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PROTOZOAN PARASITES IN COMMON CARP (*CYPRINUS CARPIO*, L. 1758) FROM CYPRINID AQUACULTURE FACILITY IN PELAGONIA REGION (BITOLA, MACEDONIA)

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Abstract: The aim of this study was to determine the protozoan parasites in common carp (*Cyprinus carpio*, L. 1758) from the largest cyprinid aquaculture facility in Macedonia, located in Pelagonia region (Bitola). This study was carried out by seasons, in a period of three years. A total of 212 specimens of common carp were examined and 134 fish (63.20%) were infected with protozoan parasites. Representatives of Protozoa were most commonly occurred during the spring (in 58.27 % of the fish examined), than in winter (55.21 %), summer (39.07 %) and the lowest in autumn (35.64 %).
In common carp from this cyprinid fish farm the presence of 4 protozoa species was established: *Myxobolus müelleri, Myxobolus encephalicus, Thelohanellus nikolskii* and *Trichodina* sp. The mean intensity with protozoan parasites in common carp was 3.60, while the prevalence 10.20 %.
Keywords: protozoa, parasites, common carp, aquaculture

1. INTRODUCTION

Ecto and endo-parasitic protozoa represent one of the most dangerous threats to the fish population. These parasites attack the fish, causing massive damage to the skin and gill epithelium (Sterud *et al*. 2003; Enayat *et al.* 2008). According these authors, even moderate infection with these organisms can cause fatal disease, because the infected fish lose appetite and stop consuming food.
Evolutionary different organisms that belong to the Protozoa may act as ecto and endoparasites in fish (Lom & Dykova, 1992). According Moraes & Martins (2004), they are the causative agents of diseases in the global aquaculture, causing damage and reduced growth of the host fish. In this way, parasitic protozoan diseases are responsible not only for great losses to the commercial fishing industry, but also for a negative social impact in developing countries where aquaculture activities contribute to food production of high nutritional value (Bondad - Reantaso *et al.* 2005). Padua & Cruz (2014) concluded that the importance of disease impact must be highlighted in order to elaborate efficient strategies for early diagnosis and fast intervention in fish farms management practices, to obtain a stable and sustainable production.

2. MATERIAL AND METHODS

A total of 212 specimens of common carp (*Cyprinus carpio*, L. 1758) from the largest cyprinid aquaculture facility in Macedonia, located in Pelagonia region (Bitola) were examined for protozoan parasites. The fish were caught using net or hook by local fishermen. The specimens were placed in plastic tanks with fishpond water and immediately transferred to the research laboratory. Fish were killed by vertebral dislocation. During the dissection, the gill filaments, the eyes, the fins, the intestines and the skin were examined under the stereomicroscope. All parasites found in each individual fish were identified and enumerated. During the study period, data on parasite species were categorized according to season.
Classical epidemiological variables (prevalence and mean intensity) were calculated according to Bush *et al.* (1997). The parasite specimens were identified using reference keys of Bykhovskaya-Pavlovskaya et al. (1964).
During the examinations at the Laboratory for fish diseases in Hydrobiological Institute in Ohrid (Macedonia), stereomicroscopes „Zeiss"- Stemi DV4 and „MBS 10", as well as light microscope „Reichart" were used.

3. RESULTS AND DISCUSSION

A total of 212 specimens of common carp (*Cyprinus carpio*, L. 1758) from the largest cyprinid aquaculture facility in Macedonia, located in Pelagonia region (Bitola) were examined and 134 fish (63.20 %) were infected with protozoan parasites. In common carp from this cyprinid fish farm the presence of 4 protozoa species was established:

1. *Myxobolus müelleri* on fins in common carp, in spring;
2. *Myxobolus encephalicus* on brain in common carp, in spring;
3. *Thelohanellus nikolskii* on fins in common carp, in summer;

4. *Trichodina* sp. on gills in common carp, in all seasons.

Data on prevalence and mean intensity with protozoan parasites (by seasons) in common carp are given in Table 1.

