

GLOCHIDIA INFECTION OF SOME ENDEMIC FISH FROM LAKE PRESPIA, N. MACEDONIA

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ABSTRACT

Large freshwater mussels (Unionida) have very long life spans, are large-bodied and produce thousands to millions of larvae (glochidia) which typically must attach to host fish tissues to complete the life cycle. It is an obligatory parasitic stage of mussel larvae. However, fewer than one in 1 million find a suitable host and survive. The degree of host specificity varies among species of unionids from specialists able to successfully parasitize only one or a few closely related fish species to generalists which can complete development on a taxonomically wide range of fish species. Freshwater mussels represent one of the most severely endangered groups of animals due to habitat destruction, introduction of nonnative species, and loss of host fishes, which their larvae (glochidia) are obligate parasites on. Glochidiosis makes damages to fish over impairing their growth, but on the other hand, freshwater mussels play an important role in fresh waters by improving water quality and filtering water out bacteria, algae and pollutants constantly, and they are also an indicator species of water quality as well.

During our parasitological examination on the fishes from the Macedonian part of the Lake Prespa in April 2022, many glochidia on the gills, skin, and fins of two endemic fishes - Prespa roach (*Rutilus prespensis*) and Prespa nase (*Chondrostoma prespense*) were found, in the range from tens to thousands at one host.

Key words: Glochidia, endemic fishes, Lake Prespa

INTRODUCTION

Lake Prespa is situated in south-western part of North Macedonia. It is divided on 2 parts: Macro and Micro Prespa. It is mesotrophic, with an average depth of 18,74 m and the biggest is 54,2 m. The lake has several small rivers as tributaries. The surface level of the lake is significantly decreased in the last decades because of long-term dried period that leads to eutrophication. The lake is formed in pliocen and is old as Lake Ohrid. The lake is typical cyprinid.

Freshwater mussels (order Unionoida) play an important role in lakes and rivers by filtering water constantly as they go, breathe and feed. They are **filter feeders** and improve water quality by filtering out bacteria, algae, organic matter and pollutants. Mussels are moving as much as 30 liters of water per day through their internal filtration systems. Mussels play a key role in aquatic environments and are considered to be "**ecosystem engineers**" because they modify aquatic habitat, making it more suitable for themselves and other organisms.

They are an **indicator of water quality** since they are sensitive to excess siltation and low dissolved oxygen levels and cannot escape these unhealthy conditions.

Freshwater mussels represent one of the most severely **endangered** groups of animals due to habitat destruction, excess sedimentation, fragmentation from dams, pollution, introduction of invasive non-native species, and more recently an intense drought in the southern plains have all contributed to destruction of mussel beds. Their population is **declined by nearly 70%** all over the world.

Loss of host fishes, which their larvae (glochidia) are obligate parasites on, endanger existence of mussels as well.

The fertilized eggs are brooded in the female's gills, where they develop into tiny larvae called "glochidia". Once mature, females may release their glochidia into the water. Large freshwater mussels (Unionida) have very long life spans, are large-bodied and produce thousands to millions of larvae (glochidia) which typically must **attach to host fish tissues** (gills, fins, skin) to complete the life cycle. It is an **obligatory parasitic stage** of mussel larvae to complete the reproductive process. However, fewer than one in 1 million find a suitable host and survive. Over a few weeks to several months the glochidia develop (metamorphose) into juvenile mussels while attached to the host fish. The degree of host specificity varies among species of unionids from specialists able to successfully parasitize only one or a few closely related fish species to generalists which can complete development on a taxonomically wide range of fish species. The young mussel releases from the fish and **falls down into the sandy sediment** of the river, where it will live more or less for 20 years.

MATERIALS AND METHODS

Fish material from a total of 27 specimens of two endemic cyprinid fish were investigated parasitologically: Prespa roach *Rutilus prespensis* and Prespa nase *Chondrostoma prespense* were subjected to a parasitological investigation, during spring 2022. Only fresh fish were subjected to routine identification, dissection, and observation methods. Cleaned parasites were separated and put in certain fixatives, prepared for determination with determined techniques of staining and clearing. For the collection of glochidia, the stereomicroscope „Zeiss Stemi 305" and microscope „Zeiss Primovert" were used. For morphological examination, permanent slides of whole individual parasite were prepared. Identification was made throughout the morphology of glochidia.

