

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## The Evaluation Of Water Quality Of Nizhniy Kaban Lake using Next-Generation Sequencing.

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### ABSTRACT

Today, bioindication is used to assess the environment, including water bodies. The method consists in studying the species of indicators inhabiting the medium under study, having been determined up to the present time visually by means of a microscope. As an alternative to the visual approach, the paper considers a method for identifying hydrobionts by the *CO1* marker gene based on the DNA-barcoding method and modern sequencing methods. The sequenced sequences of the fragment of the *CO1* hydrobiont gene of freshwater Nizhniy Kaban lake in the autumn (2016) and summer (2017) sampling periods in the fastq format are included in the international database on the NCBI's website with unique numbers *SRR5839797*(2016) and *SRR5852707* (2017). The paper presents the results of the analysis and gives an assessment of the water quality of Nizhniy Kaban lake (Kazan, Russia). Comparative analysis of metagenomic data shows that most of the animals of Lower Kaban lake are grouped near the *b*-mesosaprobic zone. By water quality Nizhniy Kaban lake can be characterized as moderately polluted and as of 2017 is transitional from *b*-mesosaprobic to *α*-mesosaprobic.

**Keywords:** bioindication, saprobity, DNA-barcoding, next-generation sequencing.

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**INTRODUCTION**

As is known, one of the highly informative methods of environmental assessment of the aquatic environment is the method of bioindication (identification of the nonspecific reaction of living organisms to changes in the conditions of their habitat) [1]. The method of bioindication has a significant advantage in comparison with chemical and physical methods, since physicochemical indicators give an assessment of water quality only at a given time, whereas the presence of indicator species of organisms makes it possible to more accurately judge the quality of water in a water body [2]. The method consists in determining the indicator organisms according to the morphological features, and the researcher's opinion may be subjective, since the identification of certain species requires the knowledge of narrow specialists because many species have very small dimensions, some species have strong sexual dimorphism, or vice versa, there are sibling species and fewer than all specialists can identify the organism at its larval stage [3].

At present, methods of molecular genetic analysis allow for instrumental identification of organisms accurate to species. Thus, for example, the DNA-barcoding method [4] is used for this purpose. Numerous DNA barcodes by kinds of organisms are accumulated in the international database of nucleotide sequences – GenBank and in Barcode of life data system (BOLD) database [5]. The DNA-barcoding method is based on a sequence of nucleotides of the DNA-barcode, which is the same inside the species and differs in different species, for example, for animals - it is a variable fragment of the *CO1* gene with a length of 600-700 base pairs [7]. Previously, we used this technique to identify zooplankton organisms with DNA-barcode *CO1* for assessing the environmental state of freshwater reservoirs by bioindication [8]. In addition, to assess the environmental state of freshwater reservoirs on the basis of modern methods of bioinformatics, we have shown the correlation between the product of the *CO1* gene and the saprobity of the indicator species of rotifers [9].

This paper provides an assessment of the water quality of Nizhniy Kaban lake (Kazan, Russia) using the *CO1* marker gene of the indicator species of hydrobionts based on the next-generation sequencing method.

**METHODS**

Sampling from Nizhniy Kaban lake (Kazan) was conducted in September 2016 and in July 2017 in accordance with standard hydrobiological methods [10] and using the Apstein network by straining 100 liters of water.

Isolation of DNA from the precipitate obtained by centrifugation of 50 ml of the sample at a rate of 10,000 g for 15 min was carried out using the FAST DNA Kit (MP biomedical) according to the manufacturer's protocol. Amplification of the isolated DNA was performed by Phusion High-Fidelity DNA polymerase (Thermo Fisher) using the primers (Table 1).

**Table 1: Primers for PCR of CO1 gene of zooplankton**

Primers	Sequences
COI_ZplankF1_I (forward primer)	5'- tcgtcggcagcgtcagatgtgtataagagacagtctasw aatcataargatattgg-3'
COI_ZplankR1_I (reverse primer)	5'- gtctcgtgggctcggagatgtgtataagagacagttcag grtgrccraaraatca-3'

Purification of the PCR products was carried out with Agencourt AMPure XP beads (Beckman Coulter), followed by a second PCR for indexing the samples (Nextera XT indices).

