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The Monitoring of Nizhniy Kaban Lake by 16s Rrna Gene Amplicon Data Set-Based Bacterial Diversity

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Abstract

The paper presents the results of research of bacterial diversity of Nizhniy Kaban Lake (Kazan, Russia) for 2016-2017, using the marker gene *16S rRNA* of hydrobionts, based on the method of next-generation sequencing. Nizhniy Kaban, Verhniy Kaban and Sredniy Kaban are included in the system of Kaban Lakes. They are located in the center of a large industrial city, and suffer anthropogenic load. According to ecological studies, Kaban Lakes are polluted. The sequences of *16S rRNA Bacteria* gene fragment of the freshwater lake Nizhniy Kaban were submitted to the international database in fastq format on the website NCBI with the numbers SRR7510984 and SRR7516469. The comparative analysis of metagenomic data showed a significant change in bacterial diversity over the years. A total of 103030 (2016) and 90402 (2017) high-quality reads were obtained; 76% (2016) and 70% (2017) of the bacterial population was classified to the genus level, while 0.25% (2017) was classified to the species level. In total, 18 species of *Bacteria* were identified. Among them, bacteria occurring in the human gastrointestinal tract, were found the most often. These types of bacteria can be a threat to human health. Therefore, the species composition of *Bacteria* community should be taken into account when assessing the ecological state of water reservoirs.

Keywords: Gene 16S rRNA, Next-generation sequencing, Freshwater lake, Bacteria

1 Introduction

Currently, the assessment of water quality is carried out using various physicochemical and biological methods. One of them is the method of isolation and identification of microorganisms. The presence of bacterial pathogens in the water reservoirs adversely affects human health. They can be the agents of such diseases as cholera, diarrhea, dysentery, hepatitis A, typhoid fever and poliomyelitis (1).

To assess the diversity of microorganisms in various environments, for example, in human intestine, bottom sediments of Lake Baikal or in the hot springs of Kamchatka, the methods of next-generation sequencing are used (2). This technology allows to accelerate the process of determining the sequences of organisms' genomes (3).

The sequencing of *16S rRNA* gene is a universal and effective approach for taxonomic characterization, as this gene is present in the genomes of all prokaryotes, and has relatively low variability (4).

Metagenomics can provide valuable information on the functional ecology of environmental communities (5). We previously used metagenomic DNA sequencing for the identification of zooplankton by *CO1* gene, and phytoplankton by *rbcL* gene, in order to assess the ecological state of the freshwater Nizhniy Kaban lake (6-7).

The work presents the results of research of bacterial diversity of Nizhniy Kaban lake (Kazan, Russia) for 2016-2017, using the marker gene *16S rRNA* of hydrobionts, based on the method of next-generation sequencing. Nizhniy Kaban, Verhniy Kaban and Sredniy Kaban are included in the system of Kaban Lakes. They are located in the center of a large industrial city, and suffer anthropogenic load. According to ecological studies, Kaban Lakes are polluted.

2 Methods

The sampling from Nizhniy Kaban Lake (Kazan, Russia) was carried out during 2016-2017, in accordance with the standard hydrobiological methods (8).

DNA was isolated from the pellet, using FAST DNA Kit (MP biomedicals), according to the manufacturer's protocol. The pellet was obtained by centrifuging of 50 ml of the sample, at a speed of 10,000 g for 15 minutes. The amplification of the isolated DNA was carried out using PhusionHigh-Fidelity DNA polemerase (ThermoFisher), and primers (Table 1). After this, the second PCR cycle was performed in order to index the samples (Nextera XT indices). Purification of PCR products was performed using

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Agencourt AMPure XP beads (Beckman Coulter). The obtained DNA libraries were sequenced on the device Illumina MiSeq (MiSeq Reagent kit v3).

Table 1: Primers for PCR of *16S rRNA* gene

Primers	Sequences
16SF_I (forward)	5'-tcgtcggcagcgtcagatgtgtataagagacagcctacgggnggcwgcag-3'
16SR_I (reverse)	5'-gtctcgtgggctcggagatgtgtataagagacaggactachvgggtatctaatcc-3'

Metagenomic data were entered into the international SRA database on the website NCBI with numbers: SRR7510984 and SRR7516469 (9). After filtering the reads by quality, trimming of sequences and removing of chimeric sequences, the obtained nucleotide sequences of *16S rRNA Bacteria* gene were aligned, using the software BLAST, in order to establish the taxonomic composition. The software Krona chart (10) and GraphPad (11) were used to build charts.

3 Results and Discussion

In 2016-2017, the next-generation sequencing method was applied with the aim to identify *Bacteria* from Nizhniy Kaban Lake.

3.1 Krona chart of the bacteria represented by 16S rRNA gene amplicon-based bacterial diversity

The percentage distribution of *Bacteria* of Nizhniy Kaban Lake by species diversity and reads for 2016 is shown in Fig. 1-2. The percentage distribution of *Bacteria* of Nizhniy Kaban Lake by species diversity and reads for 2017 is shown in Fig. 3-4. Each circle represents the phylum, class, order, family, genus, and species from the inside to the outside of the circle, respectively, indicated by the percent diversity, based on the absolute number of representative bacteria.

