Micro-scale spatial distribution of bacterioplankton in the Priplotinny (Near-dam) Reach of the Kuibyshev Reservoir in the early autumn period 2020

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Abstract. A detailed study of the distribution of bacterioplankton and photosynthetic pigments of the Priplotinny Reach of the Kuibyshev Reservoir was carried out in early autumn 2020. The bacterioplankton cell numbers were $0.8 \text{ to } 2.1 \times 10^6 \text{ cells ml}^{-1}$, and the biomass varied from 7 to 56 $\mu g \text{ C L}^{-1}$. The average chlorophyll $a$ concentration of in the integral samples was 2.91 $\mu g \text{ L}^{-1}$, varying in the range of 1.1-9.6 $\mu g \text{ L}^{-1}$ (CV 30.7%). The pheophytin content was relatively low, $19.2 \pm 10.4\%$ of chlorophyll concentration, indicating the continued active phytoplankton development in the seston in the fall of 2020. From September 17 to October 3, a weak trend for an increase in bacterioplankton abundance as well as for a decrease in the chlorophyll $a$ concentration and in the average cell volume was revealed. A significant ($p <0.05$) correlation between the Chl $a$ concentration in the total biomass of heterotrophic bacteria ($r = 0.48$) was observed. Under conditions of intense wind mixing, anthropogenic sources of pollution and the inflow of the inflow did not have a significant effect on the size structure of bacterioplankton. No pronounced local spatial complexes of stations were revealed in terms of quantitative and structural parameters of bacterioplankton and chlorophyll concentrations in the investigated part of the reservoir. In general, the concentration of pigments and the abundance and biomass of bacterioplankton correspond to the mesotrophic status of the Reach. Both the total number of bacterioplankton and the chlorophyll content in Pryplotinny Reach at the beginning of autumn 2020 are within the range of long-term monitoring values. While in the summer there is a clear long-term increasing trend of the bacterioplankton abundance, in the autumn the bacterioplankton numbers remain stable throughout the entire reservoir existence period.

1. Introduction
The study of the structural and functional organization of freshwater ecosystems and their changes under the influence of natural and anthropogenic factors is one of the main tasks in the focus of modern hydrobiology. Bacteria play a central role in global environmental processes and in Earth biogeochemistry [1]. In aquatic ecosystems, bacteria are the most important component of microbial communities responsible for the organic matter mineralization and nutrient recycling processes [2, 3]. Unlike natural lakes and rivers, the hydrological regime of hydroelectric reservoirs is regulated by human activity, and the level of anthropogenic impact is much higher than in natural water bodies. Therefore, the functioning of the microbial community in reservoirs has a number of specific features, and its study is very important.
Since the beginning of filling the Kuybyshev Reservoir in 1955-1957, bacteriological monitoring was carried out [4], but samples were usually taken at 2-3 stations on each of eight lake areas of reservoir. Although this approach provides a general picture of the bacterioplankton distribution of the reservoir, it does not allow assessing micro-scale changes in the spatial distribution of bacteria, including the effect of tributaries, local geological inhomogeneities, and point sources of anthropogenic pollution. Therefore, it is very important to apply the most detailed (spatial and temporal) sampling to study the ecology of the microbial plankton community [5] of each lake area of the Kuybyshev Reservoir. In addition, since the beginning of the 21st century, regular (monthly from April to November) monitoring studies of the Kuybyshev Reservoir have ceased. Over the last 20 years, episodic studies took place, as a rule, in the summer [6-9], therefore, in the modern period the stage of autumn restructuring of the plankton community of the Reservoir and the Near-dam Reach (Priplotinny Reach) remains unexplored.

The main objectives of this study in the near-dam area of the Kuybyshev reservoir were: 1) to determine the peculiarities of chlorophyll and bacterioplankton distribution in the early autumn; 2) to determine the main environmental factors influencing bacterioplankton abundance and its size structure in this particular period.

