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ORIGINAL ARTICLE

The effect of *Triaenophorus nodulosus* (Cestoda: Bothriocephalidea) infection on some biochemical parameters of the liver of *Perca fluviatilis*

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Abstract Natural infection of 2 to 6-year-old perch with the cestode parasites *Triaenophorus nodulosus* was shown to have minor effects on the studied components of the antioxidant defense system, nucleic acids degradation, and carbohydrate metabolism enzymes in the liver of the fish. The level of infection of 1–4 parasite larvae per fish observed in wild population of perch was shown to be moderate in terms of its effect on the health of the host fish. The activity of hepatic enzymes β-galactosidase, β-glucosidase, cathepsin D, and glutathione S-transferase showed different responses in infected males and females, which indicates different potential resistance of fish to the stress exposure between genders.

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Introduction

The study of the effect of parasites on the biochemical status of their host is important for clarifying the mutual adaptations in the parasite–host system. A parasite directly affects its host by competing with it for resources; nevertheless, there is usually a balance in the system, where parasites cannot cause major damage to the host population, since the bulk of parasites occupy its minor share (aggregated distribution) (Ieshko 1988). Hence, when assessing the health of individuals in wild species, the mere occurrence of a parasitic infection should be interpreted as a detrimental factor only after the effects produced by this parasite on both individual hosts and the entire host population have been properly investigated.

A growing number of studies are devoted to the biochemistry of parasitic worms (El-Ansary 2003; de la Torre-Escudero et al. 2012; Cui et al. 2013). However, not many of them are focused on the biochemical aspects of the life of fish helminths and their hosts' responses to infection (Izvekova 2001; Dautremepuits et al. 2003; Vysotskaya et al. 2003; Rudneva et al. 2004; Boufana et al. 2011; Frank et al. 2011; Kuklina and Kuklin 2011; Hassan et al. 2015), although they are important for improving productivity in aquaculture and preventing human parasitosis associated with the consumption of raw fish. The tapeworms *Triaenophorus nodulosus* (Pallas, 1781) (Cestoda: Bothriocephalidae) is a widespread parasite in European freshwater fish. The life cycle of the parasite involves three hosts. The first intermediate hosts are planktonic crustaceans (subclass Copepoda). The range of second

intermediate hosts is very wide, including a plethora of commercial fish species (17 families, including Percidae, Gadidae, Esocidae, Salmonidae, Osmeridae, and Thymallidae) (Kuperman 1973). Second intermediate hosts are infected with procercoids when the fish fry feed on zooplankton. Parasites penetrate the intestinal wall and move into internal organs (mainly the liver), where they get encysted and develop to the plerocercoid stage. A plerocercoid spends several years in a state similar to anabiosis until its host falls prey to the definitive host pike, *Esox* spp. (Kuperman 1973).

Previously, the local immune response around the parasite's capsule has been studied in the livers of the second intermediate hosts, *Perca fluviatilis* L., 1758 (Pronina and Pronin 1988; Dezfuli et al. 2014); however the particular effects of the infection on the overall condition of an entire fish liver have not been fully clarified. The effect of parasites on the functional state of an organ can be assessed by using a set of biochemical markers to indicate individual functional components of metabolism. It is known that the level of activity of an organism's antioxidant defense system characterizes its response to stress, including stress caused by parasitic infection (Rudneva et al. 2004; Marcogliese et al. 2010; Frank et al. 2011). The activity of lysosomal enzymes is an indicator of the stability of cellular and subcellular components and, indirectly, of the processes of growth, regeneration and degradation in the tissue. The overall intensity of metabolic processes in the infected tissue can be measured by energy and carbohydrate metabolism parameters. In this study, we investigated the effects of *T. nodulosus* plerocercoids on biochemical parameters in the liver of *P. fluviatilis* (activity of antioxidant, lysosomal enzymes, and enzymes involved in carbohydrate metabolism). The aim was to determine the biochemical response of the liver tissue to the presence of *T. nodulosus* and to identify the possible biochemical markers of the infection.

