Study of some Ectoparasites of Fishes from the Sava River as Part of Water Management in Bosnia and Herzegovina

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Abstract **- The scientific investigation of aquatic ecosystems in Bosnia and Herzegovina has become increasingly intense in recent years. Due to a deficit in studies regarding parasitology and biological control of diseases, two important fish ectoparasites (*Chilodonella cyprini* and *Ichthyophthirius multifiliis*) were investigated in 22 fish species (400 individuals) during 2017 from the middle flow of the Sava River. The prevalence of infection and infection intensity were analyzed and signs of ichthyophthiriasis were also documented. The study gives recommendations for the development of aquatic ecosystem management.**

Keywords**-ectoparasites, the Sava River, management, Bosnia and Herzegovina.**

1. Introduction

From the earliest times it has been known that fishes are a significant part of human and animal nutrition. Furthermore, fishing was one of the fundamental and effective ways to quickly find food in nature in order to meet the existential needs, which is why it became one of the important branches of economy. Fisheries development has led to an inextricable link between science and fisheries as an economic branch because the need for food research has become increasingly bigger. The researches into fish stocks and fish populations have great importance in terms of economic development and scientific achievements in different fields, especially in the field of biology, hydrobiology, and ecology. Ichthyological research in Bosnia and Herzegovina (BiH) over the last few years has gained more and more importance with new trends that include the methods of monitoring of the conditions of hydro-ecosystem and fish populations.

Current research in BiH is largely related to the exploration of the Sava River and its tributaries because the Sava River is a very significant ecosystem in BiH, characterized by a high level of biodiversity [1]. The Sava River is more than 940 km long. The river originates in Slovenia, flows through the Republic of Croatia thus making the natural border between the Republic of Croatia and BiH. In Belgrade, the Republic of Serbia, the river flows into the Danube River. The Sava belongs to the Black Sea basin.

It has been previously known that fish populations realize different forms of ecological coactions in hydro-ecosystems. One of the most important coactions is parasitism. Fish coactions with parasites of different species can often be harmful and lead to various types of fish diseases, which can be highly damaging for the fishes as the hosts. Consequently, such diseases could lead to the economic problems [2,3,4]. This study has chosen two parasites, *Chilodonella cyprinid* (Moroff, 1902; Kahl, 1931) and *Ichthyophthirius multifiliis* (Foquet, 1876), which are the causes of two important fish diseases, ichthyophtiriasis and chilodonellosis [3,5]. The problem of fish health is widespread, because there are a lot of documented cases of fish diseases such as chilodonellosis and ichthyophthiriasis in different countries [6,7,8].

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It is important to note that some foreign experts have contributed to the knowledge of the mentioned parasites. Their contributions can also be used for developing knowledge of the hydro-ecosystems in BiH. The investigation of several rivers in Croatia, including the Sava River, showed that the prevalence of infection by *Chilodonella cyprini* was 22.58%, and by *Ichthyophthirius multifiliis* it was 81.81%, which could be alarming rank for developing the aforementioned fish diseases [9].
In this study the prevalence of infection and infection intensity of *Chilodonella cyprini* and *Ichthyophthirius multifiliis* from the Sava River, BiH, were analyzed in order to gain new important data for the Agency for the Sava River District, as the main agency for aquatic ecosystem in BiH.

2. Material and methods

The study was carried out in the middle part of the Sava River on two locations marked as: Tolisa (45°3'42.44 "N; 18°38'14.07" E) and Orašje (45°2'29.53"N; 18°41'26.37"E). The length of the longitudinal profile of the survey is about 15 kilometers from the west to the east (Figure 1.).

