Biochemical response of two fish species of Gobiidae (Gobiiformes) to Cryptocotyle (Opisthorchiida, Heterophyidae) metacercariae infection from the mouth of the river Chernaya (Black Sea)

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Abstract

Metacercariae of Cryptocotyle are among the most numerous recorded trematode parasites in the estuarine biocenoses of the Azov-Black Sea basin. These parasites can massively affect body surface, gills and fins of host fishes including gobids. However, information on the influence of Cryptocotyle metacercariae on the physiological and biochemical status of gobies is extremely limited. Biochemical response of two fish species of Gobiidae - grass goby Zosterisessor ophiocephalus (Pallas, 1814) and mushroom goby Ponticola eurycephalus (Kessler, 1874) to Cryptocotyle spp. metacercariae infection at the mouth of River Chernaya (Black Sea) was investigated. Activities of superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione-S-transferase, alanine transaminase, aspartate transaminase, level of thiobarbituric acid-reactive substances were measured as parameters of biochemical response. The findings of this study demonstrate interspecific differences in fish response to parasite infections. High infection leads to an intensification of lipid peroxidation and a weakening of antioxidant protection in the muscles of mushroom goby but activation of the antioxidant enzyme system in the muscles of grass goby. The results show a greater resistance of grass goby to the infection when compared with mushroom goby.

Key words: Zosterisessor ophiocephalus, Ponticola eurycephalus, Cryptocotyle spp. metacercariae, parasitic infection, antioxidant enzymes, lipid peroxidation, aminotransferases.

Introduction

Wild fish populations are exposed to different natural and anthropogenic factors. Parasites are one of these factors. Many commercial and cultured fish species infected with parasites can be under the considerable negative impacts on their health. Host responses to parasites are varied and manifest at different levels, including immunological reactions, physiological and biochemical changes in their metabolism. Infections cause biochemical rearrangements in the infected host tissues and can bring about serious structural and functional changes in the infected organs (Mukjrakov & Silkina, 2006; Skuratovskaya et al., 2013, 2015, 2019; Eissa et al. 2014; Mozhdeghanloo & Heidarpour, 2014; Jang et al., 2017).
Trematode metacercariae are the most common parasites causing massive infections in marine and freshwater fish hosts. Metacercariae are known to have often more pathogenic effects on the host organism than those of adult forms (Okopny & Trombitsky 1984; Solovyev et al., 2010; Izvekova & Tyutin 2011). Metacercariae of Paracoenogonimus ovatus cause hyperemia and pronounced infiltration of muscle fibers as well as proliferation of connective tissue around the larval staged parasite (Kosyaev & Ksenofontova, 2012). Metacercariae family Diplostomidae with high intensity of infection can be dangerous especially for juvenile fish by slowing down the growth rate, damaging visual organs, causing curvature of the spine, tissue destruction and often death (Solovyev et al., 2010).

Metacercariae of Cryptocotyle are among the most numerous recorded trematode parasites in the estuarine biocenoses of the Azov-Black Sea basin. These parasites can massively affect body surface, gills and fins of host fishes including gobids. In the life cycle of Cryptocotyle aquatic mollusks are the first intermediate hosts, teleost fish are the second intermediate hosts. Fish-eating water birds are the definitive hosts.

All three species of Cryptocotyle – C. concavum (Creplin 1825) Lühe 1899, C. lingua (Creplin 1825) Fischesoeder 1903 and C. jejuna (Nicoll 1907) Ransom 1920, known in the Black Sea (Smogorzhevskaya, 1976), were previously found in gobiiid hosts in the mouth of River Chernaya (Black Sea) (Machkevskiy & Belousova, 2012) (Figure 1). C. concavum and C. lingua are distributed throughout the brackish waters of Ponto-Azov region while C. jejuna was previously known in the Danube Delta, off the coast of Bulgaria (Ciurea, 1924; Rădulescu, 1970) and Russia (Korch, Korch Strait) (Martynenko, 2012; Korniychuk & Martynenko, 2013). C. concavum are more than 98%, C. lingua - from 0.8 to 1.2% of the total number of metacercaria. C. jejuna is rather rare species (Naidenova, 1974).