2. Characteristic of the Kuybyshev Reservoir, its Priplotinny Reach and sampling conditions

The Kuybyshev Reservoir is the largest reservoir in Europe and third in the world by surface area [10]. It consists of eight extended lake areas separated by narrownesses. Priplotinny Reach, the widened near-dam area, also called Zhiguli Sea, located directly above the dam of the Zhiguli Hydroelectric Power Station (HPS). Some morphometric characteristics of the reservoir and its near-dam area are given in the table 1. The ecosystem of the Priplotinny Reach of the Kuybyshev reservoir is strongly affected by a number of factors, i.e. industrial and domestic effluents from the large industrial center of Togliatti; the dam of the Zhiguli HPS and its operating mode; the construction of the planned highway bridge near Klimovka village; the limestone quarries of the "Zhigulevsky building materials” plant in the Yablonevy Ovrag (an "Apple Ravine"); and the confluence of the USA River Bay.

Studies at the Priplotinny Reach were conducted on September, 17 to October, 3, 2020 at 21 stations, evenly located across the water area of the Reach and covered the entire depth range (table 2, figure 1). The air temperature during the study period varied in the range of 7-21°C. According to the website [10], the average daytime temperatures were 18.1±3.8°C, and the average nighttime temperatures were 8.9±3.1°C. The weather in September, 2020 was windy, which caused strong waves and contributed to the intensive mixing of the entire water mass. The temperature of the water column varied very slightly (table 2); the temperature difference between the surface and bottom layers did not exceed 1°C, averaging 0.23°C. The water transparency was in the range of 2.1-3.6 m; the whole water column was well aerated and weakly alkaline (table 2).

| Table 1. General characteristics of the Kuybyshev reservoir and its Priplotinny Reach [10] |
|---------------------------------|----------|----------|----------|------|--------|
| Parameters                      | Area (km²) | Volume (km³) | Length (km) | Widthmax (km) | Depthmax (m) |
| The Kuybyshev Reservoir         | 5900      | 57.3      | 510        | 40  | 41     |
| Priplotinny Reach               | 397       | 6.8       | 35         | 12  | 32     |

3. Materials and Methods

Integral water samples for counting bacterioplankton were taken on September 17 – October 3, 2020 with a Dyachenko bathometer, combining in one container equal volumes of water from different horizons: from the surface to 5 m, every meter; then up to 10 m, every 2.5 m; and after 10 m to the bottom, with an interval of 5 m. Samples were fixed with a sterile formalin solution to a final concentration of 4% and concentrated by filtration through membrane filters with a pore diameter of 0.2 μm. The total abundance of bacterioplankton was determined by epifluorescence microscopy using...
The bacterial cell sizes were estimated using the UTHSCSA Image Tools 3.00 image analysis software. The biomass in units of organic carbon was calculated based on the data on the carbon content in cells [12]. To determine the concentration of photosynthetic pigments, 0.5 L of the integral sample was filtered through a glass filter and extracted overnight in 90% (v/v) acetone in the dark at 4°C; chlorophyll (Chl) concentrations were determined spectrophotometrically using three-wave equations [13].

| Stations Depth range (m) | 1, 2, 6, 10, 13, 14, B7, H7 2-10 mean* median | 3, 4, 7, 34, 39, B3 11-15 mean* median | 5, 8, 11, 12, 15, 16, B6, 16-32 mean* median |
|--------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------------------|
| Depth (m)                | 6.5±3.0                                       | 13.2±1.5                                    | 20.3±5.7                                      |
| Temperature, surface (°C)| 15.4±0.6                                      | 15.4±0.5                                    | 15.9±0.45                                    |
| Temperature, bottom (°C) | 15.3±0.6                                      | 15.4±0.5                                    | 15.5±0.53                                    |
| Transparency (m)         | 3.08±0.41                                     | 3.28±0.18                                   | 3.14±0.67                                    |
| Turbidity (mg L⁻¹)       | 3.44±3.0                                      | 2.61±1.60                                   | 2.24±0.83                                    |
| pH                       | 8.39±0.10                                     | 8.39±0.09                                   | 8.46±0.08                                    |
| Eh                       | 185±8                                         | 179±15                                      | 174±6                                        |
| Conductivity (μS cm⁻¹)   | 316±3                                         | 323±6                                       | 315±11                                       |
| Dissolved oxygen (mg L⁻¹)| 8.03±0.18                                     | 7.88±0.36                                   | 7.95±0.21                                    |

