Fish parasites and blood parameters as bioindicators of technogenic salination of freshwater ecosystems

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Abstract. Blood parameters and the composition of parasite fauna of fishes could indicate different processes in aquatic ecosystems. This study aims to examine the parasite fauna composition and to analyse the haemotological parameters of the adult ruffe Gymnocephalus cernua in the Volim River (Perm Krai) with the high degree of water salinity under the technogenic impact from the mining of the potash-magnesium salts, as well as in the Gaiva River (the city of Perm) with natural water salinity. We found wide scope of parasite species and high infection rate, as well as the development of leucopenia, erythropenia, and dramatic morphological changes in the erythrocyte cells of ruffe in Volim River. This can be caused by high degree of salinity with its adverse effect on fish’s immunity and a wide range of species-specific intermediate hosts of parasites. The results demonstrate an opportunity to apply the parasitological and haemotological markers for the bioindication of the rivers’ technogenic salination.

1. Introduction

Haemotological parameters and the composition of parasite fauna of fishes could indicate different processes in aquatic ecosystems [9, 12, 14]. Hematopoiesis and the blood composition responsible for the adaptive processes are seen to be the most dynamic parameters of the inner environment. Changes in the habitat result in disbalance of the fishes’ physiological condition and affect the haemotological parameters [2, 15]. The parasite environment is defined by many biota components since they serve to be their binding elements. The parasites could impact the trophic chains, competition, biodiversity, and key species, thus affecting the community’s structure, which is used to indicate different ecosystem processes [16]. The purpose of the study is to examine the parasite fauna composition and to analyse the haemotological parameters of the adult ruffe Gymnocephalus cernua in the Volim River (Perm Krai) with the high degree of water salinity under the technogenic impact from the mining of the potash-magnesium salts, as well as in the Gaiva River (the city of Perm) with natural water salinity.

2. Material and methods

The object of the study is mature adult ruffe caught at the lower reach in the Volim River (26 specimens) and in the mouth of the Gaiva River (26 specimens). The material was collected at the end of August 2020 with a minnow beach seine (the Volim River) and with hooks and lines (the Gaiva River). The fishes were analysed under the methodological recommendations by I.F. Pravdin [10].

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The focus of the research was on the ruffe’s peripheral blood taken from the coccygeal artery under the V.V. Metelev procedure [8]. A Gorjaev’s count chamber was used to count the erythrocytes and leucocytes. Blood films were located and stained with Leishman stain. The erythrograms were prepared for 500 erythrocytes, while the leucograms were drawn for 200 leucocytes. The authors applied the N.T. Ivanova [4] procedure to identify the formed elements. Erythrocytes changes were compared against the L.D. Zhiteneva et al. [3]. Normal and Pathological Fish Blood Cell Atlas.

A comprehensive parasite analysis of fishes was conducted under the well-known methods [1]. The following parameters were used to characterize the fish infection rate: the extensity of invasion (EI) is the percent of infected fishes in a sample, %; abundance rate (AR) is an average number of the parasite per one host in a sample; the intensity of invasion (II) is the minimum and the maximum number of the parasites per fish in a sample.

Chemical composition of the water samples from the rivers was examined in the laboratories of the hydrochemical analysis at PSU Faculty of Geology. Parasite- and haemotology-based samplings were compared by the single-factor analysis of variance, t-criterion, and Wilcoxon test. The paper applies the significance threshold \( p=0.05 \). Aov, wilcox.test, t.test functions were used for calculations in R 3.4.3 app [17].

3. Results

During the studies, the salinity of the examined part on the Volim River (9460 mg/l) increased the Gaiva River salinity by 21.9 times (432 mg/l). Ruffe sampling was represented by the mid length 68.9 mm (Volim River) and 76.5 mm (Gaiva River) specimens matured and at their first maturity, an average weight of the fishes was 6.07 g (Volim River) and 7.34 g (Gaiva River). The analysis of the haemotological parameters revealed the significant differences in most studied parameters. The Gaiva ruffe had more absolute number of the erythrocytes and leucocytes than the Volim ruffe, while the number of the immature erythrocytes and leucocyte precursor cells was higher with the Volim ruffe (figure 1).

Both samplings showed some morphological changes in the erythrocyte cells: poikilocytosis, acentric location of the nucleus, cytoplasm vacuolization, anisocytosis, scalloped margin, nucleus deformation and double nucleus. Along with that, the Volim ruffe’s erythrocytes had more changes that the Gaiva ruffe’s erythrocytes.

Leucocyte formula included the lymphocytes, monocytes, neutrophils, eosinophils, and precursor cells (table 1). Ruffe’s blood is expressly lymphoid by nature with the lower absolute number of lymphocytes in the Volim ruffe than that in the Gaiva ruffe, while the reverse is true for the number of precursor cells, monocytes, and eosinophils.