Materials and methods

Sampling

Perch (*Perca fluviatilis*) of both sexes, aged from 2 to 6 years, were used for experiments (Table 1). Fish were netted from the northern part of Lake Ladoga, Russia (60°50′03″N, 31°33′10″E) in 2015–2016. The fish were killed by destroying the brain with a lancet, followed by cutting the abdominal wall with scissors. Livers and serosa were visually examined for the presence of capsules with larvae of *Triaenophorus nodulosus*. Thereafter, the livers were taken out, divided into several parts, pressed between two thick glass slides, and examined under a stereomicroscope. The wall shells of all the detected capsules were torn with a fine needle, and the tapeworms were retrieved and transferred to a Petri dish filled with 0.9% NaCl solution to remove mucus and tissue debris. The number of plerocercoids was determined by counting scolices. The level of the *T. nodulosus* infection in the fish was evaluated by estimating the percentage of infected fish (prevalence) and the mean number of plerocercoids per infected fish (intensity). Fish liver samples after helminth removal were frozen in liquid nitrogen and stored at − 80 °C until biochemical analysis (Table 2).

Animal experiments were carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123).

Biochemical analysis

On the day of the analysis, the frozen 0.1–0.4 g liver samples were homogenized with a Digital Disruptor Genie unit (Scientific Industries, Inc., USA) in 50 mmol Tris–HCl buffer (pH 7.5) with a fivefold dilution. The homogenate was centrifuged at 60,000×*g* for 1 h at 4 °C in a Beckman Coulter Allegra 64R centrifuge (Beckman Coulter, Inc., USA). The resultant supernatant was used for biochemical analysis.

The activity of glutathione S-transferase (GST) was determined from the rate of reduced glutathione (GSH) binding with the 1-chloro-2,4-dinitrobenzene (CDNB) substrate (Habig et al. 1974). A microplate well was injected with 0.225 µl of reaction mixture containing 1 mmol CDNB and 1 mmol GSH in 0.125 mol PBS (pH 6.5). The reaction was initiated by adding 0.025 µl of the homogenate solution, and the following rise of the solution optical density was recorded at a wavelength of 340 nm at 25 °C with a microplate reader (CLARIOstar, BMG Labtech, Germany). The relative activity of the enzyme in fish

Table 1 *Triaenophorus nodulosus* infection rates in the liver of perch from Lake Ladoga in 2015–2016

Sampling period	Infection prevalence (%)	Intensity of infection, plerocercoids/infected fish	Age of fish (years)	Length of fish AB (cm)
2015	38	1.0 ± 0.0	2–6	12–20
2016	53	1.6 ± 0.9	2–4	13–16

Table 2 Characteristics of the biological samples used for the biochemical analysis

Sampling period	Median of fish age of fish (years)/range of age (years)	Uninfected perch, number of individuals		Infected perch, number of individuals		Studied biochemical parameters
		Males	Females	Males	Females	
2015 October	$\frac{4.2}{2-6}$	6	12	8	6	GST, CAT
2016 June	$\frac{3.5}{3-4}$	7	9	14	11	GSH, RNase, DNase, Cat D, Cat B, β-glucosidase, β-galactosidase
2016 October	$\frac{3.0}{2-3}$	6	5	6	6	COX, LDH, G6PDH

tissue was expressed as the amount of the reaction product in μmol generated within 1 min, recalculated per mg of soluble protein in the tissue (μmol/min/mg protein/), where protein was determined by the spectrophotometric method through the absorbance of a peptide bond at 220 nm at 26 °C (Noble and Bailey 2009; Sukhovskaya et al. 2010).

Catalase (CAT) *activity* was determined using a modified technique of Beers and Sizer (1952). On the day of the analysis, a reaction mixture, containing 25 mmol H_2O_2 in 0.05 mol PBS (pH 7.4), was prepared. The activity of the enzyme was measured through the registration of optical density in solution at a wavelength of 240 nm at 25 °C. CAT relative activity was expressed as the amount of hydrogen peroxide decomposed within 1 min in μmol, recalculated per mg of soluble protein in the tissue (μmol/min/mg protein/), where protein was determined by the spectrophotometric method through the absorbance of a peptide bond at 220 nm at 26 °C (Noble and Bailey 2009; Sukhovskaya et al. 2010).