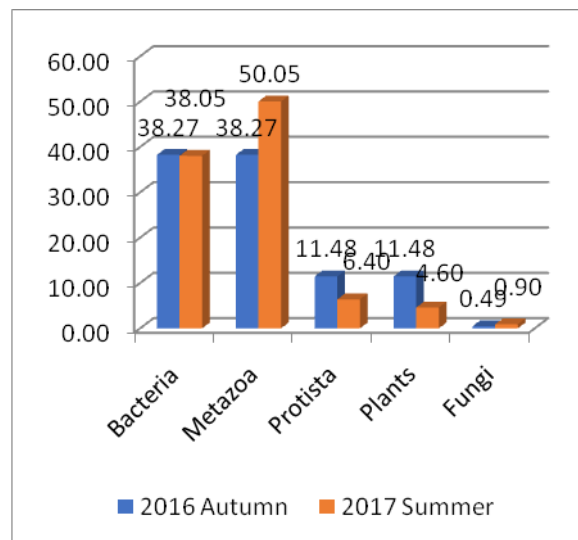
The resulting DNA libraries were sequenced on an Illumina MiSeq (MiSeq Reagent kit v3). Metagenomic data were included in the international database on the NCBI's website [6].

The obtained metagenomic data was aligned with the BLAST + program to establish the species diversity and for subsequent analysis.

**RESULTS AND DISCUSSION**

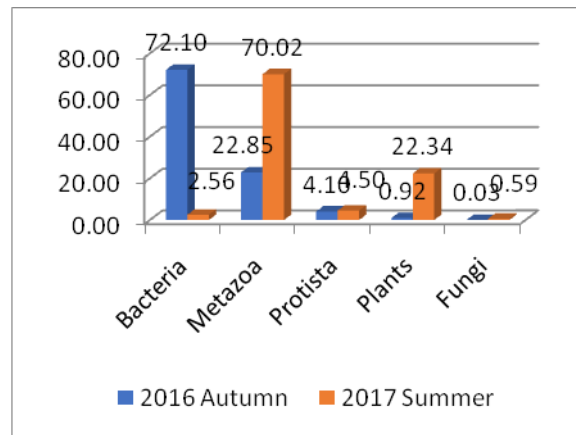
The sequenced sequences of the fragment of the *CO1* hydrobiont gene of freshwater Nizhniy Kaban lake in the autumn (2016) and summer (2017) sampling periods in the fastq format are included in the international database on the NCBI's website under numbers *SRR5839797*(2016) and *SRR5852707* (2017). After filtering the reads by quality, trimming the service sequences, and removing the chimeric sequences, the resulting nucleotide sequences were aligned with the BLAST + program to establish the taxonomic composition.

The sequencing of the sequences of the fragment of the *CO1* hydrobiont gene of freshwater Lower Kaban lake in autumn (2016) and summer (2017) helped us to identify by years respectively: 219/422 species of bacteria, 310/555 species of animals, 93/71 species of protists, 93/51 of plant species and 4/10 species of fungi. The percentage ratio of the taxonomic groups of Lower Kaban lake (autumn 2016 / summer 2017) is shown in Fig. 1.



**Figure 1: The percentage of taxons of Nizhniy Kaban lake (Autumn 2016; Summer 2017)**

The percentage of taxonomic groups of Lower Kaban lake by reads (autumn 2016 / summer 2017) is: bacteria - 72.10% / 2.56%, animals - 22.85% / 70.02%, plants - 0.92% / 22.34%, protists - 4.10% / 4.50% and fungi 0.03% / 0.59% (Figure 2).



**Figure 2: The percentage of taxons by reads of Nizhniy Kaban lake (Autumn 2016; Summer 2017)**

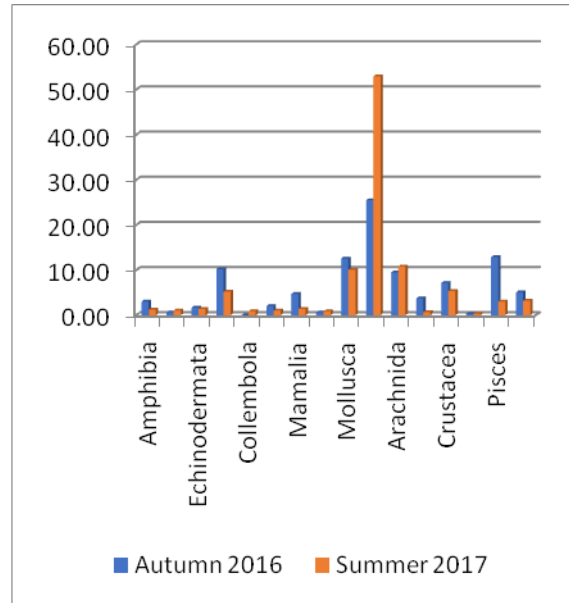
The taxonomic groups of Metazoa are analyzed further, since DNA bar-code (*CO1*) can only be used to identify animal organisms.

