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ECOLOGICAL PHYSIOLOGY AND BIOCHEMISTRY OF HYDROBIONTS

Activity of Digestive Enzymes in Perch Infected with *Triaenophorus nodulosus* (Pallas) Plerocercoids

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Abstract—It is revealed that the infection of older groups of perch with *T. nodulosus* plerocercoids reduces the activity of enzymes, ensuring the initial stages of the assimilation of protein components in fish food. The infection does not affect the activity of glycosidases. The infection changes the ratio of activities of the above groups of enzymes and possibly reduces the efficiency of fish feeding. In addition, the proportion of serine proteinases and metalloproteinases decrease, while the percentage of unidentified proteases significantly increases in the gut of infected fish.

Keywords: fishes, cestodes, *Triaenophorus nodulosus*, plerocercoid, digestive enzymes

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INTRODUCTION

Tapeworms of g. *Triaenophorus* are widespread parasites of freshwater fish. A complicated cycle of development with a change in the terminal and two intermediate hosts living in water is a characteristic of these parasites. The most common representative of the genus, *Triaenophorus nodulosus* (Pallas), possesses a wide range of secondary intermediate hosts belonging to 17 families. Some species of g. *Triaenophorus* are a serious threat for fisheries; at the plerocercoid stage they cause mass diseases and mortality in fish, mainly in fish farms [7]. Perch *Perca fluviatilis* L. is one of the intermediate hosts of *T. nodulosus*, with plerocercoids localizing in the fish liver.

The age-related dynamics of infection of the secondary intermediate hosts with *T. nodulosus* differ in various waterbodies. For instance, in Lake Baikal a high level of infection of perch yearlings was recorded, which is determined by the feeding of this fish with procercoid-infected copepods. In some cases, an increase in the infection rate was registered in the elder fish groups [9]. The authors explain this fact by specific features of perch biology in different waterbodies and by their trophic relations with the first intermediate hosts. According to these researchers, the infection of the elder fish occurs both due to the feeding of perch on zooplankton and on planktivores, including their own juvenile specimens, with the intestines filled with copepods.

It is accepted that, under optimal conditions, the effect of parasites on the physiological parameters in hosts is inconsiderable. However, the parasites may be an important factor of natural selection in specific environmental conditions or under stress. For example, in an aquatic environment, parasites are a strong biotic factor of natural selection under the acceleration of a water current or rise in temperature. A parasite-related load strongly affects tolerance to high environmental temperature and stamina in fish [22].

The data on the effects of *T. nodulosus* plerocercoids on the physiological status in the intermediate hosts are scarce and are available mainly for perch yearlings [4, 19]. The studies applying histological methods revealed that the penetration of *T. nodulosus* plerocercoids into perch liver and the formation of capsules cause weakly manifested histopathological changes related to the damage and pressing out the parenchymal cells. A bilayered capsule around the plerocercoids does not hamper their normal development and is formed in the liver 15–20 days following infection [9].

The goal of this paper is to assess the effect of the influence of infection with *T. nodulosus* plerocercoids on the activity of digestive hydrolases in perch of the elder age group.

MATERIALS AND METHODS

Specimens of perch *Perca fluviatilis* (14 specimens, body length 15.5–28.1cm, and body weight 67.8–424.4 g) sampled in September 2015 in Lake Ladoga

were studied. The intensity of infection of the fish with *T. nodulosus* was determined.

The fish were caught by net (14–30 mm mesh) and immobilized by cutting the brain with a scalpel. After that the abdominal cavity was opened on the cold surface and the intestine was removed, frozen in a Dewar flask with liquid nitrogen (–80°C), and stored before further examination.

The intestines were dissected and the chyme was discarded. To study the activity of digestive hydrolases, the homogenates of whole perch intestines were prepared in an ice bath using a glass homogenizer (Sartorius AG, Germany). The homogenates were dissolved with Ringer's solution for poikilotherms (6 g NaCl, 0.14 g KCl, 0.54 g Na_2HPO_4, 0.02 g KH_2PO_4, 0.16 g $MgSO_4$, and 0.5 mL of 10% $CaCl_2$ solution in 1 L of distilled water; pH 7.4) at 1 : 49 ratio. The homogenates were centrifuged at 9000 rpm for 5 min at a temperature of 4°C.

