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HEMATOLOGICAL PARAMETERS OF EUROPEAN PERCH (*Perca fluviatilis* Linnaeus, 1758) ASSOCIATED WITH PARASITIC INVASIONS

NGUEN THI HONG VAN, S.V. PONOMAREV, Yu.V. FEDOROVYKH ✉, Yu.N. GROZESKU, A.A. BAKHAREVA, I.V. VOLKOVA, V.I. EGOROVA, Yu.V. SERGEEVA

Astrakhan State Technical University, 16, Tatishcheva st., Astrakhan, Russia 414056, e-mail: Hongvannguyen@mail.ru, ya.panama2011@yandex.ru, jaqua@yandex.ru (corresponding author ✉), grozesku@yandex.ru, bahareva.anya@yandex.ru, gridasova@mail.ru, lekaego@mail.ru, js4444@mail.ru
ORCID:
Nguen Thi Hong Van orcid.org/0000-0002-6910-2705
Ponomarev S.V. orcid.org/0000-0002-2899-8672
Fedorovykh Yu.V. orcid.org/0000-0003-0789-1566
Grozesku Yu.N. orcid.org/0000-0002-6691-8696
Bakhareva A.A. orcid.org/0000-0002-7724-2425
Volkova I.V. orcid.org/0000-0001-8945-6669
Egorova V.I. orcid.org/0000-0002-7688-723X
Sergeeva Yu.V. orcid.org/0000-0001-8811-1378
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Abstract

European perch (*Perca fluviatilis* Linnaeus, 1758) is widespread in water basins and has a commercial value. However, in the natural environment, fish are often infected with invasive diseases, which exclude its processing for food purposes. Being in the body of fish, parasites produce toxic substances that affect various organs of fish. Widespread in the Volga Delta pathogenic species, *Eustrongylides excisus*, in large quantities occurs in perch in larval state in the abdominal cavity of the body, in the walls of the intestine, in the liver, in the calf and in the spinal muscle tissue, reaching up to 30 or more parasites per fish. Sexual maturity of the parasite occurs in the stomach of the birds. This disease is peculiar to perch species; larvae of this nematode cause granulomatous inflammatory reaction. In addition, these worms, penetrating deep into the muscles of the perch, spoil the commercial quality of fish, which leads to the culling of fish raw materials and rejection of foodstuff. This work, for the first time, compares the hematological status of infected and healthy perch of natural populations. The work aimed to assess biological and physiological effects of various parasitic invasions on European perch *Perca fluviatilis* (Linnaeus, 1758). Fish of both sexes at the age from 0 to 4 years were caught in natural reservoirs of the Lower Volga (Bolshaya Bolda and Bely Ilmen rivers, Astrakhan region) in September 2016 to June 2019. The total body length, the length to the end of the scale cover, body weight and age were determined, and the Fulton's fatness coefficient was calculated. The skin, fins, mouth, gills, eyes, heart, abdominal cavity, muscles, brain, and spinal cord of fish were examined for parasitological contamination. Blood for analysis was taken from the tail vein in vivo. Nematodes were found in more than 55 % of the caught perch. Nematodes were found in the liver (30 %), abdominal cavity (45 %), intestines (10 %), muscles (15 %), and gills (5 %). Parasites were found free within the body cavity, or encapsulated, with *Eustrongylides excisus* being the most abundant. In the infected fish, the average body weight and body condition coefficient decreased as compared to the healthy fish of the same age. However, the revealed growth retardation in the infected perch was statistically insignificant ($p > 0.05$), while the body condition coefficient was significantly lower than in uninfected fish ($p < 0.01$ for 3-year-old perch). The parasitic infestation of *P. fluviatilis* with *Eustrongylides* nematode caused symptoms of anemia, suppression of erythropoiesis (i.e., a decrease in the proportion of blast forms of the erythrocytic cell series), an increase in the proportion of neutrophils, monocytes, and lymphoblasts in the leucocyte count. In infected perch, the number of red blood cells significantly decreased ($83.01\times10^4\pm4.17\times10^4$ vs. $137.22\times10^4\pm5{,}26\times10^4/\text{mm}^3$), as well as the concentration of total protein (40.81±1.19 vs. 48.97±2.07 g/l) and blood cholesterol (5.17±0.28 vs. 6.81±0.30 mmol/l). MCH (mean corpuscular hemoglobin), erythrocyte sedimentation rate, the total number of leukocytes and platelets also increased compared to uninfected fish. The level of pathological red blood cells increased in the infected perch (9,17±0,23 % vs. 4,87±0,11 %). Changes in cell cytoplasm and nucleus, degenerative changes in the cell, changes associated with cell division were the main types of the discovered cell pathology of the infected perch.