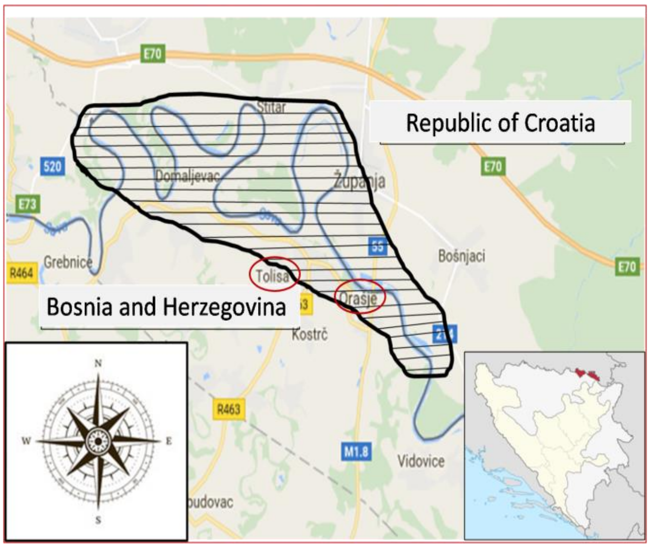

Figure 1. Graphic view of searched locations

For sampling purposes, fishing nets with the diameter of 25 mm and dimension 100x100 cm were used. In the same study, fishing nets with diameter of 50 mm and dimension of 12 m were also used. The sampling was conducted during the winter, spring, summer and autumn in the year of 2017. The total fish sample of 400 fishes and 22 species was sampled in cooperation with commercial fishermen from the Posavina region (Table 2. and 3.). After the sampling procedure, the fishes were taxonomically determined using standard methods for taxonomical determination [10,11,12]. After that procedure, microscopic analysis was performed using a customized microscope (MC50 BAT and Karl Zeiss) and the slides from the fish skin and gills were taken and used for native preparations. The number of parasites was counted in 10 microscopic fields (in work sheet and tables marked as n/10) and that was the value of infection intensity. For taxonomic determination of the parasites, the standard procedure was used [3,13]. After all determination procedures, statistical support was made by using the statistical software STATISTICA 7 for calculating the chi-square test, Kruskal-Wallis test, and Spearmans test of correlation. For the research purposes, the Agency for the Sava River District provided the data on water quality from the monitoring station Vidovice located near Orašje (Table 1.).

Table 1. Searched data of water quality (T-temperature, Cond.-electrical conductivity, mgO_2/L-saturation with oxygen)

Month	T(°C)	pH	Cond. µS/cm	mgO_2/L
Jan.	3.5	8.16	415	12.39
Feb.	7	8.04	420	11.86
Mar.	10.3	8.18	356	9.81
Apr.	17.2	7.82	432	8.96
May	15.3	8.13	417	9.54
Jun.	22.8	7.94	484	7.94
Jul.	27.1	8.01	541	7.66
Aug.	25	7.75	534	7.13
Sep.	22	7.85	512	8.19
Oct.	18	7.82	567	9.47
Nov.	11	8.07	472	9.84
Dec.	6.7	7.44	413	12.44

3. Results

All of the 400 sampled fish individuals were determined and most of them belong to the cyprinid fish family: *Abramis brama*, Linnaeus, 1758, *Abramis sapa*, Pallas, 1811, *Alburnus alburnus*, Linnaeus, 1758, *Aspius aspius*, Linnaeus, 1758, *Barbus barbus*, Linnaeus, 1758, *Blicca bjoerkna*, Linnaeus, 1758, *Carassius gibelio*, Linnaeus, 1758, *Chondrostoma nasus*, Linnaeus, 1758, *Cyprinus carpio*, Linnaeus, 1758, *Leuciscus cephalus*, Linnaeus, 1758, *Rutilus pigus virgo*, Heckel, 1852, *Tinca tinca*, Linnaeus, 1758, and*Vimba vimba*, Linnaeus, 1758.

In addition to the sampled species from the family Cyprinide, two species of the Ameiuridae family were sampled: *Ameiurus nebulosus*, Lesueur, 1819, and *Ameiurus melas*, Rafinesque, 1820.

One species from the Esocidae family was sampled and that was the species *Esox lucius*, Linnaeus, 1758. The sampled species *Neogobius gymnotrachelus*, Kessler, 1857, belongs to the Gobiidae family.

In the study, three species from the Percidae family were sampled: *Perca fluviatilis*, Linnaeus, 1758, *Sander lucioperca*, Linnaeus, 1758, and *Zingel zingel*, Linnaeus, 1766.

One species from the family Siluridae was sampled as well and that was *Silurus glanis*, Linnaeus, 1758, while collected species *Thymallus thymallus*, Linnaeus, 1758 belongs to the family Thmallydae.

Figure 2. presents the graphic view of the number of sampled species of determined fish families.

It is possible to see that the number of the sampled fish species according to the fish families does not have equal distribution, because the species from the Cyprinidae family are most dominant in the entire sample (x^2=563.99; p<0.05).

Table 2. presents the prevalence and intensity of infection by *Chilodonella cyprini*. The prevalence of infection of the total sample was 40% (Figure 4.).