The total activity of proteinases (activity of trypsin EC 3.4.21.4, chymotrypsin EC 3.4.21.1, and dipeptidases EC 3.4.13.18) was determined using 0.3% azocasein solution in a Tris buffer, pH 7.5, as a substrate [13]. A substrate with an enzymatically active preparation was incubated for 60 min at 20–22°C. The reaction was stopped by adding 1 mL of 0.3 N trichloroacetic acid (TCA) solution; the precipitate of nonhydrolyzed protein was removed by centrifugation at 9000 rpm for 5 min.

To identify various subclasses of proteinases in the gut homogenates, the following inhibitors (50 μL) were used: (1) phenylmethylsulfonyl fluoride (PMSF), an inhibitor of serine proteinases at a concentration of 100 mM in dimethylsulfoxide (DMSO); (2) ethylenediaminetetraacetic acid (EDTA), an inhibitor of metalloproteinases at a concentration of 0.5 M in 1 M NaOH.

The units of proteolytic activity (EA, ΔE_{440} g^{-1} min^{-1}) were calculated according to the equation

$$EA = \Delta \times 50 / m_к T,$$

where Δ stands for the difference between the spectrophotometer readings of the sample with substrate versus the blank sample (at a wave length of 440 nm); 50 is the final dilution of homogenate (by a factor of 50); m_i is the mass of the intestine, g; and T is the duration of incubation, min. The relevant volume of Ringer's solution was added to the blank sample instead of the enzymatically active preparation.

The amylolytic activity reflecting combined activities of the starch-hydrolyzing enzymes (α-amylase EC 3.2.1.1, glucoamylase EC3.2.1.3, and maltase EC 3.2.1.20) and the activity of sucrase (EC 3.2.1.48) was determined by an increase in hexoses according to the modified Nelson's method [11]. Upon determining amylolytic activity, a 1.8% solution of dissolvable starch was used as a substrate; upon determining sucrase, a 100 mM sucrose solution was used. Both substrates were prepared using Ringer's solution, pH 7.4. The activities of enzymes (EA) were expressed in micromoles of glucose produced after 1-min-long incubation of the enzymatically active preparation and substrate per 1 g wet tissue weight (μmol / (g min)). All biochemical tests were performed in three replicates.

The intensity of developing staining was determined on a Lambda 25 (PerkinElmer) spectrophotometer at a wavelength of 440 nm for proteinases and 560 nm for amylolytic activity and activity of sucrase.

The results are presented as means and standard errors of means ($M \pm m$). The significance of differences was assessed using a nonparametric Mann–Whitney test.

RESULTS

The plerocercoid of *T. nodulosus* was found in the liver of five perches. Body lengths and weights of both infected and uninfected fish (9 ind.) differed insignificantly: 18.73 ± 0.96 cm and 135.16 ± 21.98 g versus 20.4 ± 2.0 cm and 179.08 ± 64.79 g, respectively.

The activity of proteinases in the uninfected fish was significantly higher than in the infected (Z = 2.373, p = 0.018). In addition, the effects of inhibitors on the activity of proteinases differed depending on the infection (Fig. 1). Based on the data on the effects of inhibitors on the activity of proteinases, we calculated the shares of various subclasses of proteinases functioning in the guts of uninfected and infected perch (Fig. 2). In the infected fish, the share of serine proteinases drops from 70 to 58%; that of metalloproteinases drops from 27 to 5%. At the same time, the share of nonidentified proteinases in the infected fish increases from 1 to 36%.

Infection with *T. nodulosus* plerocercoids did not significantly affect the activity of glycosidases in the perch intestine; only the activity of amylases declined inconsiderably. The activity of sucrase remained unchanged (Fig. 3).