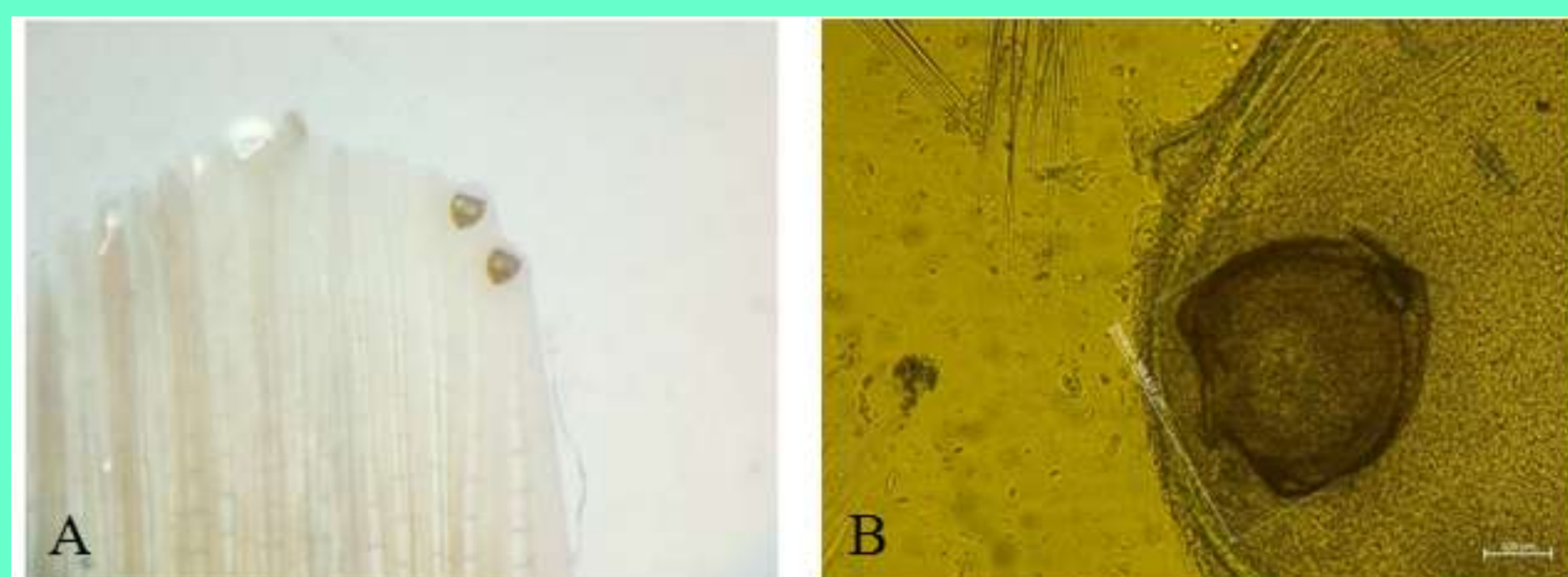


Fig. 3. Glochidia of *Anodonta cygnea* on the fins from *Rutilus prespensis* from Macedonian part of Lake Prespa – original photos: A - Glochidia found on the fin from fresh *Rutilus prespensis* specimen; B - microscopic slide of Glochidia.



Fig. 2. *Anodonta cygnea* from Macedonian part of Lake Prespa – original photo

RESULTS AND DISCUSSION

Native distribution of the swan mussel *Anodonta cygnea* (Linnaeus, 1758) is European-Siberian. It was previously found in Lake Prespa (Albrecht et al., 2012), which coincides with the findings of our parasitological investigations of fish and sampling of adult mussels from the Lake Prespa (Fig. 2). Parasitological examination was performed on the fishes from the Macedonian part of the Lake Prespa in April 2022. Two endemic cyprinid fish were investigated parasitologically: Prespa roach *Rutilus prespensis* (Karaman, 1924) – 15 specimens and Prespa nase - *Chondrostoma prespense* Karaman, 1924 - 12 specimens. Infestation with glochidia of *Anodonta cygnea* is found at 9 roaches (60%) and 8 nases (66,67%) (Fig. 4).

In this researches, many glochidia on the gills, skin and fins of two endemic fishes: Prespa roach - *Rutilus prespensis* (Karaman, 1924) and Prespa nase - *Chondrostoma prespense* (Karaman, 1924), were found, in the range from tens to thousands at one fish host (Fig. 2).

Heavy glochidia load found in our investigations induce **clear harm to the fish host** (Fig. 3).

Although the relationship between glochidium larvae of freshwater mussels Unionoida and their obligatory fish hosts has been frequently described as phoretic commensalism (glochidia benefit from transportation services by fish, with no harm to fish) or even symbiotic (both partners benefit), increasing evidence has accumulated that the **relationship is actually parasitic**.