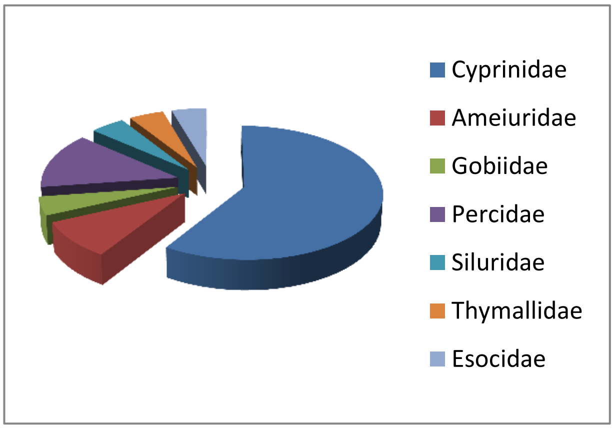

Figure 2. Graphic view of the share of sampled fish families

The mircoscopic analysis of the slides taken from the skin and the gills showed that 160 individuals out of the total 400 were infected by *Chilodonella cyprini* (Figure 3.). These were the following species: *Rutilus pigus virgo*, *Chondrostoma nasus*, *Abramis sapa*, *Blicca bjoerkna*, *Vimba vimba*, *Barbus barbus*, *Alburnus alburnus*, *Carassius gibelio* , *Esox lucius*, *Cyprinus carpio*, *Abramis brama*, *Thymallus thymallus* and *Neogobius gymnotrachelus*.

Figure 3. Chilodonella cyprini

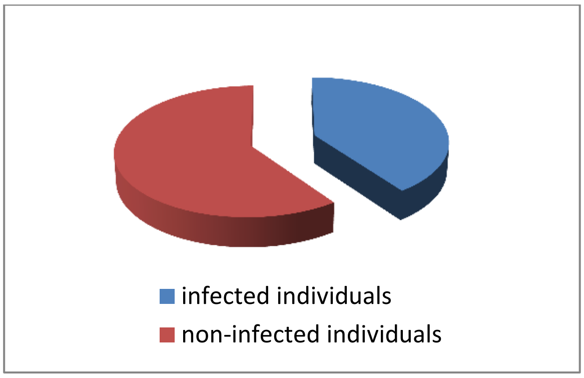

Figure 4. Infection of total sample by Chilodonella cyprini

The analysis of prevalence showed that the sampled individuals of *Abramis brama*, *Carassius gibelio* and *Chondrostoma nasus* show higher values of prevalence of infection in comparison with other infected individuals (x^2=60.26; p<0.05) (Table 2.).

Table 2. Prevalence and intensity of infection by Chilodonella cyprini (T-total fish sample; I-number of infected individuals; P (%)-prevalence of infection; n/10-intensity of infection. Dark color presents the fishes which cannot be statistically analyzed because the sample was not large enough).

Species	T	I	P(%)	n/10
A. brama	35	19	54.28	1-12
A. sapa	17	7	36.84	1-8
A. alburnus	57	17	28.82	1-6
A. melas	4	-	-	-
A. nebulosus	15	-	-	-
A. aspius	1	-	-	-
B. barbus	37	12	34.28	1-11
B. bjoerkna	25	15	60	1-15
C. gibelio	86	50	58.13	1-21
Ch. nasus	48	26	54.16	1-11
C. carpio	4	4	-	2-13
E. lucius	12	2	-	1-2
L. cephalus	1	-	-	-
N.gymnotrachelus	21	1	4.76	1
P. fluviatilis	4	-	-	-
R. pigus virgo	7	4	-	1-19
S. lucioperca	6	-	-	-
S. glanis	8	-	-	-
T. thymallus	1	1	-	1
T. tinca	1	-	-	-
V. vimba	3	2	-	1-5
Z. zingel	7	-	-	-

The Kruskal-Wallis test was used for the analysis of the intensity of infection during the seasons. The study showed equal intensity of infection during the periods of the year when infection was documented. According to the species that were analyzed, the values of the Kruskal-Wallis test were presented as follows: *Abramis brama* (H=1.12; p>0.05), *Carassius gibelio* (H=3.26; p>0.05) and *Chondrostoma nasus* (H=1.59; p>0.05). For the graphic view of seasonal dynamics of infection, the species *Abramis brama* was chosen (Figure 5.). According to the graphic view, it is evident that the highest values of infection were documented in spring and autumn but without statistical significance.