The values of coefficients of enzymatic activities in the studied groups of fish were calculated (Table 1). In the fish infected with plerocercoids, all values of these coefficients were higher than in the uninfected. However, if the A/S was only slightly higher (1.1 times), the values of A/P and S/P, were higher by factors of 5.5 and 5.2, respectively. This indicates changes in the proportions of the activities of enzymes of these groups upon infection.

DISCUSSION

Perch, the most abundant fish species in Lake Ladoga, diverges in two ecological forms: coastal and deepwater (pelagic). These forms differ in their feeding spectra, growth rate, and fecundity. By the age of 4+ to 5+, the body length of perch reaches 19–22 cm [3].

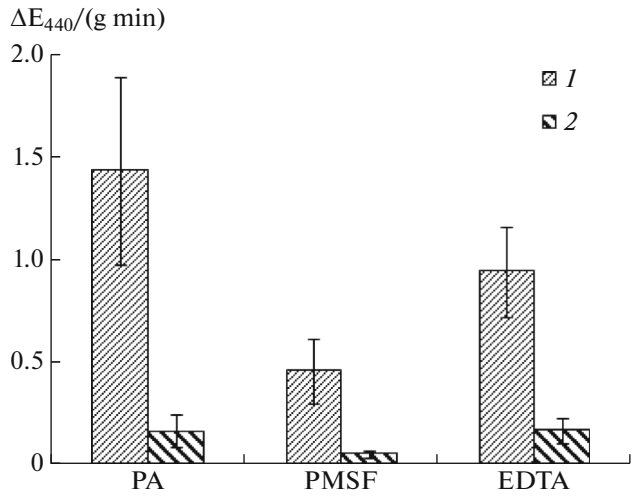

Fig. 1. Proteolytic activity (ΔE_{440} g^{-1} min^{-1}) in perch intestine depending on the infection with *T. nodulosus* plerocercoids and the influence of proteinases inhibitors on this activity: PA, total proteolytic activity; EDTA, inhibitor of metalloproteases; and PMSF, inhibitor serine proteinases. (*1*) Noninfected; (*2*) infected.

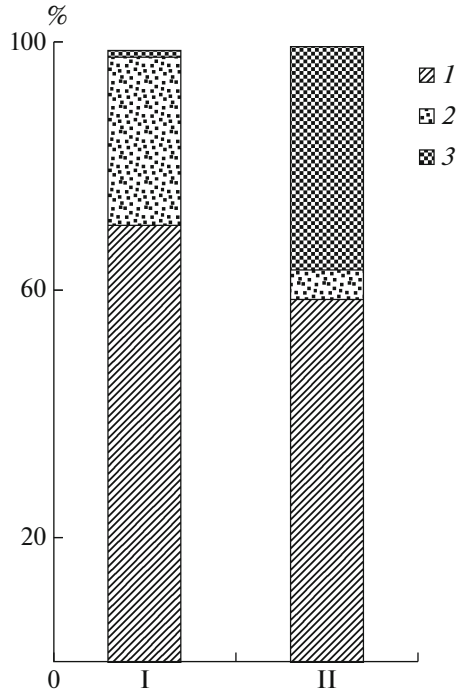

Fig. 2. Shares of various subclasses of proteinases in the intestine of perch (%) depending on the infection with *T. nodulosus* plerocercoids. (*1*) Serine proteinases, (*2*) metalloproteinases, and (*3*) unidentified proteinases. (I) Noninfected; (II) infected.

The Rybinsk Reservoir is also inhabited by two ecological forms of perch (pelagic and littoral) differing in spatial distribution, number, commercial value, and availability for fishing. The body sizes in these forms may differ; this species reaches a body length of 23–24 cm by the age of 3+ to 4+ [2].