Indeed, it is not surprising that mussel species that attach to fish for several months and that grow remarkably in size during that time, **activate fish immune system, intakes nutrients from the host, causes respiratory burden and damage to gills, reduce expression of secondary sexual traits and decreased sperm quality and may impair feeding efficiency of fish host that results with significantly lower weight of fish**. Heavy glochidia load can induce even **mortality of fish**.

The adverse effects of glochidia parasites on a host are usually density dependent. For example, a negative effect of *M. margaritifera* glochidia on the critical swimming speed of brown trout increased with the intensity of infection and was evident only when exceeded 10.000 glochidia per host. Negative growth effect of *M. margaritifera* glochidia on the fish host in natural infection intensities of less than 1.000 glochidia is also evident, although less prominent (Taubert & Geist, 2013; Chowdhury et al., 2019).

The record of glochidia of swan mussel *Anodonta cygnea* in endemic fish species from the Lake Prespa – prespa roach *Rutilus prespensis* and Prespa nase *Chondrostoma prespense* in the present study is considered the first in N. Macedonia. At the same time, both fish species represent new host for this glochidia worldwide.

The introduction of non-native species can have notable effects on the populations of native species, the ecosystem, but can also result in socio-economic consequences, caused by major damages in fisheries and aquaculture in a country. Data on non-native fish parasites are important for evaluating the health status and their general impact on native fish populations.

During the investigations that follow, we expect to find out more data about rate of infestation and the effect of glochidia on the health status of fish.

ACKNOWLEDGMENTS

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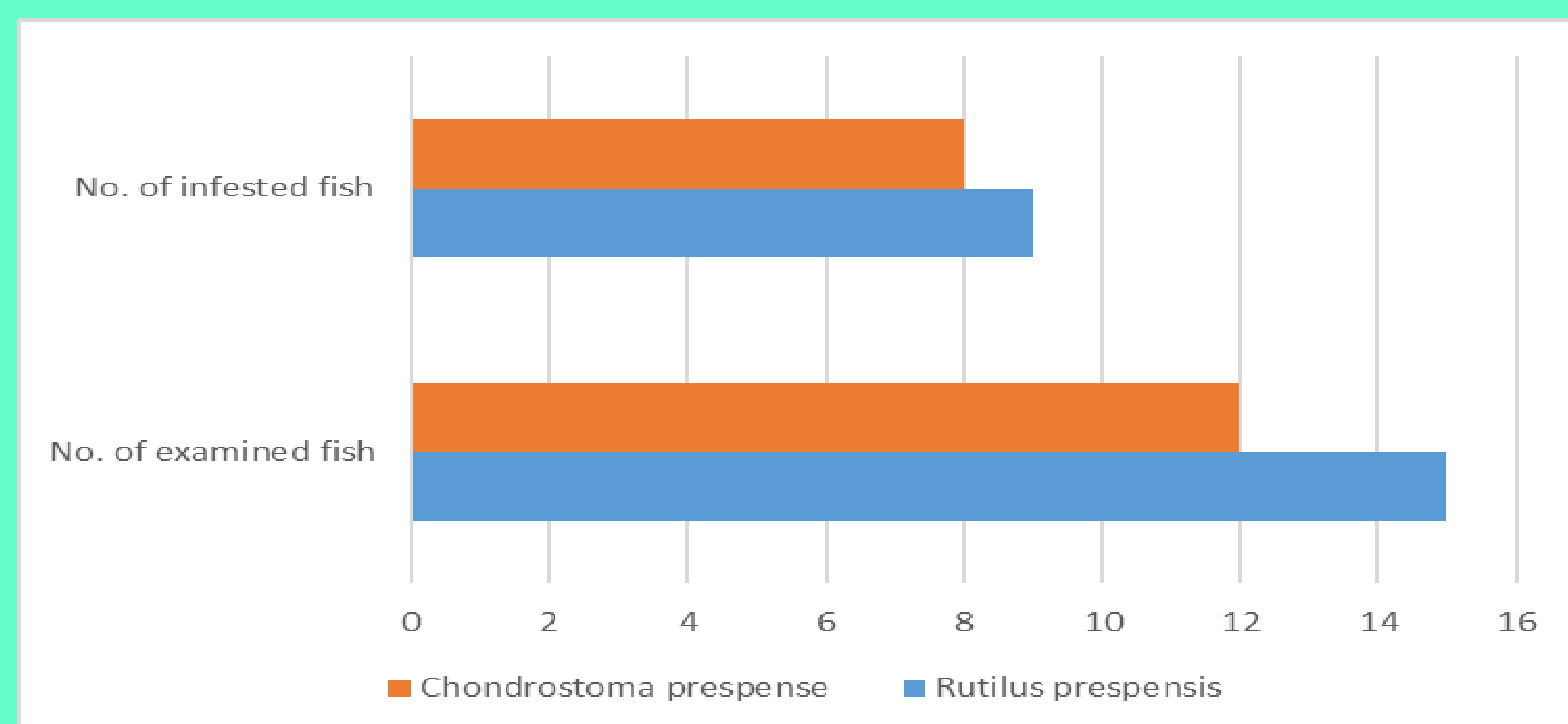