It was also found that the intensity of infection and appearance of the mentioned parasite could lead to correlation with the water temperature. The intensity of infection of *Abramis brama* was higher at the temperature of 15.3°C to 18°C (R = 0.432, p <0.05). According to the intensity of infection of *Alburnus alburnus*, it was documented that higher temperature values were optimum for this parasitic infection and the correlation between the two searched parameters was recorded (R=0.522; p<0.05). For other infected species the correlation was not found or was very low.

The analysis of the total ichthyological sample revealed the presence of *Ichthyophthirius multifiliis* (Figure 6.). The parasite was documented in 69 individuals, thus the prevalence of infection in the total sample was 17.5% (Figure 7.). The skin and gill parasite *Ichthyophthirius multifiliis* has been found on the fish skin and in one species it has been found on the skin and the gills (Table 3.). Table 3. shows that the highest prevalence of infection was found in *Abramis sapa* (70.58%), *Abramis brama* (54.28%), and *Blicca bjoerkna* (40%). There was a statistically significant difference in the prevalence of infection among these infected species (x^2 = 190.13; p <0.05).

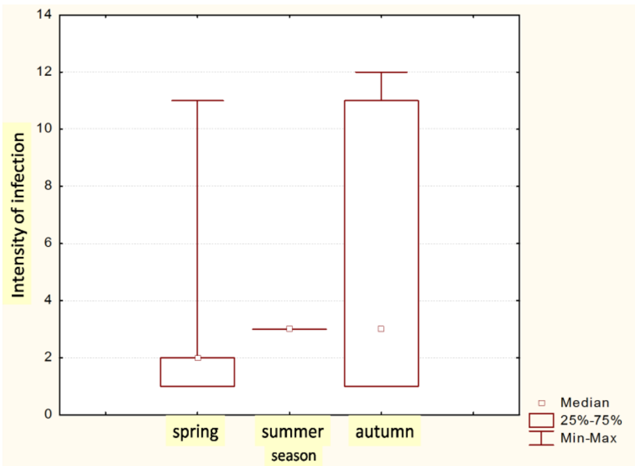

Figure 5. Graphic view of intensity of infection of Abramis brama

Table 3. Prevalence and intensity of infection by Ichthyophthirius multifiliis (T-total fish sample; I-number of infected individuals; P (%)-prevalence of infection; n/10-intensity of infection. Dark color presents the fishes which cannot be statistically analyzed because the sample was not large enough).

Species	T	I	P(%)	n/10
A. brama	35	19	54.28	1-10
A. sapa	17	12	70.58	1-10
A. alburnus	57	3	5.26	4-5
A. melas	4	-	-	-
A. nebulosus	15	-	-	-
A. aspius	1	-	-	-
B. barbus	37	2	5.40	2-4
B. bjoerkna	25	10	40	1-4
C. gibelio	86	13	15.11	1-9
Ch. nasus	48	3	6.25	1-7
C. carpio	4	1	-	3
E. lucius	12	4	-	1-7
L. cephalus	1	-	-	-
N.gymnotrachelus	21	1	4.76	3
P. fluviatilis	4	-	-	-
R. pigus virgo	7	-	-	-
S. lucioperca	6	-	-	-
S. glanis	8	1	-	2
T. thymallus	1	-	-	-
T. tinca	1	-	-	-
V. vimba	3	-	-	-
Z. zingel	7	-	-	-

The infection intensity analysis was performed in two species which were the most abundant samples, *Abramis brama* and *Carassius gibelio*. Statistically significant difference in the intensity of infection of *Abramis brama* was documented during the season (H = 7.99; p <0.05). It is evident that the intensity of infection was higher in the summer season. In the *Carassius gibelio* species, the incidence of seasonal infections was not statistically significantly varied (H = 2.86; p> 0.05). Higher values of intensity of infection were documented in summer. Figure 8. shows the graphic view of seasonal dynamic of infection of *Abramis brama*. It is evident that infection increases in summer but for other seasons of the year, the intensity of infection was equal (Figure 8.).