The concentration of reduced glutathione (GSH) was determined in accordance with the method described by Cohn and Lyle (1966) and a modified version of the technique described by Hissin and Hilif (1976). Soluble proteins were precipitated from the homogenate using 5% trichloroacetic acid. The precipitate was separated by centrifuging at 2500×*g* for 15 min. The supernatant was adjusted to pH 8.5 using 5 mol NaOH and then mixed with 0.4 mol Tris–HCl buffer (pH 8.5) containing 5 mmol ethylenediaminetetraacetic acid (EDTA). Then, 0.01% ortho-phthalaldehyde in methanol, prepared immediately before use, was added to the reaction mixture. The mixture was stirred and incubated at room temperature for 15 min, after which its fluorescence was measured (Em—420 nm, Ex—350 nm). The glutathione concentration was calculated according to the calibration curve plotted using series of solutions with different GSH concentrations in 0.4 mol Tris–HCl buffer (pH 8.5), containing 5 mmol EDTA. The relative glutathione concentration was expressed as μg GSH/mg protein, where total soluble protein was determined by the spectrophotometric method through the absorbance of a peptide bond at 220 nm at 26 °C (Noble and Bailey 2009; Sukhovskaya et al. 2010).

Cathepsin B (Cat B) *activity* was determined according to a technique described by Matsuda and Misaka (1974). Liver samples were homogenized in 0.25 mol sucrose solution with 0.01% Triton X-100 with tenfold dilution (w/v). After centrifuging at 10,000×*g* for 30 min, 0.1 ml of the supernatant was mixed with 0.2 ml of 0.065 mol Nα-Benzoyl-L-arginine ethyl ester hydrochloride in 0.1 mol acetate buffer (pH 5.0). Cathepsin B relative activity was measured at 37 °C by recording changes in optical density of solution at 525 nm and expressed in units of change in optical density ΔD_{525}/min/mg protein/, where total soluble protein was determined by the Bradford assay (Bradford 1976).

Cathepsin D (Cat D) *activity* was measured by a modified version of Anson's method (Barrett and Heathm 1977). Samples were homogenized in 0.25 mol of sucrose solution with 0.01% Triton X-100 with tenfold dilution (w/v). After centrifuging at 10,000×*g* for 30 min, 0.1 ml of the supernatant was mixed with 1.0 ml hemoglobin in 0.1 mol acetate buffer (pH 3.6). Cathepsin D relative activity was measured at 37 °C by recording changes in optical density of solution at 280 nm and expressed as ΔD_{280}/min/mg protein/, where total soluble protein was determined by the Bradford assay (Bradford 1976).

The activity of lysosomal β-glucosidase was determined as described by Pokrovsky et al. (1971). Test tubes were filled with 0.8 ml of 0.005 mol 4-nitrophenyl β-D-glucopyranoside in citrate buffer (pH 5.0), and then 0.2 ml 0.1% Triton X-100 and 0.2 ml homogenate were added. The mixture was incubated for 30 min in a water bath at 30 °C. The reaction was then terminated by adding 2 ml of 0.4 mol glycine buffer (pH 10.8). After that, the samples were centrifuged at 3600×*g* for 15 min, and the optical density of the supernatant was measured at a wavelength of 420 nm. The activity of the enzyme was expressed as nmol of 4-nitrophenol/min/mg protein/, where total soluble

protein was determined by the Lowry assay (Lowry et al. 1951).

The activity of lysosomal β-galactosidase was assayed using the method suggested by Barrett and Heathm (1977). A quantity of 0.2 ml of homogenate was added to test tubes containing 1 ml of 0.005 mol 4-Nitrophenyl β-D-galactopyranoside in citrate buffer, (pH 4.0), and incubated in a water bath for 30 min at 30 °C. The reaction was terminated by adding 1.5 ml of 3.3% trichloroacetic acid. The precipitate was separated by centrifuging at 5600×*g* for 20 min. After that, 2 ml aliquots of the supernatant were mixed with 1 ml of 0.1 mol carbonate buffer (pH 10–11) and the extinction of *p*-nitrophenol at 420 nm was measured. The relative activity of the enzyme was expressed as nmol of *p*-nitrophenol released in the reaction per unit of time (nmol of 4-nitrophenol/min/mg protein and was normalized by total soluble protein determined by the Lowry assay (Lowry et al. 1951).

