



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

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Received: 05/08/2019

Accepted: 20/11/2019

Published: 20/12/2019

Abstract

The paper presents the results of research of bacterial diversity of Sredniy Kaban Lake (Kazan, Russia) for 2016-2017, using the marker gene *16S rRNA* of aquatic organisms, based on the method of next-generation sequencing. Sredniy Kaban, Verhniy Kaban and Nizhniy Kaban are included in the system of Kaban Lakes. They are located in the center of a large industrial city, and are exposed to anthropogenic load. According to ecological studies, Kaban Lakes are polluted. The sequences of *16S rRNA* *Bacteria* gene fragment of the freshwater lake Sredniy Kaban were submitted to the international database in fastq format on the website NCBI with the numbers SRR7510929 (2016) and SRR7516240 (2017). The comparative analysis of metagenomic data showed a significant change in bacterial diversity over the years. A total of 98822 (2016) and 26046 (2017) high-quality reads were obtained; 67% (2016) and 54% (2017) of the bacterial population were classified to the genus level, while 3% (2017) was classified to the species level. In total, 15 species of *Bacteria* were identified. Among them, the dangerous bacteria *Acinetobacter*, occurring universally, were found the most often. This type of bacteria can pose 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

Poor water quality is an important problem of public health and environment. The presence of pathogens in water can adversely affect human and animal health. Contaminated water is connected with the transmission 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). High-performance sequencing technology can significantly accelerate and reduce the cost of determining the genome sequences of millions of organisms (3).

Sequencing of *16S rRNA* gene is a universal and effective approach for taxonomic characterization, as this gene is present in 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, in order to assess the ecological state of the freshwater lake Sredniy Kaban by the method of bioindication (6).

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

2 Methods

The sampling from Sredniy Kaban Lake (Kazan, Russia) was carried out during 2016-2017, in accordance with the standard hydrobiological methods (7).

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

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Phusion High-Fidelity DNA polymerase (Thermo Fisher) using the Eurogen primers (<http://evrogen.ru>) (Table 1).

Table 1 Primers for PCR of 16S rRNA gene

Primers	Sequences
16SF_I (forward)	5'- tcgtcggcagcgtcagatgtgtataagacagcctacgggng gcwgcag-3'
16SR_I (reverse)	5'- gtctcgtgggctcggagatgtgtataagacaggactachvg ggatctaatcc-3'

After this, the second PCR cycle was performed in order to index the samples (Nextera XT indices). Purification of PCR products was performed using Agencourt AMPure XP beads (Beckman Coulter).

The obtained DNA libraries were sequenced on the device Illumina MiSeq (MiSeq Reagent kit v3). Metagenomic data were entered into the international SRA database on the website NCBI with numbers: SRR7510929 and SRR7516240 (8).

After filtering the reads by quality, trimming of sequences and removing of chimeric sequences, the

obtained nucleotide sequences of 16SrRNA *Bacteria* gene were aligned, using the software BLAST+, in order to establish the taxonomic composition.

The software Krona chart (9) and GraphPad (10) 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 Sredniy Kaban Lake.

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

The percentage distribution of *Bacteria* of Sredniy Kaban Lake by species and reads for 2016 is shown in Fig. 1-2. The percentage distribution of *Bacteria* of Sredniy Kaban Lake by species 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.

