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Results of the Primary Evaluation of Processes of Natural Self-cleaning of Lake Yanisyarvi As an Object of Fishing Value

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Abstract

The initial assessment of the processes of natural self-cleaning of the lakes of the Yanisyarvi basin was carried out. The morphological, physiological and biochemical characteristics of aquatic microflora were used as the main indicators of natural self-cleaning processes in the eco-system. The dominance of the anaerobic phase of self-purification due to the enzymatic activity of obligate and facultative anaerobes was described. The proportion of anaerobes controlling intra-water geochemical processes averaged 90%, and aerobes - 10%. The morphological component was represented by a wide variety, the presence of all morphotypes with the exception of crimped forms was noted. The study of tinctorial traits, isolated isolates made it possible to establish the predominance of representatives of the Gracilicutes department, whose number, depending on the sampling station, varied from 55 to 95%. The biochemical component of the study was performed on the basis of determining the activity of isolates in 8 groups of enzymes.

Keywords: aerobes, anaerobes, natural self-cleaning, morphological diversity, facultative anaerobes, enzymatic activity

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1. Introduction

Nowadays using the initial assessment of natural self-purification processes, it becomes possible to predict the intensity of natural restoration of aquatic ecosystems [1, 2]. The leading biological component of the process are microorganisms, which have a wide variety of constitutive and inducible enzymes and moderate metabolic lability; are the main component of natural ecosystems and control the degree of degradation of natural and synthetic xenobiotics. Due to the activity of microbial communities, a cycle of nutrients, including carbon and nitrogen, occurs [3, 4]. In Karelia, due to low average annual temperatures, the rates of self-purification and biogenesis are slowed down, and the microflora experiences additional stress during the economic use of water

bodies associated with abiotic and biotic environmental factors, for example, during the implementation of fish farming.

Cage trout farming is one of the fast-growing and promising areas of aqua culture in the republic. At present, 58 fish breeding enterprises operate in the territory of Karelia. In 2018, 27.2 thousand tons of fish of different ages were grown in Karelia, and this number is growing every year [5]. This is facilitated by a moderately continental climate and an abundance of water resources.

Intensification of fisheries leads to a violation of the stability of aquatic ecosystems, and the internal waters of Karelia become threatened by local eutrophication. During artificial cultivation, a large amount of organic substances is introduced into the reservoir along with fish feed and metabolites [6, 7], the decomposition of which depends on such factors as water exchange, oxygen regime, and average annual temperature [8, 9]. These factors are not distinguished by high rates in the Northwest, which leads to pollution of the aquatic environment by accumulating organic substances and morpho-functional changes in the composition of local microflora [10, 11]. As a result, the degree of contamination of normal microflora decreases and the dose of conditionally pathogenic and pathogenic organisms that do not have the function of anatomical and physiological barrier increases. In Karelia, the process of bacterial self-purification of local water bodies has been little studied [12], in connection with this, the study of this aspect of aquatic ecology can be considered relevant for the development of methods for predicting and preventing local eutrophication in fishery areas. The goal of the work performed was to assess the state of the processes of natural self-cleaning of the reservoirs of the Yanisyarvi basin as objects of the hatchery.

2. Methods and Equipment

According to published data, the analysis of the course of natural self-purification in a reservoir is carried out according to hydrochemical (content of nutrients / nitrogen, phosphorus / and pollutants / oil products, heavy metals /), organoleptic (smell, color), hydrophysical (transparency, electrical conductivity) and hydrobiological (zoobenthos) indicators [13--15], but basic are bacteriological data, since the physiological and biochemical activity of microorganisms is the basis of trophic chains of any natural ecosystems.

The studies were conducted in the laboratory of the microbiology course at Petrozavodsk State University from 2016 to 2018. The analysis of water microflora was carried out in accordance with the requirements of the state standard ``Drinking water. Methods

of sanitary-bacteriological analysis" (GOST 18963--73, 1981; 2018). Bacterioflora was taken from Lake Bolshoy Yanisyarvi at a depth of 0.5 m into sterile containers with a volume of 2 l; In total, 32 samples of natural communities of planktonic forms of bacteria were studied. Comprehensive studies of morphological, physiological, and biochemical properties were performed to describe their role in biological self-purification processes. Morphotypes were evaluated by bacterioscopic analysis using fixed stained specimens using immersion microscopy using a Motic DMBA-300 microscope and a 2.0 MPc, 1/2 CMOS digital camera with a resolution of 1600 × 1200. As physiological parameters, the type of respiration and the peculiarities of the nutritional needs of microorganisms on the main and selective nutrient media were taken into account. Biochemical activity was studied in 8 groups of enzymes: glycolytic, proteolytic, redox (catalase, oxidase), hydrolytic, aggression enzymes (lecitovitilase, hemolysin) based on the fermentation of substrates in the composition of Giss and a set of elective media: JCA (yolk-salt agar), CA (blood agar) and others.

