

RESEARCH ARTICLE

Parasitic Copepods on Common Carp (*Cyprinus carpio*, L. 1758) from Gradche Reservoir (Macedonia)

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ABSTRACT

During the parasitological examinations of the common carp (*Cyprinus carpio*, L. 1758) from the Gradche Reservoir (Macedonia), a total of 126 fish samples were examined, from which parasite infestation was determined in 87 fish (69.05%). In this research, the following parasitic copepods were identified: *Ergasilus sieboldi*, *Ergasilus briani*, and *Lernaea cyprinacea*. *Ergasilus sieboldi* was found on gills of common carp, with a prevalence of 1.461%, while the mean intensity was 2.357. *Ergasilus briani* was found on fins of common carp, with a prevalence of 1.879%, while the mean intensity was 38.274. *Lernaea cyprinacea* was found on the fins of common carp, with a prevalence of 0.552%, while mean intensity was 2,000. Our findings of *Ergasilus sieboldi*, *Ergasilus briani*, and *Lernaea cyprinacea* in common carp are considered as first records for Macedonia. At the same time, common carp is a new host for *Ergasilus briani* in Macedonian waters.

1. Introduction

There is a growing interest in fisheries and aquaculture development around the world because of the increasing human needs for food of animal origin, as well as the fact that over 70% of the globe is covered with water. Fish is the subject of much research, not only due to its great importance in nutrition but also as a means of improving the economic situation in a country ^[1]. The degree of activity of ectoparasites and endoparasites of the body

surface and/or inside the fish body depend on the degree of water purity in combination with other environmental factors. Poor hygienic aquaculture facilities' conditions have a major contribution to the development of the parasitic disease ^[2].

There is a close relationship between parasitic communities and the level of water pollution. In polluted environments, the degree of parasites' prevalence and intensity can be an indicator of environmental quality. Because the level of water contamination can directly

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or indirectly affect the aquatic ectoparasites through the action of their intermediate host, ectoparasites that are directly exposed to water may be more sensitive to contaminants, thereby reducing their rates of survival and reproduction. The presence of ectoparasites of genders *Trichodina*, *Argulus*, and some *Dactylogyrus* mainly arises as a result of poor water quality, poor zoo - hygienic conditions, increased water temperature, or excessive settlement of fish in ponds^[3]. The parasites may be present on the skin, fins, gills, and internal organs of fish without major consequences for the host. However, under the influence of certain conditions, such as changes in aquatic parameters, stress and the introduction of exotic pathogens may increase the host acceptability to parasites and cause an imbalance of the system host/parasite/environment.

Copepods have arthropods body, composed of several segments. The segments are grouped into three parts: head, chest, and abdomen. Each segment, except the last, carries one pair of limbs. They have accessories on the head: antennae, maxilla, and mandible. There is a maxilla and 4-5 pairs of thoracic scissors on the chest. The last chest segment has a sex opening, and in females, this is where the two ovarian sacs begin. There are more than 1,500 species of copepods parasitize in fish^[4]. Gills parasites of the genera *Ergasilus* and *Lamproglana* in many high infestations can cause great damage to the fish population in fish ponds and cage farming systems. Copepods cause destruction and necrosis of tissues, feeding on the epithelium and blood of fish and causing injury and compressions of organs (skin, gills, muscles, eyes, kidneys, liver, intestines, skeleton, etc.)^[5]. Due to this, the normal function of organs, anemia, exhaustion, and weight loss occurs. Especially copepods cause big damage in young fish. A variety of different parasitic copepods can cause external infestations of freshwater and marine fish. Some members of the group are commonly referred to as fish lice. They are frequently found on the body, around the mouth, and on the gills. Parasitized fish may act lethargic. Mechanical abrasion due to the attachment and/or feeding by the copepods is common resulting in frayed fins, gill hyperplasia, and patchy epidermal damage and necrosis. Infections with secondary pathogens often occur. Most of these organisms have a direct life cycle involving several free-living and larval stages. Transmission is through contact with an infective free-swimming stage of the organism in the water column. The infective stage attaches to the fish where it goes through several larval stages before becoming

an adult.

2. Materials and Methods

This study aimed to determine the parasite fauna in common carp (*Cyprinus carpio*, L. 1758) from the Gradche Reservoir (Macedonia) (Figure 1). The Gradche Reservoir is located on Kochanska River near the village Dolno Gradche, 6 km north of Kochani. The dam is reinforced concrete, with a height of 32 m, length of the crown of 150 m, and elevation of 467 m above sea level. The Gradche Reservoir is 3.5 km long, 0.2 km wide, with a maximum depth of 29 m. The area of the Reservoir is 0.19 km², with a volume of 2.4 million m³ of water used for the water supply of the population of Kochani and irrigation of about 5761 ha of arable land in Kochansko Pole.