* mean ± standard deviation

To assess the long-term trends in changes in the abundance of bacterioplankton, data from the book [4] for the period 1957-1995 and data from our own research in 2009-2020 were used. The abundance of bacterioplankton in the Kuibyshev reservoir in 1957–1995 was determined using Erythrosine staining method (ESM) [14] instead of DAPI. We calculated the number of bacterial cells in the same sample using both staining methods to check the correctness of the comparison. It turned out that the ratio of the number of bacteria is described by the equation: N_DAPI = 1.2 N_ESM. Therefore, in this work, we used the adjusted values of the bacterial abundance in 1957-1995. Statistical data analysis was performed using software MS Excel 2019 and PAST ver. 4.03.

4. Results and Discussion

The distribution of the photosynthetic pigments and bacterioplankton over the water area of the Reach is shown in figure 1. The mean Chl a concentration in the integral samples was 2.91 µg L⁻¹, varying in the range of 1.1-9.6 µg L⁻¹ (CV 30.7%). The weighted mean Chl b concentration was 1.9% of the of the Chl a concentration, [Chl a]. On the eight stations it was not detected at all, and except for stations 4 and 5 near Usolsky and Akhtushinsky bays, it was less than 6% [Chl a]. The weighted average proportion of Chl c was almost an order of magnitude higher, 16%. This indicates that the algae synthesizing it, primarily diatoms, make a significant contribution to the composition of phytoplankton at many stations. Nevertheless, its concentration never exceeded 0.7 µg L⁻¹, averaging 0.42 ± 0.13 µg L⁻¹ (CV 13.5%). The share of pheophytin a was 19.2±10.4% of [Chl a], which indicates the predominance of actively developing phytoplankton species. A weak trend towards [Chl a] decrease from September 17 to October 3 was revealed (R² = 0.2099). No significant differences in the pigment concentrations depending on the depth of the station or its spatial location (figure 1a, table 3, 4) were found during the study period.
Figure 1. Spatial distribution of chlorophyll $a$ and pheophytin $a$ concentrations (a) and abundance and biomass of bacterioplankton (b) on the Priplotinny Reach of the Kuybyshev Reservoir. The red line shows highway bridge under construction.
Analysis of the ratio of Chl a, b and c in a sample allows us to roughly determine which phytoplankton taxa prevail in it [15, 16]. Calculations showed that from September 17 to 25 67% of the samples were dominated by Cyanobacteria, and starting from September 26, Bacillariophyta dominated in 75% of the samples, which reflects the seasonal transformation of phytoplankton. Thus, based on the changes in the chlorophyll ratio, it can be concluded with a high degree of confidence that in the Priplotinny Reach in 2020, the summer/autumn restructuring of the phytoplankton took place around September 26. In the 20th century, such process, as a rule, occurred between September 7-15 [17]. Perhaps such a temporal shift is caused by the peculiarities of weather conditions in 2020, but the impact of global climate changes cannot be excluded.

During the study, the bacterioplankton abundance varied from 0.8 to 2.1 (on average, 1.24±0.39) ×10⁹ cells L⁻¹, and the biomass – from 16.9 to 55.6 (29.8±9.4) µg C L⁻¹ (figure 1b). At all stations, bacterioplankton community was dominated by single free-floating cocci and coccobacilli (48-87 % of the total abundance and 44-84% of the total biomass); cells with linear sizes of 0.5-2 µm formed up to 95% of the total bacterial abundance and biomass. The average volume of bacterial cells was 0.124±0.026 µm³; cells with volume of 0.025-0.400 µm³ prevailed in the bacterioplankton community (figure 2a, b).