Table 1. Prevalence (E) and mean intensity (I) with protozoan parasites in common carp (*Cyprinus carpio*, L. 1758) from cyprinid aquaculture facility in Pelagonia region (Bitola, Macedonia) by seasons

Protozoa species	Spring		Summer		Autumn		Winter	
	I	E (%)	I	E (%)	I	E (%)	I	E (%)
Myxobolus müelleri	3.40	0.44	/	/	/	/	/	/
Myxobolus encephalicus	4.00	0.80	/	/	/	/	/	/
Thelohanellus nikolskii	/	/	2.44	3.08	/	/	/	/
Trichodina sp.	4.55	11.81	4.66	3.44	4.40	7.85	4.72	7.05

During this study, infection with protozoan parasites was recorded on common carp in all seasons. *Myxobolus encephalicus* and *Myxobolus müelleri* were found only in spring, while *Thelohanellus nikolskii* was detected only in summer season. Mean intensity with protozoan parasites were not varied significantly among the seasons. *Trichodina sp.* occurred in all seasons, but the greatest value of prevalence was recorded in spring (11.81).

Based on the total number of fish examined, the highest prevalence with representatives of Protozoa was determined by *Trichodina* sp. (11.81 %), followed by *Thelohanellus nikolskii* (3.08 %), *Myxobolus encephalicus* (0.80 %) and *Myxobolus müelleri* (0.44 %).

The highest mean intensity with representatives of Protozoa was determined by *Trichodina* sp. (4.72), followed by *Myxobolus encephalicus* (4.00), *Myxobolus müelleri* (3.40) and *Thelohanellus nikolskii* (2.44).

Kingdom: PROTOZOA
Phylum: MYXOZOA Grassé, 1970
Class: MYXOSPOREA Bütschli, 1881
Order: BIVALVULIDA Shulman, 1959
Suborder: PLATYSPORINA Kudo, 1919
Family: MYXOBOLIDAE Thélohan, 1892
Genus: MYXOBOLUS Bütschli, 1882
Species: *MYXOBOLUS MÜELLERI Bütschli, 1882*
Host: COMMON CARP (*CYPRINUS CARPIO*, L. 1758)
Localization: fins
Place: cyprinid aquaculture facility (Pelagonia region, Bitola)
Season: spring

Myxobolus müelleri has been detected on fins of 5 specimens of common carp, in spring season. Myxobolus müelleri has been detected in common carp for the first time. Common carp represents new host for Myxobolus müelleri in Macedonian waters and it is first recorded in this paper.

Total, the prevalence with Myxobolus müelleri in Cyprinus carpio was 0.44 %, while the mean intensity 3.40.

Myxobolus müelleri parasites on gills, fins and skin of common carp causing pathomorphological changes. The diseased fish do not take food and progressively weaken. When localizing the gills, symptoms of oxygen deficiency are noticeable (grouping along the water sources and the edges of the pool, making attempts to swallow the air), and some fish come to suffocation. Other symptoms include: scratching the bottom, clustering of fish with signs of apathy, reduced hibernation in winter, exhaustion, sunken eyes and skin discoloration.

However, major epizootics may occur in fish farms, especially in fish offspring.

Fig.1. *Myxobolus müelleri* on fins of common carp (*Cyprinus carpio*, L. 1758) (original)

Kingdom: PROTOZOA
Phylum: MYXOZOA Grassé, 1970
Class: MYXOSPOREA Bütschli, 1881
Order: BIVALVULIDA Shulman, 1959
Sub-order: PLATYSPORINA Kudo, 1919
Family: MYXOBOLIDAE Thélohan, 1892
Genus: MYXOBOLUS Bütschli, 1882
Species: *MYXOBOLUS ENCEPHALICUS Mulsow, 1911*
Host: COMMON CARP (*CYPRINUS CARPIO*, L. 1758)
Localization: brain
Place: cyprinid aquaculture facility (Pelagonia region, Bitola)
Season: spring

Myxobolus encephalicus has been detected on brain of 9 specimens of common carp, in spring season.
Total, the prevalence with Myxobolus encephalicus in Cyprinus carpio was 0.80 %, while the mean intensity 4.00.
The heavily-infected offspring with Myxobolus encephalicus manifests locomotors disorders, loss of balance and circular movements. The prognosis is difficult. The spores of the mixosporids are very resistant to the external environment and exist for more than one year. Mixosporids are usually transmitted through sick fish or food. They attack all fish age categories, and the largest epizooties occur in fishponds, especially in fish offspring.