Keywords: *Perca fluviatilis*, European perch, parasites, nematodes, hematological parameters, anemia, eustrongylidosis, blood

In terms of cultivation and catch, European perch (*Perca fluviatilis* Linnaeus, 1758) is inferior to salmon (*Salmonidae*) and cyprinids (*Cyprinidae*). The increase in its number is mainly due to the introduction and natural reproduction. For example, in Sweden, European (river) perch is widespread in many water bodies. In Denmark, about 350 thousand two-gram juveniles of this species produced annually by aquaculture methods are exported to Switzerland and Ireland. In Ireland, river perch became a potential aquaculture target as early as in 1995 [1-4]. Importantly, the volume of farmed marketable river perch over the last three years has doubled as compared to previous years. The main producers of European perch are France, Czech Republic, Netherlands, Ukraine, Tunisia, Australia [1, 5].

In Russia, until now, *P. fluviatilis* was considered trash and a low-value commercial fish. This is largely due to the fact that in natural water bodies the perch is largely infested with various parasites, especially the nematodes *Eustongylides* and *Anisakis* [6-10]. Such fish often remains sterile [11], lags behind in growth and development, and its weight is 20-25 % lower than that of uninfected individuals [12].

Changes in culinary habits, the globalization of food supplies, the development of tourism, the spread of alien species are the reasons of a threat the nematodes in fish pose to human health which should not be underestimated [13-15].

Fish farming industry enables rearing healthy river perch free from parasites. However, the farms operating without breeding their own replacement brood stock have to catch producers from natural populations for the subsequent spawning campaign. Hence, there is a need to assess fish-breeding biological parameters and physiological status of female and male perch during their appraisal.

In this study, we have established for the first time a number of hematological and biochemical parameters confirming the physiological status of infected perch from natural populations. The data obtained can be the basis for the development of the physiological and immunological indicators of the European perch both in nature and when rearing in artificial conditions, including within the framework of selection and breeding programs.

The aim of the work was to investigate how parasitic invasions affects biological and physiological characteristics of the European perch *Perca fluviatilis* L. to identify indicators that can be used to assess the health status of fish reared both for market and for the brood stock replacement.

Materials and methods. For the study carried out at the Innovation Center “Bioaquapark — the Scientific and Technical Center of Aquaculture” (Astrakhan State Technical University), the European perch *Perca fluviatilis* (Linnaeus, 1758) individuals of both sexes at the age from 0 (underyearlings) to 4 years (four-year-olds) were caught from natural reservoirs of the Lower Volga (Bolshaya Bolda and Bely Ilmen rivers, Astrakhan Province) from September 2016 to June 2019.

The absolute body length, the length to the end of the scale cover, the body weight and age of the fish were recorded, and the Fulton’s condition factor was calculated. The age was determined by the annual scale rings [16]. Parasitological examination of skin, fins, mouth, gills, eyes, heart, abdominal cavity, muscles, brain and spinal cord was performed by a standard technique [17]. Parasites were identified to species using keys and figures [18, 19]. The intensity and extensiveness of the invasion were calculated according to the description [20].