From September 17 to October 3, there was a weak tendency toward a decrease in the average cell volume (R² = 0.0959) and an increase in the total bacterioplankton abundance (R² = 0.0411); as a result, the total bacterial biomass remained almost at the same level. The influence of the depth of the station and its location on the abundance and biomass of bacterioplankton was practically absent (figure 1b, table 3, 4). The biovolume of heterotrophic bacteria significantly correlates with the concentration of Chl a (R = 0.484192, p<0.05), while bacterioplankton abundance correlates with the concentration of Chl c (R = 0.476745, p<0.05).

| Stations Depth range (m) | 1, 2, 6, 10, 13, 14, B7, H7 | 3, 4, 7, 34, 39, B3 | 5, 8, 11, 12, 15, 16, B6, 16-32 |
|-------------------------|--------------------------|---------------------|-----------------------|
| Pigments (µg L⁻¹):      |                          |                     |                       |
| Chl a                   | 3.026±2.78               | 2.17               | 1.53±0.85             | 1.28                 | 2.86±1.83 | 1.98 |
| Chl c                   | 0.47±0.14                | 0.46               | 0.33±0.14             | 0.3                  | 0.44±0.10 | 0.42 |
| Pheo a                  | 0.62 ± 0.52              | 0.5                | 0.33±0.12             | 0.3                  | 0.45±0.29 | 0.35 |
| Bacteria:               |                          |                     |                       |
| Abundance (10⁹ cells L⁻¹)| 1.46 ± 0.37              | 1.38               | 1.03±0.17             | 1.02                 | 1.18±0.46 | 0.91 |
| Biomass (µg C L⁻¹)      | 35±11                    | 35                 | 25±7                  | 26                   | 27±7      | 25   |
| Average cell volume (µm³)| 0.126±0.030              | 0.118              | 0.121±0.030           | 0.118                | 0.124±0.023 | 0.133 |

Table 3. Distribution of pigments and bacteria by groups of stations with different depths

*a mean ± standard deviation

Correlation and cluster analysis of the data revealed that, in terms of quantitative and structural parameters, bacterioplankton does not form local complexes of stations, either territorially or temporally. No significant correlations with the measured abiotic factors were also found, implying that local sources of anthropogenic impact in autumn 2020 did not significantly affect the abundance and biomass of bacterioplankton, although some differences in the size structure were observed (figure 2, c-i). Phytoplankton composition (figure 2, k-n) had a greater influence on the size structure of bacterioplankton, although the combined effect of several factors cannot be ruled out. Presumably this is due to the intensive mixing of water masses during the study period, and it can be assumed that in more calm conditions or in other seasons of the year, the differences in the quantitative and structural parameters of bacterioplankton could be more pronounced. At the level of average values, some differences are found in the studied biotic parameters by groups of stations, but due to the large variation between stations, they are not significant (tables 3, 4).
In general, the concentrations of pigments and the abundance and biomass of bacterioplankton in the Priplotinny Reach in autumn 2020 are close to those in other mesotrophic reservoirs [18-23].
ant increase in the total bacterioplankton abundance in the Priplotinny Reach in summer and autumn periods (figure 3). The trend towards an increase in the total bacterioplankton abundance in the Priplotinny Reach is clearly visible for the summer months (figure 3a). There is no such trend for the autumn period (figure 3b), possibly due to insufficient data at the beginning of the 21st century. The stable early-autumn bacterial abundance possibly reflects some seasonal features of the bacterioplankton development or the peculiarities of the mixing of the Reach at the beginning of autumn. The reasons for this phenomenon are not clear and require additional systematic studies, taking into account spatial and seasonal variability. It should be noted that for a number of other reservoirs of the Volga Cascade, a significant increase in the total bacterioplankton abundance in the long-term aspect (according to the average values for the period July-September) was found, which the authors explain by climate changes, an increase in the phosphorus load on the reservoirs and, consequently, an increase in their productivity [6, 18].

