

# 18S rRNA Identification of Hydrobionts of Nizhniy Kaban Lake & Diversity Study through Next Generation Sequencing Method

<sup>1</sup>Ludmila Frolova, <sup>2</sup>Anthony Elias Sverdrup

<sup>1-2</sup>Kazan Federal University

Email: Lucie.Frolova@gmail.com

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## Abstract

The paper presents the results of a study of the species diversity of freshwater Nizhniy Kaban Lake (Kazan, Russia) by the gene 18S *rRNA* based on the next-generation sequencing method. Nizhniy Kaban, Verhniy Kaban, and Sredniy Kaban Lakes are included in the system of urban Kaban Lakes, which are polluted lakes according to ecological studies. Sequences of the 18S *rRNA* gene fragment of the freshwater Nizhniy Kaban Lake hydrobionts were submitted to the international SRA database on NCBI website in fastq format with numbers SRR7510988, SRR7516481, SRR7465568. A total of 39505 (2016) and 22619 (2017) high-quality reads were obtained; 89.4% (2016) and 83.8% (2017) of the hydrobionts was classified to the kingdom level, while 74.7% (2016) and 81% (2017) was classified to the phylum level, 48.2% (2016) and 79.5% (2017) was classified to the class level, 80% (2017) was classified to the family level, 79.6% (2017) was classified to the genus level, and 41.9% (2017) was classified to the species level. Analysis of the metagenomic data for Nizhniy Kaban Lake shows that indicator organisms identified by the 18S *rRNA* gene are grouped near the *b*-mesosaprobity. Nizhniy Kaban Lake can be characterized as contaminated, in terms of water quality, transitional from *b*- to *a*-mesosaprobity.

## Keywords

*Saprobity, Gene 18S rRNA, Next-Generation Sequencing, Freshwater Lake, Ecology*

## Introduction

At present time, the rapid development of metagenomics is associated with the proliferation of next-generation sequencing methods. Metagenomics can provide valuable information on the functional ecology of environmental communities [1]. We previously used metagenomic DNA sequencing to analyze species diversity by marker genes *COI* of animal organisms and *rbcL* of plants to assess the ecological state of freshwater Nizhniy Kaban Lake by bioindication [2, 3]. In contrast to the marker genes *COI* and *rbcL*, the gene 18S *rRNA* is present in all eukaryotes. The ribosomal genes (*rRNA*) are one of the most conserved genes. Therefore, the systematic position of the organism and the time of discrepancy with similar species can be determined on the basis of the analysis of similarities and differences in *rRNA* sequences [4]. For eukaryotes, it is most convenient to analyze 18S *rRNA* of the three types of *rRNA*.

The paper presents the results of a study of the species diversity of the freshwater Nizhniy Kaban Lake (Kazan, Russia) according to the gene 18S *rRNA* of freshwater organisms based on the next generation sequencing method. Nizhniy Kaban, Verhniy Kaban, and Sredniy Kaban Lakes are included in the system of urban Kaban Lakes, which are polluted lakes according to ecological studies.

## Methods

Sampling from Nizhniy Kaban Lake (Kazan, Russia) was carried out in 2016-2017 in accordance with standard hydrobiological methods [5].

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

Primers	Sequences
SR1_Illumina	5'-tcgtcggcagcgtcagatgtgtataagacagattaccgcgctgct-3'
SR1r_Illumina	5'-gtctcgtggctcggagatgtgtataagacagctacctggtgatctgccagt-3'

**Table 1: Primers for PCR of 18S rRNA Gene**

A second PCR cycle was performed to index samples (Nextera XT indices). Purification of PCR products was performed using Agencourt AMPure XP beads (Beckman Coulter). The obtained DNA libraries were sequenced on an Illumina MiSeq instrument (MiSeq Reagent kit v3). Metagenomic data were submitted to the international SRA database on NCBI website with the numbers SRR7510988, SRR7516481, SRR7465568 [6]. After filtering reads by quality, trimming service sequences, and removing chimeric sequences, obtained nucleotide sequences of the 18S *rRNA* gene of hydrobionts were aligned with the BLAST program to establish the taxonomic composition. The Krona chart [7] and GraphPad [8] software were used for charts.