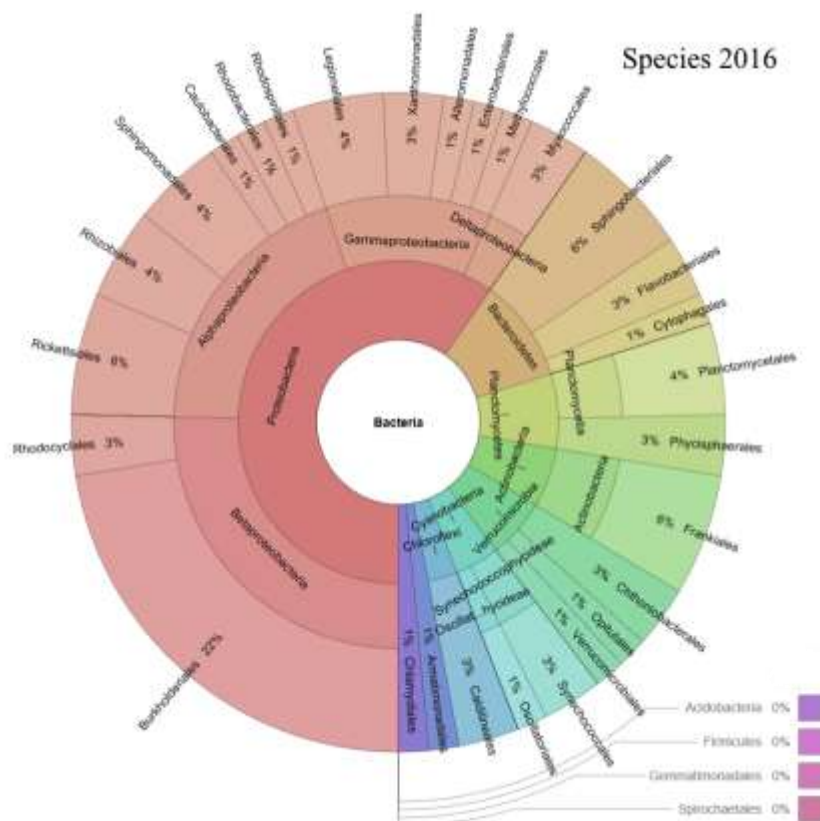


Figure 1: The percentage of 16S rRNA Bacteria species of Sredniy Kaban Lake (2016)

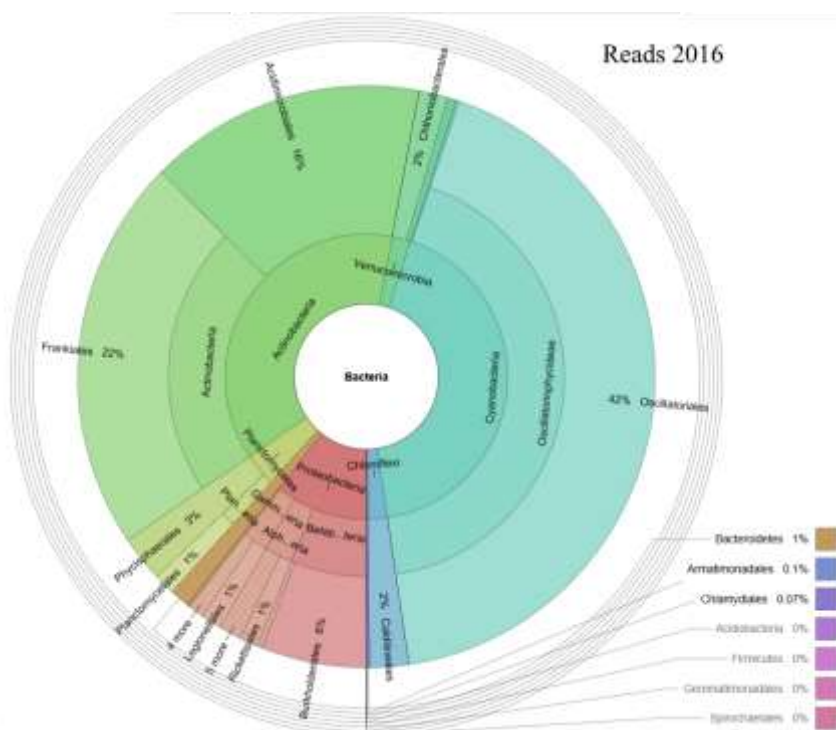


Figure 2: The percentage of 16S rRNA Bacteria reads of Sredniy Kaban Lake (2016)