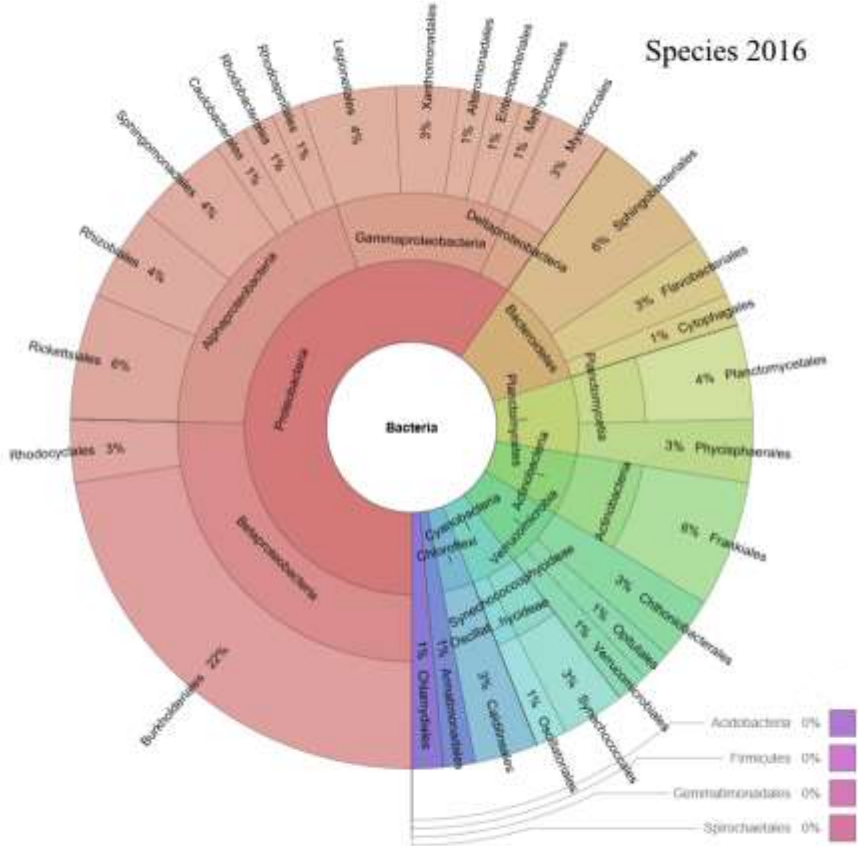

Figure 1: The percentage of species diversity *of 16S rRNA Bacteria* of Nizhniy Kaban Lake (2016)

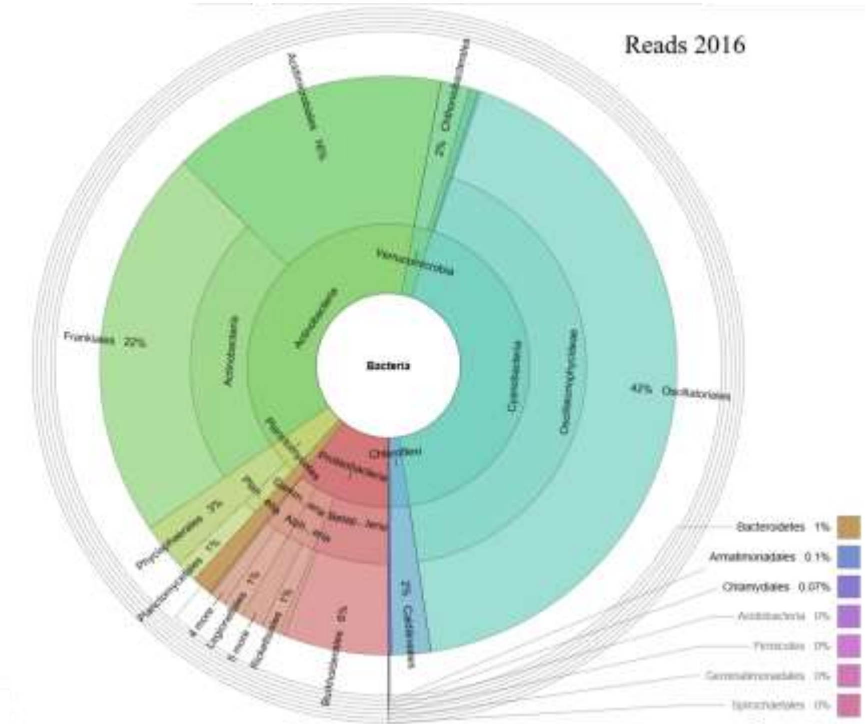

Figure 2: The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake (2016)

