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**INDO AMERICAN JOURNAL OF  
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1467373>Available online at: <http://www.iajps.com>**Research Article****THE ECOLOGICAL ESTIMATION OF LAKE VERKHNY  
KABAN USING THE NEXT-GENERATION SEQUENCING****Artur M. Husainov\*, Ludmila L. Frolova**

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**Abstract:**

*On the basis of bioindication method, the ecological state of the environment and water is estimated through the investigation of indicator species, living in the studied ecosystem, identified visually by microscope. As an alternative, the article considers the method for identification of hydrobionts in a water sample, using the marker gene COI, based on the modern sequencing methods. The results of the analysis are given in the work. The water quality of Lake Verkhny Kaban (Kazan, Russia) is estimated on the basis of DNA-barcoding, using the next-generation sequencing. Sequenced fragment sequences of COI gene of hydrobionts, living in the freshwater Lake Verkhny Kaban, were deposited in the international databases, on the web-sites NCBI and MG-RAST, respectively, with the unique numbers PRJNA394961 and mgm4779367.3 (2016); PRJNA394999 and mgm4779621.3 (2017), in the format fastq. Samples were collected in 2016 and 2017. Comparative analysis of metagenomic data shows, that most of the animal organisms of Lake Verkhny Kaban are grouped near the b-mesosaprobity. In terms of the quality, the water of Lake Verkhny Kaban can be characterized as moderately polluted, and the value of saprobity is within the b-mesosaprobity.*

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

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**INTRODUCTION:**

It is known that one of the highly informative methods of ecological estimation of aquatic environment is the use of bioindication method. It is the detection of nonspecific reaction of living organisms to the changes in the conditions of their habitat, for example pollution or purification [1]. In comparison with the chemical and physical methods of indication of water bodies, bioindication methods have a significant advantage. This is due to the fact, that physical and chemical indices give an assessment of water quality only at a given time, whereas the presence of indicator species of plants, animals, fungi or bacteria allow to estimate the quality of water in the reservoir more accurately [2]. The method consists in determining the indicator organisms, according to their morphological features. The opinion of the researcher may be subjective, and the knowledge of narrow specialists may be necessary for the identification of certain species. This is due to the fact, that many species are very small in size, some species have a strong sex dimorphism, or vice versa, there are sibling species, and not every specialist can determine the organism at the larval stage [3].

At the present time, modern methods of molecular and genetic analysis allow identifying the organisms instrumentally, correcting to the specie. For example, the method of DNA-barcoding [4] is used for this purpose. Numerous DNA-barcodes are accumulated in the international database of nucleotide sequences GenBank, according to the species of organisms [5]. The method of DNA-barcoding is based on a

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

Primers	Sequences
COI_ZplankF1_I (forward primer)	5'-tcgtcggcagcgtcagatgtgtataagagacagtctaswaatcataargatattgg-3'
COI_ZplankR1_I (reverse primer)	5'-gtctcgtgggctcggagatgtgtataagagacagttcaggrtgrccraaraatca-3'

Purification of PCR products was carried out using Agencourt AMPure XP beads (Beckman Coulter). After this, the second PCR was taken for indexing the samples (Nextera XT indices).

Received DNA libraries were sequenced on Illumina MiSeq (MiSeq Reagent kit v3). Metagenomic data were included in the international databases on the sites NCBI and MG-RAST [5,10]. Obtained metagenomic data was aligned using the program BLAST + for establishing species diversity and subsequent analysis.

**RESULTS AND DISCUSSION:**

The sequenced fragment sequences of *COI* gene of hydrobionts, living in the freshwater Lake Verkhny

sequence of DNA-barcode nucleotides, which is the same in the individuals of only one species. For example, for animals, it is a variable fragment of a gene *COI*, with length 600-700 base pairs [6]. Previously, this technology was used by us to identify zooplankton organisms by the variable fragment of gene *COI*, for estimation of ecological state of freshwater reservoirs, using the method of bioindication [7]. In addition, to assess the ecological state of freshwater reservoirs, we have shown the correlation between the product of the gene *COI* and the saprobity of the indicator species of rotifers, on the basis of modern methods of bioinformatics [8].

