

The diversity of trematodes in fishes from the middle course of the river Sava (Bosnia and Herzegovina)

Raznolikost trematoda u ribama iz srednjeg toka rijeke Save (Bosna i Hercegovina)

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ABSTRACT

The prevalence and intensity of trematodes ectoparasites in freshwater fish are presented in this paper. Parasitology researches were conducted during 2017 in the middle course of the river Sava near Orašje. All results from this study were tested with the use of nonparametric tests (χ^2 , Kruskal-Wallis test and Spearman test of correlation) in the statistical package STATISTICA 7. The total of 400 sampled specimens of different fish species were parasitologically tested. The trematodes from the class Monogenea (*Dactylogyrus*, *Gyrodactylus* and *Eudiplozoon nipponicum*) and Digenea (*Posthodiplostomum cuticola*) were found on the skin and the gills of the fish. The genus *Dactylogyrus* was found on 27 specimens, or 6.75%. Intensity of the infection for the genus *Dactylogyrus* did not show any significant deviations during different seasons. Parasites from the genus *Gyrodactylus* were found on the skin and the gills of 40 infected specimens, dominantly found on the gills. The species *Eudiplozoon nipponicum* was found in low prevalence in the total ichthyo sample of 1.75%. The presence of the species *Posthodiplostomum cuticola* is established in 18 specimens and the prevalence of the infection in total sample was 4.5%.

Key words: The Sava River, biodiversity, trematodes, fish

INTRODUCTION - Uvod

Ichthyofauna researches of river Sava have shown that it is a typical cyprinid fish river flow (reference). Parasite researches of fish living in river Sava have shown an enormous biodiversity, still data regarding fish trematodes in our freshwater are scarce. Important contribution in study of trematodes in fish from our big rivers was provided by Čanković (1963) who discovered the presence of 17 species of digenetic trematodes.

Parasitological studies enable us evaluate the value of fish as nutritional products, and to identify the parasites that are dangerous to people and animals. Because of that, study of trematodes represents a special interest, because there are species of parasites, especially trematodes that can be transferred to humans (reference). Researches of fish parasites living in river Sava have shown the presence of trematode species from classes: Monogenea and Digenea (reference). In order to analyse the current state of diversity and ecological charac-

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teristics of fish populations of river Sava, it is necessary to determine the fish species diversity in river Sava, presence of parasites in researched fish species and the seasonal dynamic of parasite appearance.

MATERIAL AND METHODS – Materijal i metode

In order to determine the presence of trematodes in fish in the middle flow of river Sava, parasitological researches were conducted on 400 fish specimens that were collected in 2017. Sampling of fish was performed on the area of middle flow of river Sava 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). Full length of longitudinal profile of research was around 15 kilometers from West towards East.

In the research, trawling nets with eyelet diameter of 25 mm and 100x100 cm dimensions were used, as well as stationary nets with eyelet diameter of 50 mm, and dimensions up to 12 meters. The parasitological researches were performed on the spot, on fresh material, because vegetative forms of trematodes die very quickly after the death of a host. Researches were focused on study of skin, fins and gills of fish.

Field researches were performed by microscope (MC50 BAT and Karl Zeiss), by taking the mucus smears from body surface from caught fish specimens and by forming the native preparations on slide glass. Samples of gill filaments for determining of ectoparasite presence were also taken. After the skin and gill samples analysis, trematode infection intensity was stated and their taxonomic affiliation was determined. Determination of trematodes found on the skin and gills was performed by using the standard methods for determination according to the literature (Bychovskaya-Pavlovskaya et al. 1962; Fijan, 2006). After the sampling, part of material was preserved in ice and transported to laboratory for further analysis (Faculty of Biology in Belgrade, Laboratory for Zoology at Faculty of Science in Tuzla). In the laboratory samples were conserved in 70% ethanol solution. The data of the water quality of the Sava river were taken from the agency for water management of Bosnia and Herzegovina. The data were analysed by using non-parametric statistical test (hi-square test, Kruskal-Wallis test and Spearman correlation test) of statistical software STATISTICA 7.