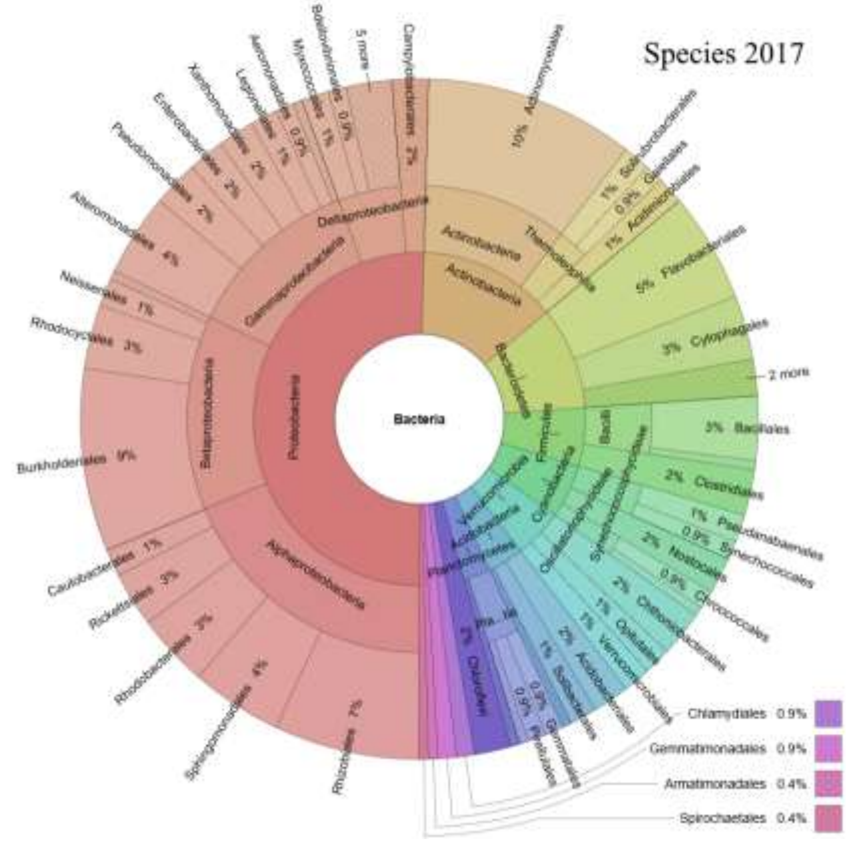

Figure 3: The percentage of species diversity *of 16S rRNA Bacteria* of Nizhniy Kaban Lake (2017)

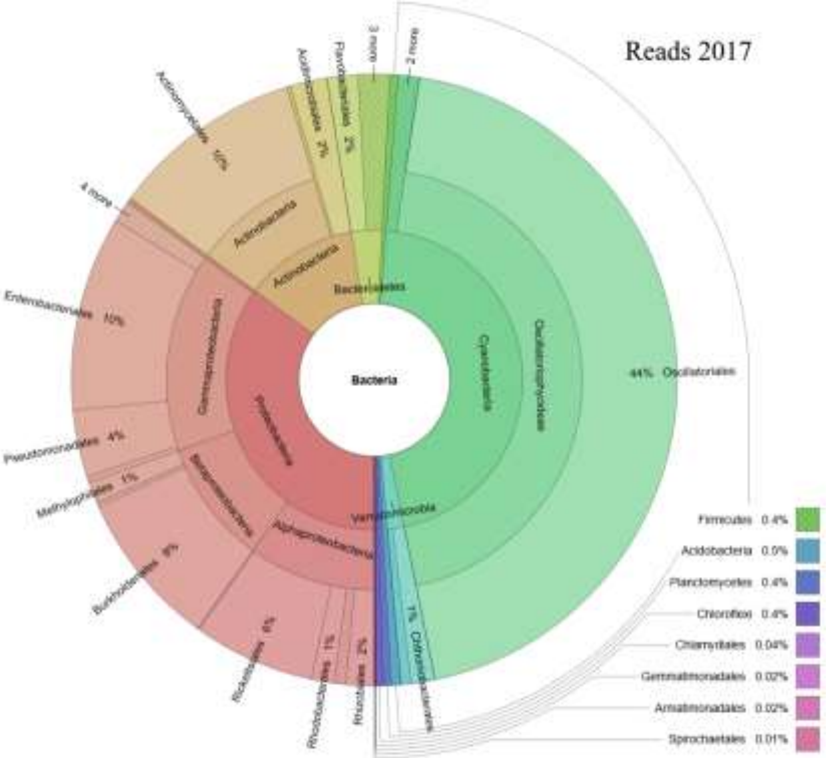

Figure 4: The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake (2017)

3.2 The percentage of 16S rRNA Bacteria of Nizhniy Kaban Lake by phylum

The percentage of species diversity of *16S rRNA Bacteria* of Nizhniy Kaban Lake by phylum (2016-2017) is shown in Figure 5. As can be seen from Fig. 5, *Proteobacteria* (56.7%/38.57%), *Bacteroidetes* (28.57%/9.28%) and *Actinobacteria* (14.29%/9.79%) are the most numerous by species diversity at the level of phylum, respectively by years.