This article provides an assessment of water quality of Lake Verkhny Kaban (Kazan, Russia), using the marker gene *COI* of indicator species of hydrobionts, on the basis of next-generation sequencing.

**METHODS:**

The samples were taken from Lake Verkhny Kaban (Kazan) in 2016 and 2017, in accordance with standard hydrobiological methods [9], and using the Apstein net, by straining 100 liters of water.

Isolation of DNA from the precipitate, obtained by the 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 a pair of primers (Table 1).

Kaban, were included in the international databases on the sites NCBI and MG-RAST, respectively, with the unique numbers PRJNA394961 and mgm4779367.3 (2016); PRJNA394999 and mgm4779621.3 (2017), in the format fastq. Samples were collected in 2016 and 2017. After filtration of reads by quality, trimming the auxiliary sequences, and removing the chimeric sequences, the resulting nucleotide sequences were aligned with the program BLAST + to establish the taxonomic composition. According to the results of sequencing of fragment

sequences of the gene *COI* of hydrobionts, living in the freshwater Lake Verkhny Kaban, carried out in 2016 and 2017, 273/2403 species of Bacteria,

335/4415 species of Metazoa, 98/253 species of Protista, 56/414 Plant species and 3/8 species of Fungi were identified (Figure 1).

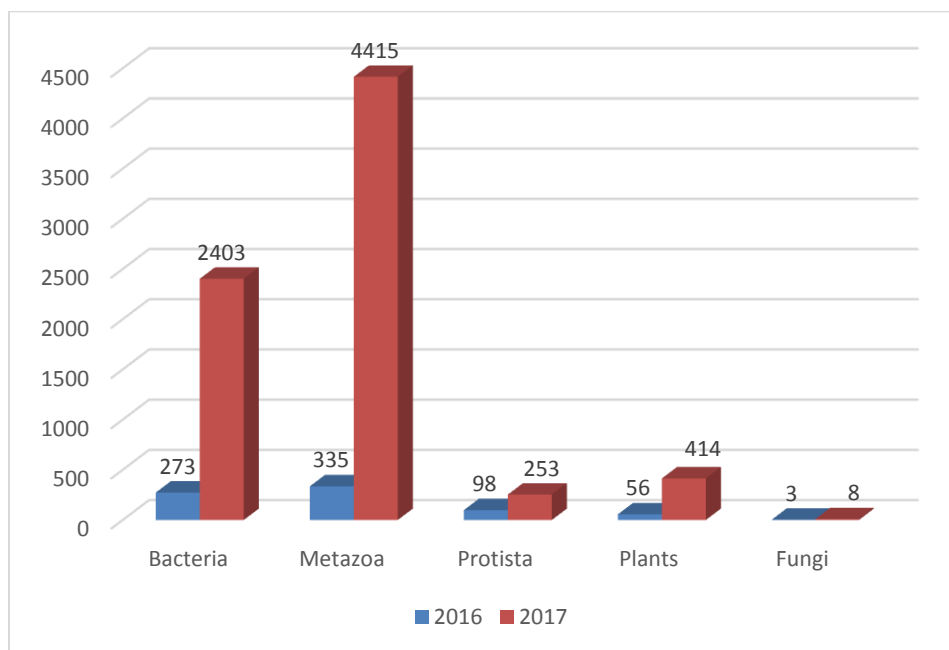


Figure 1. Species diversity of Lake Verkhny Kaban (2016; 2017)

The percentage of taxonomic groups of Lake Verkhny Kaban by the reads (2016 / 2017) is the following: Bacteria - 70.03/32.07%, Metazoa - 18.5/58.93%, Plants - 5.02/5.53%, Protista - 6.2/ 3.38% and Fungi 0.08/0.11 % (Figure 2).

