Taxonomic composition of bacterioplankton of large freshwater lakes of the Yeravninskaya depression

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Abstract. Bacterioplankton community taxonomic composition of four large freshwater lakes of the Yeravninskaya depression (Bolshoye Yeravnoye, Gunda, Isinga, and Sosnovoye) which have great fishery significance for the region has been studied. The microbial community consists in the main of bacterial phyla *Gammaproteobacteria* (22-74%), *Actinobacteria* (4-35%), *Firmicutes* (1.17-34.6%), *Bacteroidetes* (0.3-7%), *Cyanobacteria* (0.9-6.5%) and *Planctomycetes* (1.5-10.5%). Total dissolved solids and sulphates accounted for 57% of the variation in the microbial community distribution. At the genus level, *Acinetobacter* dominated (average 21.4-25.2%). *Pseudomonas*, *Exiguobacterium*, and *Massilia* were also widespread. Their predominance may indicate a possible change in the structure of the community caused by natural and anthropogenic factors. In lakes Isinga and Gunda, a large number of bacteria of the genus *Klebsiella* was observed, *Staphylococcus* and *Streptococcus* were detected. These features indicate insufficient water quality as a result of anthropogenic impact.

1. Introduction
The study of microbial diversity in aquatic ecosystems provides insight into the biogeochemical processes and ecological mechanisms that support ecosystem functioning. Freshwater lakes are important objects for the conservation of diversity and the preservation of freshwater quality. Microorganisms can be used as bioindicators of ecosystem changes [1-2]. To predict changes in ecosystems caused by climate and human activities, it is important to study microorganisms in lakes for assessing freshwater quality. The changes in the composition, number, and diversity of the microbial community are an important indicative characteristic of ecosystem disadvantage.

The Yeravninskaya depression is situated in the elevated (>900 m a.s.l.) southwestern part of the Vitim plateau. This is the area of ancient lakes of the Mesozoic Era, and modern watershed of the Vitim (the Lena River basin) and Uda (the Lake Baikal basin) Rivers. Most of the lakes are shallow (up to 5 m) and closed. They are characterised by cyclical changes in water levels associated with alternating high-water and low-water years. The lakes of the Yeravninskaya depression are ones of the most productive fish farming in Transbaikalia despite the impact of negative factors in recent dry years [3-4].

The purpose of the research was to study the taxonomic composition of the bacterioplankton of large lakes in the Yeravninskaya depression and to identify the features of the microbial composition indicating low water quality.
2. Objects and methods
In July 2019 four large freshwater lakes of the Yeravninskaya depression (Bolshoye Yeravnoye, Gunda, Isinga, and Sosnovoye) were studied (table 1). Samples were taken at 3 different points of each lake from the surface horizon into sterile dishes. The temperature and \( pH \) were measured by a portable “PH-200” pH-meter (HM Digital, China), redox potential (\( Eh \)) was measured by ORP-meter (Kelilong, China). The dissolved oxygen was determined using the Winkler method [5]. Dissolved organic matter, sulphates, nitrates, and total phosphorus were determined by classical titrimetric/photocolorimetric methods [6].

| Lake             | Area, \( k\text{m}^2 \) | Max depth, m | Description                                                                 |
|------------------|--------------------------|--------------|-----------------------------------------------------------------------------|
| Bolshoye Yeravnoye | 104                      | 5            | An enclosed fishing lake, connected by a duct with Lake Sosnovoye.          |
| Gunda            | 11                       | 5            | An enclosed fishing lake near the village of Gunda.                         |
| Isinga           | 34                       | 5            | A fishing lake near the village of Isinga, with the effluent Kholoi River.  |
| Sosnovoye        | 24                       | 5            | An enclosed fishing lake, connected by a duct with Lake Bolshoye Yeravnoye. |

