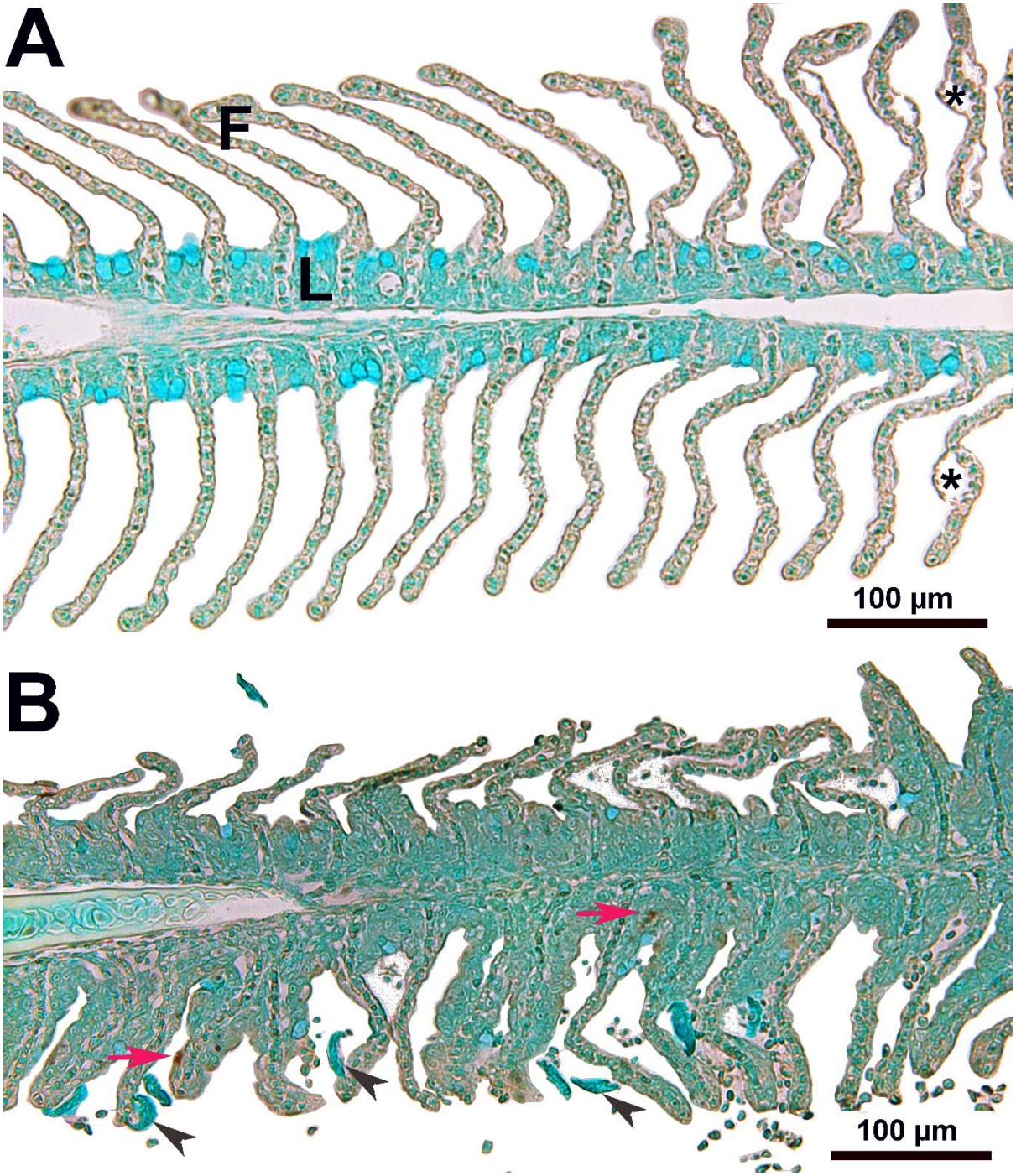


**Figure 3.** Detection of apoptosis in gill tissues of Lake Van fish. A) Normal fish gill (L: primary lamellae, F: secondary filaments, Asterix: epithelial lifting) and B) *Dactylogyrus* infected fish gill (Red arrow; apoptotic cell, Arrowhead; parasites).

