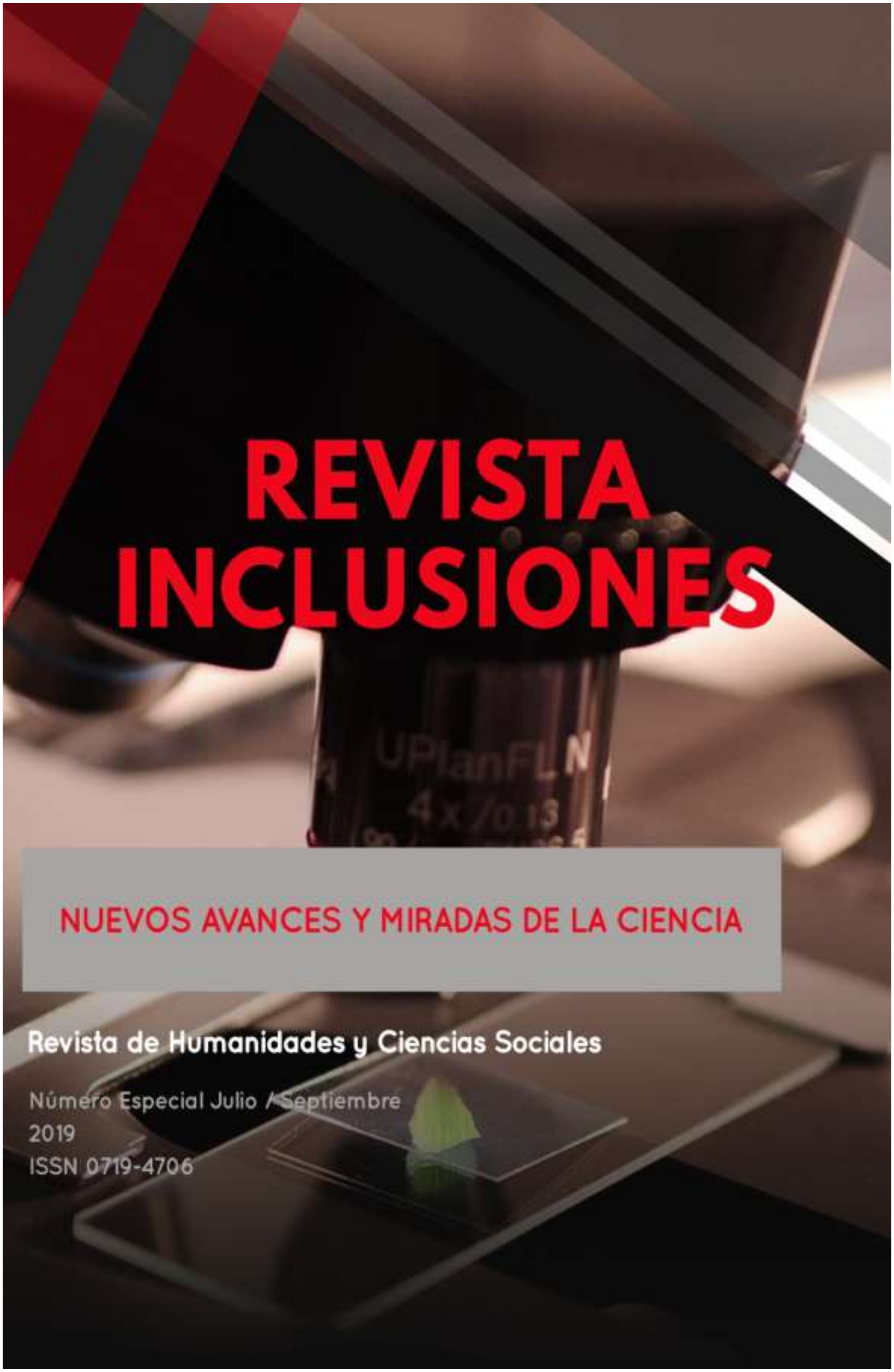

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THE NEXT-GENERATION SEQUENCYG BY RBCL GENE OF FRESHWATER ORGANISM FOR MONITORING OF SREDNY KABAN LAKE

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Abstract

The paper presents the results of analysis and an assessment of water quality of the Sredny Kaban Lake (Kazan, Russia) based on the rbcL gene of freshwater organisms by the method of sequencing a new generation. A comparative analysis of metagenomic data shows that most of the organisms of Lake Sredny Kaban are grouped by the RBCL gene near the b-mesosaprobity. Lake Sredny Kaban can be characterized as polluted, according to the quality of water, transitional to b-mesosaprobic.