3. Results

The data on the features of the participation of the detected cultures in the processes of substrate oxidation are presented in table No. 1. Representatives of aerobic, facultatively anaerobic, and anaerobic species were identified in all samples. Anaerobic microorganisms capable of a coupled process of oxidation - reduction of inorganic and organic substrates in a reservoir, accounted for the bulk of the isolates. Their percentage ratio in the samples ranged from 56 to 79%. The number of facultative anaerobes using fumarates as the ultimate oxygen acceptor was 2.8 times less than the anaerobic group and the number of cells of facultative anaerobic bacteria in the microflora of water did not exceed 36%. The proportion of aerobes in need of high concentrations of dissolved oxygen for nutrition, growth and reproduction varied from 2 to 24%.

Table 1: Variety of isolated cultures by oxidative activity.

Sample number	Number of samples	Aerobes (%)	Anaerobes (%)	Facultative anaerobes
1	8	24	64	12
2	8	2	79	19
3	8	7	73	20
4	8	8	56	36

The morphotypes of the Yanisyarvi saprophytes were extremely diverse, consisting in the abundance of spherical, rod-like, and convoluted cells (Table 2). The major types of

cells include spherical cells, and the minor ones are crimped. All isolates are represented by variants with both Gr (+) cell wall and Gr (-). The exception is vibrios and sperill-like variants for which the presence of Gr (+) forms was not detected. Spherical gram-positive cells are represented to a lesser extent (5-45%) than the negative ones (55-87%). Representatives of rod-shaped Gr (+) and Gr (-) microflora were found in all samples. The proportion of gram-positive bacteria varied from 51 to 71%, and gram-negative bacteria from 29 to 49%.

Table 2: Morphological and tinctorial features of pure Yanisyarvi saprophyte cultures.

Sample number	Spherical (%)		Rod-shaped (%)		Twisted (%)	
	gram-positive	gram-negative	gram-positive	gram-negative	gram-positive	gram-negative
1	13	87	58	42	0	100
2	5	95	64	36	0	100
3	22	78	51	49	0	100
4	45	55	71	29	0	100

In order to assess the quality of the intra-water processes and determine the degree of natural self-cleaning of Yanisyarvi in the area of trout farming, a study was made of the biochemical activity of isolates in 8 groups of enzymes (Table 3). The isolated microorganisms were characterized by a high level of oxidative, glycolytic, and proteolytic activity, which indicates the mineralization of the organic component in the reservoir and rather intensive processes of natural self-purification of the medium. However, a rather high level of occurrence of microorganisms possessing aggression enzymes (lecitovylase and hemolysin) should be noted. In 100% of cases, a persistent phenomenon of opalescence and hemolysis was recorded on media with JSA and SC. The results can be explained by the intensification of fish breeding processes, which is inevitably associated with the presence of aggressive environmental factors that provoke phenotypic changes among saprophytes, aimed at increasing the number of virulent strains that control a high degree of infection in the reservoir.

Table 3: Characterization of the biochemical activity of pure bacterial cultures.

Sample number	Catalase Heme+	Catalase Heme-	Oxidase	Hydrolase	Lecitovitilase	GGE	GPE	Hemolysin
	1	2	3	4	5	6	7	8
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+

abbreviations: GGE - a group of glycolytic enzymes, GPE - a group of proteolytic enzymes

Based on the results of the studies, it was found that in the composition of the Yanisyarvi micro flora there is a succession of microorganisms that control the intra-water processes of natural self-purification, which ensures the transformation of the main biogenic elements. Using microbiological analysis of phenotypic traits of the studied groups of heterotrophic bacterioplankton, carried out in the period from 2016 to 2018, serious changes in the structure of communities of autochthonous and allochthonous species were recorded. These changes relate to an increase in the number of both aerobic and anaerobic species, with the dominance of anaerobic forms distributed locally in the area of active functioning of trout cages. The main factors provoking the succession of unicellular objects of Yanisyarvi can be considered a low range of the internal air temperature, varying from 1.5 to 6.5 ˚C; accumulation of introduced organics coming from fish feed and metabolic products; lack of control over the epizootic state of the reservoir and accounting for the MAFAnM indicator, which explains the ratio of the parotrophic and saprotrophic representatives of bacterioplankton.