**Results and Discussion**

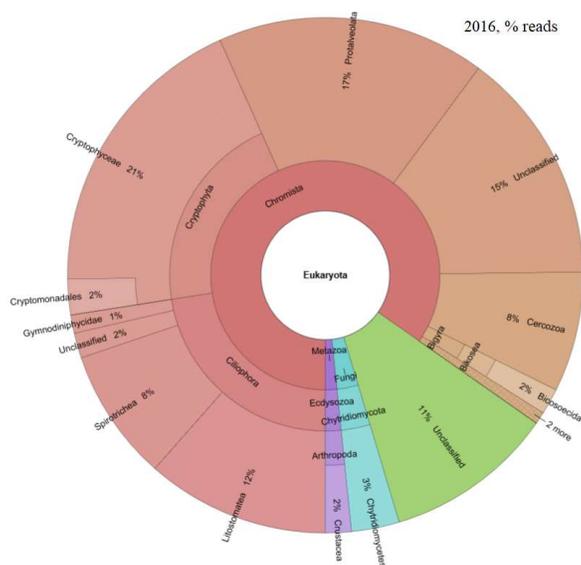
In 2016-2017 the next-generation sequencing method was used to identify hydrobiotics by *18S rRNA* gene from Nizhniy Kaban Lake. It is a lake about 2 km in length and 0.3 km in wide and has geographical coordinates of 55°46'35.6"N and 49°07'25.0" E (Fig. 1). Nizhniy Kaban Lake is located in the center of an industrial city, thus experiencing anthropogenic pressure. According to ecological studies, Nizhniy Kaban Lake is a polluted lake.



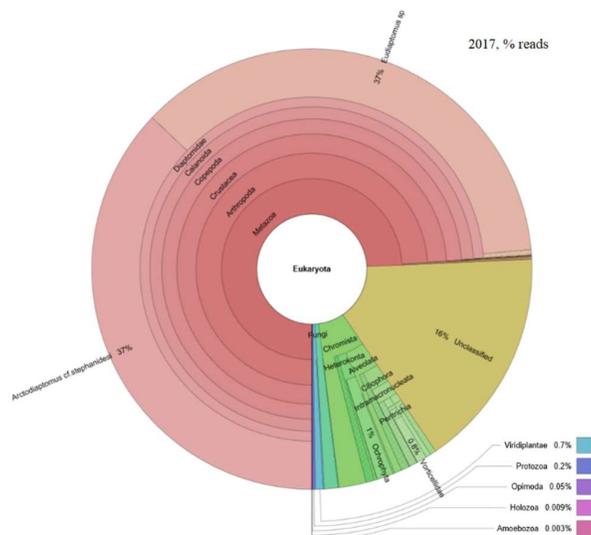
**Figure 1: The Satellite Map of Nizhniy Kaban Lake (Kazan, Russia)**  
<https://www.google.com>

*Krona chart of the hydrobiotics represented by 18S rRNA gene amplicon-based species diversity*

The percentage distribution of hydrobiotics of Nizhniy Kaban Lake by reads for 2016 is shown in Fig. 2. The percentage distribution of hydrobiotics of Nizhniy Kaban Lake by reads for 2017 is shown in Fig. 3. Each circle represents the kingdom, phylum, class, order, family, genus, and species from the inside to the outside of the circle, respectively, indicated by percent diversity.