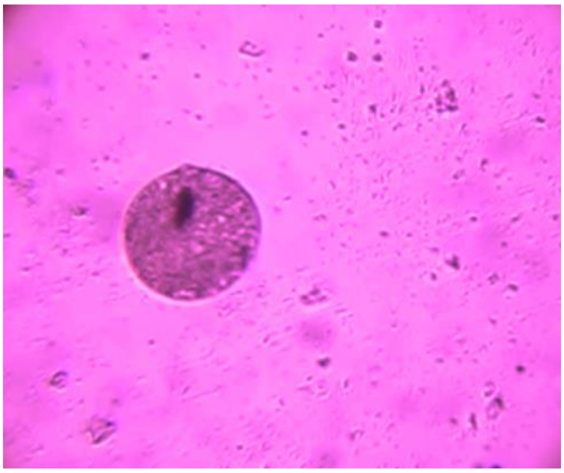

Figure 6. Ichthyophthirius multifiliis

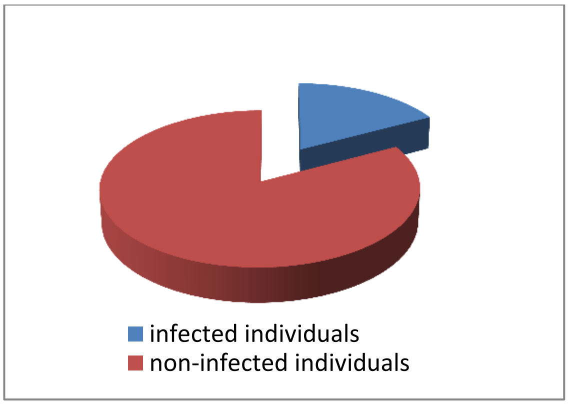

Figure 7. Prevalence of infection of the entire fish sample by Ichthiphthirius multifiliis

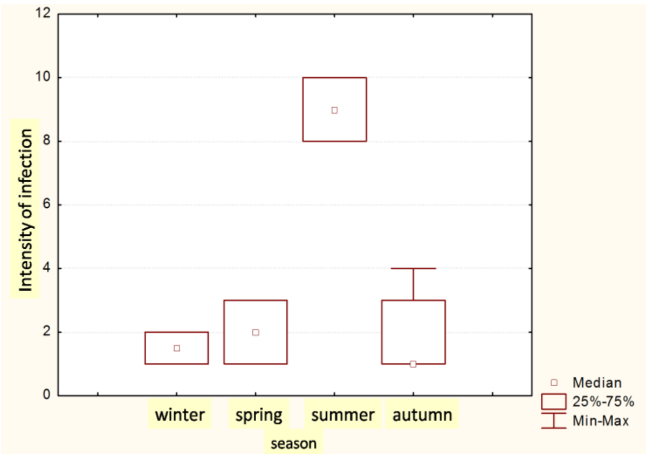

Figure 8. Seasonal dinamic of infection of Abramis brama by Ichthyophthirius mltifiliis

By analyzing the temperature and the intensity of infection, correlations were found between these variables in the *Abramis brama* type, which was also the most favorable sample for the analysis of these parameters. The infection intensity by *Ichthyophthirius multifiliis* was higher at the temperature of 15.3°C to 25°C (R = 0.409; p <0.05). In the study the signs of ichthiophthiriasis were also found, especially on the body of *Abramis brama* (Figure 9.).

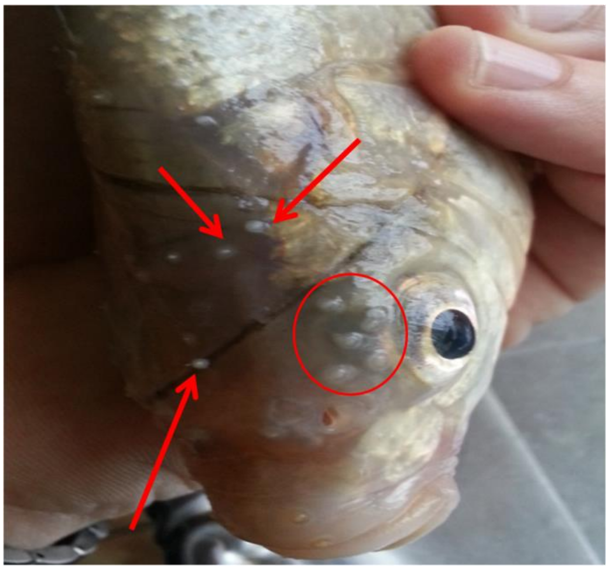

Figure 9. Ichthyophthiriasis in Abramis brama

4. Discussion

Based on our previous studies of parasites, as well as on the literature data, we assert their frequent presence on the skin during the whole year. Their almost cosmopolitan distribution, the specificity of the life cycle as well as a relative lack of specificity in terms of hosts, provide that they have to be widespread and represented in a large number of fish in the sample. The reason for this is often poorer water quality and the associated poorer fish condition, thus becoming the ideal substrate for ectoparasites from the Ciliat class.