Keywords

Bioindication – Probity – RBCL gene – Next-generation – Sequencing

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Introduction

At present, metagenomic sequencing makes it possible to significantly speed up and reduce the cost of sequencing the genomes of millions of organisms[1] compared with other sequencing methods, such as, for example, the Sanger method for DNA barcoding of animals[2] and plants. Numerous sequenced gene sequences by species of organisms are accumulated in international databases, for example, on the NCBI website. The widespread development of metagenomics is due to the proliferation of sequencing methods used in biology and medicine to assess the diversity of microorganisms in various media, for example, in the human intestine, bottom sediments of Lake Baikal or the hot springs of Kamchatka (https://biomolecula.ru). Metagenomics can provide valuable information about the functional ecology of environmental communities[3]. Metagenomic DNA sequencing can also be used to identify species present in the water column. The method of sequencing a new generation was previously used by us to assess the ecological state of freshwater bodies by the method of bioindication[4].

Bioindication[5] is based on observation of the composition and abundance of indicator species, which make it possible to more accurately assess the quality of water in a pond[6]. Indicator species and communities of organisms can live in conditions optimal for them with different content of organic matter in water. There are different zones of water pollution by saprobity: from xenosaprobic (very clean) to polysaprobic (very dirty)[7].

The exact identification of organisms is not always unambiguous, because many species have very small sizes, some species are characterized by strong sexual dimorphism or vice versa, there are twin species[8]. Modern sequencing methods allow highly accurate identification of an organism by a marker gene and can be used to assess the ecological status of water bodies[9].

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This article provides an assessment of the water quality of Lake Sredny Kaban (Kazan, Russia) using the rbcL marker gene of phytoplankton organisms based on the new generation sequencing method. Lake Sredny Kaban along with the Upper Kaban and Nizhny Kaban lakes are included in the Kaban lakes system. The lakes are located in the center of a large industrial city, experiencing anthropogenic pressure. According to environmental assessments of Lake Kaban, they are classified as polluted lakes.

Methods

Samples from Lake Sredny Kaban (Kazan, Russia) were sampled in 2016-2017. following standard hydrobiological methods[10].

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

Primers	Sequences
rbcL_AB_FI (forward)	5'-tcgtcggcagcgtcagatgtgtataagagacagtcigciaaraactayggtcg-3'
rbcL_AB_RI (reverse)	5'-gtctcgtgggctcggagatgtgtataagagacagggcatrtgccaiacrtgrat-3'
rbcL_D_FI (forward)	5'-tcgtcggcagcgtcagatgtgtataagagacaggatgatgaraayattaactc-3'
rbcL_D_RI (reverse)	5'-gtctcgtgggctcggagatgtgtataagagacagatttgdccacagtgdatacca-3'

Table 1
Primers for PCR of rbcL gene of phytoplankton

The primer pair rbcL_AB_FI (forward) and rbcL_AB_RI (reverse) were used to identify Cyanobacteria, Chlorophyta and Proteobacteria; primer pair rbcL_D_FI (forward) and rbcL_D_RI (reverse) - to identify Bacillariophyta, Cryptophyta and Haptophyta.

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Purification of the PCR products was performed using Agencourt AMPure XP beads (Beckman Coulter), after which a second PCR was set to index samples (Nextera XT indices).

The obtained DNA libraries were sequenced on an Illumina MiSeq device (MiSeq Reagent kit v3). Metagenomic data were entered into the SRA international database on the NCBI website.

The resulting metagenomic data were leveled using the BLAST program to establish species diversity and subsequent analysis.

Results and Discussion

Sequenced sequences of the rbcL gene fragment of aquatic organisms in freshwater Lake Sredne Kaban for 2016-2017. in fastq format, they are entered into the SRA international database on the NCBI website with unique numbers: SRR7470847 (2016), SRR7459787 (2017) and SRR7463947 (2017). In 2016-2017 the method of sequencing the new generation carried out the identification of organisms - Cyanobacteria, Chlorophyta and Proteobacteria; In 2017, additional studies were conducted to identify organisms - Bacillariophyta, Pyrrophyta, Cryptophyta, and Haptophyta. After filtering the quality of the reads, trimming the utility sequences and removing chimeric sequences, the resulting nucleotide rbcL gene was aligned with the BLAST program to establish the taxonomic composition.