Blood for analysis was taken from the tail vein in vivo [21]. Blood smears fixed with 95 % ethanol were stained with hematoxylin and eosin according to Romanovsky (Guidelines for conducting hematological examination of fish: collection of instructions for combating fish diseases. Part 2. Moscow, 1999) and examined under a light microscope (BIOMED 6 LED, LLC Biomed, Russia) with immersion (×100). For microphotography, a DMC-510 photo equipment (Panasonic, Japan) was used. For differentiation of the formed elements, the Ivanova's classification (1983) was applied. Morphologically altered erythrocytes were examined [21, 22]. The hemoglobin concentration was measured by the hemoglobin cyanide method using a KFK-3 photoelectric colorimeter (JSC ZOMZ, Russia). Erythrocytes (the number per 1 ml blood) were counted in a Goryaev chamber. The hemoglobin concentration per erythrocyte was calculated. Erythrocyte sedimentation rate (ESR) was assessed using a Panchenkov's apparatus (Methodological instructions for carrying out hematological examination of fish: collection of instructions for combating fish diseases. Part 2. Moscow, 1999).

The indicators of protein and lipid metabolism in healthy and invaded fish were determined by optical densitometry against calibration solutions (a KFK-3 photoelectric colorimeter, Russia). The total protein concentration was measured by the biuret reaction. In biuret test, 0.1 ml of non-hemolyzed serum was poured in tubes with 5 ml of the biuret reagent. The cholesterol concentration was measured colorimetrically as described [23].

The results were processed statistically using Microsoft Excel 2010 and JASP-1 programs (https://jasp-stats.org). The mean values (*M*), standard deviations (±σ), standard errors of the mean (±SEM) were calculated. To confirm the significance of the differences, the Student's t-test was used [24].

Results. Nematodes were found during dissection in more than 55 % of the caught river perch (Table 1). In 5 %, parasites were detected only with a microscope. In infected fish, nematodes colonized the liver (30 %), abdominal cavity (45 %), intestines (10 %), muscles (15 %), and gills (5 %). In the internal organs and in their membranes, parasites were both free and encapsulated, with *Eustrongylides excisus* being the most abundant (see Table 1).

1. Nematode infestation in European perch (*Perca fluviatilis* Linnaeus, 1758) caught from the Bolshaya Bolda and Bely Ilmen rivers (Lower Volga, Astrakhan Province, 2016-2019)

Species	Localization	Extensiveness of invasion, %	Intensity of invasion, the number of nematodes
Anisakis schupakovi	Abdominal cavity, intestines, visceral fat	51	1-19
Eustrongylides excisus	Abdominal cavity, stomach, liver, muscles, visceral fat	60	5-34
Camallanus lacustris	Visceral fat, intestines	30	1-7

Nematodes of *Eustrongylides* spp. inhabit muscles, body cavities, and internal organs of fish [25, 26], including in *P. fluviatilis* [9, 27]. Pathohistological changes in *P. fluviatilis* upon invasion of this parasite are muscle degeneration and necrosis which spread to the sarcoplasm, basal lamina of the sarcolemma, endomysial cells of connective tissue, and capillaries [27]. Immunohistochemical staining revealed numerous proliferating cells in the thickness of the capsule and in the immediate vicinity of the larva of *Eustrongylides* sp., which, as the authors suggest, indicated the initiation of restoration of the muscle damaged by the nematode [27]. Guagliardo et al. [25] described similar histopathological sings, muscle atrophy and chronic inflammatory response in Galaxias maculatus.

Our observations showed that *P. fluviatilis* infestation with *Anisakis schupakovi*, *Eustrongylides excisus*, and *Camallanus lacustris* decreases the average body weight and body condition coefficient compared to healthy fish of the same age. However, the revealed growth retardation in sick individuals was statistically insignificant ($p > 0.05$), while the body condition coefficient in infected fish was significantly lower than in uninfected ($p < 0.01$) (Table 2).