High intensities of Cryptocotyle metacercariae infections can lead to serious pathologies, reduction of weight and, thus, resulting lower condition factor of host fish (Korniychuk & Martynenko, 2009; Goncharov et al., 2017). However, information on the influence of Cryptocotyle metacercariae on the physiological and biochemical status of gobies is extremely limited. Therefore, more studies are required to understand the actual mechanisms of host adaptations to the parasites.

The aim of the present study was to investigate biochemical response of grass goby Zosterisessor ophiocephalus (Pallas, 1814) and mushroom goby Ponticola eurycephaatus (Kessler, 1874) to Cryptocotyle metacercariae infection.
Materials and methods

Fish were caught at the mouth of the River Chernaya in Sevastopol Region of the Black Sea in September 2012, 2013.

Parasitological examination. Parasitological analysis of fish was carried out. As a result of the study of helminths by our colleagues, the data of which were kindly provided to us, Acanthocephala (in small numbers or a bit more than a dozen) and Trematoda (in small numbers) were identified in intestines of a few fish specimens. We studied Cryptocotyle spp. metacercariae, which were found on the skin and fins, and myxosporean species Kudoa nova Naidenova, 1975, which was found in fish muscles.

The myxosporeans were analyzed using the compressor method. Muscle samples were flattened and examined under a binocular microscope, then wet smears were made from tissue samples and seen under a microscope MBI-3 at a magnification x600. Myxosporeans K. nova were identified in muscles in the form of diffuse infiltration at an intensity of infection of units, tens, in several cases hundreds of spores in a smear. The prevalence of K. nova was 21 % for P. eurycephalus and 7 % for Z. ophioccephalus.

To detect metacercariae of Cryptocotyle parasitological analysis of the skin and fins of gobid hosts were carried out according to the standard method (Bykhovskaya-Pavlovskaya, 1985). Metacercarial cysts were counted under MBS-10 binocular microscope at the magnification x28. All fish body was screened. In this study the effect of each species of three Cryptocotyle spp. on the host organism was not separately isolated. The total number of metacercariae of the genus on the skin and fins of fish was calculated.

To exclude the influence of K. nova on biochemical parameters in muscles, non-infected specimens with K. nova were used for biochemical analysis.

Biochemical analysis. After parasitological analyses fish non-infected with K. nova were subdivided in three groups depending on the intensity of Cryptocotyle metacercariae infection. Non-infected specimens were included in the first (I) group. Individuals with intensity of infection 1–20 parasites per infected host were included in the second (II) group and fish specimens infected with 50–120 parasites per infected host were included in the third (III) group. It was hardly possible to distinguish separately a group of individuals with the intensity of infection between 20 and 50 parasites per infected host since only one specimen of mushroom goby and six specimens of grass gobies were found.

Muscle samples from each fish were collected and homogenized in the ice-cold 0.85% solution of NaCl. Homogenates were centrifuged at 8000 g for 15 min and then supernatant was used for biochemical analyses. Superoxide dismutase (SOD) activity was assayed spectrophotometrically based on inhibition of the reduction of nitroblue tetrasonium with NADH mediated by phenazine methosulfate under basic conditions (Nishikimi et al., 1972). Catalase (CAT) activity was measured by the method involving the reaction of hydroperoxide reduction (Asatiani, 1969). Peroxidase (PER) activity was detected by spectrophotometric method using benzidine reagent (Litvin, 1981). Glutathione reductase (GR) activity was assayed spectrophotometrically by reaction of the NADPH degradation (Pereslegina, 1989). Glutathione-S-transferase (GST) activity was determined spectrophotometrically by the conjugate 1-chloro-2,4-dinitrobenzene using as substrate at the presence of reduced glutathione (Pereslegina, 1989). Alanine transaminase (ALT), aspartate transaminase (AST) activities were also determined spectrophotometrically with 2,4-dinitrophenylhydrazine using the standard kit (Filicit – Diagnosis (Ukraine)). Thiobarbituric acid-reactive substances (TBARS) concentration was determined spectrophotometrically according to the standard procedure based on the formation of colored complex with thiobarbituric acid (Stalnaya & Garishvily, 1977). Total soluble protein concentration was quantified spectrophotometrically by using the kits of the Filicit-Diagnosis. All biochemical parameters were calculated per mg protein.