In terms of the diversity of species we identified by years 2016/2017: 9/7 species of amphibians, 2/6 species of sponges, 5/8 species of echinoderms, 30/29 species of cnidarians, 0/5 species of columbolus, 6/6 species of rotifers, 14/8 species of mammals, 2/5 species of millipedes, 37/56 species of mollusks, 75/294 species of insects, 28/60 species of arachnids, 11/4 bird species, 21/30 species of crustaceans, 1/2 species of reptiles, 38/17 species of fish and 15/18 species of worms.

The results of the sequenced sequences of the fragment of the *CO1* hydrobiont gene of freshwater Nizhniy Kaban lake in autumn (2016) and summer (2017) by diversity of species of *Metazoa* are given in percentage in Table 2 and are shown in Fig. 3.

**Table 2: Species diversity of Metazoa in the percentage of Nizhniy Kaban lake**

Taxon	Autumn 2016	Summer 2017
<i>Amphibia</i>	3.06	1.26
<i>Porifera</i>	0.68	1.08
<i>Echinodermata</i>	1.70	1.44
<i>Cnidaria</i>	10.20	5.23
<i>Collembola</i>	0.00	0.90
<i>Rotifera</i>	2.04	1.08
<i>Mamalia</i>	4.76	1.44
<i>Myriapoda</i>	0.68	0.90
<i>Mollusca</i>	12.59	10.09
<i>Insecta</i>	25.51	52.97
<i>Arachnida</i>	9.52	10.81
<i>Aves</i>	3.74	0.72
<i>Crustacea</i>	7.14	5.41
<i>Reptilia</i>	0.34	0.36
<i>Pisces</i>	12.93	3.06
<i>Worms</i>	5.10	3.24



**Figure 3: The percentage of species diversity of Metazoa in of Nizhniy Kaban lake (Autumn 2016; Summer 2017)**

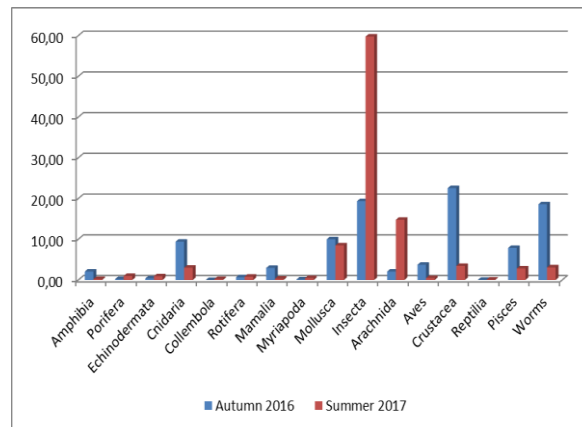
In terms of the number of organisms we identified by years 2016/2017: 49/13 reads of amphibians, 5/51 reads of sponges, 9/48 reads of echinoderms, 222/157 reads of cnidarians, 0/12 reads of columbolus, 16/43 reads of rotifers, 70/16 reads of mammals, 3/25 reads of millipedes, 236/441 reads of mollusks, 457/3109 reads of insects, 49/769 reads of arachnids, 88/25 reads of bird, 533/180 reads of crustaceans, 1/4 reads of reptiles, 186/148 reads of fish and 439/161 reads of worms.

The results of the sequenced sequences of the fragment of the *CO1* hydrobiont gene of freshwater Nizhniy Kaban lake in autumn (2016) and summer (2017) by number of organisms of *Metazoa* are given in percentage in Table 3 and are shown in Fig. 4.

**Table 3: The quantity of Metazoa organisms (the quantity of reads) in the percentage of Nizhniy Kaban lake**

Taxon	Autumn 2016	Summer 2017
<i>Amphibia</i>	2.07	0.25
<i>Porifera</i>	0.21	0.98
<i>Echinodermata</i>	0.38	0.92
<i>Cnidaria</i>	9.39	3.02
<i>Collembola</i>	0.00	0.23
<i>Rotifera</i>	0.68	0.83
<i>Mamalia</i>	2.96	0.31
<i>Myriapoda</i>	0.13	0.48
<i>Mollusca</i>	9.99	8.48
<i>Insecta</i>	19.34	59.77
<i>Arachnida</i>	2.07	14.78
<i>Aves</i>	3.72	0.48
<i>Crustacea</i>	22.56	3.46
<i>Reptilia</i>	0.04	0.08
<i>Pisces</i>	7.87	2.85