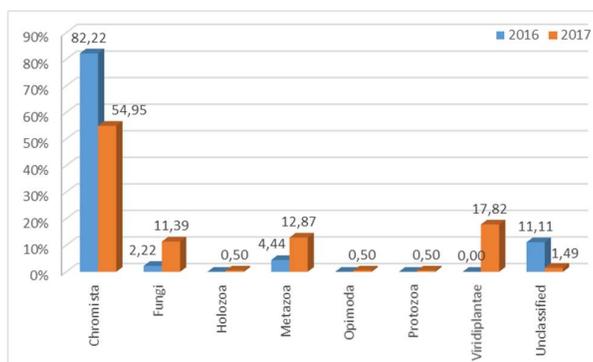
**Figure 2: The Percentage of 18S rRNA Hydrobiotics by Reads of Nizhniy Kaban Lake (2016)**



**Figure 3: The Percentage of 18S rRNA Hydrobionts by Reads of Nizhniy Kaban Lake (2017)**

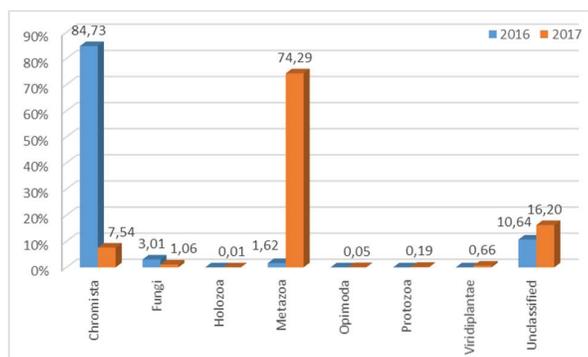
*The percentage of 18S rRNA hydrobionts of Nizhniy Kaban Lake by kingdom*

The percentage of species diversity of 18S rRNA hydrobionts of Nizhniy Kaban Lake by kingdom (2016-2017) shown in Figure 4. As can be seen from Fig. 4, the most numerous at the kingdom level, respectively, by year are *Chromista* (82.22% / 54.95%), *Metazoa* (4.44% / 12.87%) and *Viridiplantae* - 17.82% (2017) by species diversity.



**Figure 4: The Percentage of Species Diversity of 18S rRNA Hydrobionts of Nizhniy Kaban Lake by Kingdom (2016-2017)**

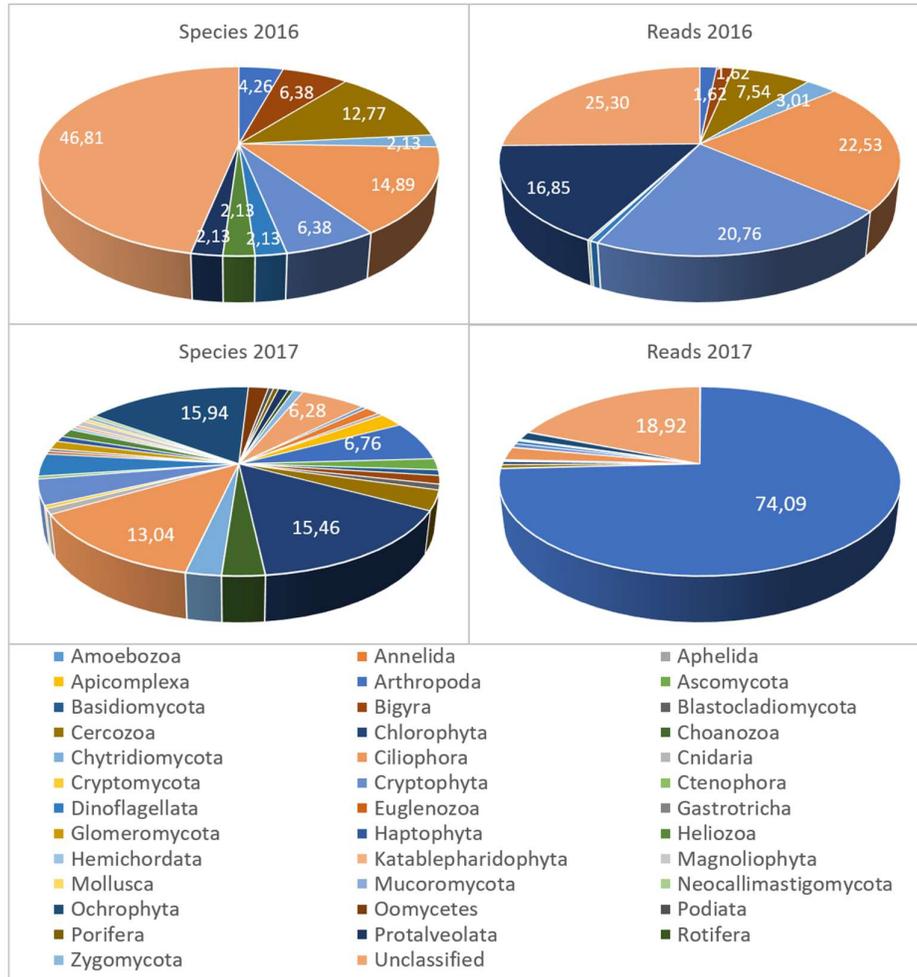
The percentage of reads of 18S rRNA hydrobionts of Nizhniy Kaban Lake by kingdom (2016-2017) shown in Figure 5. As can be seen from Fig. 5 among the hydrobionts, the most numerous according by reads are *Chromista* (84.73% / 7.54%) and *Metazoa* – 74.29% (2017).