Statistical analysis. Mean values +/- SEM (standard error of the mean) were determined. The significance of the difference between the samples was evaluated with the use of Mann-Whitney U-test. The difference was considered significant at the significance level p ≤ 0.05. The statistical analysis was carried out with the use of software programs Past 3 and Microsoft Office Excel 2016.

Results

Cysts of trematode Cryptocotyle spp. were found in 89% grass gobies and 70% mushroom gobies. Intensity of infection ranged 1–125 for grass gobies, 1–120 for mushroom gobies.

Total length and the intensity of Cryptocotyle metacercariae infection of grass goby and mushroom goby from different groups are given in Table 1.
Table 1. Total length and the intensity of infection of grass goby *Z. ophiocephalus* and mushroom goby *P. eurycephalus* from different groups.

| Parameter                      | Groups       |
|--------------------------------|--------------|
|                                | I            | II           | III           |
| Zosterisessor ophiocephalus    |              |              |               |
| Length, cm                     | 9.4 ± 0.98   | 9.5 ± 1.35   | 8.2 ± 0.42    |
| Intensity of infection, spec.  | -            | 8.56 ± 1.41  | 90.0 ± 1.47   |
| Ponticola eurycephalus         |              |              |               |
| Length, cm                     | 6.4 ± 0.35   | 6.5 ± 0.49   | 7.6 ± 0.57    |
| Intensity of infection, spec.  | -            | 9.61 ± 1.47  | 84.0 ± 10.69  |

Interspecific differences in the biochemical response of fish infected with metacercariae of *Cryptocotyle* were found and values of biochemical parameters in muscle samples of mushroom goby are given in Table 2. GST activity was significantly lower in the specimens with high intensity of infection (III group) than that of non-infected ones (Group I) ($P<0.05$). On the other hand, TBARS concentration in individuals from Group III was significantly higher when compared with the values of fish from groups I and II ($P<0.05$). No differences in ALT, AST, SOD, CAT and GR activities were observed between infected and non-infected gobiids (Table 2).

Table 2. Biochemical parameters in muscle tissues of mushroom goby *P. eurycephalus* non-infected and infected with metacercariae of *Cryptocotyle* spp. (mean ± SEM, * indicates significant differences when compared with group I, ■ indicates significant differences when compared with group II, $P<0.05$).

| Parameter                      | Groups       |
|--------------------------------|--------------|
|                                | I            | II           | III           |
|                                | (n = 11)     | (n = 18)     | (n = 9)       |
| ALT, μmol−mg protein−h         | 0.074 ± 0.007| 0.081 ± 0.007| 0.115 ± 0.019 |
| AST, μmol−mg protein−h         | 0.11 ± 0.015 | 0.102 ± 0.008| 0.134 ± 0.018 |
| SOD, arbitrary units mg protein−min | 8.24 ± 1.87 | 7.3 ± 2.63   | 5.48 ± 1.12   |
| CAT, mg H$_2$O$_2$−mg protein−min | 0.029 ± 0.004| 0.025 ± 0.002| 0.033 ± 0.007 |
| GR, nmol NADPH−mg protein−min  | 1.13 ± 0.43  | 1.22 ± 0.17  | 0.97 ± 0.32   |
| GST, nmol conjugate−mg protein−min | 19.85 ± 4.06 | 12.44 ± 1.49 | 9.68 ± 2.0* |
| TBARS, nmol−mg protein         | 0.56 ± 0.07  | 0.49 ± 0.03* | 0.79 ± 0.08* |

Values of biochemical parameters in muscle samples of grass goby were also determined and are given in Table 3. SOD and CAT activities were significantly higher in fish from Group III than those of Group I and II and a similar result was obtained in GR activity in specimens from Group III when compared with Group I ($P<0.05$). No differences in ALT, AST, GST activities and TBARS concentration were found between three groups of fish (Table 3).

The attempt was also made to study the possible influence of the detected intestinal helminths on the biochemical parameters of host muscles. But no biochemical response was found.