According to the results of the sequencing of the rbcL gene Cyanobacteria and rbcL Proteobacteria (Bacteria), rbcL Chlorophyta (Viridiplantae) of the fresh-water Lake Middle Kaban for 2016/2017. identified respectively: 62/34 species of Bacteria and 53/14 species of Viridiplantae. The percentage of taxonomic groups (Kingdom) of Lake Sredny Kaban by species (2016-2017), respectively: 53.91% / 46.09% Bacteria, 70.83% / 29.17% Viridiplantae (Fig. 1).

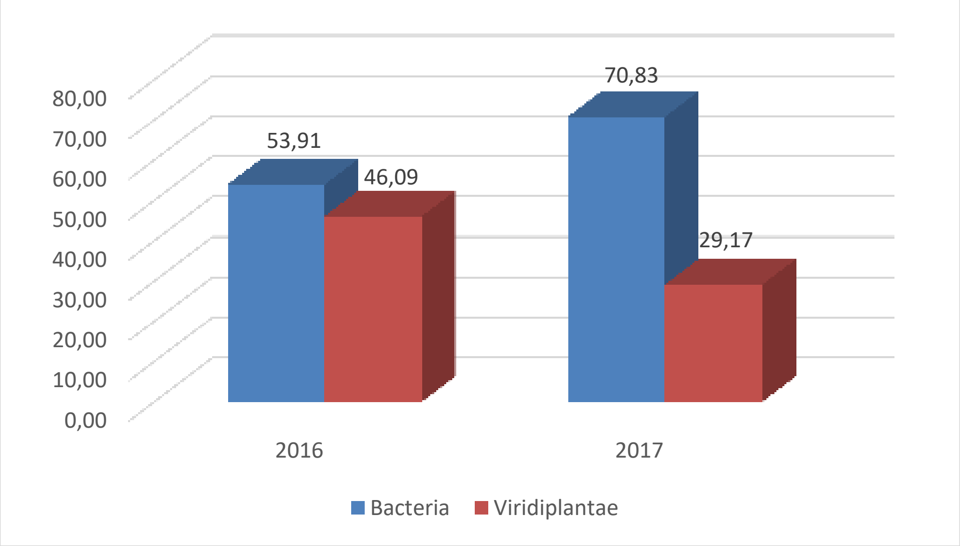

Figure 1
The percentage of the taxon (Kingdom) by species diversity
of Sredniy Kaban Lake (2016; 2017)

The percentage ratio of taxonomic groups (Kingdom) of Lake Sredny Kaban on reads is respectively: 97.90% / 98.43% Bacteria, 2.10% / 1.57% Viridiplantae (Fig. 2).