Figure 1. Gradche Reservoir
(web site of Kochani Municipality)

This parasitological study was carried out by seasons, during 2018. A total of 126 specimens of common carp from Gradche reservoir were examined for parasitological investigations. Only fresh fish were subjected to routine identification, dissection, and observation. Cleaned parasites were separated, put in appropriate fixatives, and prepared for determination using determining techniques of staining and clearing^[6-9]. Parasites on native smears were observed under a light microscope at the magnification of $\times 200$ and $\times 400$. The parasite specimens were identified using the reference keys^[7,10,11]. During the examinations at the Department for Fish Diseases in the Institute of Hydrobiology in Ohrid (Macedonia), Zeiss stereomicroscopes (Stemi DV4), as well as a light Reichart microscope, were used. Classical epidemiological variables (prevalence and mean intensity) were calculated^[12].

Data of prevalence and mean intensity (by seasons) with determined parasite species are given in Table 1.

Table 1. Prevalence (P) and mean intensity (I) with parasitic copepods in common carp (*Cyprinus carpio*, L. 1758) from Gradche Reservoir, by season

Parasite species	Spring		Summer		Autumn		Winter	
	I	P (%)	I	P (%)	I	P (%)	I	P (%)
<i>Ergasilus sieboldi</i>	2.889	1.794	/	/	/	/	1.400	0.441
<i>Ergasilus briani</i>	/	/	/	/	38.274	1.879	/	/
<i>Lernaea cyprinaceae</i>	/	/	/	/	/	/	2.000	0.552

3. Results and Discussion

During the parasitological examinations of the common carp from the Gradche Reservoir (Macedonia), a total of 126 fish samples were examined, from which parasite infestation was determined in 87 fish (69.05%). In this research, the following parasitic copepods were identified: *Ergasilus sieboldi*, *Ergasilus briani*, and *Lernaea cyprinaceae*.

Ergasilus sieboldi was found on gills of 14 specimens of common carp in winter, with a prevalence of 1.461%, while the mean intensity was 2.357. The prevalence with *Ergasilus sieboldi* by seasons was as follows: spring is 1.794% and winter is 0.441%, while the mean intensity: spring is 2.889 and winter is 1.400.

The Ergasilidae family is one of the largest families of Copepods that parasitize fish. Only adult females are parasitic, with a larval stage, while adult males are plankton^[13]. *Ergasilus* is the largest genus of the Ergasilidae family that includes more than 180 species^[14]. *Ergasilus sieboldi* is an ectoparasite that lives in large numbers on the gills of freshwater fish, causing a disease called ergasillosis. *Ergasilus sieboldi* parasitizes the gill filaments of fish from families: Cyprinidae, Salmonidae, Percidae, Siluridae, and others and it is very common in marine and freshwaters throughout Europe and Asia^[11].

The body of *Ergasilus sieboldi* has a pear-shaped form, with a length of 1.1 mm - 1.5 mm and width 0.4 mm - 0.7 mm. There is a pair of hooks on the head with which it is fastened to the gill filaments (Figure 2). Females have two elongated eggs bags on the body back, of approximately the same length as the body, which is especially pronounced in the summer period and contains 50 eggs - 140 eggs.

Larvae emerge from the eggs that immediately attack other fish, and reach the adult stage in 2 - 8 weeks^[15]. After fertilization, males die. During one year, under favorable conditions, several generations of shrimp are developing. This is especially pronounced in the summer period.

The females usually find both infest their hosts after mating and are then susceptible to metamorphosis in which adult forms change the shape of the body and increase before egg production begins^[16].

The presence of this copepod in fish gills causes mechanical tissue irritation, so in the beginning, there is epithelial hyperplasia and fusion of gills, and later lead to inflammation and necrotic changes. Severely injured fish are anemic, have pale gills, and weaken. The parasite constricts blood vessels in the gills, causing difficulty breathing. Deaths are quite common in infected offspring. These parasites are more common in lakes, where the disease can reduce natural fish production by up to 50%^[17].

According to data from the previous parasitological research in Macedonia, *Ergasilus sieboldi* was identified in *Alburnus albidus alborella* from Lake Ohrid^[8]. *Ergasilus sieboldi* was identified in the following fish species of natural lakes in Macedonia: *Leuciscus cephalus albus*, *Barbus meridionalis petenyi*, *Alburnus albidus alborella*, *Scardinius erythrophthalmus*, *Rutilus rubilio*, *Pachychilon pictus*, *Barbus cyclolepis prespensis*, *Alburnus alburnus belvica*, *Alburnoides bipunctatus prespensis* and *Tinca tinca*^[17-19]. *Ergasilus sieboldi* was found in common carp from Tikvesh Reservoir^[20]. According to literary reviews from surrounding countries and the world, the presence of *Ergasilus sieboldi* in common carp was determined in waters in Serbia^[21], in fishponds in the Czech Republic^[22], in fishponds in Iraq^[23], and in fishponds in Iran^[24]. In Turkey, data on the appearance of *Ergasilus sieboldi* in common carp were published from Lake Iznik^[25,26], from the Dalyan Lagoon^[27], and from the Lake Karacaören Dam^[28].