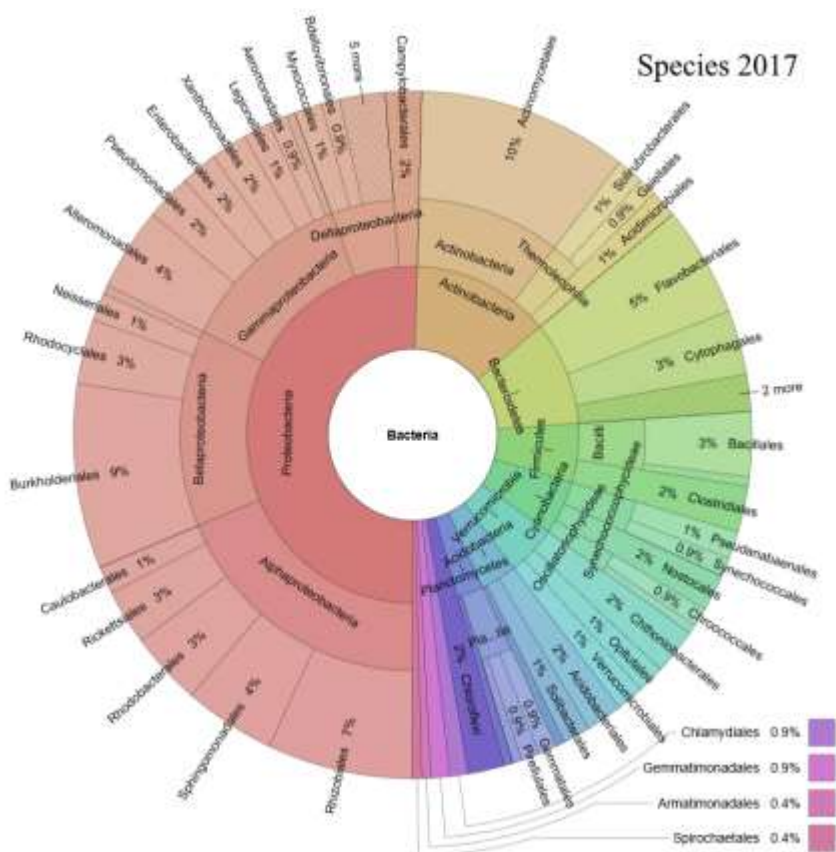


Figure 3: The percentage of 16S rRNA Bacteria species of Sredniy Kaban Lake (2017)

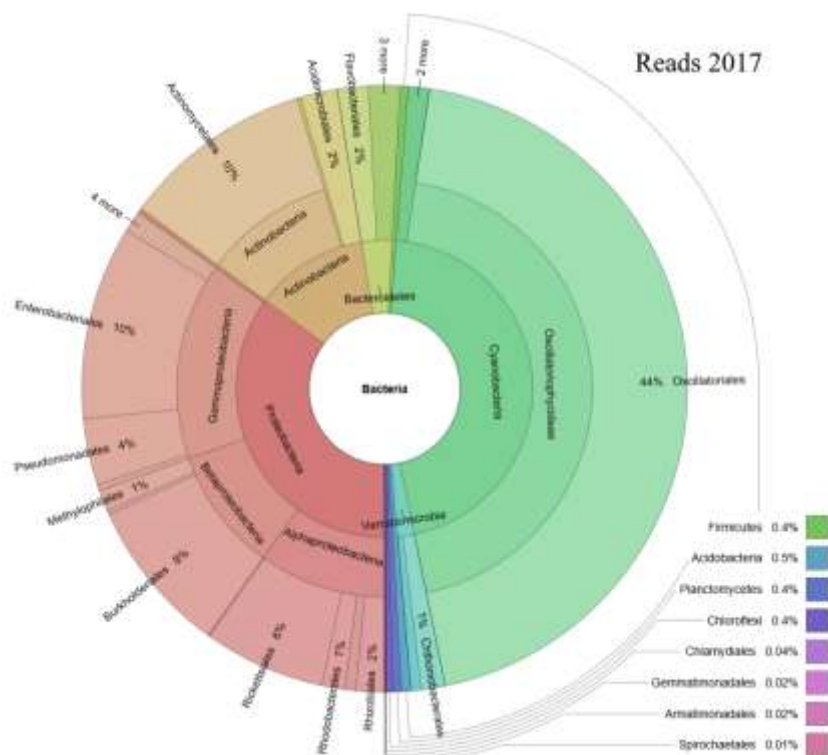


Figure 4: The percentage of 16S rRNA Bacteria reads of Sredniy Kaban Lake (2017)

The percentage of 16S rRNA Bacteria of Sredniy Kaban Lake by phylum. The percentage of species diversity of 16S rRNA Bacteria of Sredniy Kaban Lake by phylum (2016-2017) is shown in Figure 5. As can be seen from Fig. 5, *Proteobacteria* (59.7%/50.43%), *Actinobacteria* (5.97%/13.48%) and *Bacteroidetes* (10.45%/10%) are the most numerous by species diversity at the level of phylum, respectively by years.