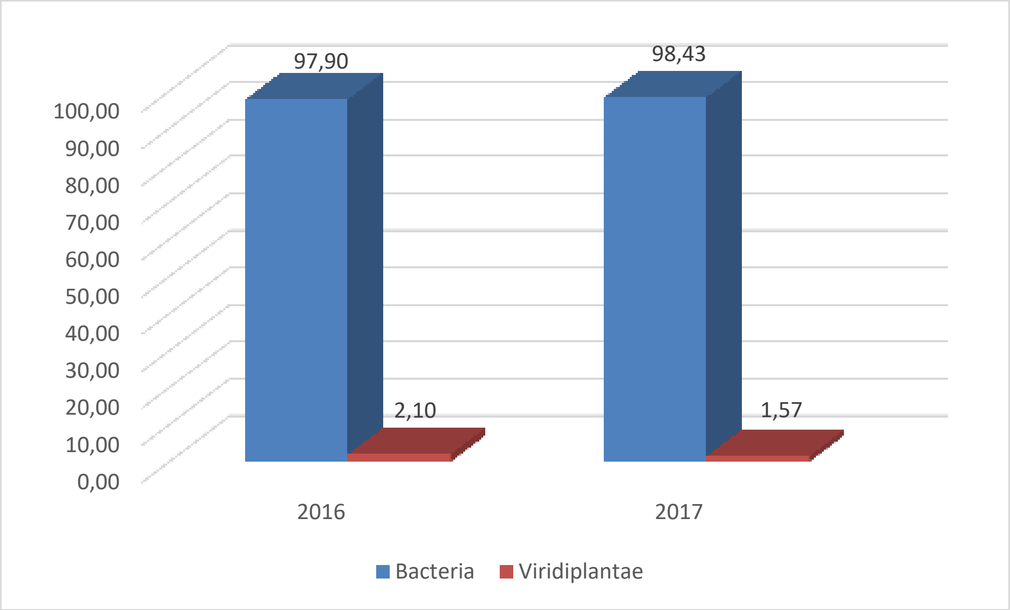

Figure 2
The percentage of the taxon (Kingdom)
by reads of Sredniy Kaban Lake (2016; 2017)

From Fig.1-2, it can be seen that Bacteria prevail by species and read. The greatest contribution is made by the bacteria Oscillatoria Nigro-Viridis (25% / 0%) and Planktothrix agardhii (70% / 93%), which are indicators of b-mesosaprobity.

According to the diversity of phytoplankton species of Lake Sred Kaban, respectively, by year 2016/2017: 23/17 Cyanobacteria species, 29/15 Proteobacteria species, 48/14 Chlorophyta species, respectively. The percentage of the diversity of phytoplankton species of Lake Sredny Kaban for 2016-2017. respectively, it is: 22.11% / 34.48% of Cyanobacteria species, 31.73% / 41.38% of Proteobacteria species, 46.15% / 24.14% of Chlorophyta species (Figure 3).

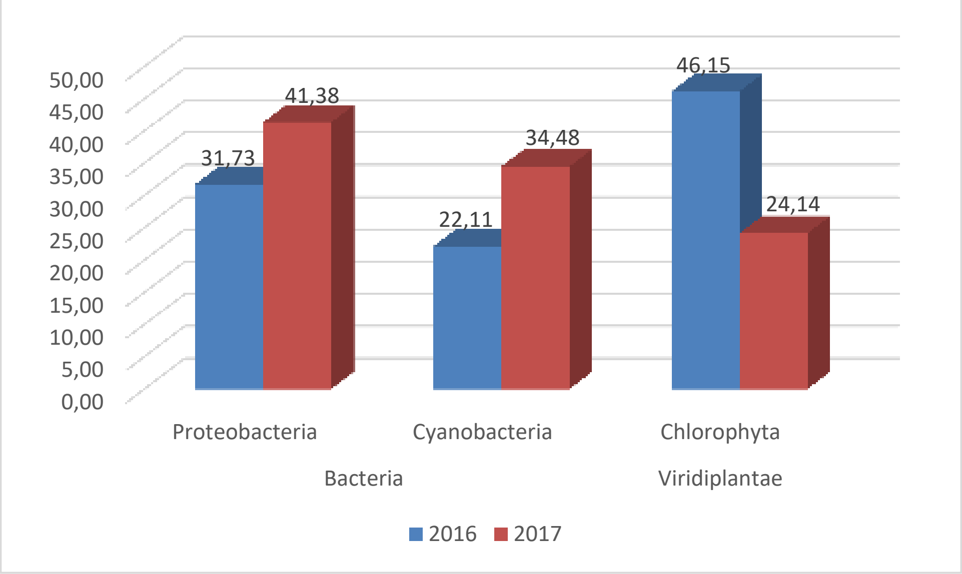

Figure 3
The percentage of species diversity of *rbcL* hydrobionts
of Sredny Kaban Lake (2016; 2017)

The percentage of the number of reads in the rbcL gene of hydrobionts of the freshwater Lake Sredny Kaban is, respectively, by year: 96.36% / 98.27% of Cyanobacteria reads, 1.58% / 0.47% of Proteobacteria reads, 2.07% / 1.26% of Chlorophyta reads (Fig. 4).