2. Biological parameters in European perch (*Perca fluviatilis* Linnaeus, 1758) of different ages invaded and uninvaded with parasitic nematodes (*M*±SEM, Bolshaya Bolda and Beliy Ilmen rivers, Lower Volga, Astrakhan Province, 2016-2019)

Indicator	Two-year-olds		Three-year-olds	
	invaded (n = 17)	uninvaded (n = 7)	invaded (n = 17)	uninvaded (n = 7)
Body weight, g	47.21±4.19	54.33±5.14	91.77±7.85	106.20±10.06
Absolute body length, cm	15.36±0.32	16.89±0.41	19.68±0.60	20.11±0.72
Length to the end of the scale cover, cm	13.34±0.41	13.92±0.37	16.92±0.47	17.30±0.56
Fulton's condition factor	1.99±0.08	2.01±0.07	1.89±0.09*	2.05±0.10

* The differences between infected and uninfected fish are statistically significant at $p < 0.01$.

In infected fish, a decrease in the hemoglobin concentration (42.35±2.07 g/l) was detected compared to healthy fish (48.40±2.35 g/l), however, these differences were insignificant ($p > 0.05$) (Table 3).

3. Hematological and biochemical parameters in European perch (*Perca fluviatilis* Linnaeus, 1758) invaded and uninvaded with parasitic nematodes (*M*±SEM, Bolshaya Bolda and Bely Ilmen rivers, Lower Volga, Astrakhan Province, 2016-2019)

Indicator	Invaded fish (n = 17)	Uninvaded fish (n = 7)
Hemoglobin, g/l	42.35±2.07	48.40±2.35
Erythrocyte sedimentation rate (ESR), mm/h	4.50±0.12**	3.34±0.22
Total erythrocyte counts, $\times 10^4$/mm^3	83.01±4.17**	137.22±5.26
Mean corpuscular hemoglobin, MCH, pg	51.03±2.12*	28.34±1.91
Total protein, g/l	40.81±1.19**	48.97±2.07
Cholesterol, mmol/l	5.17±0.28**	6.81±0.30

*, ** The differences between infected and uninfected fish are statistically significant at $p < 0.05$ and $p < 0.01$m respectively.

These data are consistent with available publications. In *Cyprinus carpio* with parasitic invasion, a significant decrease in the hemoglobin concentration was also reported [28]. In hematological studies of *Sander lucioperca* inhabiting the Anzali wetlands (Iran), the hemoglobin concentration increased compared to uninfected fish, but the differences were also insignificant [29]. Many authors reported that the European perch has significant resistance to abiotic and biotic environmental factors, including parasitic invasion [30-34], therefore, the blood hemoglobin concentration changes ambiguously during infection.

The blood erythrocyte counts in infected perch significantly decreased and amounted to $83.01\times10^4\pm4.17\times10^4$ cells/mm3, while in healthy fish this indicator was $137.22\times10^4\pm5.26\times10^4$ cells/mm3 ($p < 0.01$). In the works of other researchers, a decrease in the erythrocyte counts was noted not only in the carp, but also in the African catfish *Clarias gariepinus*, when infected with parasites, which indicated anemia [35]. Due to the decrease in the number of red blood cells, the mean corpuscular hemoglobin (MCH) increased. Differences in this indicator between infected and healthy river perch were significant (51.03±2.12 vs. 28.34±1.91 pg, $p < 0.01$). The ESR also increased (4.50±0.12 vs. 3.34±0.22 mm/h, $p < 0.01$). An increase in ESR, firstly, is a consequence of a decrease in the number of erythrocytes, and secondly, it indicates the development of an inflammatory process in the fish under the influence of parasites [36, 37]. Study of the *Eustrongylides* sp. invasion in the *Channa punctatus* showed that the mean erythrocyte count, hematocrit and hemoglobin concentration were significantly higher ($p < 0.01$) in uninfected fish, while the MCH value significantly increased ($p < 0.01$) in infected fish [26].

In our study, the concentration of total blood protein in healthy perch

P. fluviatilis was 48.97±2.07 g/l while in infected individuals it decreased to 40.81±1.19 g/l ($p < 0.01$). In *C. punctatus*, significant differences in total protein and glucose concentrations were also noted between fish uninfected and infected with *Eustrongylides* sp. [26]. The same authors showed a significant ($p < 0.01$) increase in the activity of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and the cholesterol level in fish infected with *Eustrongylides* sp. compared to uninfected fish [26].