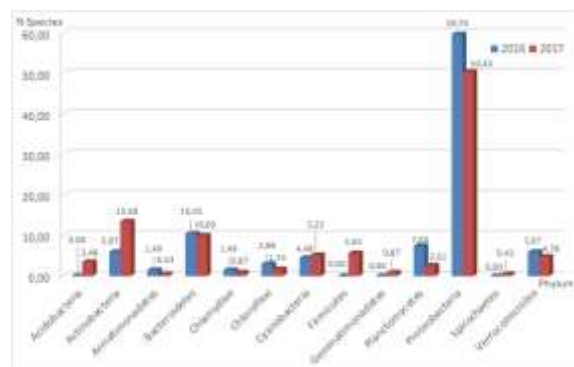


Figure 5: The percentage of species diversity of 16S rRNA Bacteria of Sredniy Kaban Lake by phylum (2016-2017)

The percentage of 16S rRNA Bacteria reads of Sredniy Kaban Lake by phylum (2016-2017) is shown in Figure 6. As can be seen from Fig. 6, *Cyanobacteria* (42.59%/45.53%), *Actinobacteria* (37.41%/12.37%) and *Proteobacteria* (10.67%/35.12%) are the most numerous by reads.

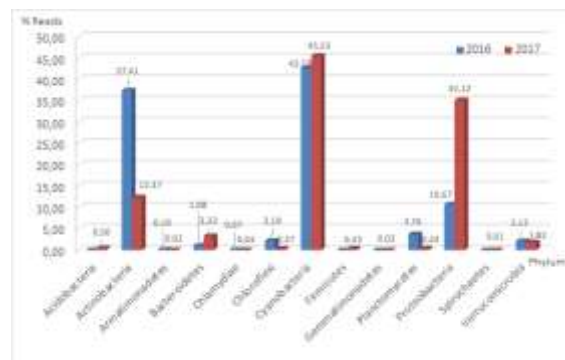


Figure 6: The percentage of 16S rRNA Bacteria reads of Sredniy Kaban Lake by phylum (2016-2017)

3.2 The percentage of 16S rRNA Bacteria of Sredniy Kaban Lake by class

The percentage of species diversity of 16S rRNA Bacteria of Sredniy Kaban Lake by class (2016-2017) is shown in Fig. 7, parts 1-2. As can be seen from Fig. 7, *Alphaproteobacteria* (19.4%/18.7%), *Betaproteobacteria* (25.37%/13.48%) and *Gammaproteobacteria* (11.94%/12.17%) are the most numerous by species.

The percentage of 16S rRNA Bacteria reads of Sredniy Kaban Lake by class (2016-2017) is shown in Fig. 8, parts 1-2. As can be seen from Fig. 8, *Oscillatoriothymiceae* (42.42%/44.41%), *Actinobacteria* (21.91%/10.2%), *Acidimicrobiia* (15.51%, 2016), *Gammaproteobacteria* (15.33%, 2017) are the most numerous by species.

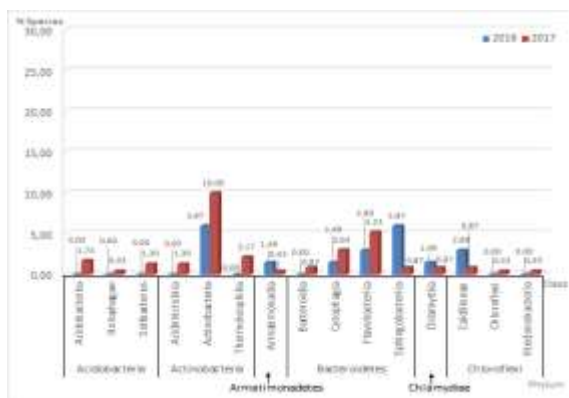


Figure 7: part 1. The percentage of species diversity of 16S rRNA Bacteria of Sredniy Kaban Lake by class (2016-2017)

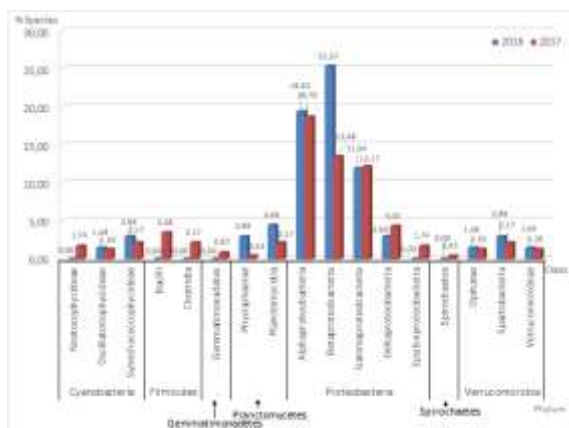


Figure 7: part 2. The percentage of species diversity of 16S rRNA Bacteria of Sredniy Kaban Lake by class (2016-2017)