RESULTS AND DISCUSSION – Rezultati i diskusija

Ichthyoparasitological researches of middle flow of river Sava were performed during four seasons in 2017. Researches were performed on 400 specimens of different fish species. According to taxonomic affiliation, most of sampled fish (13) were of Cyprinidae family. Other species participate less in the sample. Three species are from Percidae family, two are from Ameiuridae family and other species in the sample are: Esocidae, Gobiidae, Siluridae and Thymallidae families with one species each. Research has proven presence of ectoparasites from Monogenea and Digenea classes from Trematoda group in the researched specimens of fish.

In Monogenea class, presence of parasites from *Dactylogyrus*, *Gyrodactylus* genera and *Eudiplozoon nipponicum* species was proven, while from Digenea class, *Posthodiplostomum cuticola* species was determined. These parasites, which were found on skin and gills, can cause negative consequences to fish health, such as increased mucus production on skin and gills, which can cause serious metabolic problems of the host (Fijan, 2006; Skenderović, 2015).

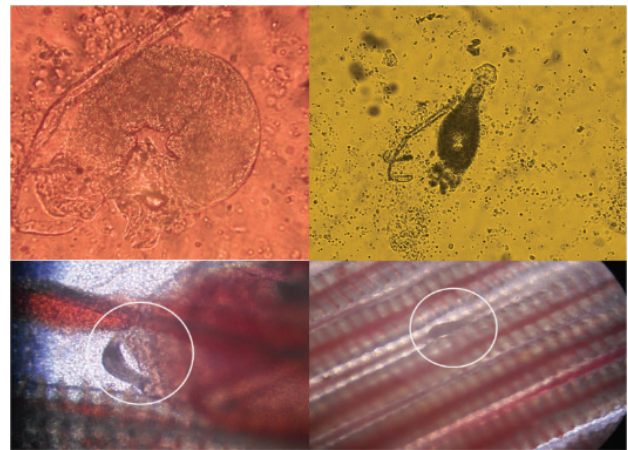


Figure 1. *Dactylogyrus* sp.

Slika 1. *Dactylogyrus* sp.

From total of 400 researched different species fish specimens, *Dactylogyrus* genus parasites were determined on bodies of 27 specimens of fish, which relates to 6.75% of ichthyo sample, whereby it is not possible to determine that fish population of researched region are highly infected by parasites from this genus. (Figure 1.). By parasitological research of gills and skin of fish, *Dactylogyrus* genus parasites were found in following fish species: *Abramis brama*, *Ballerus sapa*, *Barbus barbus*, *Blicca bjoerkna*, *Carassius gibelio*, *Cyprinus carpio*, *Silurus glanis*, *Rutilus virgo* and *Vimba vimba*.

A representation of prevalence and infection intensity of sampled fish specimens which had the determined parasites (Table 1). In analysis of *Abramis brama* species, parasite was found on skin and gills in same ratio, as well as in species *Ballerus sapa*. Infection analysis in common barbel and white bream shows parasite infection on the gills. On prussian carp, parasite occurs on skin and gills in the same ratio, while on the infected specimens of common carp parasite was found only on skin. In infected catfish specimens parasite was found on the skin. On specimens of heckel and vimba bream, parasite was found only on the gills.

Table 1. Prevalence and infection intensity by species from *Dactylogyrus* genus in total ichthyosample (*species is excepted from statistical analysis due to low sample number)

Tabela 1. Prevalencija i intenzitet infekcije vrsta iz roda *Dactylogyrus* u ukupnom uzorku (*vrsta je izuzeta od statističke analize zbog malog broja uzoraka)

No:	Fish species	Total	Infected	Prevalence %	Infection intensity
1.	<i>Abramis brama</i>	35	8	22,85	1-7
2.	<i>Ballerus sapa</i>	17	2	11,76	5-12
3.	<i>Barbus barbus</i>	37	1	2,7	3
4.	<i>Blicca bjoerkna</i>	25	2	8	1-3
5.	<i>Carassius gibelio</i>	86	8	9,3	1-5
6.	<i>Cyprinus carpio</i> *	4*	1*	25*	2
7.	<i>Silurus glanis</i> *	8*	2*	25*	7-10
8.	<i>Rutilus virgo</i> *	7*	2*	28,5*	2
9.	<i>Vimba vimba</i> *	3*	1*	33,3*	1

By analysing the infection prevalence, it was determined that prevalence was uneven, meaning that infection of *Abramis brama* species was statistically much higher when compared to other values of infection prevalence ($\chi^2=20,30$; $p<0,05$) (Picture 2).