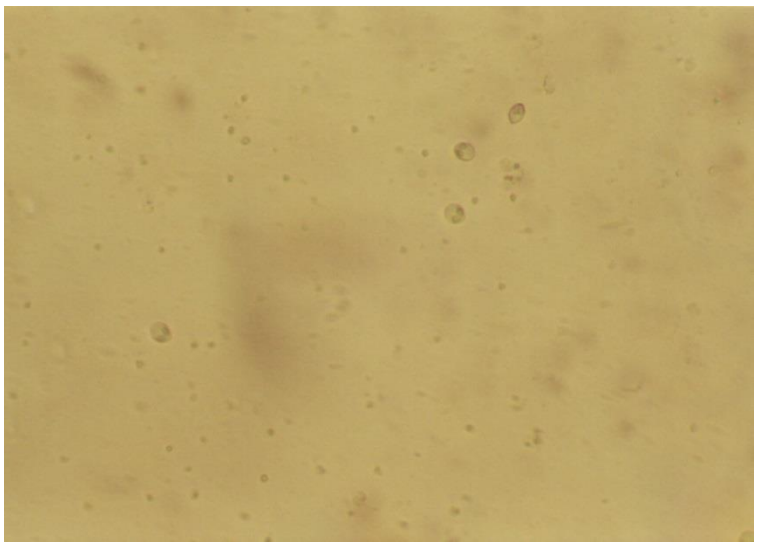

Fig. 2. *Myxobolus encephalicus* on brain of common carp (*Cyprinus carpio*, L. 1758) (original)

Kingdom: PROTOZOA
Phylum: MYXOZOA Grassé, 1970
Class: MYXOSPOREA Bütschli, 1881
Order: BIVALVULIDA Shulman, 1959
Sub-order: PLATYSPORINA Kudo, 1919
Family: MYXOBOLIDAE Thélohan, 1892
Genus: THELOHANELLUS Kudo, 1933
Species: *THELOHANELLUS NIKOLSKII* Achmerov, 1960
Host: COMMON CARP (*CYPRINUS CARPIO*, L. 1758)
Localization: fins
Place: cyprinid aquaculture facility (Pelagonia region, Bitola)
Season: summer

Thelohanellus nikolskii has been detected in fins of 35 specimens of common carp, in summer season.
Total, the prevalence with Thelohanellus nikolskii in Cyprinus carpio was 3.08 %, while the mean intensity 2.44.
Thelohanellus nikolskii causes a disease in one-year-old carp, called telochannelosis. It forms large (3 - 4 mm), round, cystoid plasmodium on the fins. The spores are elliptical, with a thick wall (19 × 12 μm). Plasmodium growth may cause cartilage fragmentation and in severe cases, the fins broken. As a result, locomotor disorders and microbiological contamination occur.
Disease can also occur in 90% of carp offspring, and losses depend on its size. One of the initial symptoms is the appearance of fins dark discoloration, and later, deformations of the fins rays and thickening of their epithelium. In severe cases, the fins fall off, and as a result, the fish lose their locomotors ability. Often, this disease can result with the appearance of secondary bacterial and mycosis infections, as well as, fish mortality.

Kingdom: PROTOZOA
Phylum: CILIOPHORA (Doflein, 1901) Copeland, 1956
Class: OLIGOHYMENOPHOREA de Puytorac et al. 1974
Sub-class: PERITRICHIA Stein, 1859
Order: MOBILIDA Kahl, 1933
Family: TRICHODINIDAE Claus, 1874
Genus: TRICHODINA Ehrenberg, 1830
Species: *TRICHODINA SP. (Cyprinus carpio)*
Host: COMMON CARP (*CYPRINUS CARPIO*, L. 1758)
Localization: gills
Place: cyprinid aquaculture facility (Pelagonia region, Bitola)
Season: spring, summer, autumn, winter