Activity of lysosomal nucleases. Ribonuclease (RNase) and deoxyribonuclease (DNase) activities were assayed using the methods described by Levitsky et al. (1973) and Pokrovsky et al. (1971). The substrates were 0.1% RNA or DNA solutions (Sigma-Aldrich) in 0.2 mol acetate buffer (pH 5.2 and 5.0, respectively). Substrates with the homogenate were incubated for 15 min at 30 °C. The undegraded nucleic acids were then removed by precipitation in 0.5 mol perchloric acid solution or 0.25% uranylacetate in 0.5 mol perchloric acid (for DNA and RNA, respectively). The quantity of low-molecular products from nucleic acid hydrolysis was determined from the supernatant's optical density at 260 nm. The relative activity of the enzymes was expressed in units of optical density, ΔD_{260}/min/mg protein/, where total soluble protein was determined by the Lowry assay (Lowry et al. 1951).

Assays of the activity of enzymes involved in energy and carbohydrate metabolism. Tissue samples were weighed, homogenized in 0.05 mol Tris–HCl buffer (pH 7.5) and purified from tissue fragments by centrifugation (13,500×*g*, 5 min). The *cytochrome c oxidase* (COX) activity was determined in accordance with the method described by Smith (1955). COX activity was measured based on the oxidation of cytochrome *c* by COX, which was determined in reaction mixture (0.05 mmol reduced cytochrome C in 0.1 mol PBS, pH 7.0), from the decrease in absorbance of solution at 550 nm during 5 min at 25 °C after addition of homogenate. The total activities of *lactate dehydrogenase* (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) were assayed using the method described by Bergmeyer (1983). The activity of LDH was determined in a reaction mixture (100 mmol PBS (pH 7.5), 5 mmol NADH, 23 mmol pyruvate-Na) after addition of homogenate based on the change in absorbance of solution at 340 nm during 5 min at 25 °C. The activity of G6PDH was determined in a reaction mixture (50 mmol Tris–HCl (pH 7.5) with 15 mmol NADP+, 40 mmol glucose-6-phosphate) after addition of homogenate based on the change in absorbance at 340 nm during 5 min at 25 °C. The relative catalytic activity of the selected enzymes was reported in μmol of substrate/min/g protein, where total soluble protein was determined by the Bradford assay (Bradford 1976).

Statistical data treatment

Statistical analyses were performed with Past 3.10 and Microsoft Excel. Differences between samplings were estimated by two-way nonparametric analysis of variance (PERMANOVA). Post hoc analyses were performed using the nonparametric Mann–Whitney U test. Correlations between the investigated indices were analyzed using Spearman's rank correlation coefficient. Differences were considered significant at $p \leq 0.05$.

Results

The rate of *Triaenophorus nodulosus* infection in the examined perch from Lake Ladoga is shown in Table 1. A comparison of the infection prevalence and intensity between different years (Table 1) showed that the prevalence of *T. nodulosus* in perch was higher in 2016 compared to 2015 (mean 1.6 and 1.0 plerocercoid per fish, respectively), probably due to the lower average age of the fish in the 2016 sample.

According to the correlation analysis, the intensity of the infection in individual fish did not relate to the levels of the investigated biochemical indices in the liver, probably due to relatively low infection intensity in the studied fish (maximum four cysts in one specimen). Also, a moderate negative correlation was detected between GST activity and fish age ($r = -0.34$, $p = 0.03$), similar to the one previously demonstrated in pike muscles (Borvinskaya et al. 2011).

The levels of some studied biochemical parameters have been shown to vary among fish of different sexes and ages; therefore, these factors were taken into account in the analysis. Accordingly, β-glucosidase activity was higher in males compared to females ($p = 0.02$) (Fig. 1A, Table 3). It was demonstrated that there is an interaction between sex and the intensity of infection in their effect on the activity of GST, β-glucosidase and cathepsin D (Fig. 1, Table 3). The activities of β-glucosidase, β-galactosidase and cathepsin D were considerably higher in uninfected males than in uninfected females or infected males. GST activity was lower in infected female fish compared to uninfected females.