For DNA isolation, water of about 500 ml was concentrated on nitrocellulose membrane filters with a diameter of 0.22 µm, and further treated with PowerSoil DNA Kit (MOBIO, USA). Sequencing analysis of the V3–V4 variable regions of the 16S rRNA gene was done on a MiSeq sequencer (Illumina, United States) in the Core Centrum ‘GenomicTechnologies, Proteomics and Cell Biology’ in ARRIAM. Each sample was sequenced in three parallels, demonstrating high convergence and validity of the data [7]. Bioinformatic data processing of the taxonomic composition of bacterial communities was carried out using the RDP Pipeline service (https://pyro.cme.msu.edu). To reveal potential associations among environmental and sequencing data sets canonical correspondence analyses (CCA) were conducted using Excel data analysis add-on XLSTAT (Addinsoft) [8].

3. Results and discussions

3.1. Aquatic characteristics
The water temperature was 21-26°C, \( pH \) – 8.4-9.0, oxygen content – 8.6-12.2 mg/l (table 2). The total dissolved solids (TDS) varied from 276 mg/l (Isinga) to 592 mg/l (Sosnovoye). The water in all studied lakes is of bicarbonate-sodium type. Bicarbonates were up to 326 mg/l in Lake Sosnovoye, in the other lakes – 140-213 mg/l. The sodium ion content was 53-110 mg/l. TDS in the studied lakes increased in comparison with previous studies [3], that might be caused by recent low-water years. The high values of dissolved organic matter 19-38 mg/l were observed with the maximum for Lake Sosnovoye. The water of Lake Sosnovoye was turbid and had a greenish colour at the time of sampling, indicating an increase in organic matter production and blooms. The total phosphorus content did not exceed 0.13 mg/l, nitrate content was quite low 1.93 mg/l.

3.2. Taxonomic composition of bacterioplankton
We identified operational taxonomic units (OTUs) in sequencing reads; they vary from 241 to 415 (table 2). The Shannon diversity index was also determined for all samples and ranged from 3.10 to 4.62. The Shannon index values were at the level of diversity in the surface water of Lake Baikal [9-10]. According to this index, the highest bacterioplankton species diversity was observed in Lakes Bolshoye Yeravnoye (average 4.06) and Sosnovoye (average 4.21). The Shannon index was lower in the Isinga (average 3.46) and Gunda (average 3.71).
Table 2. Characteristics of the water in large lakes of the Yeravninskaya depression.

| Lake, site                  | Depth, m | t, °C | pH  | О₂, mg/l | TDS, mg/l | Org C, mg/l | NO₃⁻, mg/l | SO₄²⁻, mg/l | Total P, mg/l |
|-----------------------------|----------|-------|-----|----------|-----------|-------------|------------|-------------|---------------|
| Bolshoye Yeravnoye 1       | 7.0      | 21.6  | 8.4 | 9.0      | 347.25    | 19.0        | 1.07       | 0.66        | 0.08          |
| Bolshoye Yeravnoye 2       | 4.0      | 21.7  | 8.5 | 8.6      | 287.84    | 19.2        | 0.83       | 0.66        | 0.07          |
| Bolshoye Yeravnoye 3a      | 0.5      | 26    | 8.5 | 9.3      | 270.31    | 19.0        | 1.17       | 0.66        | 0.035         |
| Gunda 1                    | 4.4      | 21.1  | 8.9 | 9.6      | 369.49    | 18.0        | 0.77       | 0.66        | 0.035         |
| Gunda 2                    | 9.4      | 21.2  | 8.7 | 9.9      | 367.32    | 18.1        | 0.90       | 0.66        | 0.061         |
| Gunda 3a                   | 0.5      | 22.0  | 8.8 | 10.6     | 360.42    | 15.1        | 0.53       | 0.66        | 0.026         |
| Isinga 1                   | 3.2      | 21.4  | 9.0 | 12.2     | 276.13    | 22.5        | 1.93       | 0.49        | 0.035         |
| Isinga 2                   | 3.1      | 21.3  | 8.5 | 9.6      | 284.19    | 21.0        | 1.70       | 0.49        | 0.044         |
| Isinga 3a                  | 0.5      | 21.5  | 9.5 | 11.8     | 277.69    | 21.5        | 1.80       | 0.49        | 0.041         |
| Sosnovoye1                 | 1.5      | 21.1  | 8.6 | 9.6      | 592.18    | 34.5        | 1.30       | 1.64        | 0.13          |
| Sosnovoye 2                | 4.9      | 21.5  | 8.7 | 9.8      | 577.90    | 38.0        | 1.23       | 1.81        | 0.092         |
| Sosnovoye 3a               | 0.5      | 22.3  | 8.7 | 9.0      | 581.19    | 37.0        | 1.57       | 2.47        | 0.07          |