Trichodina sp. has been detected on gills of 117 specimens of common carp, in all seasons. Total, the prevalence with Trichodina sp. in Cyprinus carpio was 35.67 %, while the mean intensity 4.55.
Trichodina are the protozoa of the phylum Ciliophora that cause parasitic disease in fish, called trichodiniasis, manifesting whitish deposits of the fish skin and gills. Mainly, they parasitize in freshwater fish throughout the world.
Trichodinids can be found parasitizing both freshwater and marine fishes on the body surface, buccal cavity and gills. Nevertheless, relatively few of them have become endoparasites in the intestine, kidney and urinary bladder of their hosts (Lom & Dykova, 1992). Proliferation of the group in the environment seems to be associated with bad water quality, total number of bacteria and ecological aspects of the fish species. In this way, the use of trichodinids as an indicator for eutrophication in brackish-water environments was suggested (Palm & Dobberstein, 1999). The trichodinas are attached to the fish body using a special fixing device - an adhesive disk. The parasite moves through the water using the eyelashes that are arranged in two rows along the edges of the upper and lower sides of the body. These parasites infect the fish skin, gills and fins, causing tissues irritation. In addition, the authors state that one of the symptoms is a disorder of the general fish health condition, anxiety, loss of appetite and dyspnea.
According Basson & Van As (2006), trichodins generally have a low degree of specificity to a host. Most species are commensal, causing little or no damage to their host, while many of them are important ectoparasites, which, if present in large numbers, can cause serious epithelial damage to the fish skin and gills.
Their reproduction in fish farms has been related to high stocking density, high organic matter contents and increased water temperature. Yemmen et al. (2011) noticed that some trichodinid species were found to be suppressed with increased water temperature.

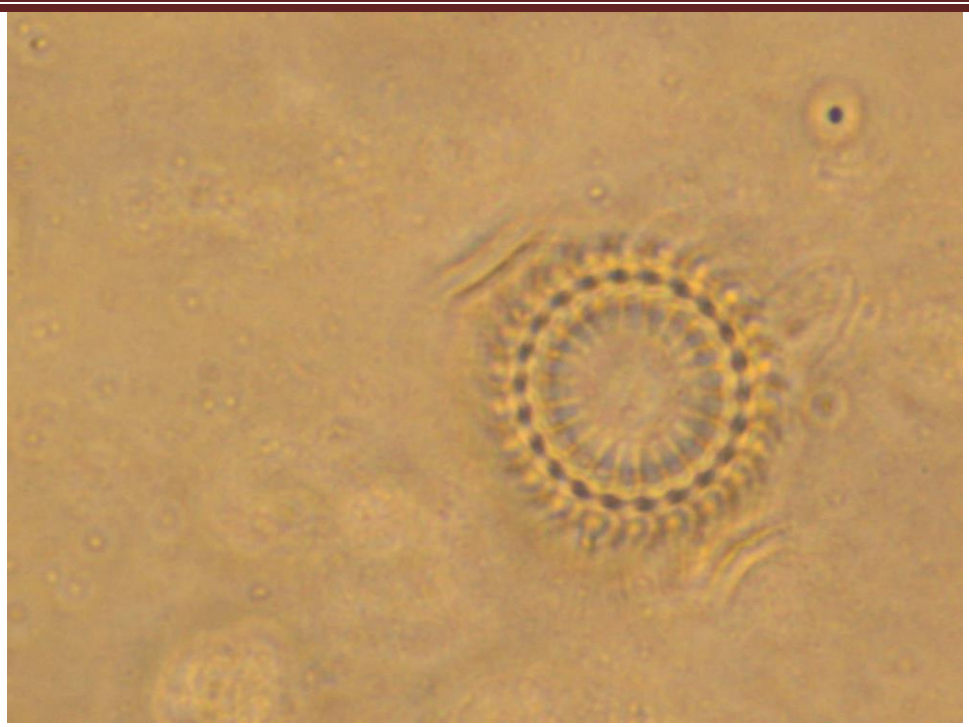

Fig.3 *Trichodina sp.* on gills of common carp (*Cyprinus carpio*, L. 1758) (original)

4. CONCLUSION

In common carp (Cyprinus carpio, L. 1758) from the largest cyprinid aquaculture facility in Macedonia, located in Pelagonia region (Bitola) the presence of 4 protozoa species was established: Myxobolus müelleri, Myxobolus encephalicus, Thelohanellus nikolskii and Trichodina sp.