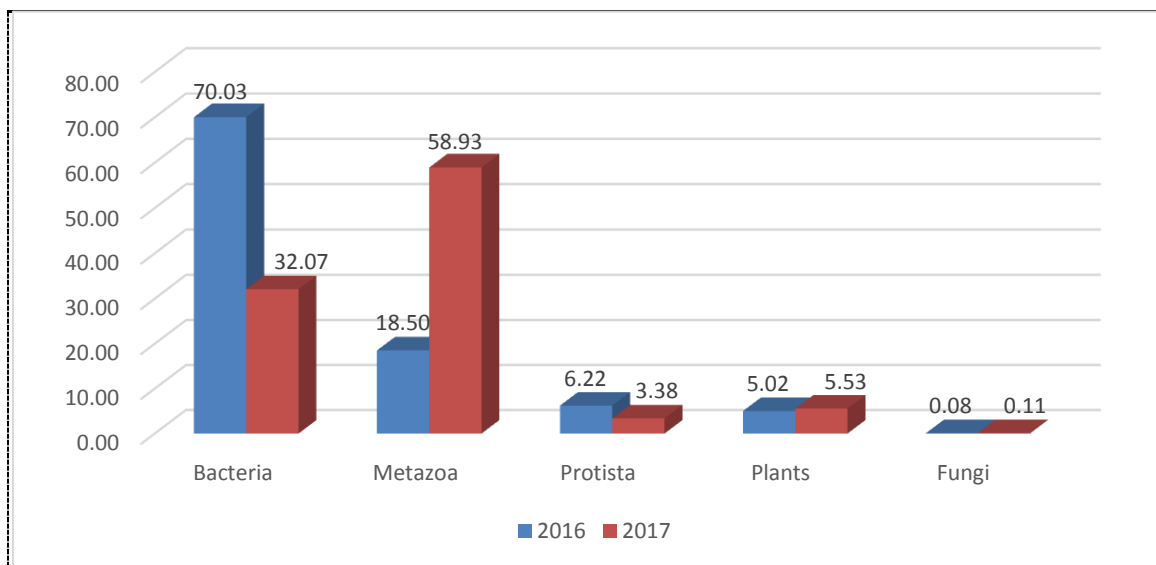


Figure 2. The percentage of taxons of Lake Verkhny Kaban by reads (2016; 2017)

The taxonomic groups of Metazoa are analyzed below, since DNA barcode (*COI*) can only be used to identify animal organisms.

The following variety of species was identified, by the years 2016/2017, respectively: 7/3 species of *Amphibia*, 6/3 species of *Porifera*, 3/5 species of *Echinodermata*, 22/11 species of *Cnidaria*, 1/2 species of *Collembola*, 12/23 species of *Rotifera*, 10/13 species of *Mammalia*, 5/4 species of *Myriapoda*, 36/46 species of *Mollusca*, 97/131

species of *Insecta*, 40/48 species of *Arachnida*, 9/6 *Aves* species, 19/29 species of *Crustacea*, 2/1 *Reptilia* species, 29/12 *Pisces* species and 13/14 species of *Worms*.

The results of sequencing of fragment sequences of the gene *COI* of hydrobionts, living in the freshwater Lake Verkhny Kaban, carried out in 2016 and 2017, by the variety of species Metazoa are shown in percentage terms in Table 2 and are presented in Figure 3.

Table 2. Species diversity of Metazoa of Lake Verkhny Kaban in percentage terms

Taxon	2016	2017
<i>Amphibia</i>	2.25	0.85
<i>Porifera</i>	1.93	0.85
<i>Echinodermata</i>	0.96	1.42
<i>Cnidaria</i>	7.07	3.13
<i>Collembola</i>	0.32	0.57
<i>Rotifera</i>	3.86	6.55
<i>Mammalia</i>	3.22	3.70
<i>Myriapoda</i>	1.61	1.14
<i>Mollusca</i>	11.58	13.11
<i>Insecta</i>	31.19	37.32
<i>Arachnida</i>	12.86	13.68
<i>Aves</i>	2.89	1.71
<i>Crustacea</i>	6.11	8.26
<i>Reptilia</i>	0.64	0.28
<i>Pisces</i>	9.32	3.42
<i>Worms</i>	4.18	3.99