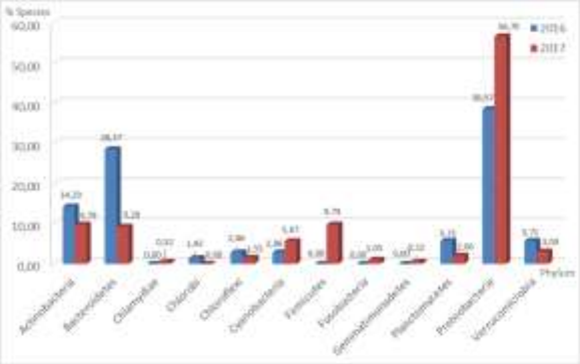

Figure 5: The percentage of species diversity of *16S rRNA Bacteria* of Nizhniy Kaban Lake by phylum (2016-2017)

The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake by phylum (2016-2017) is shown in Figure 6. As can be seen from Fig. 6, *Cyanobacteria* (75.63%/75.19%) and *Proteobacteria* (3.68%/19%) are the most numerous by reads.

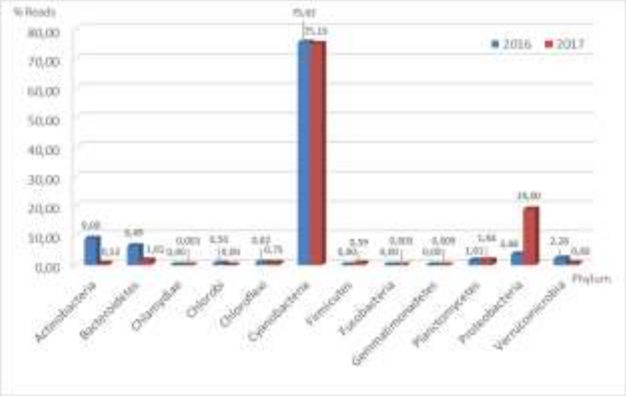

Figure 6: The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake by phylum (2016-2017)

3.3 The percentage of 16S rRNA Bacteria of Nizhniy Kaban Lake by class

The percentage of species diversity of *16S rRNA Bacteria* of Nizhniy Kaban Lake by class (2016-2017) is shown in Fig. 7, parts 1-2. As can be from Fig. 7, *Alphaproteobacteria* (14.3%/21.1%), *Betaproteobacteria* (15.71%/16.49%) and *Gammaproteobacteria* (5.71%/14.43%) are the most numerous by species diversity. The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake by class (2016-2017) is shown in Fig. 8, parts 1-2. As can be seen from Fig. 8, the species *Oscillatoriophycideae* (75.60%/68.15%) is the most numerous by reads among *Bacteria*.

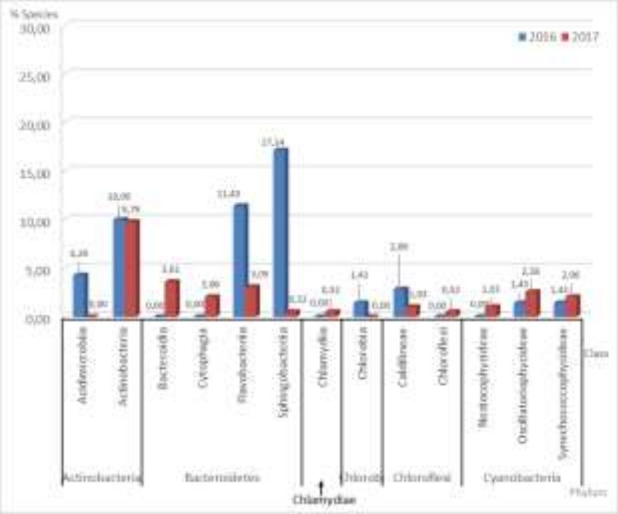

Figure 7: part 1. The percentage of species diversity of *16S rRNA Bacteria* of Nizhniy Kaban Lake by class (2016-2017)

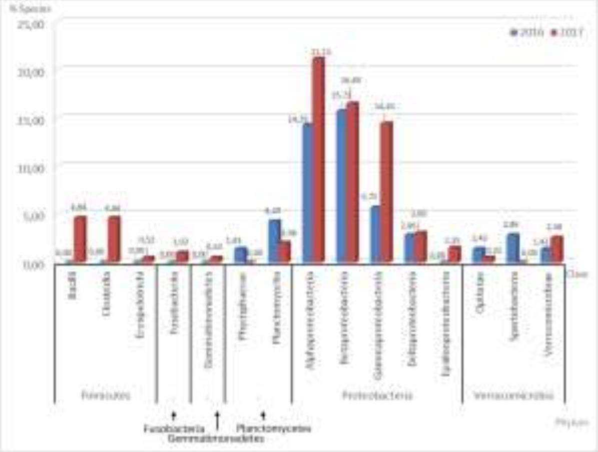

Figure 7: part 2. The percentage of species diversity of *16S rRNA Bacteria* of Nizhniy Kaban Lake by class (2016-2017)