Discussion

It is known that the nature of metabolic changes caused by helminthic infections is determined both by the effect of parasites on a host organism and characteristics of host responses. The effect of helminthic infections on metabolic processes depends on the taxonomic status of both partners, the life cycle of
BIOCHEMICAL RESPONSE OF TWO GOBIID FISHES TO METACERCARIAE INFECTION

parasites, the intensity and duration of infection (Izvekova & Tyutin, 2011; Skuratovskaya et al., 2013; Eissa et al., 2014).

Table 3. Biochemical parameters in muscle tissues of grass goby Z. ophiocephalus non-infected and infected with metacercariae of Cryptocotyle spp. (mean ± SEM, * indicates significant differences when compared with group I, ** – indicates significant differences when compared with group II, P< 0.05).

| Parameter                  | Groups                      |
|----------------------------|-----------------------------|
|                            | I (n = 10)                  | II (n = 17)                  | III (n = 9)                  |
| ALT, μmol mg protein/h     | 0.086 ± 0.014               | 0.091 ± 0.007               | 0.06 ± 0.013                |
| AST, μmol mg protein/h     | 0.143 ± 0.024               | 0.143 ± 0.02                | 0.096 ± 0.015               |
| SOD, arbitrary units mg protein/min | 4.24 ± 0.44 | 3.52 ± 0.76 | 6.76 ± 0.53* ** |
| CAT, mg H2O2 mg protein/min | 0.02 ± 0.001                | 0.02 ± 0.002                | 0.046 ± 0.002* ** |
| GR, nmol NADPH mg protein/min | 1.08 ± 0.35                | 1.28 ± 0.37                | 2.38 ± 0.48*    |
| GST, nmol conjugate mg protein/min | 11.74 ± 0.91  | 8.41 ± 1.14 | 13.75 ± 2.21  |
| TBARS, nmol mg protein     | 0.49 ± 0.03                 | 0.41 ± 0.04                 | 0.43 ± 0.03                |

Host adaptations to parasites are due to the activity of protective systems aimed at reducing the negative influence and the destruction of toxic metabolites. Antioxidant (AO) system is one of the defense systems protecting organisms from oxidative stress caused by biotic and abiotic factors. The balance of lipid peroxidation and antioxidant activity characterizes the adaptive capabilities of living organisms while a shift in the balance results in pathological changes including damage of molecular and cellular structures. The level of TBARS is a well-characterized biomarker of oxidative stress manifested in pathogen enhance the formation of reactive oxygen species in organism (Bello et al., 2000; Mukjrakov & Silkina, 2006; Skuratovskaya et al., 2013, 2015, 2019; Rudneva et al., 2016). Parasites affect the metabolism of infected fish partly by inducing oxidative stress, which is manifested in the enhancement of peroxide and free radical processes. The host response is characterized by enhanced synthesis of reactive oxygen species aimed at parasite elimination. This process can both inhibit and stimulate antioxidant enzyme activities (Bello et al., 2000; Mukjrakov & Silkina, 2006; Skuratovskaya et al., 2013, 2015, 2019; Mozhdeeganloo & Heidarpour, 2014).

In this study the increase of TBARS concentration in the muscles of the highly infected specimens of mushroom goby is probably due to the impairment of lipid metabolism induced by the enhancement of free radical and peroxide processes and the weakening of individual links of antioxidant protection, as evidenced by the decrease of GST activity. At the same time, the increase in the activity of SOD, CAT and GR in muscles of highly infected grass gobies can indicate the activation of antioxidant protection against parasitic infection. Reported interspecies differences in the biochemical response can be associated with a higher resistance of grass goby to metacercariae of Cryptocotyle infection as compared with mushroom goby.