**Figure 5: The Percentage of Reads of 18S rRNA Hydrobionts of Nizhniy Kaban Lake by Kingdom (2016-2017)**

*The percentage of 18S rRNA hydrobionts of Nizhniy Kaban Lake by phylum*

The percentage of species diversity and reads of *18S rRNA* hydrobionts of Nizhniy Kaban Lake by phylum (2016-2017) shown in Figure 6. As can be seen from Fig. 6, the most numerous at the phylum level for 2016 are *Ciliophora* (14.89%), *Cercozoa* (12.77%), *Bigyra* and *Cryptophyta* (6.38%), and Unclassified (46.81%) by species diversity; 2017 - *Ochrophyta* (15.94%), *Chlorophyta* (15.46%), *Ciliophora* (13.04%) and Unclassified (6.28%).



**Figure 6: The Percentage of Species Diversity and Reads of *18S rRNA* Hydrobionts of Nizhniy Kaban Lake by Phylum (2016-2017)**

*The percentage of 18S rRNA hydrobionts of Nizhniy Kaban Lake by species*

The species diversity of hydrobionts in 2017 is 41.9% of reads from the total number of organisms. Ten hydrobionts are included on a top by reads - *Arctodiatomus cf.stephanidesi* (88.46%), *Cryptomonas curvata* (2.97%), *Leiocephalium pseudosanguineum* (0.96%), *Paraphysomonas foraminifera* (0.92%), *Onychodiatomus sanguineus* (0.77%), *Vorticella aequilata* (0.58%), *Coleps hirtus* (0.46), *Stephanodiscus parvus* (0.42%), *Poteriochromonas malhamensis* (0.23%), *Fragilaria rumpens* (0.21%) (Fig 7).