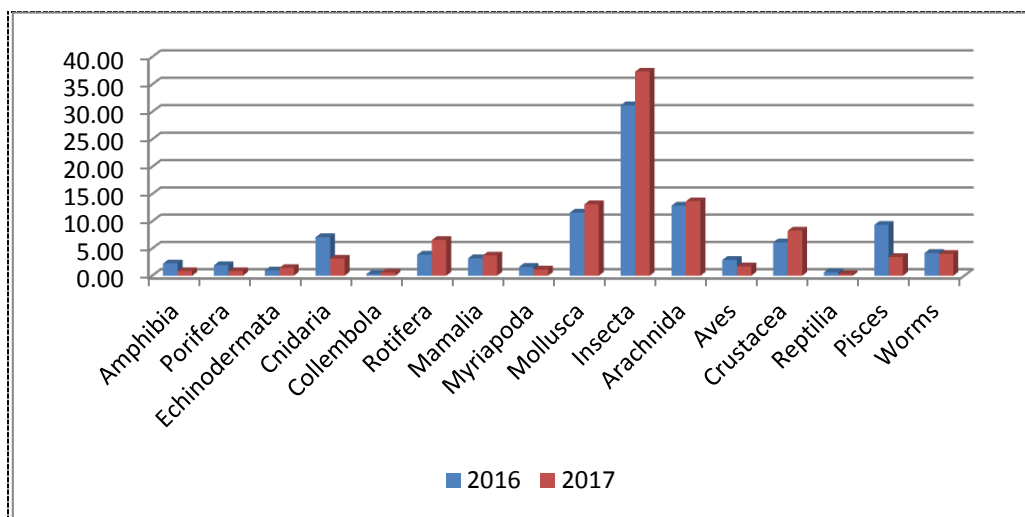


Figure 3. Species diversity of Metazoa of Lake Verkhny Kaban in percentage terms (2016; 2017)

The following quantity of organisms was identified, by the years 2016/2017, respectively: 16/47 reads of *Amphibia*, 35/21 reads of *Porifera*, 3/10 reads of *Echinodermata*, 74/90 reads of *Cnidaria*, 37/4 reads of *Collembola*, 278/1246 reads of *Rotifera*, 30/114 reads of *Mammalia*, 9/5 reads of *Myriapoda*, 143/326 reads of *Mollusca*, 423/1393 reads of *Insecta*, 77/527 reads of *Arachnida*, 29/9 reads of *Aves*, 129/322 reads of *Crustacea*, 2/1 reads of *Reptilia*, 107/36 reads of *Pisces* and 100/97 reads of *Worms*.

The results of sequencing of fragment sequences of the gene *COI* of hydrobionts, living in the freshwater Lake Verkhny Kaban, carried out in 2016 and 2017, by the quantity of Metazoa organisms are given in percentage terms in Table 3 and are shown in Figure 4.

Table 3. The quantity of Metazoa organisms (the quantity of reads) of Lake Verkhny Kabanin percentage terms

Taxon	2016	2017
<i>Amphibia</i>	1.07	1.11
<i>Porifera</i>	2.35	0.49
<i>Echinodermata</i>	0.20	0.24
<i>Cnidaria</i>	4.96	2.12
<i>Collembola</i>	2.48	0.09
<i>Rotifera</i>	18.63	29.33
<i>Mammalia</i>	2.01	2.68
<i>Myriapoda</i>	0.60	0.12
<i>Mollusca</i>	9.58	7.67
<i>Insecta</i>	28.35	32.79
<i>Arachnida</i>	5.16	12.41
<i>Aves</i>	1.94	0.21
<i>Crustacea</i>	8.65	7.58
<i>Reptilia</i>	0.13	0.02
<i>Pisces</i>	7.17	0.85
<i>Worms</i>	6.70	2.28