Our results are consistent with other data demonstrating prooxidant-antioxidant changes in fish infected with parasites and interspecies differences in the biochemical response on parasite infection. A significant increase of lipid peroxidation, but no difference in CAT and SOD activities were observed in muscle tissues of the freshwater fish Rhamdia quelen infected with metacercariae of Clinostomum detruncatum (Bello et al., 2000). The analysis of prooxidant-antioxidant parameters in tilapia Oreochromis niloticus infected with metacercariae of Diplostomum tilapiae and Heterophyes sp. showed a significant increase in SOD, CAT, GR, glutathione peroxidase activities and malondialdehyde concentration in liver and muscle tissues (Eissa et al., 2014). A decrease in total antioxidant activity and an increase of lipid peroxidation were shown in the blood and liver of freshwater bream Abramis brama infected with plerocercoids of Ligula intestinalis (Mikrjakov & Silkina, 2006), and in gill tissues of goldfish Carassius auratus parasitised with Dactylogyrus spp. (Mozhdeeganloo & Heidarpour, 2014). Comparative study of biochemical parameters in muscle tissues of round goby Neogobius melanostomus with different intensity of infection with metacercariae of Cryptocotyle spp. showed a decrease of CAT, peroxidase, GR activities and an increase of TBARS concentration in highly infected fish (intensity of infection – 100–360 specimens). The results revealed that the high infection resulted in a shift in the prooxidant-antioxidant balance towards...
intensification of lipid peroxidation and reduction of the antioxidant enzyme activities (Skuratovskaya et al., 2019).

The effects of experimental infections with larvae (cercariae) of two trematode species - *Ornithodiplostomum* sp. developing in the liver, and *O. ptychocheilus* developing in the brain on induction of oxidative stress in fathead minnows *Pimephales promelas* were evaluated (Stumbo et al., 2012). For *Ornithodiplostomum* sp. lipid peroxidation concentration in liver tissue increased 5 days after exposure and remained higher than controls until the end of the experiment at 28 days. For *O. ptychocheilus* liver lipid peroxidation concentration was higher than controls at 5 days, but not thereafter. These experimental results support those from field studies, indicating that the lipid peroxidation assay may be an effective biomarker for parasite-induced oxidative stress in fish, and that the nature of the oxidative stress response is species and/or tissue specific (Stumbo et al., 2012).

Violations in the lipid metabolism of fish infected with metacercariae of *C. concavum* were also found (Shchepkina, 1981). In the liver and muscles of highly infected individuals of round goby *Neogobius melanostomus* (intensity of infection – 130–250 specimens) the concentration of triglycerides and total lipids were significantly lower when compared with the low infected fish (intensity of infection – 5–20 specimens) (Shchepkina, 1981).

In our study, no differences in the ALT and AST activities in the muscles of non-infected and infected mushroom goby and grass goby were observed. ALT and AST play the main role in protein metabolism and are widely used as biomarkers of various conditions of organisms, including assessing parasitic influences. An absence of differences in aminotransferase activities suggests that metacercariae of *Cryptocotyle* do not affect protein metabolism in muscle tissues of studied fish in the present study. The results are consistent with data obtained on round goby *Neogobius melanostomus* infected with metacercariae of *Cryptocotyle* spp. No differences in the ALT and AST activities in muscle tissues were observed between low infected and highly infected fish (Skuratovskaya et al., 2019). Increase in aminotransferase activities was shown in the blood of African catfish *Clarias gariepinus*, tilapia *Oreochromis niloticus* (El-Seify et al. 2011) and carp *Cyprinus carpio* (Ali & Ansari, 2012) infected with monogenea indicated liver and muscle damages in their hosts. Infected carp had higher levels of plasma AST and ALT than non-infected and control conspecifics. In this case, increased levels of ALT indicate liver impairment, and AST is a biomarker of cellular disintegration and energy collapse during activity (Slavik et al. 2017).

To sum up, the findings of this study demonstrate interspecific differences in the biochemical response of fish to metacercariae of *Cryptocotyle* spp. infections. High infection leads to an intensification of lipid peroxidation and a weakening of antioxidant protection in the muscles of mushroom goby but activation of the antioxidant enzyme system in the muscles of grass goby. The results show a greater resistance of grass goby to the infection when compared with mushroom goby.

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**References**

Ali, H. & Ansari, K.K. (2012) Comparison of hematological and biochemical indices in healthy and monogenean infected common carp, *Cyprinus carpio*. Annals of Biological Research, 3(4), 1843–1846.