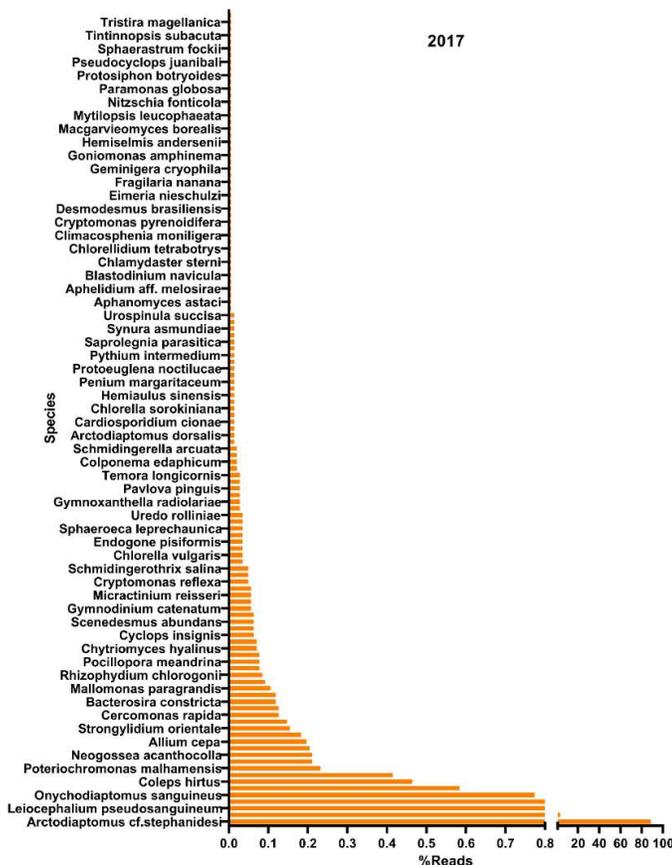


Figure 7: The Species of Hydrobionts and Reads of Nizhny Kaban Lake (2017)

122 hydrobionts of Nizhny Kaban Lake were identified by 18S rRNA gene. Among them, there are 14 hydrobionts with saprobities in Sladechek's list [9]. Table 2 shows the saprobionts of Nizhny Kaban Lake.

Species	Saprobity	Reads%
<i>Cryptomonas curvata</i> <sup>1</sup>	b	2.970
<i>Vorticella aequilata</i> <sup>2</sup>	p	0.580
<i>Coleps hirtus</i> <sup>3</sup>	b-a	0.460
<i>Eudiaptomus graciloides</i> <sup>4</sup>	o	0.120
<i>Cyclops insignis</i> <sup>5</sup>	o-b	0.063
<i>Keratella cochlearis</i> <sup>6</sup>	b-o	0.060
<i>Tintinnidium fluviatile</i> <sup>7</sup>	o-b	0.035
<i>Chlorella vulgaris</i> <sup>8</sup>	p-a	0.035
<i>Botryococcus braunii</i> <sup>9</sup>	o-b	0.007
<i>Sphaeroeca volvox</i> <sup>10</sup>	a	0.007
<i>Nitzschia fonticola</i> <sup>11</sup>	o-b	0.007
<i>Dinobryon divergens</i> <sup>12</sup>	b	0.007
<i>Cyclotella meneghiniana</i> <sup>13</sup>	a-b	0.007
<i>Chlamydomonas reinhardtii</i> <sup>14</sup>	a	0.007

Table 2 : Hydrobionts with Saprobities in Sladechek's List of Nizhny Kaban Lake

<sup>1</sup> Freshwater algae. They are not known to produce any toxins [10]. *Cryptomonas* are also photolithotrophs that contribute to oxygenic carbon fixation making them highly critical to the carbon levels of freshwater environments [11].

<sup>2</sup> Freshwater species of *Ciliata*. *Vorticella* habitats may include moist soil, mud and plant roots. They are known to feed on bacteria and can also form extracellular associations with mosquitoes, nematodes, prawns, and tadpoles [12].

<sup>3</sup> The *C. hirtus* does not possess algal symbionts and feeds directly on other microbes [13]. *Coleps* feeds on bacteria, algae, flagellates, living and dead ciliates, animal and plant tissues [14]. *Coleps* uses toxicysts, which are organelles containing poison that it uses to capture its prey from its oral area.

<sup>4</sup> Invertebrate animals. *E. graciloides* is only found at pH <5.5 when conductivity is above 2 mS / m [15].

<sup>5</sup> Freshwater invertebrates. It lives along the plant-covered banks of stagnant and slow-flowing bodies of water, where it feeds on small fragments of plant material, animals or carrion. *Cyclops* has the capacity to survive unsuitable conditions by forming a cloak of slime [16].

<sup>6</sup> *Keratella cochlearis* is a rotifer [17]. The planktonic animal occurs worldwide in freshwater and marine habitats.

<sup>7</sup> *Tintinnidium fluviatile* - unicellular eukaryotes. A review of the ecology of the fresh-water Tintinnina indicated that water temperature seems to be the most essential ecologic factor [18].