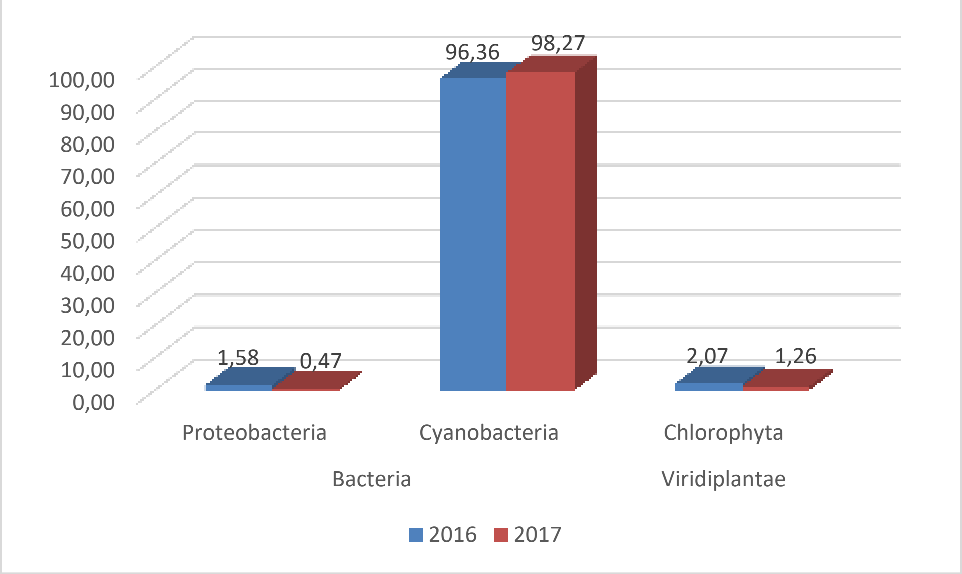

Figure 4
The percentage of *rbcL* hydrobionts reads of Sredniy Kaban Lake (2016; 2017)

From fig. 3-4 it can be seen that by species diversity in 2016. Chlorophyta prevails (predominantly b-a-mesosaprobity), and in 2017. Proteobacteria (p-saprobic); Reads are dominated by Cyanobacteria (o-b-mesosaprobic).

In 2017 Additional sequencing of the rbcL gene fragment Bacillariophyta, rbcL Cryptophyta and rbcL Haptophyta of the freshwater Lake Sredny Kaban was carried out.

Percentage ratio of taxonomic groups of Lake Sredny Kaban by types and reads for 2017 accordingly, it is: 8.77% / 7.77% Cryptophyta, 1.75% / 11.95% Haptophyta, 89.47% / 80.28% Bacillariophyta (Fig. 5). As can be seen from fig. 5 Bacillariophyta organisms significantly dominate by the diversity of species and the number of reads over other algae. IndicatorIndicator organisms of Bacillariophyta characterize the reservoir as b-o-saprobic.

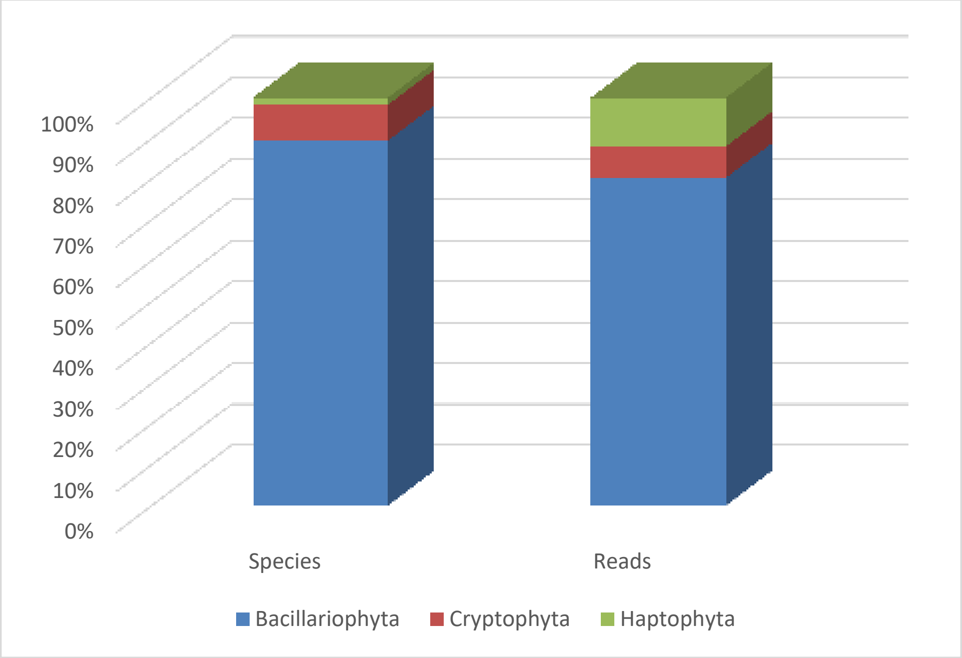

Figure 5
The percentage of taxon diversity and reads of hydrobionts
of Sredny Kaban Lake (2017)

The sequencing results of the rbcL gene fragment Bacillariophyta, rbcL Cryptophyta and rbcL Haptophyta of the freshwater Lake Sredny Kaban (2017) are shown in Fig. For the diversity of species and abundance of organisms Fig. 6.