## DISCUSSION

This study is the first on *Dactylogyrus* infection and pathology of endemic fish in Lake Van, which is among the largest alkaline lakes in the world. As a result of histopathological examinations, hyperplasia, hypertrophy, lamellar detachment, fusion in the secondary lamellae, hemorrhage, necrosis, and epithelial desquamation were detected in the Lake Van fish infected with *Dactylogyrus*. It has been observed that in intense parasitic infection, necrosis and shedding are more common in the gills, increasing epithelial cell proliferation in the regions where the parasites attach to the gills, causing hyperplasia and, as a result, lamellar fusion. The histopathological findings observed in the present study are similar to

those of studies performed in different fish species (Abdelmonem et al. 2010; Santos et al. 2017; Ramudu et al. 2020; Kumari and Nomani 2021). Lamellar separation in the gills was observed in both groups. Although this lesion was reversible, it was quite severe in parasitized gills. Blood congestion and aneurysm may be caused by sudden and intense blood flow to the gills, as stated previously (Rosety-Radriguez et al. 2002). Therefore, it can be inferred that the oxygen deficiency in the tissue caused by parasitic infection in Lake Van fish causes damage to the pillar cells.

It is known that parasitic infection increases apoptosis in animals (Bienvenu et al. 2010; Bosurgi and Rothlin 2021). In a study conducted in zebra mussels, apoptosis was observed only in circulating hemocytes as a result of ectoparasite infection, while no or few apoptotic cells were observed in infected tissues (Minguez et al. 2013). Similarly, in the present study, the number of apoptotic cells in the infected fish gills was very low. In the Lake Van fish, apoptosis may have occurred in immune cells in the blood.

No fish with gills infected with Dactylogyrus were found among the fish caught in the lake. This may have been due to the salty, alkaline, and high pH water of Lake Van (Table 1). In addition, one of the most important factors affecting the presence and density of monogeneans is temperature. Depending on the species, temperature demands also differ (Öztürk and Özer 2014). The lake water temperature, which fell to +4°C in winter, may have caused the elimination of parasites in the gills, and the water temperature, which increased to 19.9°C during the breeding migration, may have caused an increase in the number of parasites.

The present study was carried out in a limited number of fish gills, to further understand the impact of parasites on fish, the examination of other tissues of the fish caught in the lake environment in terms of parasite infection is suggested. *Dactylogyrus* is a genus of helminths represented by more than 900 species worldwide (Kumari and Monari 2021). Despite the diversity of fish in Turkey, the number of *Dactylogyrus* species is quite low when compared to other countries (Soylu 2009). This may be due to insufficient research on fish parasites. Most *Dactylogyrus* parasites infect cyprinids and often have high host specificity. Therefore, this Dactylogyus parasite is thought to be a new species as it is also observed for the first time in Lake Van fish, may be a new species.

Lamellar fusion seen in the gills as a result of parasite infection causes surface reduction and consequently a decrease in oxygen uptake. Advanced histopathological lesions cause the death of adult fish and larvae. In addition, parasite infections negatively affect osmoregulation in the gills (Oğuz and Kaval Oğuz 2020). According to the results obtained, the histopathological changes seen as a result of infection in Lake Van fish increase the effect of stress factors such as hunting pressure, hunger, fish density during migration, and swimming against the flow direction.

## FUNDING

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## ETHICAL CONSIDERATIONS

All of the animal experimental procedures were performed in accordance with the animal study protocols approved by the Animal Researchers Local Ethic Committee of Van Yüzüncü Yıl University (protocol no: 2020/12) and the Republic of Turkey Ministry of Agriculture and Forestry (08/03/2019-20122).

## DECLARATION OF COMPETING INTEREST

No potential conflict of interest was reported by the authors.

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***ROLE OF AUTHORS:*** *ANE, ZABEA and AP - carried out the histology analyzed the data; EKO - wrote and revised the manuscript. ARO - conceived the study, wrote, revised the manuscript, and designed the experiment; All authors read and approved the final manuscript.*