Taxon	Autumn 2016	Summer 2017
Worms	18.58	3.09



**Figure 4: The quantity of Metazoa organisms (the quantity of reads) in the percentage of Nizhniy Kaban lake (Autumn 2016; Summer 2017)**

The analysis of the metagenomic data on the *CO1* gene fragment of the animal organisms of Nizhniy Kaban lake (autumn 2016) identified 13 organisms classified as indicators as per V. Sladechek’s list of the indicator organisms [11]:

- 10 rotifers:

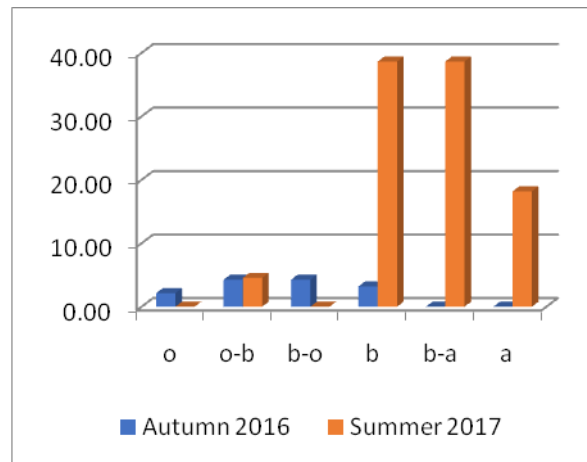
- 3 organisms of *Asplanchna sieboldi* referring to *o-b*-mesosaprobic zone with an indicator weight of 1.50;
- 4 organisms of *Keratella cochlearis* - to the *b-o*-saprobic zone with an indicator weight of 1.55;
- 3 organisms of *Brachionus plicatilis* - to the *b*-mesosaprobic zone with an indicator weight of 2.00;
- 1 mollusk - *Dreissena polymorpha* referring to *o-b*-mesosaprobic zone with an indicator weight of 1.50;
- 2 crustaceans of the same species of *Mesocyclops leuckarti* referring to the *o*-saprobic zone with an indicator weight of 1.25.

The analysis of the metagenomic data on the *CO1* gene of the animal organisms of Nizhniy Kaban lake (summer 2017) identified 44 organisms classified as indicators as per V. Sladechek’s list of the indicator organisms [10]:

- 19 rotifers:

- 17 organisms of *Brachionus calyciflorus*. referring to *b-a*-mesosaprobic zone with an indicator weight of 2.50;
- 2 organisms of *Philodina megalotrocha* referring to *o-b*-mesosaprobic zone with an indicator weight of 1.50;
- 17 crustaceans of the same species of *Gammarus roeseli* belonging to *b*-mesosaprobic zone with an indicator weight of 2.25
- 8 worms of the same species of *Helobdella stagnalis* - to the *a*-mesosaprobic zone with an indicator weight of 2.60.

The percentage of species of hydrobionts by zones of saprobity is shown in Fig. 5.



**Figure 5: The percentage of hydrobionts saprobity of Nizhniy Kaban lake (Autumn 2016; Summer 2017)**

A comparative analysis of metagenomic data shows that most of the animals of Lower Kaban lake are grouped around the *b*-mesosaprobic zone (Fig. 5); in summer 2017, animal organisms prevailed (70.02%) compared with bacterial organisms (2.56%), whereas in autumn 2016, bacterial organisms (72.10%) prevailed in Lower Kaban lake compared to animal organisms (22.85%) (Fig. 2), which is due to the flowering of water during this period.

#### SUMMARY

As a result of the use of modern methods of next-generation sequencing, molecular-genetic and bioinformational analysis, Lower Kaban lake can be characterized by its water quality as moderately polluted and its saprobity index lies within the *b*-mesosaprobic contamination zone. As of 2017, Nizhniy Kaban lake is transitional from *b*-mesosaprobic to *a*-mesosaprobic.

#### CONCLUSIONS

The method of bioindication is one of the main parts of the assessment of the environmental state of water bodies, which is carried out according to the recommendations developed in the last century. Since that time, science has moved far ahead. Advancements in molecular biology have improved the identification of organisms by marker genes and proteins, which made it possible to accurately identify even "controversial" species. This has directly increased the effectiveness and reliability of assessing the environmental status of water bodies. The obtained results are of great practical interest in the field of monitoring of water bodies in particular, and the environment in general.

#### ACKNOWLEDGEMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

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