| Table 1. Correlation of the leucocytes in the ruffe’s peripheral blood. |
|--------------------------|--------------|------------------|
| Parameter               | Volim River  | Gaiva River      |
|                        | Relative number, % | 9.7±0.31        | 5.9±0.25*     |
| Precursor cells         | Absolute number thousand/1µl | 9.56±0.382      | 7.62±0.382*   |
| Neutrophils             | Relative number, % | 2.4±0.24        | 2.4±0.17      |
|                        | Absolute number thousand /1 µl | 2.43±0.262      | 3.05±0.215    |
| Eosinophils             | Relative number, % | 1.0±0.14        | 0.2±0.08*     |
|                        | Absolute number thousand /1 µl | 0.95±0.145      | 0.31±0.093*   |
| Monocytes               | Relative number, % | 5.9±0.39        | 2.7±0.23*     |
|                        | Absolute number thousand /1 µl | 5.86±0.438      | 3.34±0.282*   |
| Lymphocytes             | Relative number, % | 81.0±0.69       | 88.8±0.32*    |
|                        | Absolute number thousand /1 µl | 79.67±1.656     | 113.08±2.187* |

*significant differences under the Student’s t-test \( p<0.05 \).
Figure 1. The number of the erythrocytes (a), leucocytes (b), a share of the immature erythrocytes (c) and leucocyte precursor cells (d), as well as the number of the erythrocytes with poikilocytosis (e) and cytoplasm vacuolization (f) in the Gaiva and Volim ruffe.

The Volim and Gaiva fish parasite fauna was represented by 8 and 4 parasite organisms respectively (table 2). The Volim ruffe were mainly positive for trematode cysts Ichthyocotylurus platycephalus which were found in the ureters (all specimens), kidneys (20 specimens), bladders (2 specimens), and intestines (3 specimens). Cysts I. platycephalus were also abundant among the parasites of the Gaive ruffe but were found in 17 out of the tested specimens and localized in the ureters. Trematode metacercariae Diplostomum spathaceum were the second common parasites and were spotted in the eye lens and vitreous body in 23 out of 26 specimens of the Volim ruffe and in the eye lens of 13 Gaiva fishes. Besides, 7 specimens of the Volim ruffe were positive for trematode metacercariae Tylodelphys clavata in their eye vitreous body and the eye lens while only one Gaiva
ruffe’s eye vitreous body was infected with the same parasite. The Volim fishes were also found positive for other types of parasites (*Bunodera luciopercae*, *Camallanus truncatus*, *Posthodiplostomum brevicaudatum*, *P. clavata*, *Triaenophorus nodulosus*) or the Gaiva ruffe were infected with the parasites (*Rhipidocotyle campanula*).

**Table 2.** The Volim and Gaiva ruffe’ organs infected by *Gymnocephalus cernua*

| Localization          | Parasites (EI; AR; II) | Volim River       | Gaiva River       |
|-----------------------|------------------------|-------------------|-------------------|
| stomach               | *Bunodera luciopercae* 23%; 1.3; 1-2 |                    |                   |
|                       | *Camallanus truncatus* 12%; 1; 1-1    |                   |                   |
|                       | *Triaenophorus nodulosus* 35%; 2.5; 1-4 |                   |                   |
|                       | *Ichthyocotylurus platycephalus* 12%; 8.3; 2-18 | | |
| intestine             | *Bunodera luciopercae* 15%; 2.3; 2-3    |                   |                   |
|                       | *Camallanus truncatus* 4%; 2; 2-2     |                   |                   |
|                       | *Triaenophorus nodulosus* 27%; 4; 4-4   |                   |                   |
| urinary bladder       | *I. platycephalus* 8%; 6; 1-11        |                   |                   |
| ureters               | *I. platycephalus* 100%; 12.9; 2-26    |                   |                   |
| kidneys               | *I. platycephalus* 77%; 7.6; 2-18      |                   |                   |
|                       | *Diplostomum spathaceum* 12%; 1.7; 1-2 |                   |                   |
| eye vitreous body     | *Tylodelphys clavata* 38%; 1.9; 1-2     |                   |                   |
|                       | *Posthodiplostomum clavata* 35%; 1.8; 1-4 |   |                   |
|                       | *P. brevicaudatum* 8%; 1.0; 1          |                   |                   |
|                       | *Rhipidocotyle campanula* 11%; 1; 1     |                   |                   |
| eye lens              | *Diplostomum spathaceum* 88%; 2.3; 1-6  |                   |                   |
|                       | *Tylodelphys clavata* 23%; 2; 2-2       |                   |                   |

Notes: EI – extensity of invasion; AR – abundance rate; II – intensity of invasion.

**4. Discussion**

A high degree of salinity with its adverse effect on fishes immunity and a wide range of species-specific intermediate hosts determine a wide scope of parasite species and a high Volim ruffe infection rate, as well as the development of leucopenia, erythropenia, and dramatic morphological changes in the erythrocyte cells. Harmful stress factors, including technogenic salination, trigger extensive infection with helminths among fishes due to their immunosuppression which is manifested in lower resistance to the parasite, bacterial, and virus invasions [7, 13, 14]. This can be supported by a higher rate of erythropoiesis (more young forms of erythrocytes), more precursor cells and monocytes in the white blood, which is likely to be an active adaptive response.

On the other hand, *B. luciopercae*, *C. truncatus*, *T. nodulosus* which were found in the Volim ruffe and which require copepods for their life cycle [11, 5] speak for the dominating role of large benthic cyclops *Megacyclops viridis*, *Eucyclops serrulatus*, *Macrocyclops albidus* in zooplankton biomass against the overall depletion in the Volim plankton species [6].

The comparative analysis revealed the differences in the rivers by their species, infection rates, parasite localizations, as well as ruffe’s blood characteristics, which demonstrates an opportunity to apply the parasitological and haematological markers for the bioindication of the rivers’ technogenic salination.

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