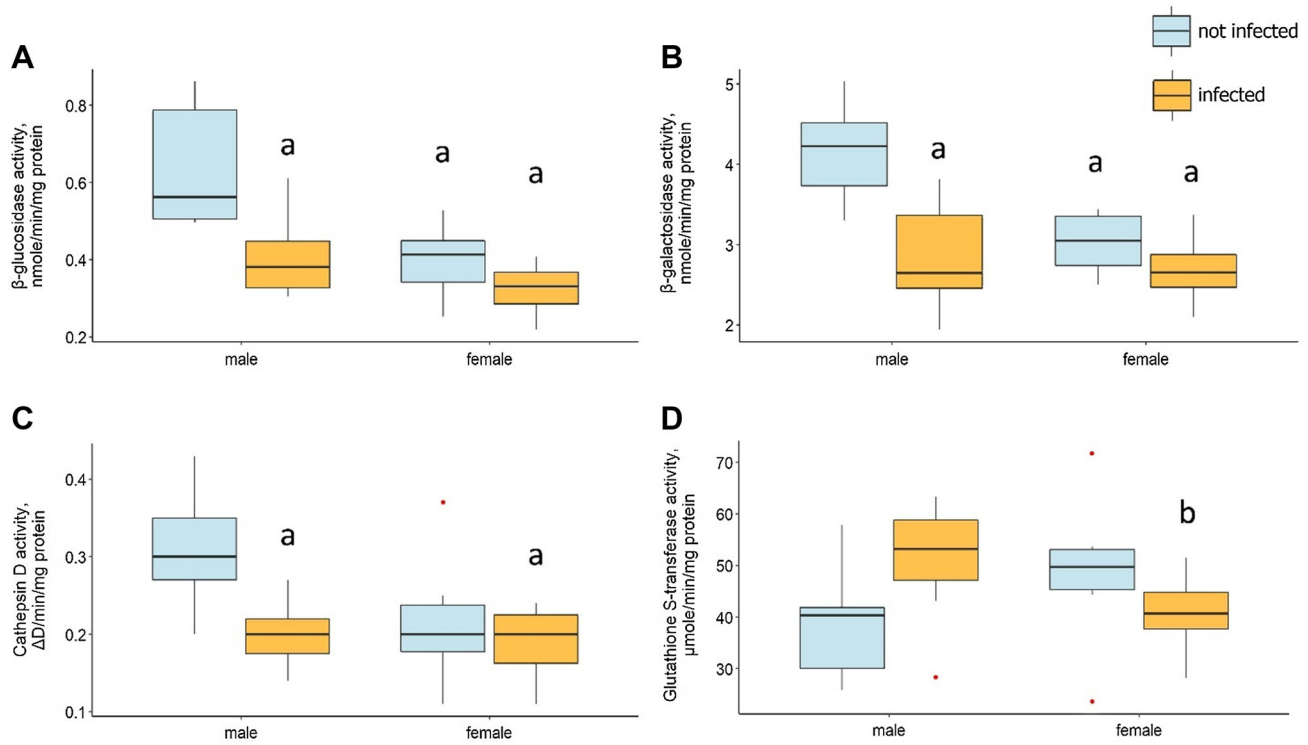

Fig. 1 Activity box plot of data of activity of β-glucosidase galactosidase (**A**), β-galactosidase glucosidase (**B**), cathepsin D (**C**) and Gglutathione S-transferase (**D**) in the liver of perch not infected (1) and infected (2) with *T. nodulosus* (median ± semi-interquartile range). **D** Liver of perch not infected and infected with *T. nodulosus*. a—The difference is statistically significant compared to uninfected males (p value < 0.05). b—The difference is statistically significant compared to uninfected females (p value < 0.05)

Table 3 Values of biochemical parameters in the liver of perch infected and not infected with *T. nodulosus* (median ± semi-interquartile range)