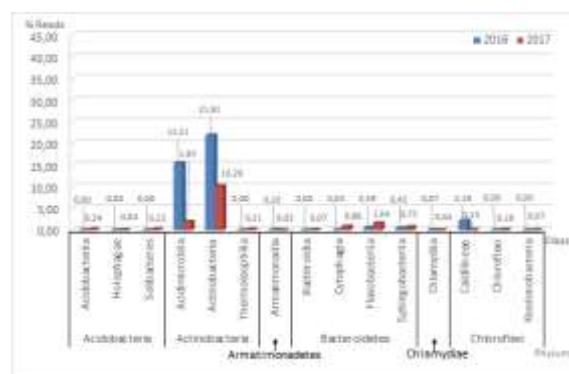


Figure 8: part 1. The percentage of 16S rRNA Bacteria reads of Sredniy Kaban Lake by class (2016-2017)

3.3 The percentage of 16S rRNA Bacteria of Sredniy Kaban Lake by order

The percentage of species diversity and reads of 16S rRNA Bacteria of Sredniy 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* (1.49%/42.42%), *Frankiales*

(5.97%/21.91%), *Burkholderiales* (22.39%/5.88%); in 2017: *Oscillatoriales* (0.43%/44.39%), *Enterobacteriales* (1.74%/10.3%), *Burkholderiales* (8.7%/8.29%), *Actinomycetales* (10%/10.20%).

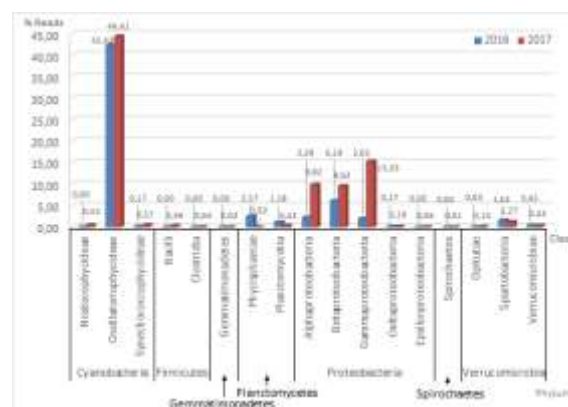


Figure 8: part 2. The percentage of 16S rRNA Bacteria reads of Sredniy Kaban Lake by class (2016-2017)

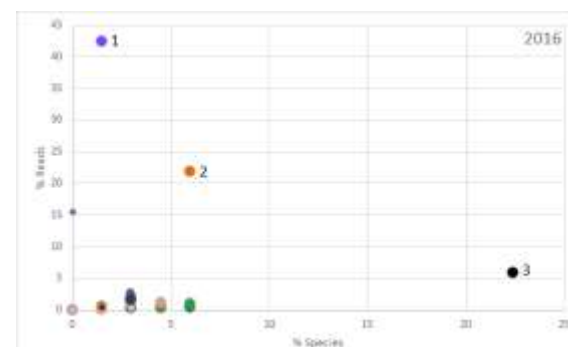


Figure 10: The percentage of species diversity and reads of 16S rRNA Bacteria of Sredniy Kaban Lake by order (2016): (1 – *Oscillatoriales* (1.49%/42.42%), 2 – *Frankiales* (5.97%/21.91%), 3 – *Burkholderiales* (22.39%/5.88%))

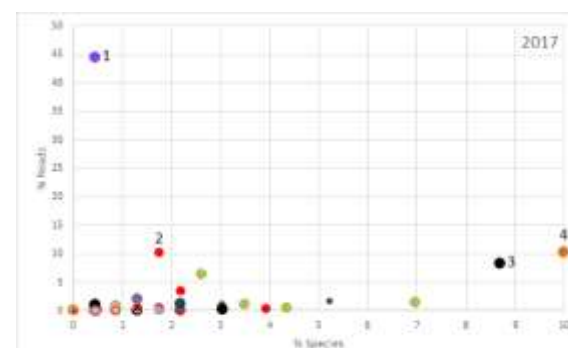


Figure 11: The percentage of species diversity and reads of 16S rRNA Bacteria of Sredniy Kaban Lake by order (2017): (1 – *Oscillatoriales* (0.43%/44.39%), 2 – *Enterobacteriales* (1.74%/10.3%), 3 – *Burkholderiales* (8.70%/8.29%), 4 – *Actinomycetales* (10.00%/10.20%))