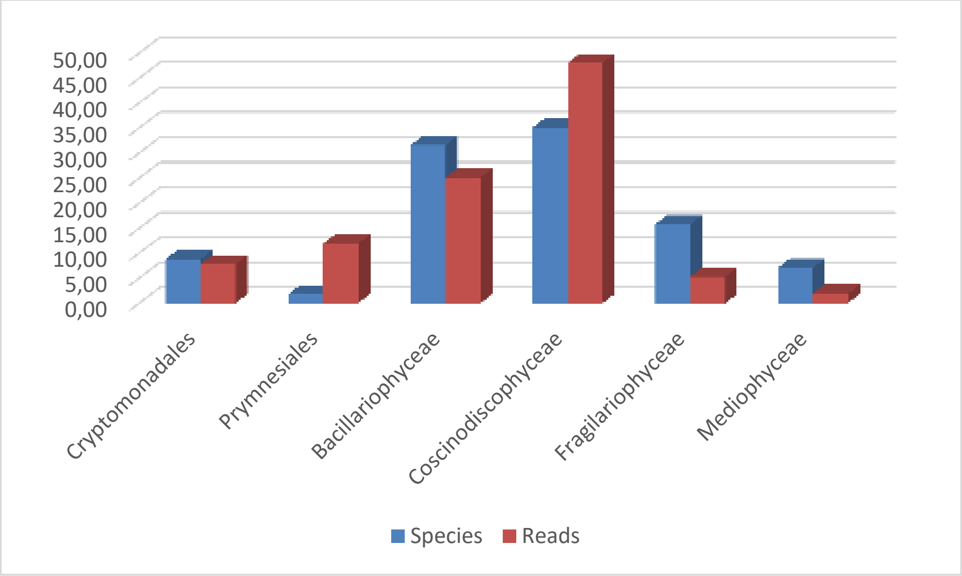

Figure 6
The percentage of species diversity and reads of hydrobionts
of Sredniy Kaban Lake (2017)

Figure 6 shows that Bacillariophyta: Bacillariophyceae (b-saprobity) and Coscinodiscophyceae (a-b-saprobity) dominate in the diversity of species, and in addition to them, Haptophyta: Prymnesiales.

From the analysis of metagenomic data on the rbcL gene fragment Cyanobacteria, rbcL Chlorophyta and rbcL Proteobacteria of Lake Sredny Kaban (2016), 12 species were identified that have the status of the indicator from the indicator organisms:

- 4 species of Cyanobacteria:

- Oscillatoria Nigro-Viridis, Planktothrix agardhii - b-mesosaprobity;
- Oscillatoria tenuis - a-mesosaprobity;
- Phormidium autumnale - b-a-mesosaprobity;

- 1 species of Proteobacteria:

- Thiocystis violascens - p-probity;

- 7 Chlorophyta species:

- Actinastrum hantzschii, Coelastrum pseudomicroporum, Micractinium pusillum, Botryococcus braunii - b-mesosaprobity;
- Ankistrodesmus falcatus - b-a-mesosaprobity;
- Chlorella vulgaris, Chlorella pyrenoidosa - p-a-saprobity.

From the analysis of metagenomic data on the rbcL gene Cyanobacteria, rbcL Chlorophyta of Lake Sredny Kaban (2017), 10 species were identified that have the status of the indicator from the lists of indicator organisms:

- 6 Cyanobacteria species:

- Anabaena crassa - o-b-mesosaprobity;
- Lyngbya aestuarii - b-o-mesosaprobity;
- Microcystis aeruginosa, Oscillatoria Nigro-Viridis, Planktothrix agardhii - b-mezosaprobity;
- Oscillatoria tenuis - a-mesosaprobity;

- 1 species of Proteobacteria:

- Thiocystis violascens - p-probity;

- 3 species of Chlorophyta:

- Phacotus lenticularis - b-mesosaprobity;
- Ankistrodesmus falcatus - b-a-mesosaprobity;
- Chlorella Vulgaris - p-a-mesosaprobity.