Biochemical parameter	Liver of uninfected perch		Liver of infected perch	
	Males	Females	Males	Females
Glutathione S-transferase (GST)	40 ± 6	53 ± 6	50 ± 4	41 ± 4[b]
Catalase (CAT)	63 ± 8	39 ± 14	48 ± 13	79 ± 19
Reduced glutathione (GSH)	0.11 ± 0.02	0.09 ± 0.03	0.09 ± 0.11	0.18 ± 0.02
Cathepsin B (Cat B)	0.44 ± 0.11	0.35 ± 0.07	0.48 ± 0.11	0.38 ± 0.08
Cathepsin D (Cat D)	0.33 ± 0.04	0.20 ± 0.03	0.20 ± 0.02[a]	0.20 ± 0.03[a]
β-Galactosidase	4.23 ± 0.39	3.05 ± 0.31[a]	2.65 ± 0.45[a]	2.65 ± 0.21[a]
β-Glucosidase	0.56 ± 0.14	0.41 ± 0.05[a]	0.38 ± 0.06[a]	0.33 ± 0.04[a]
Deoxyribonuclease (DNase)	0.022 ± 0.001	0.021 ± 0.003	0.021 ± 0.004	0.021 ± 0.001
Ribonuclease (RNase)	0.022 ± 0.005	0.018 ± 0.003	0.023 ± 0.008	0.018 ± 0.002
Cytochrome *c* oxidase (COX)	5.06 ± 0.28	3.05 ± 0.59	4.16 ± 1.01	5.03 ± 1.30
Lactate dehydrogenase (LDH)	38 ± 7	36 ± 3	37 ± 8	42 ± 2
Glucose-6-phosphate dehydrogenase (G6PDH)	47 ± 12	40 ± 5	30 ± 8	49 ± 16

[a]The difference is statistically significant compared to uninfected males

[b]The difference is statistically significant compared to uninfected females

The comparison of the studied biochemical parameters in the livers of fish with and without *T. nodulosus* plerocercoids showed there were no statistically significant differences for a majority of the indices, including the antioxidant defense (CAT, GSH), lysosomal proteases (Cat B) and metabolic enzymes (LDH, COX and G6PDH) (see Table 3).

Discussion

Hosts respond to parasitic infections in a variety of ways at different levels of organization, including immunological responses and physiological and biochemical alterations (Shishova-Kasatochkina and Leutskaya 1979; Sidorov et al. 1989; Sajid and McKerrow 2002; Dzik 2006). At the biochemical level, adaptations of the host aim to reduce the negative effect of the parasites' toxic excretions, as well as the byproducts of their own defensive reactions.

One universal, non-specific response to a variety of negative impacts, including parasitic invasion, is the intensification of oxidative reactions. To reduce the effects of the resulting oxidative stress, cells possess an antioxidant defense system that protects them against the harmful actions of reactive oxygen species (Hermes-Lima 2004; Jiang et al. 2014). This system includes both low-molecular compounds (glutathione, ascorbic acid, tocopherol, etc.) and antioxidant enzymes (catalase, glutathione peroxidase, glutathione S-transferase, superoxide dismutase, etc.) (Cecarini et al. 2007). The activity of antioxidant defense enzymes is known to be modified due to parasitic infections in fish (Dautremepuits et al. 2003; Rudneva et al. 2004).

We found no reliable modifications in the activity of commonly-recognized antioxidant defense markers, such as CAT and GSH, in the livers of perch infected with *T. nodulosus* plerocercoids compared to the fish bearing no helminth larvae in the liver. The components of antioxidant defense, in general, were shown to function normally. The fact that there was no GSH depletion for the neutralization of free radicals and reactive oxygen compounds also proves that the metabolic processes remained stable.

Hepatic GST activity in infected perch females was shown to be 20% lower than in uninfected females. A similar reduction in hepatic GST activity under helminth infection has been previously revealed in fish (Galtier et al. 1983; Skálová et al. 2007; Frank et al. 2011). It is believed that the parasites release special substances to suppress the host's immune response and antioxidant defense system. Thus, in perch females, a reduction in the activity of GST alone, while the rest of the antioxidant defense indices remain unchanged, is an indication of low peroxidation in the liver, possibly due to the suppression of antioxidant defense in host tissues by *T. nodulosus*.