The microbial community of the lakes at phyla level was dominated by *Gammaproteobacteria* (48-74%), *Actinobacteria* (4-35%), *Firmicutes* (1.17-34.6%), *Bacteroidetes* (0.3-7%), *Cyanobacteria* (0.9-6.5%), *Planctomycetes* (1.5-10.5%) (figure 1).

The distribution of these phyla is typical for freshwater microbial communities located in different geographic zones of the world [11]. In the previously studied large freshwater lakes of the Baikal territory – Kotokel, Gusinoe, Shchuchye, and in the coastal area of Lake Baikal, a wide distribution of phyla *Bacteroidetes, Cyanobacteria, Verrucomicrobia* was observed [12]. In the lakes of the Yeravninskaya depression, the share of phyla *Planctomycetes* and *Firmicutes* has increased. In some points of Gunda, Sosnovoye, Bolshoye Yeravnoye the phylum *Deinococcus-Termus* was found (0.25-9.3%), but was not typical for Lake Isinga.

Table 3. Bacterioplankton species diversity estimates.

| Lake, site                  | Reads | OTUs | Shannon Index |
|-----------------------------|-------|------|---------------|
| Bolshoye Yeravnoye 1        | 22,038| 271  | 3.88          |
| Bolshoye Yeravnoye 2        | 18,360| 195  | 3.68          |
| Bolshoye Yeravnoye 3a       | 22,617| 401  | 4.62          |
| Gunda 1                     | 27,129| 241  | 3.54          |
| Gunda 2                     | 22,713| 267  | 3.66          |
| Gunda 3a                    | 23,272| 266  | 3.95          |
| Isinga 1                    | 26,576| 410  | 3.92          |
| Isinga 2                    | 31,823| 249  | 3.36          |
| Isinga 3a                   | 29,002| 281  | 3.10          |
| Sosnovoye1                  | 28,440| 415  | 4.13          |
| Sosnovoye 2                 | 29,310| 352  | 4.18          |
| Sosnovoye 3a                | 29,415| 365  | 4.31          |

* in-shore
Figure 1. Taxonomic composition of bacterioplankton of lakes of the Yeravninskaya depression at the phylum level.

Canonical correspondence analysis (CCA) demonstrated potential relationships between the composition of microbial community and the environmental parameters measured in the study (figure 2). Among the parameters, total dissolved solids (TDS) and sulphates were the major components of the first axis in the ordination plot, explaining 57% of the total variance, whereas water temperature, pH, nitrates, and phosphates were major components of the second axis, explaining 16% of the total variance.

Figure 2. CCA plot showing the relationships between bacterial communities and environmental parameters in four lakes.
At the genus level, *Acinetobacter* was widespread and dominated (from 11.2 to 34.8% at different points, on average 21.4-25.2%). Bacteria of the genera *Pseudomonas*, *Exiguobacterium*, and *Massilia* were also widespread (table 3). *Acinetobacter* and *Pseudomonas* have flexible ecological strategies and wide substrate specificity. Like the rapid growth of weeds, these bacteria can dominate natural ecosystems under favourable conditions, suppressing the development of other genera [13]. *Acinetobacter* were found in various habitats including soils and freshwater bodies exposed to high anthropogenic pressure [14-15]. The main substrates for these bacteria are hydrocarbons and polysaccharides. The genus *Massilia* was found in recreational freshwaters including the microbial community of Lake Baikal water [15-16]