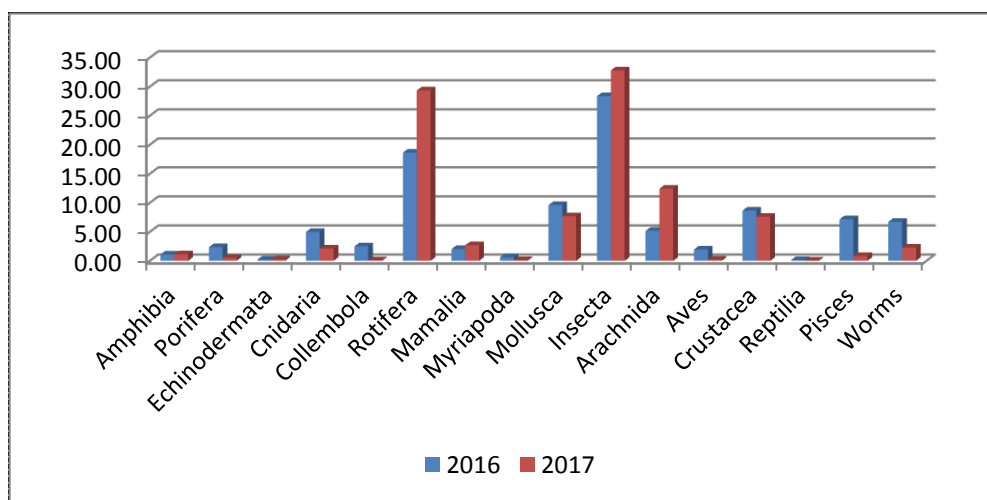


Figure 4. The quantity of Metazoa organisms (the quantity of reads) of Lake Verkhny Kabanin percentage terms (2016; 2017)

From the analysis of metagenomic data, based on the fragment of *COI* gene of animal organisms of Lake

Verkhny Kaban in 2016, 159 organisms were identified. They have the status of indicators, and

belong to the list of indicator organisms of V. Sladechek [11]:

- 153 Rotifers:
  - 5 organisms of *Brachionus calyciflorus*, belonging to the *b-a*-mesosaprobity, with indicator weight of 2.50;
  - 9 organisms of *Keratella cochlearis*, belonging to the *b-o*-saprobity, with indicator weight of 1.55;
  - 103 organisms of *Brachionus plicatilis*, belonging to the *b*-mesosaprobity, with indicator weight of 2.00;
  - 2 organisms of *Brachionus sericus*, 1 organism of *Philodina citrina*, 21 organisms of *Polyarthra dolichoptera*, 12 organisms of *Synchaeta kitina*, belonging to the *o*-saprobity, with indicator weights from 0.90 to 1.40;
  - 1 organism *Philodina megalotrocha* - belonging to the *o-b*-mesosaprobity, with indicator weight of 1.50;
- 1 Mollusc – *Unio crassus*, belonging to the *b-o*-mesosaprobity, with indicator weight of 1.50;
- 5 Insects of the same species *Baetis alpinus*, belonging to the *x*-saprobity, with indicator weight of 0.20.

From the analysis of metagenomic data, based on the fragment of *COI* gene of animal organisms of Lake Verkhny Kaban in 2017, 748 organisms were

identified. They have the status of indicators, and belong to the list of indicator organisms of V. Sladechek [11]:

- 691 Rotifers:
  - 305 organisms of *Brachionus calyciflorus*, belonging to the *b-a*-mesosaprobity, with indicator weight of 2.50;
  - 1 organism of *Brachionus rubens*, belonging to the *a*-mesosaprobity, with indicator weight of 3.25;
  - 74 organisms of *Brachionus plicatilis*, belonging to the *b*-mesosaprobity, with indicator weight of 2.00;
  - 24 organisms of *Keratella cochlearis*, belonging to the *b-o*-saprobity, with indicator weight of 1.55;
  - 5 organisms of *Trichocerca capucina*, 1 organism of *Macrotrachela quadricornifera*, 1 organism of *Philodina citrina*, belonging to the *o*-saprobity, with indicator weights from 1.00 to 1.15;
  - 280 organisms of *Philodina megalotrocha*, belonging to the *o-b*-mesosaprobity, with indicator weight of 1.50;
- 57 Crustaceans:
  - 27 organisms of *Daphnia magna*, belonging to the *a-p*-saprobity, with indicator weight of 3.40;
  - 30 organisms of *Thermocyclops oithonoides*, belonging to the *o*-saprobity, with indicator weight of 1.30.

The percentage of hydrobionts by saprobity is shown in Figure 5.

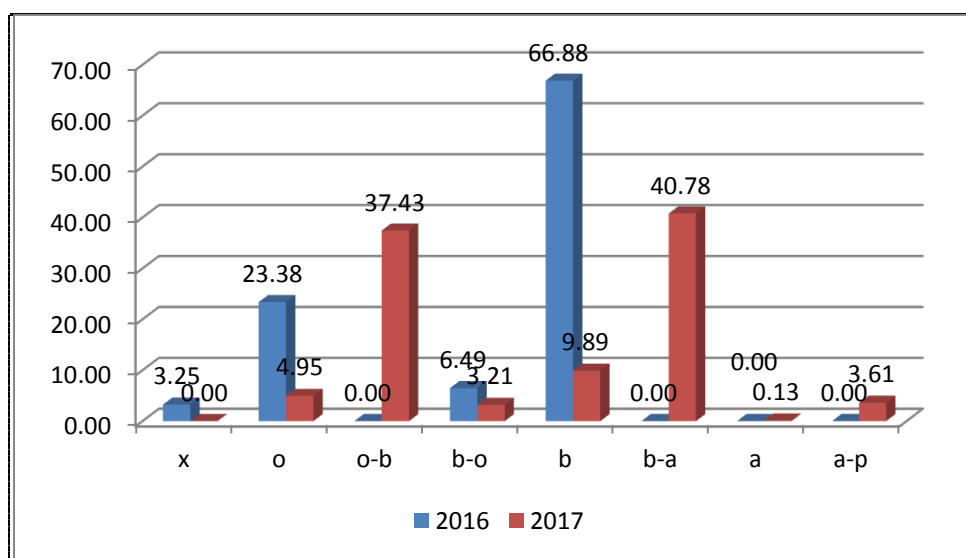


Figure 5. The percentage of hydrobionts saprobity of Lake Verkhny Kaban

(2016; 2017)

The comparative analysis of metagenomic data shows, that most of the animal organisms of Lake

Verkhny Kaban are grouped around the *b*-mesosaprobity (Fig. 5). Animal organisms prevailed

(58.93%) in 2017, compared with bacterial organisms (32.07%). Whereas in 2016, bacterial organisms (70.3%) prevailed in Lake Verkhny Kaban, compared to animal organisms (18.5%) (Fig. 2), that was due to the blooming of water.

#### SUMMARY:

Thus, as a result of the use of modern methods of next-generation sequencing, molecular and bioinformation analysis, Lake Verkhny Kaban in terms of water quality can be characterized as moderately polluted, and the value of *b*-mesosaprobity. The obtained results coincide with the results of studies, carried out by the ecologists on Lake Verkhny Kaban during the indicated periods, according to which Lake Verkhny Kaban is at the stage of transition from *b*-mesosaprobity to *o*-saprobity.

#### CONCLUSIONS:

The method of bioindication is an integral part of the assessment of ecological state of water bodies, which is carried out according to the recommendations, developed in the last century. During this time, science and practice have moved far ahead. Progress in molecular biology has improved the identification of organisms by marker genes and proteins. The inventions give the positive results, and increase the effectiveness and reliability of assessment the ecological status of water object. The obtained results are of great practical interest in the field of monitoring of water bodies, in particular, and the environment as a whole.

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