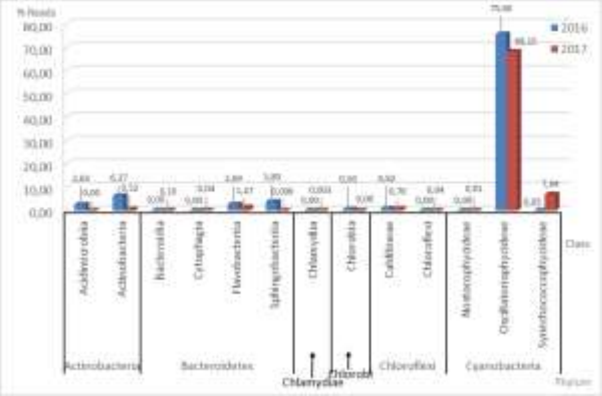

Figure 8: part 1. The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake by class (2016-2017)

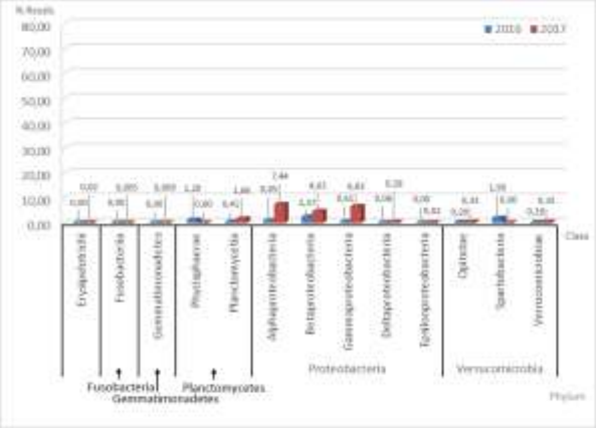

Figure 8: part 2. The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake by class (2016-2017)

3.4 The percentage of 16S rRNA Bacteria of Nizhniy Kaban Lake by order

The percentage of species diversity and reads of *16S rRNA Bacteria* of Nizhniy Kaban Lake by order (2016-2017) is shown in Fig. 9-11. As can be seen from Fig. 9-11, the following orders were of the greatest importance in terms of species diversity and/or reads, in 2016: *Oscillatoriales* (1.43%/75.60%), *Sphingobacteriales* (17.14%/3.79%), *Burkholderiales* (14.29%/2.05%), *Flavobacteriales* (11.43%/2.69%); in 2017: *Oscillatoriales* (1.03%/67.63%), *Burkholderiales* (10.31%/4.08%), *Actinomycetales* (8.25%/0.47%).

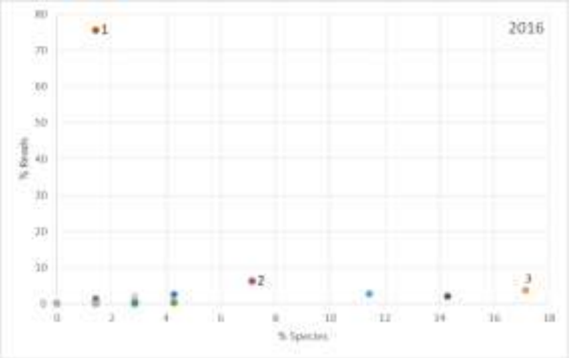

Figure 10: The percentage of species diversity and reads of *16S rRNA Bacteria* of Nizhniy Kaban Lake by order (2016)

1 – *Oscillatoriales* (1.43%/75.60%), 2 – *Frankiales* (7.14%/6.25%),
3 – *Sphingobacteriales* (17.14%/3.80%)