Fig 4. Infestation of cyprinid fish *Rutilus prespensis* and *Chondrostoma prespense* from Lake Prespa with glochidia of *Anodonta cygnea*

LENGTH-WEIGHT RELATIONSHIPS OF FIVE NATIVE, ENDEMIC AND THREE ALIEN, INTRODUCED FISH SPECIES FROM LAKE PRESPA



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Introduction

The length-weight relationship and the parameters derived from it have important application in fish biology, fish ecology and fisheries management (Le Cren, 1951; Froese, 2006). Variance in weight-length relationships are depending on the season, the population, or annual differences in environmental conditions (Froese, 2006). The b parameter of the length-weight relationship is indicator for the fish growth. When $b=3$ it indicates isometric growth, in which weight increases as the cube of length. When $b>3$ the fish is increasing in weight (presumably also in volume) at a greater rate than required to maintain constant body proportions, and vice versa when $b<3$ (Ricker 1979). Fulton's Condition Factor (K) is a mean for comparing the weight and length in a particular fish sample, suitable for comparing different individual fish of the same species, and can indicate differences related to sex, season, or place of capture (Ricker, 1975). Although this factor assumes that the investigated fish is growing without any change of form ($b=3$), it is the most popular and widely used condition factor in ichthyological investigations today.

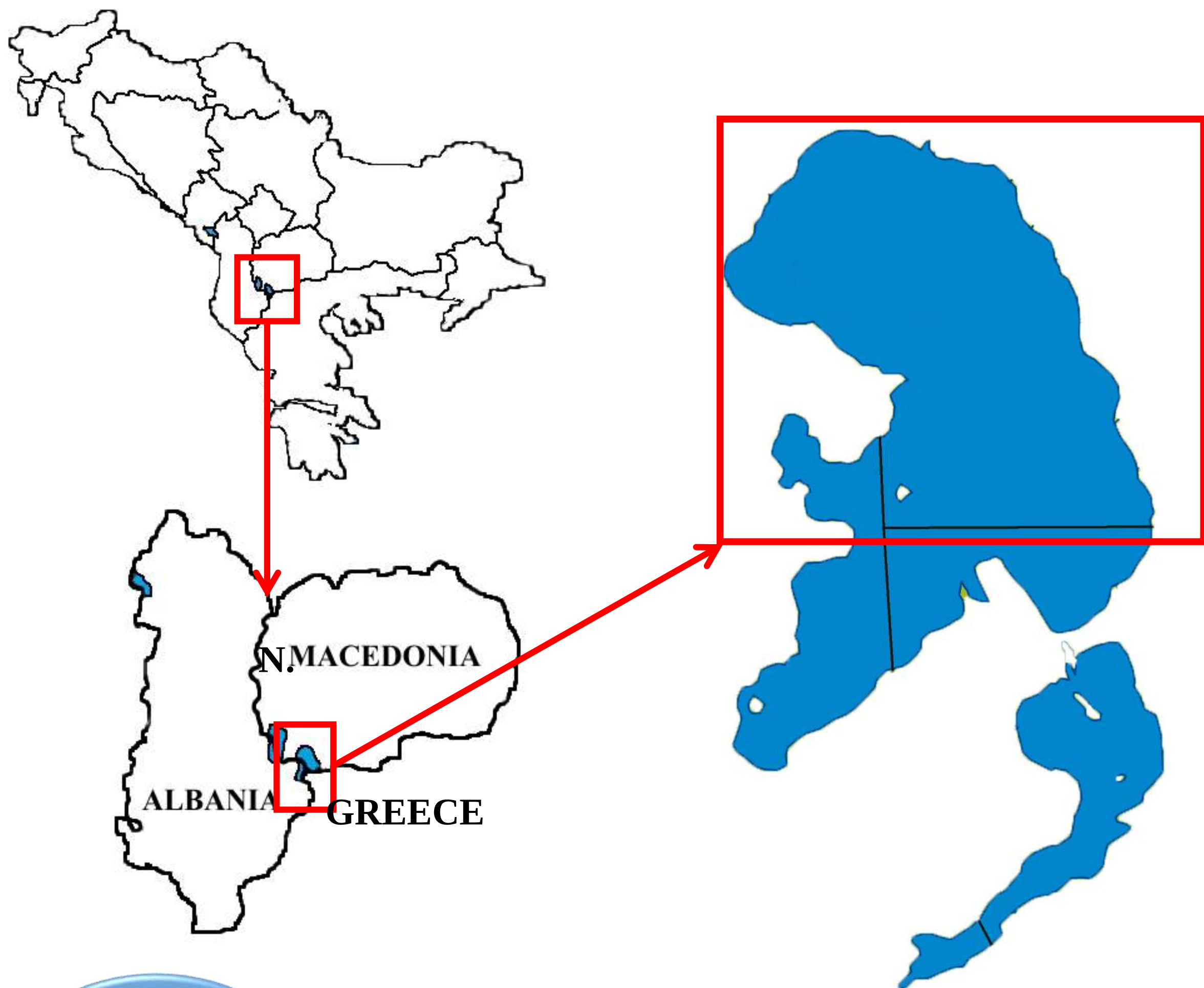
Regarding that, aim of this study was to investigate the length-weight relationships for 8 fish species from Lake Prespa, including *Alburnus belvica* (Karaman, 1924), *Barbus prespensis* (Karaman, 1924), *Chondrostoma prespense* (Karaman, 1924), *Rutilus prespensis* (Karaman, 1924), *Squalius prespensis* (Fowler, 1977) all of which are native endemic species found only in Lake Prespa, and, *Cyprinus carpio* (Linnaeus, 1758), *Carrasius gibelio* (Bloch, 1782) and *Lepomis gibbosus* (Linnaeus, 1758),, alien fish species that are introduced in different periods in history.