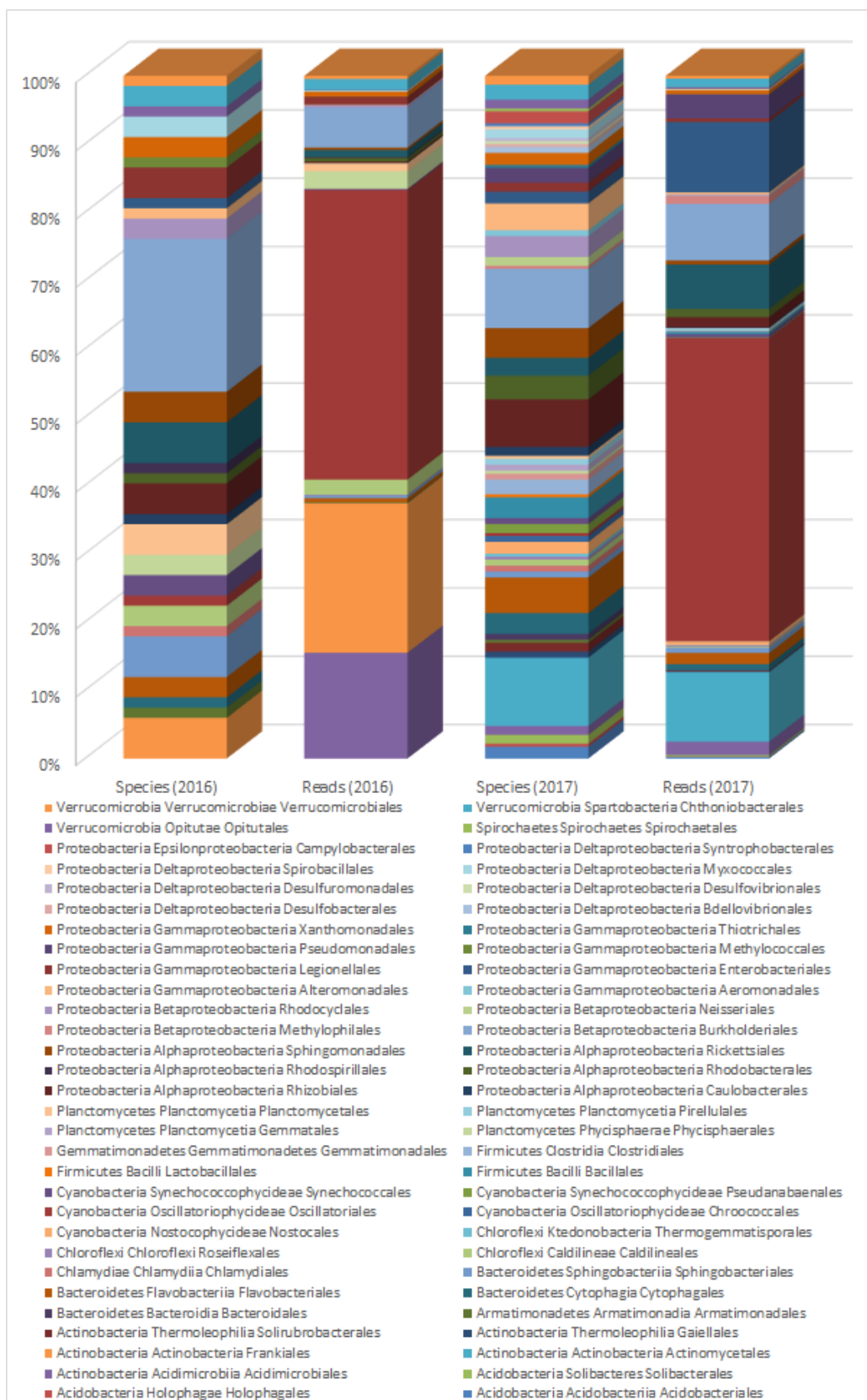


Figure 9: The percentage of species diversity and reads of 16S rRNA Bacteria of Sredniy Kaban Lake by order (2016-2017)