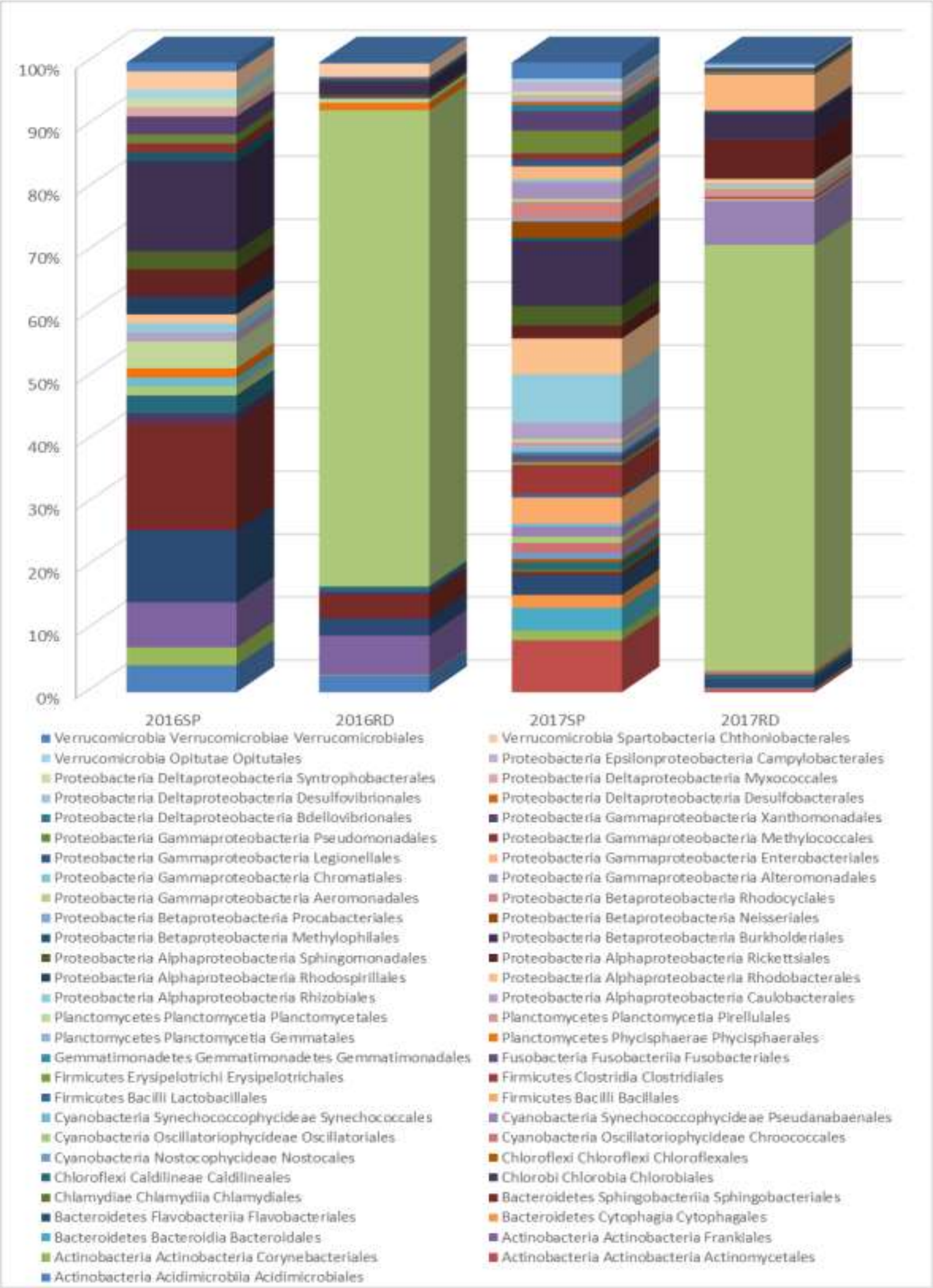

Figure 9: The percentage of species diversity and reads of *16S rRNA Bacteria* of Nizhniy Kaban Lake by order (2016-2017)

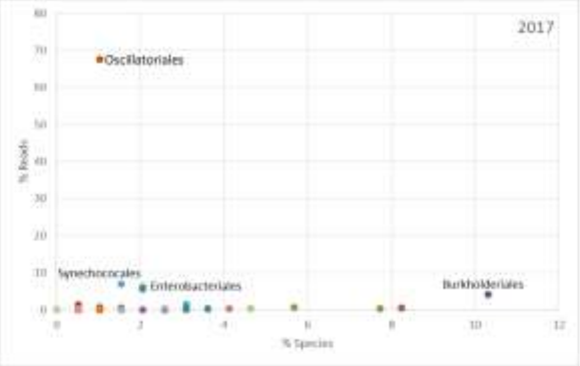

Figure 11: The percentage of species diversity and reads of *16S rRNA Bacteria* of Nizhniy Kaban Lake by order (2017)

1 – *Oscillatoriales* (1.03%/67.63%), 2 – *Pseudoanabaenales* (1.54%/6.95%),
3 – *Enterobacteriales* (2.06%/5.6%), 4 – *Burkholderiales* (10.31%/4.08%)

3.5 The percentage of 16S rRNA Bacteria of Nizhniy Kaban Lake by family

The total number of identified *Bacteria* by family is 26/93 families, respectively for 2016/2017. The families by reads are shown in Fig. 12, among them 11% (2016), and 17% (2017) are not classified. The following families are the most represented: *Microcoleaceae* - 38% (2016), 42.5% (2017), *Sporichthyaceae* - 5.78% (2016); *Enterobacteriaceae* - 9.85% (2017), *Comamonadaceae* - 5.54% (2017).

3.6 The percentage of 16S rRNA Bacteria of Nizhniy Kaban Lake by genus

The percentage of *16S rRNA Bacteria* of Nizhniy Kaban Lake by genus (*2016-2017*) is shown in Fig.13. As can be seen from Fig. 13 - 12.4% of identified *Bacteria* genera were unique for 2016, 19.4% genera were common for 2016-2017, and 68.2% genera were unique for bacterial community of 2017. *Flavobacterium* (1.4%/0.65%), *Fluviicola* (0.32%/0.42%), *Lewinella* (0.4%/0.3%), *Limnobacter* (0.03%/0.15%), *Limnohabitans* (0.11%/0.02%), *Mycobacterium* (0.11%/0.04%), *Opitutus* (0.19%/0.08%), *Phenylobacterium* (0.03%/0.02%), *Planctomyces* (0.05%/0.01%), *Planktothrix* (69.82%/42.40%), *Polynucleobacter* (0.40%/0.06%), *Prosthecobacter* (0.16%/0.03%), *Rickettsia* (0.08%/0.01%), *Sediminibacterium* (0.22%/0.63%), *Zymomonas* (0.22%/0.02%) were the common genera for 2016-2017.