This study was conducted on Lake Prespa, Republic of North Macedonia. Lake Prespa is a mesotrophic water body, located on the Balkan Peninsula, and it is shared, and it lies on the borderline of three countries: N.Macedonia, Albania and Greece. It has a unique aquatic wildlife and represents a hotspot of endemism.

Materials and Methods

Total of 3386 specimens were sampled using gillnets with mesh size of 18, 20, 24 and 26 mm knot to knot. The sampling took place in different seasons in the years 2021 and 2022. The nets were planted in the littoral zone of the lake from 0-10 meters depth, at dusk and were left overnight in the water, staying in the water approximately 12 hours. After lifting of the nets the fish were immediately processed. First they were determined and divided by species, and total length (to nearest 1 mm) and body weight (to nearest 0,1 g) were measured using a ruler and laboratory scale, respectively.

For estimation of the length-weight relationship, the function $W=aL^b$ was used (Ricker 1975), and the parameter b, was tested by a t-test at the 0.05 significance level, to verify if it was significantly different from 3. Fulton's condition factor was calculated for every fish using the formula $K = (\text{weight (g)} / (\text{length (cm)})^3) \times 100$ (Ricker, 1975).



Autochthonous Fish Species



Alburnus belvica (Karaman, 1924)



Barbus prespensis (Karaman, 1924),



Chondrostoma prespense (Karaman, 1924)



Rutilus prespensis (Karaman, 1924)



Squalius prespensis (Fowler, 1977)

Allochthonous Fish Species



Cyprinus carpio (Linnaeus, 1758)



Carrasius gibelio (Bloch, 1782)



Lepomis gibbosus (Linnaeus, 1758)

* Фотографии

Results

species	n	Length(cm)		Weight(g)		Regression parameters				Condition Factor	
		min	max	min	max	b	95% CL of b	a	95% CL of a	r ²	avg±st.dev.
<i>Alburnus belvica</i>	15	15.5	21.1	30	95.5	3.2963	2.3695 - 4.2232	0.0041	0.0003 - 0.0595	0.8195	0.98 ± 0.14
<i>Barbus prespensis</i>	38	15.6	25.4	45	161.3	2.4702*	2.2724 - 2.6678	0.0535	0.0298 - 0.0962	0.9470	1.12 ± 0.10
<i>Chondrostoma prespense</i>	331	14.4	27.6	22.7	200	3.0743	2.9898 - 3.1588	0.0075	0.0059 - 0.0097	0.9396	0.94 ± 0.06
<i>Rutilus prespensis</i>	1952	12.5	21.1	23	120.3	3.0098	2.9601 - 3.0595	0.0119	0.0103 - 0.0136	0.8785	1.22 ± 0.09
<i>Squalius prespensis</i>	15	17.9	27	68.4	254.6	3.1196	2.6976 - 3.5417	0.0078	0.0021 - 0.0289	0.9515	1.14 ± 0.09
<i>Cyprinus carpio</i>	124	10.3	28	14.2	330.4	2.9531	2.8839 - 3.0224	0.0158	0.0131 - 0.0190	0.9832	1.40 ± 0.10
<i>Carassius gibelio</i>	18	11.1	14	21.6	40	2.8482	2.4427 - 3.2537	0.0229	0.0083 - 0.0632	0.9327	1.56 ± 0.08
<i>Lepomis gibbosus</i>	890	8.7	17.1	13	122.3	3.3486*	3.2859 - 3.4113	0.0095	0.0082 - 0.0110	0.9253	2.19 ± 0.15