The percentage of the saprobity of species by the rbcL gene Cyanobacteria, rbcL Proteobacteria, and rbcL Chlorophyta (2016-2017) is shown in Fig.7. Figure 7 shows a significant predominance of the percentage ratio of species in b-mesosaprobity in both 2016 and 2017, and an increased value in p-a-saprobity in 2016.

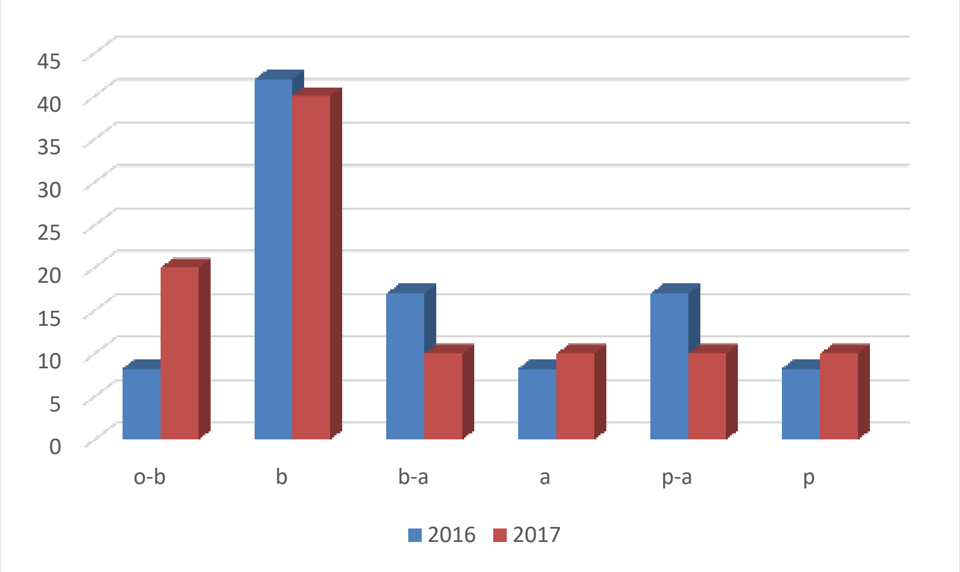

Figure 7
The percentage of hydrobionts saprobity by species
of Sredny Kaban Lake (2016; 2017)

The percentage of the number of reads by rbcL gene Cyanobacteria, rbcL Proteobacteria, and rbcL Chlorophyta by saprobity (2016, 2017) is shown in Fig.8.

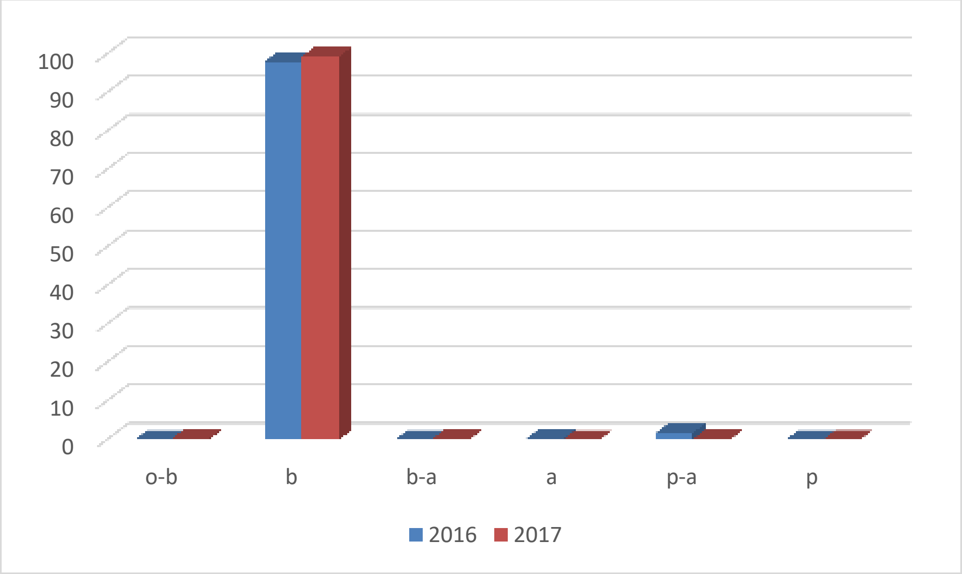

Figure 8
The percentage of hydrobionts saprobity by reads
of Sredny Kaban Lake (2016; 2017)