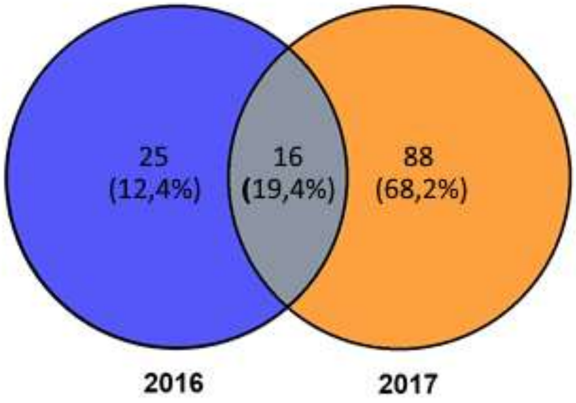

Figure 13: The percentage of *16S rRNA Bacteria of* Nizhniy Kaban Lake by genus (2016-2017)

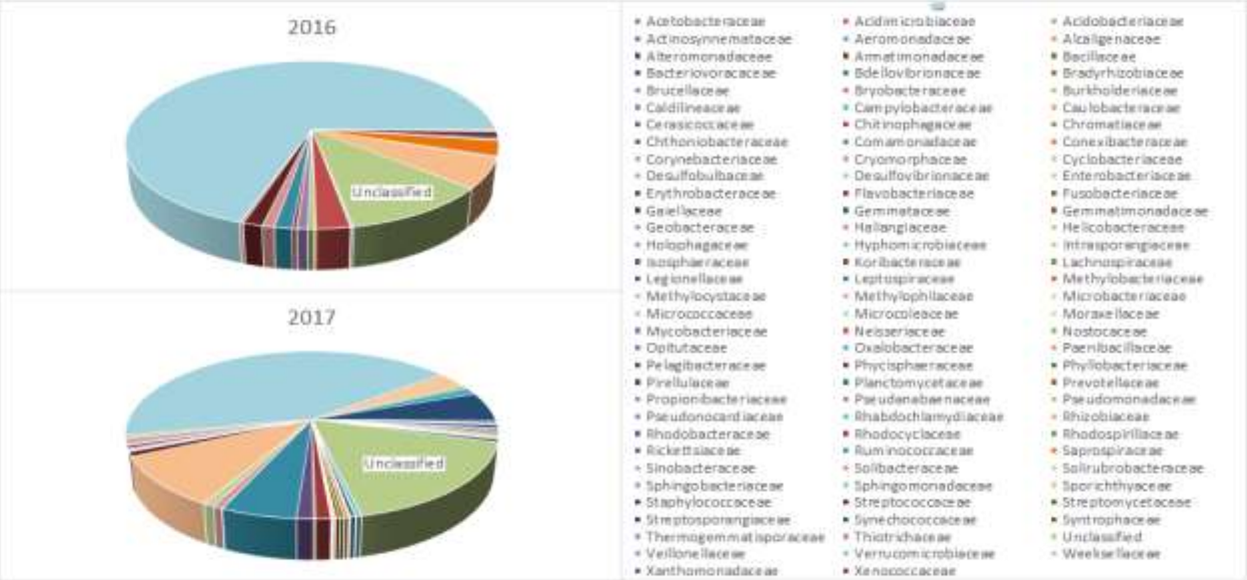

Figure 12: The percentage of *16S rRNA Bacteria* reads of Nizhniy Kaban Lake by family (2016-2017)

Table 2: Summary of *Bacteria* identified to the species level from *16S rRNA* gene-based metagenomic study of freshwater from Nizhniy Kaban Lake (2017)

Phylum	**Class**	**Order**	**Family**	**Species**	**%**
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agromyces mediolanus[1]	0.0379
				Candidatus Aquiluna rubra[2]	0.0263
			Micrococcaceae	Micrococcus luteus[3]	0.0032
			Propionibacteriaceae	Propionibacterium acnes[4]	0.0042
	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella aerofaciens[5]	0.0221
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides uniformis[6]	0.0126
			Porphyromonadaceae	Parabacteroides distasonis[7]	0.0074
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus epidermidis[8]	0.0021
	Clostridia	Clostridiales	Peptococcaceae	Desulfosporosinus meridiei[9]	0.0042
			Ruminococcaceae	Faecalibacterium prausnitzii[10]	0.0274
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Cetobacterium somerae[11]	0.0011
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas diminuta[12]	0.0358
	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax paradoxus[13]	0.0158
	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter rhizosphaerae[14]	0.0021
			Pseudomonadaceae	Pseudomonas veronii[15]	0.0116
		Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas mexicana[16]	0.0074
	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Sulfuricurvum kujiense[17]	0.0011
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecobacter debontii[18]	0.0284

[1] Aniline-assimilating bacteria. They occur in the soil; there are cases of human infection (12).

[2] It can be found in fresh and salt water (13).

[3] It is an obligate aerobe, widely distributed in the environment. It can be found in soils, dust, water and air. It is also a part of the normal microflora of the skin surface of humans and mammals (14).

[4] It occurs on the skin and in the gastrointestinal tract of humans and animals. It can cause the skin diseases in humans (15).