In some similar studies, a very high level of fish ectoparasites was documented and these studies include two very important ciliat taxa: *Chilodonella cyprini* and *Ichthyopthirius multifiliis* [14,15,16]. These taxa were documented with a significant different prevalence of infection, which is in agreement with the current ectoparasitic study.

The species *Chilodonella cyprini* occurs with a relatively high extensity, i.e., prevalence of infections mainly on cyprinid fish. Although there is the evidence that this species can parasitize both on the skin and on the gills, it has been found only on the skin in this research. Bauer has classified this type of ectoparasite that prefers low temperatures and has proven that under experimental conditions the fastest propagation occurs at the temperatures between 5 and 10°C, while the temperatures over 20°C have a lethal impact on the parasite [17]. For that temperature condition the prevalence of infection was high during the spring and autumn in some species, although the correlation between temperature values and intensity of infection was low. Some later research has shown that the optimum for the breeding of this species is at quite higher temperatures of 12-18°C, which can be explained by climate changes, global warming and faster response of species to change self in different ecological factors [18]. Bream, as a host, was present in the samples throughout the year and its extensity and intensity of infection shows a typical double curve with spring and autumn peaks during the lower temperature period. The values of the intensity of infection with this species are relatively low and were in line with similar researches [19,20], where it was shown that the values rarely exceeded the rank of ten individuals in ten microscopic fields.

The parasitic species *Ichthyophthyrius multifiliis* shows greater number in the first half of the year. Although there is not much literature data on seasonal dynamics, some data speak about two types of ichthyophthiriasis, i.e. the disease that shows up in summer and lasts for 1-3 weeks and ends with the death of the fish, and the spring disease, which is more dangerous because it lasts all winter and infects more fishes [21]. Information about the optimum temperature for the development of this species is also different. According to some authors, the maximum intensity and extensity of infection occurs in spring months at water temperatures between 10°C and 15°C [20]. The research conducted by Bauer shows that this species is best multiplied at high water temperatures (20-25°C), while other authors found experimental conditions for parasitic death at temperatures above 25°C after ten hours [17, 22]. The same authors indicate the longest lifespan at temperatures between 16 and 22°C, which is consistent with our results suggesting that this temperature is the most optimum for the life cycle of this parasite [22].

In the current study the signs of ichthyophthiriasis were also found in the *Abramis brama* species. This is an indication for some important problems of fish health as some authors argue that ichtyophthiriasis can have death consequences [2,3,4]. For that reason, it is necessary to establish the permanent monitoring of aquatic ecosystems in BiH based on ecological and biological studies as well as on the studies of parasitical coactions and fish health.

The development of fishing management requires the usage of more tools: flow, water quality, habitat and biological tools [23]. Some authors from BiH argue that analysis of some physico-chemical parameters and bioindicators can show the real condition of hydro-ecosystems and could be used for assessment of water quality. For that reason it is very important to understand that fish condition and health have a very important impact on the economy. BiH is a very rich country in terms of biodiversity and power of ecosystems and river ecosystems are one part of that richness. For that purpose this study should be used for developing a program for fish protection and monitoring, such as in Croatia and Serbia, which means study of river ecosystems and parasitical coaction to find out something more about fish protection and to reveal the way how to make a better ecological condition. The appearance of ichthyophtiriasis as a very harmful fish disease should be an alarm for state agencies to develop the programs and projects which should include study, monitoring and publication as well as collaboration with other scientists. It is also very important to establish a state level organization or, institute for ecosystem conservation and protection in BiH with all the necessary study parts based on biological science.

5. Conclusion

In the sample of 400 fish individuals and 22 fish species, the parasitic taxa *Chilodonella cyprini* and *Ichthyophtirius multifiliis* were documented with statistically significant prevalence of infection in some fish hosts. The analysis of infection intensity showed that these two parasitic species are present throughout the year. In this study, ichthyophthiriasis as a very harmful fish disease was also recorded especially on the bream. Finally, the study gives recommendations on how to improve fish health and management through a better system of organization.

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