The lysosomal proteases, cathepsin B and cathepsin D, are involved in the degradation and rearrangement of proteins inside cells (Nemova and Bondareva 2005; Lysenko et al. 2011), and their activation points to massive protein damage; for instance, due to oxidative stress. In addition, cathepsins participate in antigen presentation and are, thus, important actors in the immune response to infection. The upregulation of cathepsin D expression in the liver and other organs of fish was observed in a series of experiments where various fish were infected with pathogenic bacteria (Jia and Zhang 2009; Feng et al. 2011; Liu et al. 2012; Azizan et al. 2014).

In this study, hepatic cathepsin D activity was reduced in infected perch males; whereas in females, the activity of the enzyme did not change. Cathepsin B activity also did not vary among the studied fish. The minor lysosomal protease activity alteration in the infected fish agrees with the non-activation of antioxidant defense components, and indirectly suggests there was no significant oxidation-induced damage to proteins and membranes. As cathepsins B and D play important roles in the organism's immune response to pathogenic infections, signs of the inflammatory process were not observed in the hepatic tissue. According to histological studies, infection of perch with *T. nodulosus* usually induces only a slight and local immune response (Pronina and Pronin 1988; Dezfuli et al. 2014), which does not affect the overall functioning of the organ. A possible reason for the host's lack of aggressive response to the infection is that the parasite can suppress the immune response by secreting specific inhibitors of proteases and other biologically active molecules (Robinson et al. 2009; Lysenko et al. 2011; Chen et al. 2017).

Glycolytic lysosomal enzymes (β-glucosidase and β-galactosidase) cleave carbohydrate substrates (glycolipids, polysaccharides), detaching the carbohydrate component from glycoproteins and proteoglycans in cellular membranes (van der Spoel et al. 2000; Spiro 2002; Winchester 2005). These enzymes were found to be involved in the response to *T. nodulosus* infection in male perch, who exhibited a reduction in hydrolase activity when infected; while in females, no significant differences were observed. This modification of activity may point to a rearrangement of the metabolic pathways of carbohydrate degradation and utilization of alternative energy sources in fish males, as well as the slightly different energy demand of the two sexes (Fokina et al. 2015).

The noticeable variability of activity of β-galactosidase, β-glucosidase, cathepsin D, and glutathione S-transferase was found in the liver of control individuals (not infected) between fish of different sexes. In particular, variability was detected between males and females in the liver of perch in the controls (not infected fishes). It can be summarized that infection does not have such a strong effect that could lead to changes in the biochemical activity of these indicators beyond their natural variability. It can be assumed that the identified biochemical changes are insufficient to significantly affect the survivability of the healthy fishes, but may play a role in the selective survival of fish of one of the sexes under severe stress.

Acidic lysosomal nucleases (DNases and RNases) contribute to apoptotic cell death processes by fragmenting the nuclear material of cells (Miró et al. 1999; Counis and Torrigli 2000; Torriglia et al. 2000; Stoka et al. 2006). These enzymes also recycle nucleic acids damaged by reactive oxygen species during oxidative stress (Vysotskaya and Nemova 2008). In our study, the activity of lysosomal nucleases in perch livers was not modified by *T. nodulosus* larval infection, suggesting it probably had no effect on the stability of nucleic acids in hepatocytes.

Helminths are known to utilize their host's carbohydrates as their main source of energy (Barrett 2009), with saccharides depletion being a possible result. Carbohydrates can also be utilized by the fish themselves to supply energy for the operation of their defense systems. One can, therefore, expect that the parameters of carbohydrate metabolism in fish organs will be modified considerably in the case of a parasitic infection (Hassan et al. 2015).