Infection intensity in season periods did not show statistically significant deviations. As analysis models, two statistically interesting species were chosen: *Abramis brama* and *Carassius gibelio* because these two species had the highest number of infected specimens. In analysis of infection intensity of species *Abramis brama* no statistically significant deviations were determined in autumn and spring periods when the infection was determined ($H=1,45$; $p>0,05$) (Picture 2). In analysis of

species *Carassius gibelio*, results have also shown that there are no differences in infection intensity in seasons when infection was determined ($H=1,14$; $p>0,05$).

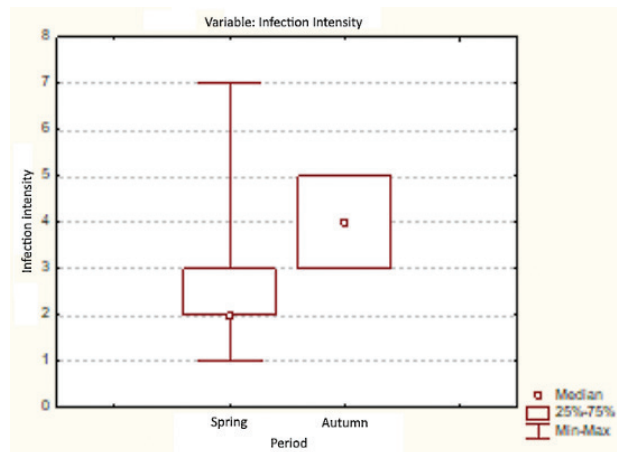


Figure 2. Infection intensity by species from genus *Dactylogyrus* over the season of research for *Abramis brama* species

Slika 2. Intenzitet infekcije vrsta roda *Dactylogyrus* tokom sezone istraživanja vrsta *Abramis brama*

In analysis of infection intensity and water temperature for species *Abramis brama* no statistically significant correlation was found between these parameters ($R=0,456$; $p>0,05$). Infection was determined on temperatures of 17,2°C and 18°C. In analysis of infection intensity and temperature for species *Carassius gibelio* negative, statistically insignificant correlation was determined ($R=-0,362$; $p>0,05$). Infection was determined in a very wide temperature values spectrum, from 10,3°C to 27,1°C.

Species from *Gyrodactylus* genus, Nordmann, 1832, represent parasites form Monogenea class which are common causers of fish diseases called gyrodactylosis. Gyrodactylosis is a gill and skin disease. These species reproduce quickly and they can have up to 2000 offspring in a single month. Bad environmental conditions in the hydro ecosystem serve to the development of these parasites, especially in fish spawn but also in adult fish (Fijan, 2006).

Out of total 400 sampled fish, *Gyrodactylus* parasites were determined in 40 fish specimens, whereby the prevalence of infection of total sample is 10%, which means that significantly higher number of specimens was not infected by parasites from this genus.

Gyrodactylus parasites are determined of following fish species: *Abramis brama*, *Ballerus sapa*, *Alburnus alburnus*, *Barbus barbus*, *Blicca bjoerkna*, *Carassius gibelio*, *Chondrostoma nasus*, *Esox lucius*, *Rutilus virgo*, *Sander lucioperca* and *Vimba vimba*. Parasites were found on skin and gills of infected fish specimens, where the higher number is found on gills.



Figure 3. *Gyrodactylus* sp.

Slika 3. *Gyrodactylus* sp.

Table 2. Prevalence and infection intensity by *Gyrodactylus* genus parasites in total ichthyosample (*species is excepted from statistical analysis due to low sample number)

Tabela 2. Prevalencija i intenzitet infekcije vrsta iz roda *Gyrodactylus* u ukupnom uzorku (*vrsta je izuzeta od statističke analize zbog malog broja uzoraka)

No:	Fish species	Total	Infected	Prevalence %	Infection intensity
1.	<i>Abramis brama</i>	35	5	14,28	1-12
2.	<i>Ballerus sapa</i>	17	2	11,76	4-5
3.	<i>Alburnus alburnus</i>	57	3	5,26	1-7
4.	<i>Barbus barbus</i>	37	4	10,81	1-13
5.	<i>Blicca bjoerkna</i>	25	4	16	1-5
6.	<i>Carassius gibelio</i>	86	13	15,11	1-5
7.	<i>Chondrostoma nasus</i>	48	4	8,33	2-6
8.	<i>Esox lucius</i>	12*	2*	16,66*	2
9.	<i>Rutilus virgo*</i>	7*	1*	14,28*	1
10.	<i>Sander lucioperca</i>	6*	1*	16,66*	2
11.	<i>Vimba vimba*</i>	3*	1*	33,33*	1

Based on microscopical analysis of skin and gills of fish, prevalence and infection intensity is shown for each infected species (Table 2). By infection prevalence analysis of optimal samples for statistical testing, equal infection distribution in all tested species was determined ($\chi^2=7,75$; $p>0,05$).