*Significant difference of the value for parameter b from 3 (t-test; $p<0.05$)

Conclusions

Our study gave also gave an update to the LWR data for the above mentioned fish species. Nevertheless, taking in consideration the uniqueness of the Lake Prespa ecosystem and, the economical importance of some of this fish species, we can say that LWR investigations regarding these species are scarce. This kind of data is of great importance for fishery research and management, and can have application in conservation biology and ecology. Also our study can provide valuable data for FishBase, in which none of the investigated species from Lake Ohrid have LWR description presented.

References

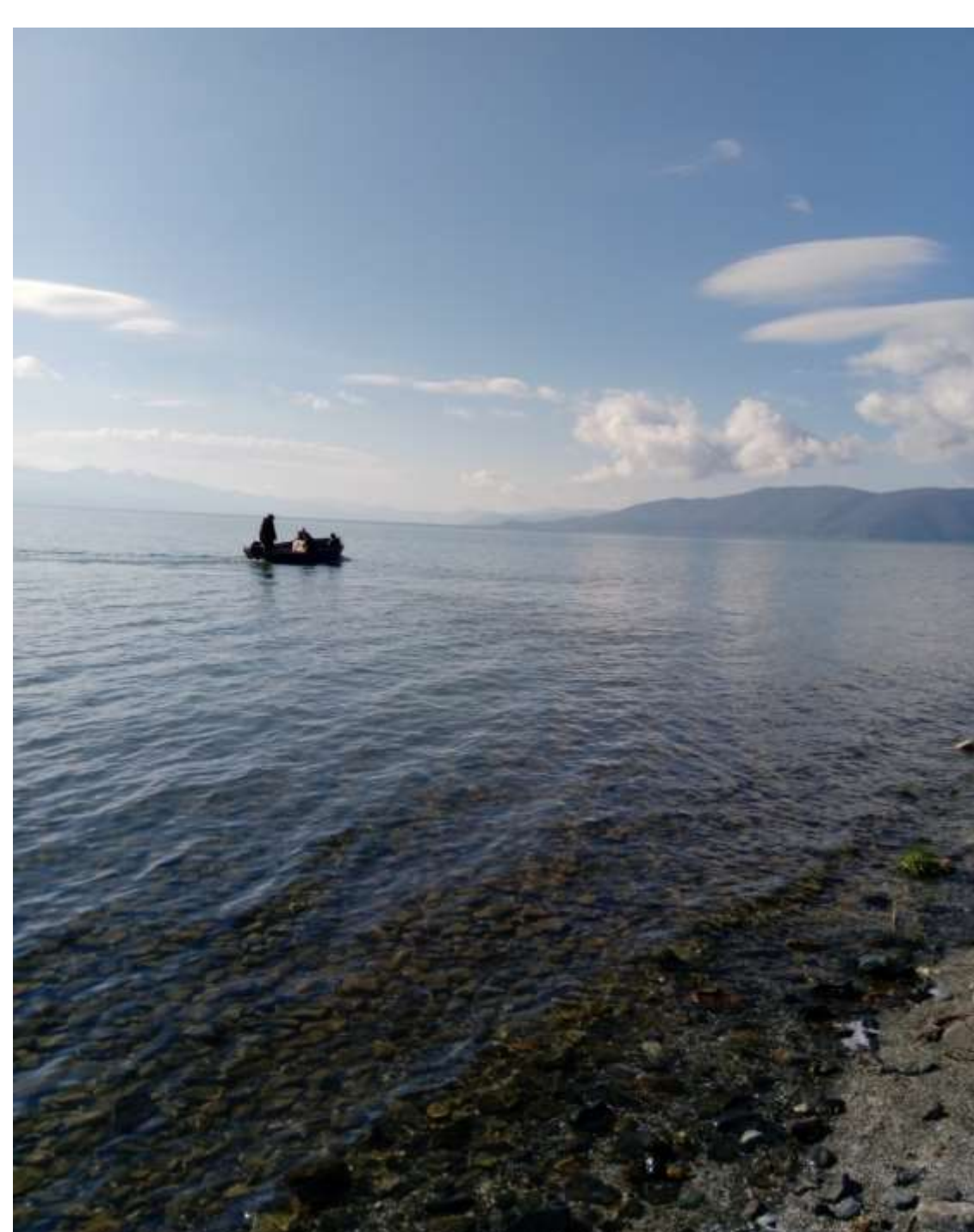
- Froese R. 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology*. 22, 241–253.
- Le Cren, E. D. 1951. The length–weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*. 20: 201–219.
- Ricker W.E., 1975. Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada*, 191 . 381 p.
- Ricker. W. E. 1979 Growth rates and models. In: W. S. Hoar. D. J. Randall and J. R. Brett(Eds.), *Fish Physiology Vol. VIII Bioenergetics and Growth*. Academic Press. London, pp. 677-743.
- *Ljubo Stefanov© so Spirkovski Z, Ilik-Boeva D, Talevski T, Paliqi A, Kapedani E (2012). The fishes of Prespa. Skopje, Macedonia, UNDP.