4. Discussion

The data obtained are confirmed by analogous studies performed on fishery reservoirs located in the Muezersky district - lakes Hedo and Mui [7]. The aim of this work was to analyze the state of heterotrophic groups of bacteria capable of growth at different temperature ranges. In parallel, the species biodiversity of spore crops and sanitary-indicative microorganisms was taken into account. A change in the ratio of species and ecological groups of bacteria has been established, which, according to the authors, is associated with the dominance of allochthonous microflora over autochthonous. In addition, a seasonal change in the number of micromycetes was revealed, which showed abundant growth in the spring. The authors explain this correlation of ecological and physiological groups of microorganisms by the intensification of eutrophication processes with the favorable sanitary status of the studied water areas.

As a result of the work done, it is advisable to consider the planned introduction of microbiological monitoring at fish farms in order to prevent the irreversible consequences associated with the eutrophication of water bodies, an increase in the degree of their integrity and the development of epizootic situations.

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Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Kulikovsky, K.L., Toloknova, A.N. (2013). Improving methods and information-measuring systems for determining the ability of a reservoir to self-cleaning. *Izvestiya Vysshikh Uchebnykh Zavedenii. Volga region. Technical science*, vol. 25, pp. 71--80.

[2] Kalitina, E.G., Mikhailik, T.A., Semkin, P.Yu., Barabanshchikov, Yu.A., Zorin, S.A. (2015). Feetures of microbiological composition for water from the Razdolnaya River (southern Pri-morye). *Izvestia PINRO.* vol. 180. pp. 187--197.

[3] Netrusov, A. I., Kotova, I. B. (2006). *Microbiology.* Moscow, M: Academy Publ. Center.

[4] Sidorova, N. A., Obukhova, E. S. (2012). The main groups of heterotrophic bacterioplankton of fishery water bodies of Karelia. *Materials of the scientific and practical Internet conference «Actual problems of hydrobiology and ichthyology in the context of biological invasions in freshwater ecosystems»* Kazan, pp. 87-90.

[5] Agriculture Report for the Republic of Kazakhstan (2018).

[6] Kitaev, S.P., Ilmast, N.V., Sterligova, O. P. (2006). *Methods for assessing the nutrient load from trout farms on aquatic ecosystems.* Petrozavodsk: Karelian Scientific Center of the Russian Academy of Sciences. pp. 103-112.

[7] Obukhova, E.S. (2013). Ecological features of pseudomonads in the composition of autoflora of rainbow trout in the conditions of Karelia. PhD Dissertation, Petrozavodsk.

[8] Mikheev, V.P. (1982). *Cage cultivation of commercial fish*. Moscow, M.: Light and food industry, pp. 109-111.

[9] Ryzhkov, L. P., Kuchko, T. Yu., Kuchko, Ya. A. (2000). *Growing trout in cages: methodological recommendations*. Petrozavodsk: Publishing House of PetrSU.29 p.

[10] Sidorova, N.A. Parshukov, A.N. (2014). Microbiocenosis of rainbow trout in cage farms of northern Karelia. *Scientific notes of the Petrozavodsk State University*. vol. 1. pp. 29-33.

[11] Ed. Kalina, G.P., Chistovich, G.N. (1969). *Sanitary Microbiology.* Moscow, M.: Medicine.

[12] Parshukov, A.N., Sidorova, N.A. (2010). Bacterial self-cleaning of fishery reservoirs. *Scientific notes of Petrozavodsk State University*. №.6. pp. 14-16.

[13] Nabeeva, E.G. (2010). Assessment of restoration and self-purification of heterogeneous aquatic ecosystems according to macrozoobenthos indicators. PhD Dissertation. Nizhny Novgorod.

[14] Kondakova, G.V. (2007). *Bioindication. Microbiological indicators.* Yaroslavl: Yaroslavl State University, pp. 39-45.

[15] Sidorova, N. A. (2013). *Sanitary-biomedical research in fish farming.* Petrozavodsk: Publishing House of PetrSU. p. 21.