In analysis of infection intensity according to research season, no statistical significance was proven for *Abramis brama* species, whereby it can be claimed that infection is present in same intensity in spring and autumn. ($H=0,52$; $p>0,05$) (Figure 4).

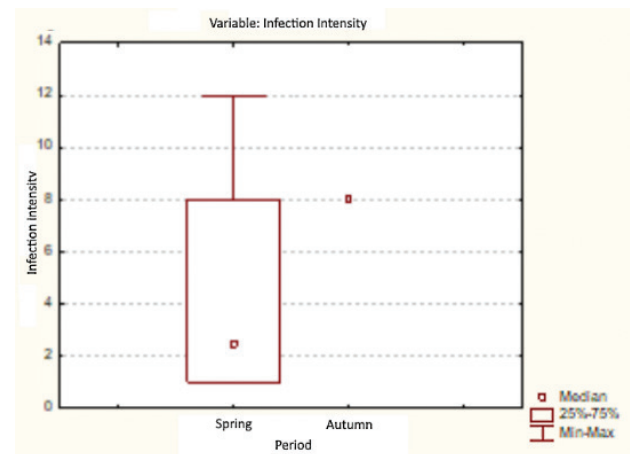


Figure 4. Infection intensity by parasites from *Gyrodactylus* genus for *Abramis brama* species for periods when the infection was determined

Slika 4. Intenzitet infekcije parazitima roda *Gyrodactylus* za vrstu *Abramis brama* u periodima kad je infekcija determinisana

Similar results were determined for infected specimens of *Carassius gibelio* species. Equal distribution of infection intensity was determined in periods when the infection was determined. ($H=2,75$; $p>0,05$) (Figure 5). There

was no statistically significant correlation between the mentioned ecological factors and infection intensity parameters ($R=0,230$; $p>0,05$). Infection was determined in temperature range from $10,3^{\circ}\text{C}$ to $27,1^{\circ}\text{C}$, which represents a wide range of variation in temperature. Infection of *Abramis brama* species, was determined in temperature range from $17,2^{\circ}\text{C}$ to 18°C .

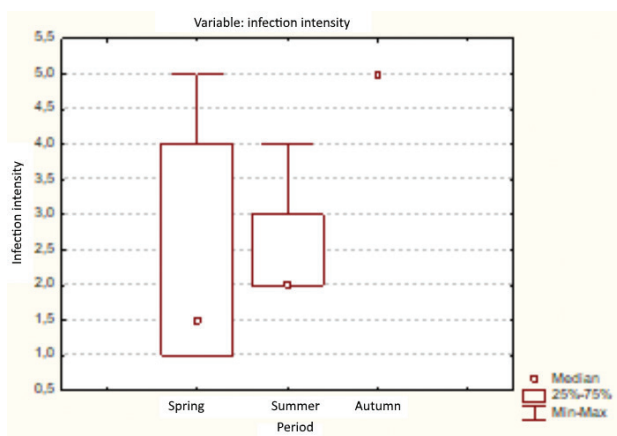


Figure 5. Infection intensity by parasites from *Gyrodactylus* for *Carassius gibelio* species for periods when infection was determined

Slika 5. Intenzitet infekcije parazitima roda *Gyrodactylus* za vrstu *Carassius gibelio* u periodima kad je infekcija determinisana

Species from *Gyrodactylus* and *Dactylogyrus* genera occur with with highest frequency in the researched fish, these parasites are interesting because of their vivipary and polyembryony, but the factor that triggers the sudden appearance of these parasites is still not known. (Scholz, 1999; Bakke et al. 1990; Lund and Heggebergerget, 1992). *Eudiplozoon nipponicum* (Goto, 1891) is a parasite species from Monogenea class. It causes fish disease called Diplozoonosis.