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

Phylum	Class	Order	Family	Species	%
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	<i>Edaphobacter modestum</i> ¹	0.0064
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	<i>Agromyces mediolanus</i> ²	0.0593
				<i>Candidatus Aquiluna rubra</i> ³	0.0572
			Micrococcaceae	<i>Micrococcus luteus</i> ⁴	0.0032
			Propionibacteriaceae	<i>Propionibacterium acnes</i> ⁵	0.0244
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	<i>Flavobacterium succinicans</i> ⁶	0.0064
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	<i>Staphylococcus epidermidis</i> ⁷	0.0074
	Clostridia	Clostridiales	Ruminococcaceae	<i>Faecalibacterium prausnitzii</i> ⁸	0.0053
Proteobacteria	Alpha-Proteobacteria	Caulobacteriales	Caulobacteraceae	<i>Brevundimonas diminuta</i> ⁹	0.0530
		Rickettsiales	Rickettsiaceae	<i>Orientia tsutsugamushi</i> ¹⁰	0.0053
	Beta-proteobacteria	Burkholderiales	Comamonadaceae	<i>Variovorax paradoxus</i> ¹¹	0.0095
		Neisseriales	Neisseriaceae	<i>Chitinilyticum litopenaei</i> ¹²	0.0074
	Gamma-proteobacteria	Pseudomonadales	Moraxellaceae	<i>Acinetobacter rhizosphaerae</i> ¹³	3.0645
	Epsilon-proteobacteria	Campylobacteriales	Helicobacteraceae	<i>Sulfuricurvum kujiense</i> ¹⁴	0.0117
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	<i>Prostheobacter debontii</i> ¹⁵	0.0222

¹ It is a type species of the genus *Edaphobacter*; it was initially isolated from alpine soil, rich in calcium carbonate (11).

² Aniline-assimilating bacteria. They occur in the soil; there are cases of human infection (12).

³ It can be found in fresh and salt water (13).

⁴ 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).

⁵ It occurs on the skin and in the gastrointestinal tract of humans and animals. It can cause the skin diseases in humans.

⁶ Phosphite-assimilating bacteria. They were isolated from the intestines of zooplankton *Daphnia magna* (14).

⁷ It is a gram-positive bacteria, one of more than 40 species of the genus *Staphylococcus* (15). It is a part of the normal microflora of human skin, and mucous membranes (less often) (16).

⁸ It is one of the most common and important commensal bacteria of human intestinal microbiota (17).

⁹ It was isolated from clinical samples of patients with mucoviscidosis. It is used as a potential bioremediator of marine oil pollution (18).

¹⁰ *O. tsutsugamushi* is a mite-borne bacterium, which causes the life-threatening human disease scrub typhus (19).

¹¹ It can be found everywhere. It is abundantly present in environments, which are contaminated with organic compounds or heavy metals (20,26,30-32).

¹² It was isolated from surface water of an aquaculture pond, containing Pacific white shrimp. It is susceptible to ampicillin, chloramphenicol, kanamycin, nalidixic acid, novobiocin, rifampicin and tetracycline; resistant to erythromycin, gentamicin, penicillin G and streptomycin (21,33,34).

¹³ Gram-negative bacteria, 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 (22,25).

¹⁴ 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 (23, 28).

¹⁵ It was isolated from fresh water (24, 27, 29).

3.4 The percentage of 16s rrna bacteria of sredniy kaban lake by family

The total number of identified *Bacteria* by family is 37/94 families, respectively for 2016/2017. The families by species are presented in Fig. 12 a), b). The families by reads are shown in Fig. 12 c), d); among them 10% (2016), and 17% (2017) are not classified. The following families are the most represented: *Microcoleaceae* – 38% (2016), 42.5% (2017), *Sporichthyaceae* – 20.5% (2016), *Acidimicrobiaceae* – 14% (2016); *Enterobacteriaceae* – 9.8% (2017), *Pelagibacteriaceae* – 5.7% (2017).

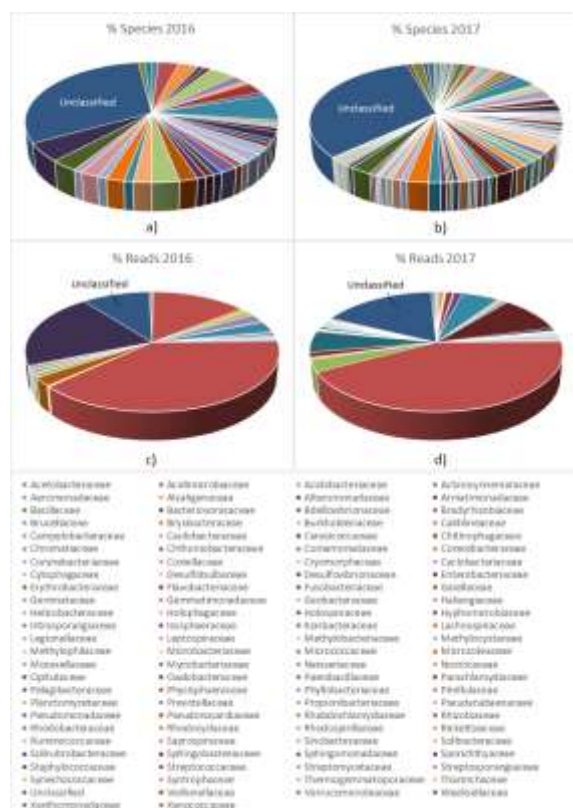


Figure 12: The percentage of species diversity and reads of 16S *rRNA* *Bacteria* of Sredniy Kaban Lake by family (2016-2017)