Asatiani, V.S. (1969) *Enzyme analysis methods*. Moskow, Nauka, 740 pp. (in Russian)

Bello, A.R., Fortes, E., Bello-Klein, A., Bello, A.A., Lesuy, S.F. & Robaldo, R.B. (2000) Lipid peroxidation induced by *Clinostomum deterinatum* in muscle of the freshwater fish *Rhamdia quelen*. *Diseases of Aquatic Organisms*, 42, 233–236. https://doi.org/10.3354/dao042233
BIOCHEMICAL RESPONSE OF TWO GOBIID FISHES TO METACERCARIAE INFECTION

Bykhovskaya-Pavlovskaya, I.E. (1985) Fish parasites: a study guide. Methods of Zoological research-practice. Leningrad, Nauka, Leningradskoe otdeleineie, 123 pp. (in Russian)

Ciurea, J. (1924) Heterophide`s de la faune parasitaire de Roumanie. Parasiotologia, 16, 1–21.

Eissa, I.A.M., Derwa, H.I., Mona, I., Ramadan, R.A., Mona, Z. & Nashwa, M. (2014) Use of enzyme activities as biomarkers for oxidative stress induced by metacercarial affections in some cultured tilapia species. Life Science Journal, 11(3), 284–289. https://doi.org/10.7537/marslsj110314.42

El-Seify, M.A., Zaki, M.S., Desouky, A.R.Y., Abbas, H.H., Hady, O.K.A. & Zaid, A.A.A. (2011) Study on clinopathological and biochemical changes in some freshwater fishes infected with external parasites and subjected to heavy metals pollution in Egypt. Life Science Journal, 8(3), 401–405. https://doi.org/10.7537/marslsj080311.64

Goncharov, S.L., Soroka, N.M., Priyma, O.B. & Dubovyi, A.I. (2017). Distribution of trematodes Cryptocotyle (Trematoda, Heterophyidae) in fish of the family Gobiidae in the estuary waters and the Black Sea in Southern Ukraine. Vestnik zoologii, 51(5), 393–400. https://doi.org/10.1515/vzoo-2017-0046

Izvekova, G.I. & Tyutin A.V. (2011). Occurrence of partenites in mollusks and the influence that Metacercaria of Apophallus muehlingi (Jagerskiold, 1898) and Posthodiplonstomum cuticola (Nordmann, 1832) has on some biochemical parameters in fish. Inland Water Biology, 4(3), 367–372. https://doi.org/10.1134/S1995082911030114

Jang, Y.-H., Subramanian, D., Won, S.H. & Heo, M.S. (2017) Immune response of olive flounder (Paralichthys olivaceus) infected with the myxosporean parasite Kudoa septempunctata. Fish & Shellfish Immunology 67, 172–178. https://doi.org/10.1016/j.ssi.2017.06.019

Korniychuk, Yu.M. & Martinenko. I.M. (2013) Trematodes Cryptocotyle jejuna in clams Hydrobia acuta of the Kerch Strait. In abstract book: XV Conference of the Ukrainian Science Association of Parasitologists, Kiev, p. 58. (in Russian)

Kosyaev, N.I. & Ksenofontova, V.A. (2012) Paracoenogonimosis fish: vet-sanitary examination. Scientific notes KGAVM named NE Bauman, 212, 60–63. (in Russian)

Litvin, F.F. (1981) Laboratory manual of physicochemical methods in biology. Moscow: Moscow State University, 240 pp. (in Russian)

Machkevskiy, V.K. & Belousova, Yu.V. (2012) Cryptocotyle Metacercariae (Trematoda: Heterophyidae) from gobies (Pisces) in the Black River estuary (Black Sea, Crimea). In: Nigmatulin, Ch.M. (Eds.) Proceedings of the V All-Russian conference with international participation on theoretical and marine parasitology. Kaliningrad, AtlantNIRO, pp. 135–138. (in Russian)

Martynenko, I.M. (2012) Finding a Cryptocotyle jejuna (Nicoll, 1907) in the Kerch Strait. Proceedings of Conference of young zoological scientists. Kiev, Zoological Institute of Ukraine, pp. 21. (in Russian)

Mikrjakov, V.R. & Silkina N.I. (2006) Characteristics of lipid peroxidation in the system host-parasite at the case Ligula intestinalis L. (Cestoda, Pseudophyllidae) – Abramis brama (L.). Biology of inner waters, 4, 63–66. (in Russian)

Mozhdeganloo, Z. & Heidarpour, M. (2014). Oxidative stress in the gill tissues of goldfishes (Carassius auratus) parasitized by Dactylogyrus spp. Journal of Parasitic Diseases, 38(3), 269–272. https://doi.org/10.1007/s12639-013-0239-z

Naidenova, N.N. (1974) Parasitic fauna of fish of the goby family of the Black and Azov seas. Kiev: Naukova Dumka, 184 pp. (in Russian)

Nishikimi, M., Rao, N.A. & Jagik, K. (1972) The occurrence of superoxide anion in the reaction of reduced phenazine. Biochemical and Biophysical Research Communications, 46(2), 849–854.