HISTOPATHOLOGICAL ANALYSIS OF LIVER AND GILLS IN FISH POPULATION FROM LAKE PRESPA

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Lake Prespa – april 2021

There has been conducted a histological analysis of the samples of liver from the natural population of fish, which inhabits Prespa Lake. The caught specimens of fish were with satisfactory external look, active and without any external changes.

The analysis of the histological preparations of liver from the investigated specimens indicated presence of many types of tissue lesions. The obtained results revealed pathological changes in the liver tissue including an inflammatory processes, fibrosis, bile duct epithelium necrosis on a level of hepatic bile tract.

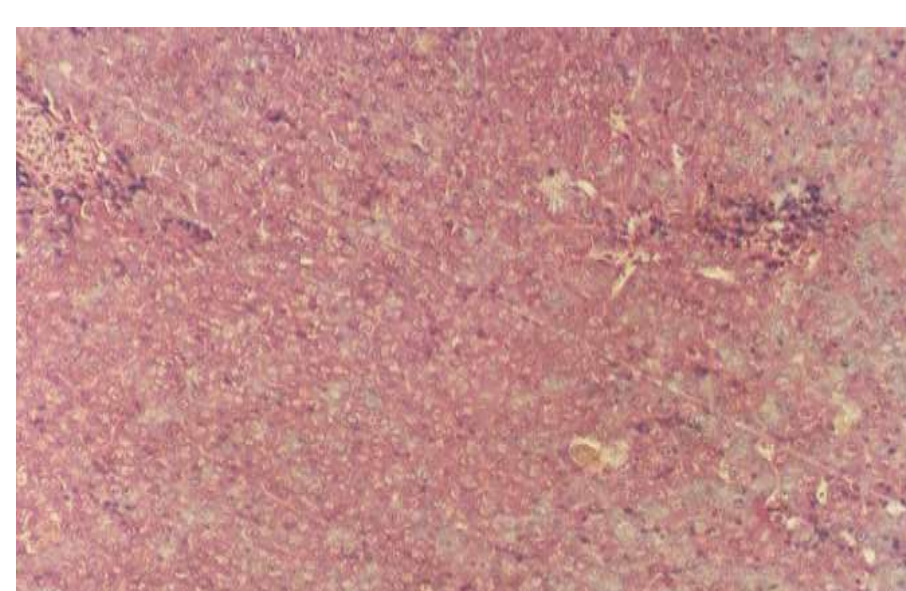


Figure 1. Necrosis and inflammation on the level of hepatocellular oarenhima

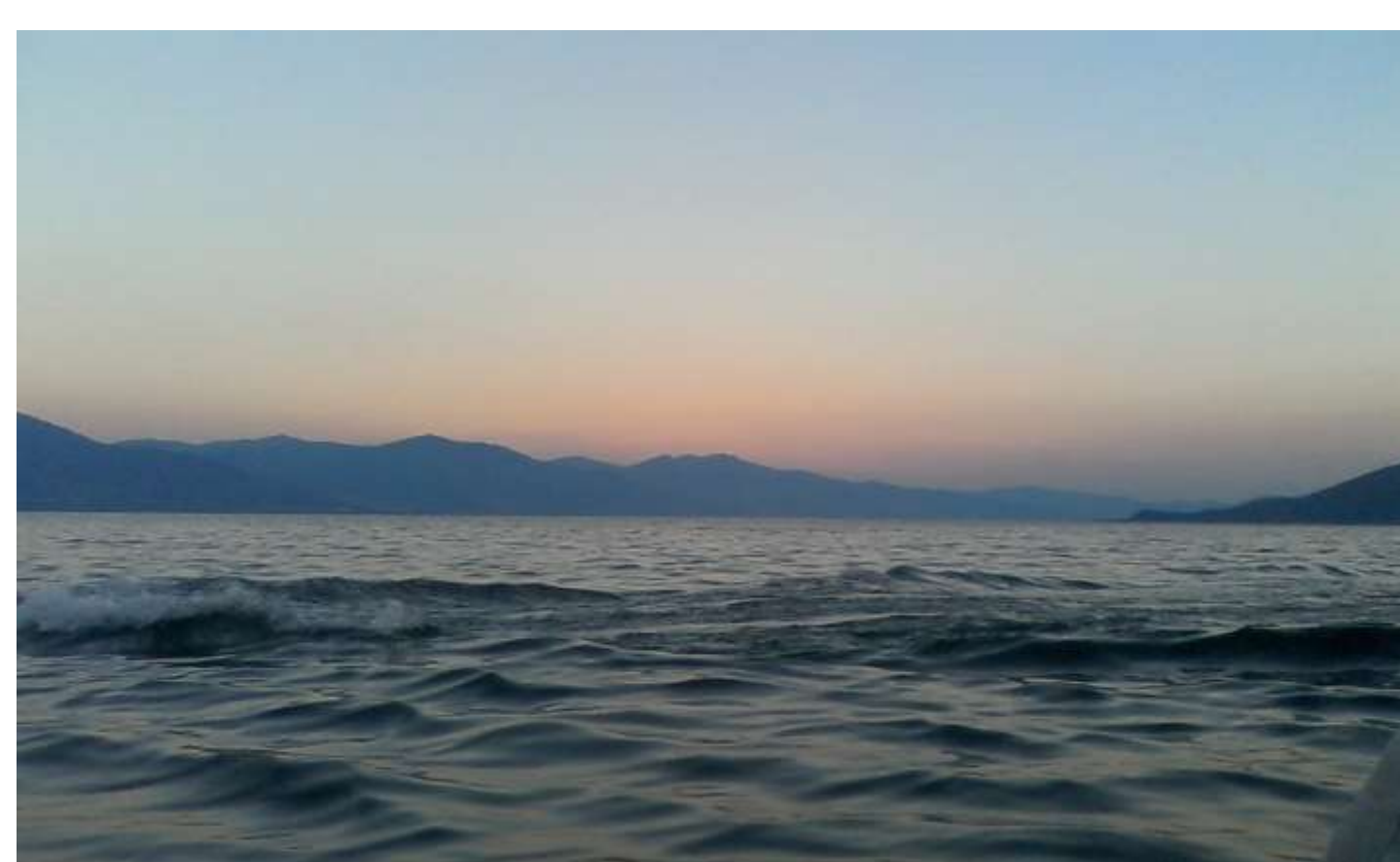
ABSTRACT

Histopathological biomarkers can be indicators for the effects on organisms by various anthropogenic pollutants and represent reflection of the overall health of the entire population in the ecosystem. Some structural lesions in liver and gills tissue have been accepted as valid biomarkers of anthropogenic stress at fish. Histopathology is the method of detecting chronic effect of exposure in the various tissues and organs to environmental stressors.

Liver and gill samples of fish individuals collected from Lake Prespa were isolated and processed by standard paraffin procedure for making histological preparations and microscopic analysis. Based on standard histopathological analysis some lesions in liver and gill tissues were detected.

KEY WORDS: histopathology, liver, gills, Lake Prespa

During period of 2020- 2021, in the Prespa Lake have been caught total of 43 pieces of fish, which were classified in 6 different species (*Barbus prespensis*, *Chondrostoma prespense*, *Alburnus belvica*, *Rutilus prespensis*, *Cyprinus carpio* and *Squalius prespensis*). Out of each specimen there have been dissected a piece of the liver for histological analysis, which, thereafter has been processed on the standard procedure for development of histological preparation. They have been analyzed under a microscope and the registered tissue lesion has been photographed.



Lake Prespa



Lake Prespa – september 2020

Histopathology provides a rapid method to detect effects of irritants in various organs. The exposure of fish to contaminants is likely to induce a number of lesions in different organs. Liver is suitable organs for histological examination

In order for the quality of the water to be increased, there should be undertaken some measures for prevention from alohtone pollution, as well as there should be conducted continuous monitoring of the quality of the water in the Prespa Lake .



Figure 2. Gills destroyed