The results of microscopic examination of blood smears showed that the proportion of blast and young cells of the erythrocyte lineage decreased in infected perches (0.47±0.04 %) compared to healthy perches (6.36±0.23 %). This indicates the inhibition of erythropoiesis under the stress effect of the parasite. The proportion of pathologically altered erythrocytes in blood smears increased to 9.17±0.23 % in infected fish vs. 4.87±0.11 % in healthy fish ($p < 0.01$). This increase was mainly due to poikilocytes and schizocytes. This appeared to result from a decrease in the strength of the cell membrane which was a symptom of anemia. Chiocchia et al. [38] showed that under parasitic invasion, swelling of erythrocytes occurred due to a change in the osmotic gradient.

Many works have shown that, upon parasite invasion, the most significant changes occur in the leukocyte formula and blood leukocyte counts [26, 33, 39, 40]. Our study revealed an increase in the number of leukocytes up to 252.12±12.25 cells per 1000 erythrocytes in infected fish vs. 125.4±4.28 cells per 1000 erythrocytes in healthy fish, which is associated with the role of leukocytes in the body, since they are responsible for the protective function of the immune system during parasite invasion [28, 41]. In addition, the number of platelets per 1000 erythrocytes decreased. In the leukocyte formula, shifts towards neutrophils and monocytes occurred (Table 4).

4. Microscopic examination of blood smears of European perch (*Perca fluviatilis* Linnaeus, 1758) invaded and uninvaded with parasitic nematodes (*M*±SEM, Bolshaya Bolda and Bely Ilmen rivers, Lower Volga, Astrakhan Province, 2016-2019)

Trait, indicator	Invaded fish (*n* = 17)	Uninvaded fish (*n* = 7)
	Erythropoietic cells, %	
Erythroblasts	0.32±0.27	0.45±0.33
Oxyphilic normoblasts	0.13±1.32*	4.73±1.01
Polychromatophilic normoblasts	0.02±0.71	1.18±0.54
	Abnormal erythrocytes, %	
Poikilocytosis	5.9±0.15	3.98±0.14
Pointy end	0.79±0.11	0.59±0.02
Pear-shaped	0.30±0.03	0.28±0.04
Multifaceted	4.11±0.09	3.11±0.78
Nuclear shift to the periphery	1.18±0.02**	0.70±0.13
Schizocytosis	1.82±0.07**	0.19±0.06
Oligochromasia	1.22±0.12	–
Nucleus deformation	0.17±0.05	–
Total proportion of abnormal cells	9.17±0.23**	4.87±0.11
	Leukocyte formula, %	
Lymphocytes	75.77±0.98**	90.2±0.54
Neutrophils	3.57±0.32**	0.79±0.11
Monocytes	7.55±0.46**	1.57±0.26
Lymphoblasts	7.27±0.67	5.86±0.37
Myelocytes	3.27±0.74**	0.79±0.12
Promyelocytes	2.57±0.28**	0.79±0.12
Leukocytes per 1000 erythrocytes	252.12±12.25**	125.4±4.28
Thrombocytes per 1000 erythrocytes	2.10±2.01	7. 1±1.89

Note. Dashes indicate that the abnormality was not seen.
*, ** The differences between infected and uninfected fish are statistically significant at $p < 0.05$ and $p < 0.001$, respecetively.

Thus, in more than half of the cases, parasite invasion was found in perches caught from natural reservoirs of the Lower Volga. *Eustrongylides excisus* was the most frequent species of the detected nematodes. In fish infected with parasitic nematodes, the blood erythrocyte counts, the total protein concentration, and the cholesterol level significant decreased. Infected fish also showed an increase in the mean corpuscular hemoglobin values, ESR, the total number of leukocytes and platelets compared to uninfected fish. Analysis of the blood formed element in infected perch showed suppression of erythropoiesis, which is a sign of anemia. An increase in the proportion of neutrophils, monocytes, and lymphoblasts in the leukocyte formula indicate an increase in leukopoiesis, including neutrophilia in the fish affected by parasite invasion. The resulting informative indicators have the prospect of being used to assess the physiological and immunological state of river perch in aquaculture and when performing selection and breeding work.

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