Cycle of life of these parasites happens in three phases; free swimming organism phase, the phase where parasite attaches itself to gills of the host fish, and the phase where male and female merge into one individual creating a special structure called "living X".

This species is widely spread freshwater fish parasite, which damages gill apparatus by sucking blood, which consequentially causes fish anemia (Fijan, 2006). *Eudiplozoon nipponicum* species was determined in a very low prevalence in total ichthyo sample, 1.75%. This species was found in following host fish species: *Abramis brama*, *Barbus barbus*, *Ameiurus nebulosus* and *Blicca bjoerkna*. Discovery of this species is significant because it is the first time that this species was found in river Sava. (Nedić and affiliates, 2016).

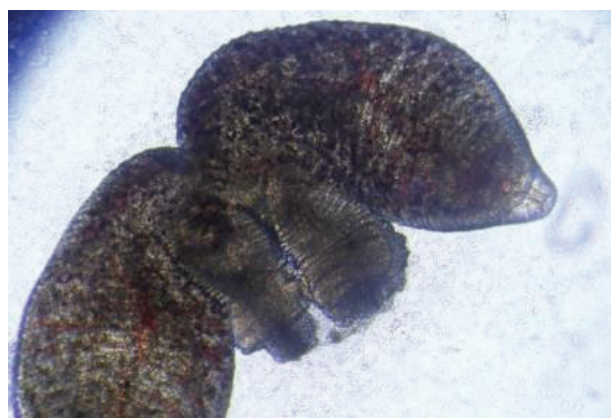


Figure 6. *Eudiplozoon nipponicum*

Slika 6. *Eudiplozoon nipponicum*

Table 3. Prevalence and infection intensity by *Eudiplozoon nipponicum* species in total ichthyo sample (*species is excepted from statistical analysis due to low sample number)

Table 3. Prevalencija i intenzitet infekcije vrste *Eudiplozoon nipponicum* u ukupnom uzorku (*vrsta je izuzeta od statističke analize zbog malog broja uzoraka)

No:	Species	Sampled	Infected	Prevalence %	Infection intensity
1.	<i>Abramis brama</i>	35	3	8,57	1-2
2.	<i>A. nebulosus</i> *	5*	1*	6,66*	1
3.	<i>Barbus barbus</i>	37	1	2,70	2
4.	<i>Blicca bjoerkna</i>	25	2	8	1-2

By analysing the infection prevalence, no statistical significance was determined ($\chi^2=3,26$; $p>0,05$). Infection intensity analysis, as well as the analysis of temperature relation as an ecological factor was not performed due to very low prevalence and intensity of infection.

Posthodiplostomum cuticola, Nordmann, 1832 is a fluke species systemised in Digenea class. It causes disease, scientifically marked as posthodiplostomosis. Transitional hosts in its life cycle are snails from *Planorbis* and *Anisus* genera, and other transitional hosts are Cyprinidae (Fijan, 2006).

In total ichthyo sample, presence of *Posthodiplostomum cuticola* species was determined in 18 specimens, with prevalence of 4.5%, whereby the significance between infected and noninfected specimens was determined. By

microscopical analysis, presence of parasites was determined predominantly on gills of infected specimens and in lesser quantity on skin of following species: *Abramis brama*, *Ballerus sapa*, *Barbus barbus*, *Blicca bjoerkna* and *Carassius gibelio*. The prevalence and infection intensity of infected species by *Posthodiplostomum cuticola* is shown in Table 4.

Table 4. Prevalence and infection intensity by *Posthodiplostomum cuticola* species in total ichthyo sample

Table 4. Prevalencija i intenzitet infekcije vrste *Posthodiplostomum cuticola* u ukupnom uzorku

No:	Species	Sampled	Infected	Prevalence %	Infection intensity
1.	<i>Abramis brama</i>	35	5	14,28	2-4
2.	<i>Ballerus sapa</i>	17	1	5,88	2
3.	<i>Barbus barbus</i>	37	3	8,10	1-3
4.	<i>Blicca bjoerkna</i>	25	2	8	1-4
5.	<i>Carassius gibelio</i>	86	7	8,13	1-3

By analysing the infection prevalence, statistically even distribution of infection in all infected species was determined ($\chi^2=4,52$; $p>0,05$). By analysing the infection according to season periods in *Carassius gibelio* species, there was no statistical significance ($H=1,80$; $p>0,05$) although it can be noted that infection in this species occurs on higher temperature, between 10,3°C and 27,1°C.