In our study, the assessment of carbohydrate and energy metabolism enzyme indices failed to reveal any changes in the livers of perch infected with *T. nodulosus* larvae. There was no modification of activity of cytochrome *c* oxidase, lactate dehydrogenase (usually activated when glucose reserves are depleted) or glucose-6-phosphate dehydrogenase, which supplies the cell with energy and reducing equivalents; these, in turn, are channeled to the neutralization of oxidation products. Our results showed there were no significant changes in the rates of aerobic and anaerobic metabolism in perch when the liver contained one or two *T. nodulosus* plerocercoids. Accordingly, at this level of intensity of infection, the consumption of the host's carbohydrates by dormant larvae of the parasite is minor. Data from histochemical studies confirm that, when the infection rate is low (1–2 plerocercoids), the glycogen content in the fish's liver does not change. By contrast, with high infection intensities, hepatic glycogen stores are depleted, especially in cells closest to the cyst (Pronina and Pronin 1988). At the same time, a substantial reduction in hepatic glycogen and glucose stores has been previously reported by Izvekova (2001) by biochemical assay in yearlings of perch infected with *T. nodulosus*. However, in that study, the intensity of the infection in the examined fish was not provided, so it is difficult to assess how strong the negative impact was.

It was previously demonstrated that the main pathological changes in perch liver occur when the larvae penetrate into the organ, because this is when the parenchyma is physically damaged (Pronina and Pronin 1988).

Perch can become infected in the very first week after hatching, when the fry actively feed on procercoid-infected plankton (Kuperman 1973). After 2 years of age (and when more than 10 cm long), perch change their diet to greater consumption of benthos and prey. As a result, the rates of *T. nodulosus* infection in the fish gradually decrease with age, since, after several years of staying encysted, the parasites become resorbed; fish may still get re-infested when preying on other plerocercoid-infected fish or by consuming plankton-feeders with procercoid-bearing copepods in their intestines (Ieshko 1988; Pronina and Pronin 1988). The perch population surveyed in this study was aged 2–6 years; hence, the majority of the examined cysts had been in the liver for prolonged time periods. The fish in our study were probably past the most dangerous infection phases. After these phases, the host–parasite relationship reaches equilibrium, which is no longer associated with modifications in the investigated enzymatic systems.

The analysis of the investigated biochemical markers in the perch livers confirmed that the studied fish population is in a stable and balanced host–parasite relationship with *T. nodulosus*. The lack of pronounced response from components of the antioxidant defense system, energy metabolism enzymes, and lysosomal nucleases is probably due to the low infection intensity and pathogenic effect. The low level of infection intensity (not more than four plerocercoids per fish) observed in Lake Ladoga is typical for wild perch populations, unlike in other species (e.g., rainbow trout, burbot) where infection intensities may be from tens to hundreds of parasites (Kuperman 1973). The immune responses in the perch livers were limited to the immediate vicinity of the parasite's capsule, shown previously (Pronina and Pronin 1988; Dezfuli et al. 2014). Together with the low infection rate, this indicates a high degree of mutual adaptation of the perch and the encysted *T. nodulosus* to each other. One of the possible mechanisms of low immune reactivity of plerocercoids is the release of biologically-active molecules that stimulate the production of a fibrous capsule by the host and suppress the immune response (Liu et al. 2006; Hewitson et al. 2009; Robinson et al. 2009; Coakley et al. 2016; Chen et al. 2017). As the perch is one of the most common second intermediate hosts for *T. nodulosus,* this adaptation is probably resulting in the prolonged co-evolution of these two species.

Conclusions

In this study, *Triaenophorus nodulosus* infection in 2 to 6-year-old perch from Lake Ladoga (infection intensity: 1–4 plerocercoids) was shown to have minor effects on the studied components of the antioxidant defense system, lysosomal enzymes, and carbohydrate metabolism enzymes in the liver of the fish. Hence, this level of infection can be regarded as moderate in terms of its effect on the health of the host fish. The variation in biochemical

reactions in response to the parasitic infection in male and female perch confirms the different resistance to the potentially stressful conditions between genders. Lysosomal β-glucosidase, β-galactosidase, cathepsin D, and GST enzymes are presumably involved in the host's adaptation to co-existence with the parasite. These parameters need to be studied further to determine their potential as markers of infection.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical standards All procedures followed were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) and the 8th Edition of the Guide for the Care and Use of Laboratory Animals (NRC 2011). All guidelines for use of animals were followed. This article does not contain any studies with human.

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