Some researchers studying the large number of perches in the Lake Baikal basin have noted a trend toward a decrease in body length and weight in the fish of various ages infected with *T. nodulosus* when compared with uninfected individuals [9]. If the group sample was small, the differences were inconsiderable; however, with an increase in the number of studied fish, the difference became more pronounced. It is likely that the absence of length–weight differences between uninfected and infected perch revealed in the present study relates to the small sample size. In the lakes of Karelia, perch, as one of the main food items in pike ration (along with ruff), plays a leading role in sustaining the number of *T. nodulosus*, and predatory (piscivorous) perches of elder age groups serve as reservoir hosts for *T. nodulosus* [1].

The plerocercoids localize in the liver, where the parasites incapsulate by overgrowing connective tissues around them. The production of a capsule serves as a protective response of a host to the invasion of a parasite [7]. According to certain published data [19], the plerocercoids in the perch liver may survive for no more than 2 years. It was noted that the level of mutual adaptation of the plerocercoids to the host perch differs in different populations of the latter, and this difference is determined by the type of helminth circulation in the specific ecosystems [9]. The response of the host is of direct but not proportional relation to the infection intensity: the less specific a parasite is to a host, the harder the pathological changes that it triggers are, even at low infection intensity [8]. Owing to the high activity of the capsule walls formed around a

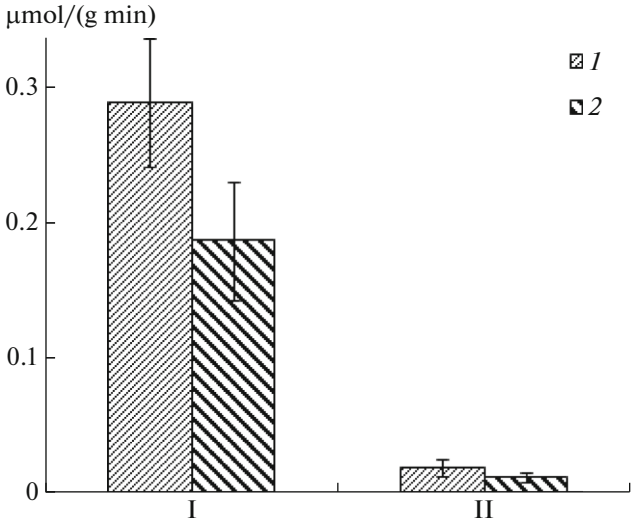

Fig. 3. Activities of glycosidases (μmol/(g · min)) in the intestine of perch (%) depending on the infection with *T. nodulosus* plerocercoids. (I) Activities of amylases; (II) sucrase activity. (*1*) Noninfected; (*2*) infected.

Table 1. Values of the coefficients of enzymatic activities in perch noninfected and infected with *T. nodulosus* plerocercoids

Fish group	A/P	S/P	A/S
Noninfected	0.20	0.01	16.43
Infected	1.11	0.06	17.53
Ci/*Cn*	5.6	6.0	1.1

A/P, ratio of activities of amylases to activities of proteases; S/P, activity of sucrase to activities of proteases; A/S, activities of amylases to activity of sucrase; and *Ci*/*Cn*, ratio of values of coefficients of activities of enzymes.

parasite in the host liver, plus to the abundance of capillaries, the capsule serves as a semipermeable cover that, on the one hand, provides favorable conditions for the feeding, growth, and development of a parasite; on the other hand, it reliably protects from the effect of the host tissue [7].

It was revealed [4] that the infection of the perch yearlings with *T. nodulosus* plerocercoids seriously affects the state of the host liver. The invasion leads to a 42% increase in the liver weigh and a 42 and 34% decrease, respectively, in glucose and glycogen contents, indicating the intensive use of carbohydrates in the fish in response to the invasion.

A decrease in the activities in perch infected with plerocercoids accords to the data [19] on the effect of infection on the activities of hydrolases in the intestine of perch yearlings. It was shown that, in the yearlings infected with *T. nodulosus* plerocercoids, the activities of proteolytic and glycolytic enzymes are lower than in noninfected ones. The decrease in the activities of digestive hydrolases is especially noticeable in the foregut.