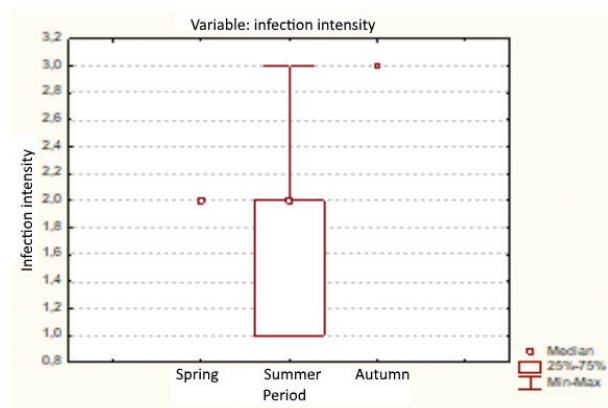


Figure 7. Seasonal dynamic of *Posthodiplostomum cuticola* infection in *Carassius gibelio* species

Slika 7. Sezonska dinamika infekcije *Posthodiplostomum cuticola* na vrsti *Carassius gibelio*

Highest number of infected specimens was determined during the summer season. Ichthyoparasitological research of Modrac hydroaccumulation have shown that *Posthodiplostomum cuticola* is represented in the same percentage (11.42%) in researched fish during summer and spring (Skenderović et al. 2012).

By analysing the infection intensity and temperature as a ecological factor, very low negative correlation was determined which was not close to statistical significance ($R=-0,148$; $p>0,05$), but as it was mentioned earlier, infection was determined on higher water temperatures where it is notable that given parasite prefers higher temperatures in wider ecological valence. Highest prevalence was determined in *Abramis brama* species, more than 15%. Some researches have shown that infection prevalence values can be over 60% in some species in hydro accumulations (Skenderović, 2015). Earlier researches have shown that infection by this parasite is noted with different prevalence oriented on Cyprinidae species (Zrnčić et al. 2009). According to data obtained by Skenderović and affiliates (2011) *Posthodiplostomum cuticola* is represented in 63.3% in researched fish in river Spreča.

CONCLUSION - Zaključak

During the ichthyoparasitological research of fish of middle flow of river Sava, presence of parasites from Trematoda group was determined. Four trematode species, from Monogenea class, presence parasites from *Dactylogyrus*, *Gyrodactylus* genera and *Eudiplozoon nipponicum* was determined, while from Digenea class, *Posthodiplostomum cuticola* was determined. Researches have shown that species from *Gyrodactylus* and *Dactylogyrus* genera appear with the highest frequency in studied fish. *Eudiplozoon nipponicum* was determined in a very low prevalence in total ichthyo sample, 1.75%. By using microscopical analysis, dominant presence of *Posthodiplostomum cuticola* species was determined on gills of infected specimens. Highest number of infected specimens was determined during the summer for the researched period.

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SAŽETAK

U ovom radu prikazana je prevalencija i intenzitet ektoparazita trematoda u slatkovodnoj ribi. Parazitološka istraživanja provedena su tokom 2017. godine u srednjem toku rijeke Save kod Orašja. Svi rezultati ove studije testirani su upotrebom neparametrijskih testova (χ^2 , Kruskal-Wallisov test i Spearmanov test korelacije) u statističkom paketu STATISTICA 7. Ukupno je uzorkovano 400 uzoraka različitih vrsta riba parazitološki testirano. Trematodi iz klase Monogenea (*Dactylogyrus*, *Gyrodactylus* i *Eudiplozoon nipponicum*) i Digenea (*Posthodiplostomum cuticola*) pronađeni su na koži i škragama riba. Rod *Dactylogyrus* pronađen je na 27 primjeraka ili 6,75%. Intenzitet infekcije za rod *Dactylogyrus* nije pokazao značajnija odstupanja tokom različitih sezona. Paraziti iz roda *Gyrodactylus* pronađeni su na koži i škragama 40 zaraženih primjeraka, dominantno na škragama. Vrsta *Eudiplozoon nipponicum* pronađena je u maloj prevalenciji u ukupnom uzorku riba od 1,75%. Prisustvo vrste *Posthodiplostomum cuticola* utvrđeno je u 18 primjeraka, a prevalencija infekcije u ukupnom uzorku bila je 4,5%.