Okopny, N.S. & Trombitsky, I.D. (1984) On mechanism of pathogenesis in fishes during the infection with trematodes of the genus Diplostomum (Trematoda/Diplostomidae). Parazitologiya, XVII(3), 228–232. (in Russian)

Pereslegina, I.A. (1989) The activity of antioxidant enzymes of saliva in healthy children. Laboratornoe Delo, 11, 20–23. (in Russian)

Rădulescu, I. (1970) Cheie dichotomică pentru cunoaşterea stărilor de boală la pestii din Marea Neagră. Bul Letr Cercet Proiect Piscic, 23, 66–96. (in Romanian)
Rudneva, I.I., Skuratovskaya, E.N., Chesnokova, I.I., Shaida, V.G. & Kovyrshina, T.B. (2016) Biomarker response of Black Sea scorpion fish *Scorpaena porcus* to anthropogenic impact. In: Kovács, A. & Nagy, P. (Eds) *Advances in Marine Biology*. Vol. 1. USA, Nova Science Publishers, p. 111–146.

Shchepkina, A.M. (1981) On the effect of metacercariae of trematode *Cryptocotyle convavum* on the lipid contents in tissues of round goby. *Parazitologiya*, XV(2), 185–187. (in Russian)

Skuratovskaya, E.N., Kovyrshina, T.B. & Chesnokova I.I. (2019) Influence of *Cryptocotyle* spp. metacercariae (Creplin, 1825) on some biochemical parameters of round goby *Neogobius melanostomus* (Pallas, 1814). *Bulletin of the European Association of Fish Pathologists*, 39(1), 24–30.

Skuratovskaya, E.N., Yurakhno, V.M. & Zav’yalov A.V. (2013) The influence of parasitic invasion on the Black Sea whiting *Merlangius merlangus euxinus* (Gadidae) morphophysiological and biochemical parameters. *Vestnik zoologii*, 47(4), 309–317. doi: https://doi.org/10.2478/vzoo-2013-0032

Skuratovskaya, E.N., Zav’yalov, A.V. & Rudneva, I.I. (2015) Response of the antioxidant system of Black Sea whiting *Merlangius merlangus euxinus* (Nordmann, 1840) to parasitic nematode *Hysterothylicium aduncum* (Rudolphi, 1802) infection. *Bulletin of the European Association of Fish Pathologists*, 35(5), 170–176.

Slavík, O., Horký, P., Douda, K., Velíšek, J., Kolářová, J. & Lepič, P. (2017) Parasite-induced increases in the energy costs of movement of host freshwater fish. *Physiology & Behavior*, 171, 127–134. https://doi.org/10.1016/j.physbeh.2017.01.010

Smogorzhevskaya, L.A. (1976) *Helminths of waterfowl and marsh birds of the fauna of Ukraine*. Kiev, Naukova dumka, 416 pp. (in Russian)

Solovyev, M.M., Kashinskaya, E.N. & Glupov, V.V. (2010) Infectiousness with the metacercarias of Diplostomidae family and the activity of digestive enzymes in young Siberian dace *Leuciscus leuciscus baicalensis* (Dyb) in the Kargat River in the Basin of Lake Chany. *Contemporary Problems of Ecology*, 3(5), 555–561.

Stalnaya, I.D. & Garishvily, T.G. (1977) Method of malonic dyaldehyde determination using thiobarbituric acid. In: Orecovich (Eds.), *Current Methods in Biochemistry*, Moscow: Medicine Publ., pp. 66–68. (in Russian)

Stumbo, A.D., Goater, C.P. & Hontela A. (2012). Parasite-induced oxidative stress in liver tissue of fathead minnows exposed to trematode cercariae. *Parasitology*, 139, 1666–1671. https://doi.org/10.1017/S0031182012001023