Figure 8 shows a significant predominance of the percentage of the number of reads for the rbcL gene of Cyanobacteria, rbcL of Proteobacteria, and rbcL of Chlorophyta by b-mezosprobnosti in 2016-2017. From the analysis of additional metagenomic data on the rbcL Haptophyta gene of Lake Sredny Kaban (2017), no phytoplankton organisms with indicator status from the list of indicator organisms were detected. From the analysis of additional metagenomic data on the rbcL gene Bacillariophyta and rbcL Cryptophyta of Lake Sredny Kaban (2017), 11 species have been identified that have the status of the indicator from the indicator organisms:

- 1 species of Cryptophyta

- Cryptomonas curvata - b-mesosaprobity;

- 10 species of Bacillariophyta:

- Amphora ovalis, Coscinodiscus radiatus, Cyclostephanos dubious, Cyclotella atoms, Gomphonema clever, Nitzschia fonticola - o-b-mesosaprobate;
- Amphora pediculus, Cyclotella meneghiniana, Cymbella cistula, Ulnaria delicatissima var.angustissima - b-mezosaprobnost.

The percentage of species and reads by rbcL gene Bacillariophyta and rbcL Cryptophyta by saprobity (2017) is shown in Fig.9. Figure 9 shows a slight predominance of the percentage ratio of species of hydrobionts in o-b-mesosaprobity and a significant predominance of a percentage of the number of hydrobiont readings in b-mesosaprobes in 2017.

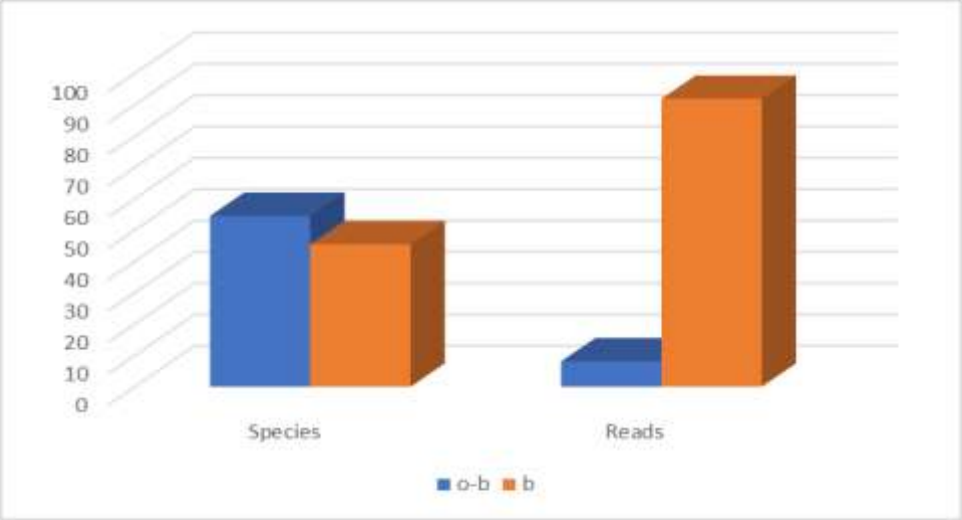

Figure 9
The percentage of hydrobionts saprobity
by species and reads of Sredniy Kaban Lake (2017)

The results obtained for the rbcL gene Bacillariophyta, rbcL Cryptophyta of indicator species complement the results for the rbcL gene Cyanobacteria, rbcL Chlorophyta and rbcL Proteobacteria, the indicator species of which indicate mainly the b-mesosaprobity of Lake Sred Kaban.

Summary

According to the results of research using modern methods of sequencing a new generation, molecular and bioinformatic analysis of water quality, Lake Sredny Kaban is a transition to b-mesosaprobic and can be characterized as polluted.

Conclusion

New generation sequencing methods can be successfully used to assess the ecological status of water bodies. The use of modern methods of molecular biology for bioindication gives positive results, increasing the efficiency of assessing the ecological status of water bodies. The results obtained are of great practical interest in the field of monitoring water bodies, in particular, and the environment in general.

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