Figure 2. *Ergasilus sieboldi* (whole parasite) on gills in common carp (*Cyprinus carpio*) from Gradche reservoir (original)

Ergasilus briani was found on fins of 18 specimens of common carp in autumn, with a prevalence of 1.879%, while the mean intensity was 38.274. Our findings of *Ergasilus briani* in common carp are considered as the first record for Macedonia. At the same time, common carp is a new host for *Ergasilus briani* in Macedonian waters.

The length of *Ergasilus briani* ranges from 0.76 mm - 0.98 mm, and the width from 0.22 mm - 0.24 mm (Figure 3). The segment of the V pair of legs sometimes is not separated from the sex segment. Antenna II has short members, and the penultimate one is wide, so the length exceeds the width by about 2.5 times. The last member is a strong curved. There is a thorn on the basal limb, which is usually absent in other species. On the extremity of the exopodite, on an IV pair of legs, there are 4 hairs (others species have 5). Egg bags do not exceed the length of the copepod.

This widespread species differs from the companion species *Ergasilus sieboldi* in the shorter last member (claw) of the antenna II, in the wider and shorter limbs, the absence of hairs on the abdominal segments, and the micro-location of the gills. *Ergasilus briani* is usually found among gill filaments, compared with *Ergasilus sieboldi* that usually parasitizes on the outer surface of the gills.

The life cycle of *Ergasilus briani* includes several free-swimming stages before the adult female becomes a parasite. These larval stages can live for several weeks in water and feed on algae. Females – parasites live approximately one year and overwinter on fish. The life cycle of *Ergasilus briani* is temperature-dependent, with reproduction beginning in spring at a temperature of about 8 °C, continuing until late autumn, and ending in winter.

Ergasilus briani is found on the gills of cyprinid fish, but may also be found in other fish species, often together with *Ergasilus sieboldi*. Its life cycle covers multiple stages, but only adult females parasitize. This parasite is attached between the gill filaments of its host, using both special antennas that serve this purpose.

The presence of a large number of parasites of this species can cause serious gill damage leading to respiratory disorders and normal gill function, resulting in hyperplasia and necrosis. Infested fish become less tolerant of changes in the environment. It leads to poor condition, weight loss, and in the offspring even to mortality. The insertion of the antennae deep into the gill tissue also can cause blood vessels to constrict and rupture.

According to the literary data, the infestation with *Ergasilus briani* is seasonal and generally occurs during the summer or late autumn, which is in correlation with

our findings on this parasite in the autumn. *Ergasilus briani* attacks the smaller fish, with a body length of less than 10 cm. The transmission of *Ergasilus briani* can occur through infected water, plants, or fishing equipment.

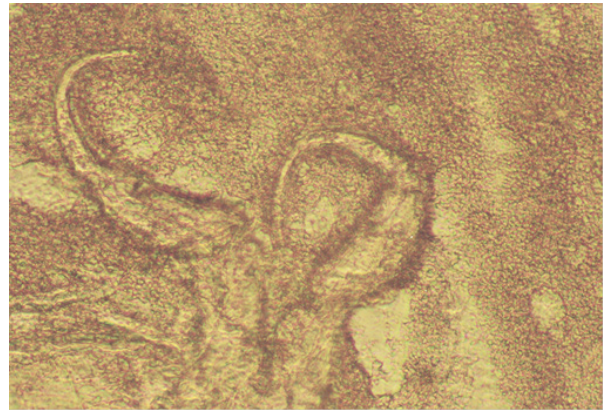


Figure 3. *Ergasilus briani* (hooks) on gills in common carp (*Cyprinus carpio*) from Gradche reservoir × 200 (original)

Lernaea cyprinacea was found on the fins of 5 specimens of common carp in winter, with a prevalence of 0.552%, while the mean intensity was 2.000. Our findings of *Lernaea cyprinacea* in common carp are considered as the first record for the Gradche reservoir.

The female's body reaches a length of 9 mm - 21 mm, while the male is smaller and moves up to 0.7 mm. There are four so-called cephalic horns on the head, of which the anterior two are cylindrical and the other two are T-shaped. The parasite is fixed to the muscles of the host with their help. It can be found on the surface of the whole fish body, but most often parasitizes on the fins and around the oral cavity.