In many fish species, the pancreas is closely fused with liver. This is why both organs are considered hepatopancreas, even though their cells are independent from each other. The cells of the pancreas secrete proteolytic enzymes in the inactive form (trypsinogen, chymotrypsinogen and A and B procarbopeptidases), as well as amylase, lipase, and nucleases in the active states, into the intestine [10]. In addition, in fish, like in other animals, membrane digestion plays an in important role in the digestion of food. This digestion takes place on the structures of the brush border of enterocytes with the participation of the pancreatic and specifically membrane enzymes adsorbed from the intestine cavity [12]. The hydrolysis of proteins and polysaccharides takes place mainly in the intestine cavity, while the hydrolysis of disaccharide takes place predominantly on the structures of intestine mucosa. It is found that sucrase is closely bound to the membrane of the enterocyte brush border and up to 97–100% of its activity is manifested in the fish intestine mucosa [12]. A decline in the activities of hydrolases both in yearlings and adult perch may be related to the impaired functioning of hepatopancreas in the infected fish and, consequently, to the decrease in the synthesis and secreting of the zymogens of proteolytic enzymes and amylase to the intestine. However, infection with plerocercoids does not affect the activity of sucrase, specifically the membrane enzyme.

An inhibitory analysis of the enzymes is one informative tool allowing for the assessment of the contribution of various subclasses of proteinases in the total proteolytic activity in the digestive tract [23–25]. With this method, differences in the spectra of proteinases in noninfected and infected fish and the prevalence of activities of serine proteinases (trypsin, chymotrypsin, and elastase) were revealed [24]. The data presented in [14, 15, 20, 21, 24] indicate the considerable variability of the share of serine proteinases in the total proteolytic activity in various fish species. A decrease in the share of serine proteinases, a slight decline in the activities of amylases (pancreatic enzymes), and a lack of changes in the sucrase activity in the infected perch confirm the above suggestion on the dependence of the activities of intestinal hydrolytic enzymes on the impaired functioning of hepatopancreas. As was revealed in some fish species, infection with adult cestodes change the spectrum of proteinases [6].

The inhibition of the total proteolytic activity by EDTA indicates the presence of proteinases that require metal ions for functioning. The data on the activities of metalloproteinases for various fish species differ, varying from trace amounts to quite large values [17, 23].

In addition, in the infected adult perch, like in the yearling studied earlier [19], the proportion of the activities of enzymes changes. However, in most cases, in yearlings the value of G/P coefficient (the ratio of the activities of glycosidases including the activities of pancreatic amylases and sucrase to the activities of proteinases) decreases twofold [19], indicating a stronger effect of infection with *T. nodulosus* on the synthesis of glycosidases when compared to proteinases in the fish of this age. On the other hand, in infected adult perch, the values of the A/P and S/P coefficient increase considerably. In our opinion, this relates to the age-related changes in perch feeding. In Lake Ladoga, perch turns to predation at the age of 7+, while younger fish exhibit mixed feeding [3]. In the Rybinsk Reservoir, younger perch feeds mainly on various invertebrates and turns to feeding on fish at the age of 2+ to 4+ (depending on habitat) [2].

It is likely that the more pronounced effect of infection with plerocercoids on the activities of proteinases when compared to the activities of amylases in adult perch relates to the higher sensitivity of its proteolytic enzymes to the impact of various factors. It was revealed [5] that the activities of proteinases in fish

upon infection with cestodes change more strongly than the activities of glycosidases. In addition, it was shown that intestinal proteinases in some fish species are more sensitive than glycosidases to the influence of organic pollutants [18] and heavy metals [16].

CONCLUSIONS

The infection of elder perch with *T. nodulosus* plerocercoids decreases the activities of enzymes providing the initial stages of assimilation of the protein components in the fish food, but does not affect the activities of glycosidases. In turn, this leads to the changes in the proportion of the activities of these enzyme and presumably decreases the efficiency of fish feeding. In addition, it was revealed that the shares of serine proteinases and metalloproteinses decrease, while the share of the unidentified proteinases in the intestine of infected fish rose considerably. The data of the present study adds to knowledge on the regularities of parasite–host relations and on the influence of infection with plerocercoids on the intermediate host.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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