### Table 3. Average of dominant genera abundance in bacterioplankton of the lakes of the Yeravninskaya depression, %.

| Genus of bacteria | Bolshoye Yeravnoye | Gunda | Isinga | Sosnovoye |
|-------------------|---------------------|-------|--------|-----------|
| *Acinetobacter*    | 24.99               | 22.13 | 21.40  | 25.17     |
| *Pseudomonas*      | 5.40                | 2.78  | 13.60  | 8.49      |
| *Exiguobacterium*  | 12.10               | 2.74  | 11.5   | 4.27      |
| *Massilia*         | 8.60                | 2.60  | 0.00   | 8.00      |
| *Klebsiella*       | 0.00                | 13.10 | 2.90   | 0.00      |
| *Microbacterium*   | 5.00                | 3.23  | 6.37   | 0.00      |
| *Sphingomonas*     | 0.37                | 1.77  | 4.13   | 0.00      |

*Firmicutes* were represented by the class *Bacilli*, genera *Exiguobacterium*. The share of the phylum *Firmicutes* was 1.2-11.2%, and reached 34.6% only in one sample ‘Isinga3’. *Exiguobacterium* are known to be able to survive in a wide range of temperatures, which makes them particularly interesting to study: we found them in habitats with different temperature conditions. In addition, the strains are tolerant to high levels of UV radiation, high contents of halogens and heavy metals; they can grow in a wide pH range (5-11). In the recreational polluted waters of fresh lakes in the United States, the share of the genus *Exiguobacterium* increased [15].

*Klebsiella* was found in samples from Lakes Gunda – 12.28-28.05%, and Isinga 15.4%. *Klebsiella* is a genus of opportunistic bacteria widely distributed in the environment including soil and water [17]. Bacteria of *Klebsiella* are able to form polysaccharide capsules allowing them to be resistant to adverse environmental conditions. It belongs to the *Enterobacteriaceae* which is an indicator of probable fecal contamination. In Lakes Isinga and Gunda, where a high share of *Klebsiella* was registered, the increased total numbers of bacteria were found and coliform bacteria were detected [18]. *Staphylococcus* (1.13%) and *Streptococcus* (1.67%) were also found in Lake Isinga. It can probably be explained by intensive cattle grazing on the shores of Isinga and Gunda. *Staphylococcus aureus* has previously been found in almost half of the samples taken from freshwater beaches [19].

### 4. Conclusion

Bacterioplankton community composition of four large freshwater lakes of the Yeravninskaya depression has been studied. The bacterioplankton species diversity was as high as for Lake Baikal, according to the values of Shannon diversity index. Diversity in Lake Sosnovoye and Lake Bolshoye Yeravnoye was higher than in Lake Isinga and Lake Gunda. The taxonomic composition mainly consists of the bacterial phyla typical of freshwater lakes: *Gammaproteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, and *Planctomycetes*. According to canonical correspondence analysis, parameters such as TDS and sulphate content were responsible for 57% of the variation in microbial community distribution, while water temperature, pH, nitrate and phosphate content – for 16% of the variation. The features of the taxonomic composition of bacterioplankton
have been identified, which reveal prognostic risks as a result of interrelated environmental and anthropogenic (social) factors. *Acinetobacter*, *Pseudomonas*, *Exiguobacterium* and *Massilia* dominated in genera composition. Their predominance may indicate a possible change in the structure of the community caused by natural and anthropogenic factors. In Lakes Isinga and Gunda, a large number of bacteria of *Klebsiella* genus was registered, as well as *Staphylococcus* and *Streptococcus*. These features indicate a decline in water quality as a result of anthropogenic impacts.

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