Parasitic copepods of freshwater fish, cause a disease called lerneosis. About 110 species of parasites are described, from 14 different genera belonging to the family Lernaeidae^[13]. The genus *Lernaea* includes more than 40 parasite species. *Lernaea cyprinacea* is the most common species, found in waters across North America, Europe, Asia, South Africa, and Eastern Australia^[29]. *Lernaea cyprinacea* is a cosmopolitan copepod parasite in many species of freshwater fish^[30]. This parasite has a wide range of hosts and is registered in more than 100 fish species, from 25 families and 10 orders. *Lernaea cyprinacea* has been identified in more than 45 cyprinid fish species, which in Europe are major hosts of this parasite^[29].

The life cycle of *Lernaea cyprinacea* consists of nine stages, including three free-floating stages of nauplius, five copepod stages, and one stage of the adult form. As soon as in the body of the host fish are formed male

and female adult forms, the male dies and the female metamorphoses.

The front of the body of the metamorphosed adult female attaches to the host tissue, where it lays eggs, while the rest of the body is released into the water. Free-swimming larval spawns hatch from the eggs, which are about four days pass into infectious copepod larvae, which are usually attached to the gills of the host. Depending on the temperature, for about one week the copepods turn into an adult form, with optimal development of T from 28 °C - 36 °C and poor development of T below 20 °C. Adult males die within 24 hours, and the females remain attached to the same host or swim in search of a new host ^[29].

Infection with these parasites can cause serious pathological effects on their host - fish. The most common histopathological changes in infected adult cyprinids are manifested by severe degeneration and necrosis of the skin and muscles. Extensive edema, hemorrhage, leucocyte infiltrations, and melano-macrophages occur on the dermis and hypodermis. One of the consequences is damage and necrosis of the gill epithelium, while adult females usually cause hemorrhage, muscle necrosis, and severe inflammation, which are the entry point for secondary bacterial and fungal infections ^[31]. The larval stage usually infests the gills of the host, while the main pathogenic effect is caused by adults females, which use their claws to penetrate deep into the skin and body muscles causing sores, blood clots, and a decline in the scales. In severe cases, changes are seen throughout the skin, and the parasite can penetrate deep into the muscles and even into the body cavity. Fish weaken, and deaths occur because of anemia and secondary infections with bacteria and mycoses. Once lernosis occurs, it is very difficult to eradicate ^[13].

According to the data from previous parasitological research in Macedonia, the presence of *Lernaea cyprinacea* was first established in common carp from the fish farm Zabeni – Bitola ^[32]. Also, the presence of *Lernaea cyprinacea* was determined in common carp in waters in Macedonia ^[18]. According to the literary reviews from surrounding countries and the world, data on the presence of *Lernaea cyprinacea* in common carp has been published in waters in Serbia ^[21], in fishponds in Romania ^[33], in rivers in Spain ^[34], in waters in Mozambique ^[35], in fishponds in Iran ^[36,37], in fishponds in Iraq ^[23,38], in fishponds in Egypt ^[39] and in waters in southern California ^[40].

Lernaea cyprinacea causes high mortality in freshwater fish. The pathological effect of *Lernaea cyprinacea* is greater in smaller fish because the attachment organs penetrate much deeper into the fish body, often causing damage to internal organs ^[29]. This is in correlation with

our research, where we concluded that adult forms of *Lernaea cyprinacea* are particularly harmful to young fish due to their relatively large size and the method of attachment and feeding. The presence of *Lernaea cyprinacea* complicates consuming food and slowing down the growth of diseased fish ^[41].

4. Conclusions

Parasites are important components in an ecosystem through their diverse effects on the dynamics of the host population, interactions with the community, and the structure of the biocenosis. Many diseases in fish prevent their productivity and fertility, leading to catastrophic consequences in fisheries and aquaculture. The health of a population depends on disease control and maintaining a healthy relationship between living organisms and their environment.

In terms of intensive fish farming, there is a very high density of susceptible fish in a limited space, and their physiological and health status is not always satisfactory, so there are a large number of susceptible individuals, with excellent conditions for the spread of mass parasitic infections. On the other hand, the fish in open waters regularly has a large number of parasites species, especially with the complex life cycle, as animals that occur as intermediate hosts of parasites (snails, worms, and crabs) are present in the wild.

Preventive measures for the elimination of parasitic diseases consist in the destruction of the parasites themselves or destroying those aquatic organisms that appear as their transitory hosts. Regarding this, permanent ichthyoparasitological controls of the fish health status should be made.

In most parasitic diseases, if stressful moments are avoided and optimal conditions of the water environment are ensured, as well as adequate health and physiological status, the fish may tolerate the invasion of parasites. Severe forms of parasitic infections are often resulting from the adverse influence of the environment.

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Conceptualization, analysis and writing - Dijana Blazhekovikj - Dimovska

Writing - review - Stojmir Stojanovski

Conflict of Interest

There is no conflict of interest.

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