Myxobolus müelleri has been detected in common carp from Macedonian waters for the first time. At the same time, common carp represent new host for Myxobolus müelleri and it is first recorded in this paper.

Based on the total number of fish examined and parasite species found, mean intensity with protozoan parasites in common carp was 3.60, while the prevalence 10.20 %.

During this research, we established that protozoa represent a major problem in cyprinid aquaculture facilities where, high temperatures and the content of organic matter accelerate the life cycle of the parasites and promote their dissemination.

In order to control outbreaks of diseases in fish farms it is fundamental to first characterize carefully the hygienic-sanitary state of the facility. This includes being aware of disease diagnosis history, recognition of pathogen dispersal and/or transmission modes involved.

Adequate management include not handling fish when abiotic factors such as water temperature and pH are exceeding limits generally considered safe for the fish species, avoid translocation of fingerlings from one place to another without proper care, always use good quality feeds, monitor regularly the quality of the water entering the farm, avoid water sharing among ponds and disinfect fish handling equipment.

REFERENCES

Basson L, Van As JG. (2006): Trichodinidae and other ciliophorans (Phylum Ciliophora). In: Woo PTK. Fish diseases and disorders. 2nd ed. Cab International; p. 154-182. vol. 1. Protozoan and Metazoan infections.

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R et al. (2005): Disease and health management in Asian aquaculture. Vet Parasitol; 132(3-4): 249-272.

Bush A.O., K.D. Lafferty, J. M. Lotz, A.W. Shostak (1997): "Parasitology meets ecology on its own terms: Margolis et al. Revisited". J. Parasitology., 83: pp. 575- 583.

Bykhovskaya-Pavlovskaya I. E., A. V. Gusev, M. N. Dubinina, N. A. Izyumova, T. S. Smirnova, I. L. Sokolovskaya, G. A. Shtein, S. S. Shulman, V. M. Epstein (1964): "Key to Parasites of Freshwater Fish of the U.S.S.R". Leningrad.

Enayat S.R. Mohamed M. El-Naggar, Nagwa M. Ali (2008): "Trichodinid ectoparasites (Ciliophora: Peritrichida) infecting the Nile cichlid fishes Sarotherodon galilaeus and Tilapia Zillii at Dakahlia province, Egypt". Egypt J. Zool., 51: 199 -219.

Lom J, Dyková I. (1992): Protozoan parasites of fishes. Developments in Aquaculture and Fisheries Science. Amsterdam: Elsevier Science; 315 p. vol. 26.

Moraes F.R, Martins M.L. (2004): Condições predisponentes e principais enfermidades de teleósteos em piscicultura intensiva. In: Cyrino JEP, Urbinati EC, Fracalossi DM, Castagnolli N, editors. Tópicos especiais em piscicultura de água doce tropical intensiva. São Paulo: TecArt; p. 343-383.

Pádua SB, Cruz C. (2014): Health challenges in tilapia culture in Brazil. Aquaculture Asia Pacific, 10 (2): 37-39.

Palm HW, Dobberstein RC. (1999): Occurrence of trichodinid ciliates (Peritricha: Urceolariidae) in the Kiel Fjord, Baltic Sea, and its possible use as a biological indicator. Parasitol Res 1999; 85(8-9): 726-732.

Sterud E., Simolin P., Kvellestad A. (2003): "Infection by Parvicapsula sp. (Myxozoa) is associated with mortality in seacaged Atlantic salmon Salmo salar in northern Norway". Diseases of Aquatic Organisms, 54(3): 259-63.

Yemmen C, Ktari MH, Bahri S. (2011): Seasonality and histopathology of Trichodina puytoraci Lom, 1962, a parasite of flathead mullet (Mugil cephalus) from Tunisia. Acta Adriat; 52(1): 15-20.