[5] *Collinsella aerofaciens*, a rod-shaped nonmotile obligate anaerobe, is the most abundant actinobacterium in the gastrointestinal tract of healthy humans. An altered abundance of *C. aerofaciens* may be linked with several health disorders, including irritable bowel syndrome (16).

[6] Bacteroides spp are the non-spore forming gram-negative bacilli, which are the part of the human resident flora (17).

[7] *Parabacteroides distasonis* belong to the main intestinal microbiota of healthy people. At the same time, these bacteria can cause some infections (18).

[8] It is a gram-positive bacteria, one of more than 40 species of the genus *Staphylococcus* (19). It is a part of the normal microflora of human skin, and mucous membranes (less often) (20).

[9] *Desulfosporosinus meridiei* sp. nov., is a spore-forming sulfate-reducing bacterium, isolated from gasolene-contaminated groundwater (21).

[10] It is one of the most common and important commensal bacteria of human intestinal microbiota (22).

[11] *Cetobacterium somerae* is a gram-negative, microaerotolerant, non-spore-forming and rod-shaped bacterium from the genus *Cetobacterium,* which has been isolated from human feces (23).

[12] It was isolated from clinical samples of patients with mucoviscidosis. It is used as a potential bioremediator of marine oil pollution (24).

[13] It can be found everywhere. It is abundantly present in environments, which are contaminated with organic compounds or heavy metals (25).

[14] Gram-negative bacteria; they are chemorganotrophs with oxidative metabolism. They are saprophytes, and universal in occurrence. They may be the cause of many infectious processes, including meningitis, septic disease in humans, and septicemia, abortion in animals. In February 2017, WHO ranked acinetobacteria among the most dangerous bacteria, due to their resistance to existing antibacterial drugs (26, 38, 39).

[15] *Pseudomonas veronii* is a gram-negative, rod-shaped, fluorescent, motile bacterium, isolated from natural springs in France. It may be used for bioremediation of contaminated soils, as it has been shown to degrade a variety of simple aromatic organic compounds (27, 37).

[16] *Pseudoxanthomonas mexicana* is a species of mesophilic, motile, strictly aerobic, gram-negative, non-spore-forming, rod-shaped bacteria with one polar flagellum, first isolated from human urine, riverside urban soil and anaerobic digester (28, 31, 35, 36).

[17] It is a facultative anaerobe, chemolithoautotrophic sulfur-oxidizing bacterium, typical representative of the genus. The cells have the shape of curved rods, they are mobile, and have a single polar flagellum (29, 33, 34).

[18] It was isolated from fresh water (30, 32).

3.7 The percentage of 16S rRNA Bacteria of Nizhniy Kaban Lake by species

The species diversity of *Bacteria* in 2017 was 0.25% of the total number of organisms by reads. Table 2 shows the classification of bacterial organisms in Nizhniy Kaban Lake. The percentage of species diversity by reads of *16S rRNA Bacteria* of Nizhniy Kaban Lake for 2017 is shown in Fig.14.

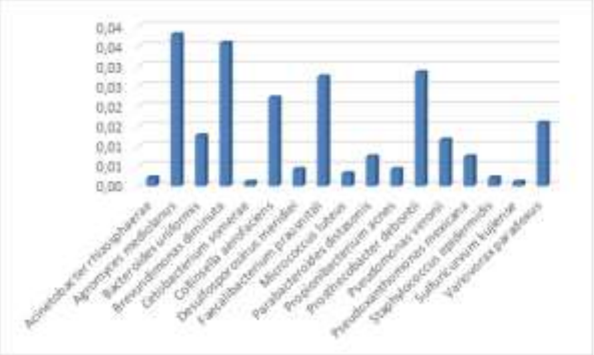

Figure 14: The percentage of species diversity by reads of *16S rRNA Bacteria* of Nizhniy Kaban Lake (2017)

4 Summary

According to the results of the study, using the modern method of next-generation sequencing, the bacterial profile of Nizhniy Kaban Lake for 2016-2017 was characterized. The comparative analysis of metagenomic data showed a significant change in bacterial diversity over the years. A total of 103030 (2016) and 90402 (2017) high-quality reads were obtained; 97.8% (2016) and 98.4% (2017) of the bacterial population were classified to the phylum, while 97.5% (2016) and 95.9% (2017) were classified to the class level, 92.4% (2016) and 95.4% (2017) were classified to the order level, 89.2% (2016) and 87.1% (2017) were classified to the family level, 76.4% (2016) and 70.1% (2017) were classified to the genus level, and 0.25% (2017) was classified to the species level. In total, 18 species of *Bacteria* were identified. Among them, bacteria occurring in the human gastrointestinal tract, were found the most often. These types of bacteria can be a threat to human health. Therefore, the species composition of *Bacterial* community should be taken into account when assessing the ecological state of water reservoirs.

5 Conclusions

The results obtained are of great practical interest in the field of monitoring of water reservoirs. The method of next-generation sequencing can be successfully used for the control of water reservoirs, in particular, and for the assessment of ecological state of water reservoirs, in general.

Acknowledgements

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