<sup>8</sup> Green eukaryotic microalgae, which has been present on earth since the Precambrian period [19].

<sup>9</sup> *Botryococcus braunii* is a green planktonic microalga. The species is notable for its ability to produce high amounts of hydrocarbons, that are typically around 30–40% of their dry weight [20, 21].

<sup>10</sup> Choanoflagellata is a type of unicellular eukaryote. *Sphaeroeca volvox* is the type species of the genus *Sphaeroeca* of *Choanoflagellata* [22].

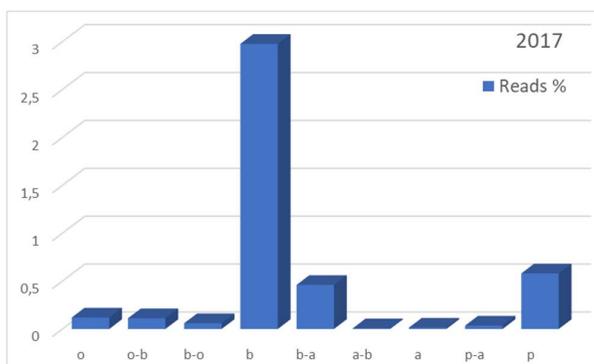
<sup>11</sup> A freshwater species of unicellular diatoms [23]. An important factor for the development of diatoms is temperature, the degree of illumination, and the quality of light [24].

<sup>12</sup> *Dinobryon* is a type of microscopic algae. *Dinobryon* is mixotrophs, capable of obtaining energy and carbon through photosynthesis and phagotrophy of bacteria. *D. divergens* blooms in lakes and ponds. Such blooms may produce volatile organic compounds that produce odors and affect water quality [25, 26].

<sup>13</sup> *Cyclotella* is a centric diatom. *Cyclotella* occurs in stagnant waters. The nutrient content does not matter. They live both floating (plankton) and on the ground (benthos). *C. meneghiniana* was firstly described by Kützing [27].

<sup>14</sup> *Chlamydomonas reinhardtii* is a single-cell green algae. *C. reinhardtii* is a well-studied biological model of the organism, partly due to its ease of culturing and the ability to manipulate its genetics. When illuminated, *C. reinhardtii* can grow photoautotrophically, but it can also grow in the dark if supplied with organic carbon [23].

Fig. 8 shows the percentage of saprobionts of Nizhniy Kaban Lake by reads for 2017.



**Figure 8: The Percentage of Reads by Hydrobionts Saprobity of Nizhniy Kaban Lake (2017)**

As can be seen from Fig. 8, most indicator organisms are grouped near *b*-mesosaprobity, which, in accordance with the classification of water quality, is characterized as polluted.

### Summary

Based on the results of the study, the next-generation sequencing method was used to identify hydrobionts by *18S rRNA* gene for Nizhniy Kaban Lake for 2016-2017.

A total of 39505 (2016) and 22619 (2017) high-quality reads were obtained; 89.4% (2016) and 83.8% (2017) of the hydrobionts was classified to the kingdom, while 74.7% (2016) and 81% (2017) was classified to the phylum level, 48.2% (2016) and 79.5% (2017) was classified to the class level, 80% (2017) was classified to the family level, 79.6% (2017) was classified to the genus level, and 41.9% (2017) was classified to the species level.

A total of 122 species were identified, among them there are 14 species with saprobity from Sladeczek's list. Algae *Cryptomonas curvata* of *b*-mesosaprobity is the most numerous by reads. *Cryptomonas* are also photolithotrophs that contribute to oxygenic carbon fixation making them highly critical to the carbon levels of fresh water environments [11]. *b*-mesosaprobity indicates that the water is polluted.

### Conclusions

Usage of the next-generation sequencing method allows the identification of a large number of hydrobionts. The method can be used for bioindication for assessing the ecological state of water reservoirs. This is great practical interest in the field of monitoring of water reservoirs.

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