3.5 The percentage of 16S rRNA Bacteria of Sredniy Kaban Lake by genus

The percentage of 16S rRNA *Bacteria* of Sredniy Kaban Lake by genus (2016-2017) is shown in Fig. 13. As can be seen from Fig. 13, at the level of genus, 12.4% of groups were unique for the bacterial community in 2016; 19.4% of groups were common for 2016-2017, and 68.2% were unique for the bacterial community in 2017. Common genera for 2016-2017 were the following: *Dechloromonas* (0.13%/0.1%), *Flavobacterium* (0.13%/0.64%), *Hyphomicrobium* (0.06%/0.02%), *Legionella* (0.66%/0.19%), *Limnobacter* (0.38%/0.15), *Limnochabitans* (0.63%/0.02%), *Mycobacterium* (0.18%/0.04%), *Opitutus* (0.03%/0.08%), *Phenylobacterium* (0.03%/0.02%), *Planctomycetes* (0.03%/0.01%), *Planktothrix* (38.34%/42.40%), *Polynucleobacter* (1.10%/0.06%), *Rickettsia* (0.72%/0.01%), *Sediminibacterium*

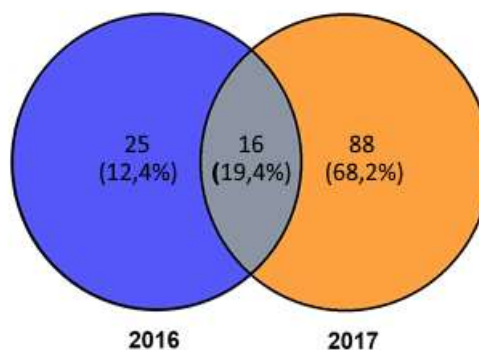


Figure 13: The percentage of 16S rRNA *Bacteria* of Sredniy Kaban Lake by genus (2016-2017)

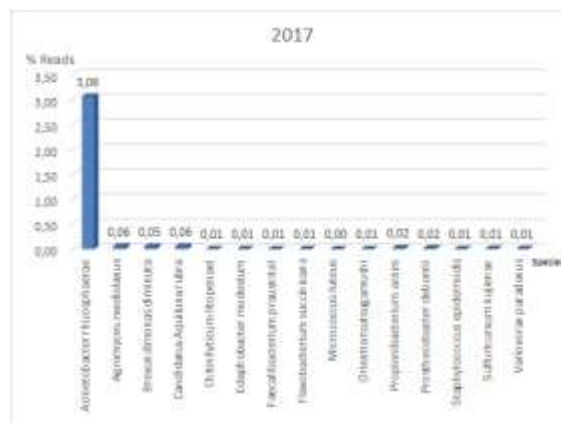


Figure 14: The percentage of species diversity by reads of 16S *r*RNA *Bacteria* of Sredniy Kaban Lake (2017)

4 Summary

According to the results of the study, using the modern method of next-generation sequencing, the bacterial profile of Sredniy 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 98822 (2016) and 26046 (2017) high-quality reads were obtained; 97% (2016) and 99% (2017) of the bacterial population were classified to the phylum, while 96% (2016) and 98% (2017) were classified to the class level, 94% (2016) and 97% (2017) were classified to the order level, 90% (2016) and 83% (2017) were classified to the family level, 67% (2016) and 54% (2017) were classified to the genus level, and 3% (2017) was classified

to the species level. In total, 15 species of *Bacteria* were identified. Among them, the dangerous bacteria *Acinetobacter*, occurring universally, were found the most often. This type of bacteria can pose a threat to human health. Thus, according to bacterial diversity, Sredniy Kaban Lake is estimated as contaminated. The percentage of species diversity by reads of 16S rRNA *Bacteria* of Sredniy Kaban Lake for 2017 is shown in Fig. 14.

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

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University. The authors would like to thank Dr. S. Malanin and E. Boulygina, the scientists of Kazan Federal University, for their assistance in experimental work.

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