Our results are quite consistent with the long-term data of A I Ivatin [4] on changes in the total bacterioplankton abundance in the Priplotinny Reach of the Kuibyshev Reservoir in September and do not exceed the average long-term values (figure 3). The trend towards an increase in the total bacterioplankton abundance in the Priplotinny Reach is clearly visible for the summer months (figure 3a). There is no such trend for the autumn period (figure 3b), possibly due to insufficient data at the beginning of the 21st century. The stable early-autumn bacterial abundance possibly reflects some seasonal features of the bacterioplankton development or the peculiarities of the mixing of the Reach at the beginning of autumn. The reasons for this phenomenon are not clear and require additional systematic studies, taking into account spatial and seasonal variability. It should be noted that for a number of other reservoirs of the Volga Cascade, a significant increase in the total bacterioplankton abundance in the long-term aspect (according to the average values for the period July-September) was found, which the authors explain by climate changes, an increase in the phosphorus load on the reservoirs and, consequently, an increase in their productivity [6, 18].

**Table 4.** Distribution of pigments and bacteria by groups of stations with different spatial location

| Location          | highway bridge under construction | Left bank  | Central part | Right bank | Usa River estuary |
|-------------------|-----------------------------------|------------|--------------|------------|------------------|
| Stations          | B7, B6, B3, H7                    | 1, 2, 6, 10, 13, 15, 39 | 3, 7, 11     | 4, 5, 34, 12, 14, 16 | 8                |
| Pigments (µg L⁻¹):|                                   |            |              |            |                  |
| Chl a             | 0.53±0.11                         | 0.45±0.12  | 0.27±0.04    | 0.39±0.14  | 0.49             |
| Chl c             | 5.21±3.56                         | 2.25±1.17  | 1.51±0.41    | 1.91±1.2   | 2.63             |
| Pheo a            | 0.22±0.24                         | 0.61±0.54  | 0.35±0.1     | 0.54±0.26  | 0.63             |
| Bacteria:         |                                   |            |              |            |                  |
| Abundance (10⁹ cells L⁻¹) | 1.26±0.29              | 1.22±0.37  | 0.95±0.19    | 1.29±0.49  | 1.92             |
| Biomass (µg C L⁻¹) | 35±7                              | 30±12      | 25±5         | 30±10      | 23.91            |
| Average cell volume (µm³) | 0.153±0.03          | 0.127±0.014| 0.107±0.03   | 0.115±0.022| 0.093            |

**Figure 3.** Long-term dynamics of bacterioplankton abundance in the Priplotinny Reach in summer (June-August) (a) and autumn (September-October) (b) periods.

**5. Conclusion**

The early autumn period is characterized by a decrease in water temperature and an increased intensity of mixing, which smooth out local differences between the stations of the reservoir. Summer-autumn restructuring of the phytoplankton community occurs simultaneously with hydrological changes: summer cyanobacterial complexes are replaced by autumnal diatoms-dominated ones. According to
the changes in the ratio of chlorophylls concentrations, in the studied part of the Kuybyshev Reservoir in 2020, the change of the summer phytoplankton complex to the autumn one occurred around September 26, that is, with an approximately ten-day later then in the second half of the 20th century. The relatively low pheophytin fraction indicates that phytoplankton continued to develop actively in the seston in autumn 2020.

During the period of our study, the distribution of photosynthetic pigments over the water area of the Priplotinny Reach could be influenced, in particular, by the lateral inflow, and, in addition, the movement of local "blooming spots" of cyanobacteria, caused by the wind and current, during the period of their dominance in phytoplankton. At the same time, the bacterial abundance and biomass were distributed more evenly, and during the period of our study, no regular dependences on the studied environmental factors were revealed. The size structure of bacterioplankton was more variable and could be influenced by both phytoplankton composition and local influences, including increased turbidity due to bottom sediments resuspension in the area of the bridge construction. However, these differences are small, and the influence of individual factors cannot be confirmed or disproved by statistical methods.

The concentration of Chl a and Chl c significantly correlated, respectively, with the biomass and the abundance of heterotrophic bacteria. These correlations are obviously related to the dependence of the rate of bacterial growth on the photoautotrophic production of organic matter.

Although in the summer the quantitative development of bacterioplankton in the Priplotinny Reach has increased significantly since the filling of the Kuybyshev Reservoir, in the autumn this trend is very insignificant, which may be due to both seasonal factors and the peculiarities of mixing of the water reservoir.

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