Ciliofauna Species Wealth of the Khabarovsk Central City Pond

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Abstract. The paper describes the composition of the Ciliata species of the Khabarovsk Central Pond. In the course of the inventory, 43 Ciliata species belonging to 2 subtypes and 11 classes have been identified, the most numerous of which are Spirotrichea and Oligohymenophorea. Also, 20 species have not previously been recorded on the territory of the Middle Amur Region. By the frequency of occurrence, the vast majority of species (23 representatives) are observed in the solitary samples (up to 15 %). The background species include ciliates such as Coleps hirtus, Strombidium viride, Stylonychia mytilus complex, Paramecium caudatum, and Uronema marinum. It has been found that in the reservoir being studied, the average size (up to 200 μm) ciliates dominate. By the ecotope preferred, the ‘transitional’ cluster representatives prevail, which indicates the possible widespread colonization of the reservoir by protozoa. This is due to the shallow depth of the Central Pond and, therefore, the lack of clear boundaries between individual zones.

1. Introduction
Free-living ciliates (Ciliophora) constitute an essential part of the protozoan complex of any water reservoir. Ciliates being the primary consumers play an important role in the transformation of organic matter in nature. On the one hand, protists are an integral component of the hydrobiont (fish fry, rotifers, etc.) nutrition, and on the other hand, they participate in the organic matter decomposition. Recently, ciliates have been actively used in various biological monitoring systems [1-3].

Currently, the fauna of freshwater Ciliata is characterized by the largest number of taxa and according to today’s estimates, the number of species in them varies from 3,000 to 30,000 [4]. Such a wide scatter in the scholars’ estimates is due to the emergence of modern protozoa identifying means (electron scanning microscopy, silver impregnation, etc.).

Ciliates are eurybionts capable to inhabit fresh and marine water reservoirs, soil, karst lakes, geothermal springs, etc. A special group is represented by Ciliata epibionts, which can be found on the integuments of various hydriobionts (daphnia, ostracods, oligochaetes) and in the stomachs of ruminants [5-7]. The ciliocenoses of technogenic reservoirs on the Khabarovsk Territory have not virtually been studied, except for the A.V. Zhukov’s work on aeration tanks of water treatment facilities [8].
The research objective is to identify the species composition and morpho-ecological features of the Khabarovsk Central Pond Ciliata.

2. Material and Techniques

The central ponds are a cascade of three reservoirs, which are now actively used by the population for recreation, catamaran boat tours, and ship modeling competitions. The hydrobiological material was sampled from the ‘middle’ pond during the vegetative season in 2013-2015, and 2018. To do this, small wide-necked glass vessels and the ‘fouling glass’ technique modified by the author [9] were used. In total, 94 samples have been processed during the study.

To identify the Ciliata species composition, the water was enriched with a nutrient medium (hay infusion) and held in a dimly lit place at room temperature. The ciliofauna was studied under Leica DME (Germany), Motic BA300 (China), and Mikmed-6 (Russia) microscopes using Webbers MyScope 300M and MC-6.3 digital cameras. In the case of a low protozoan cell number, the samples were centrifuged. The linear dimensions of the ciliates were determined using a micrometer eyepiece scale and software supplied with digital cameras.

Living ciliates were observed using micro-aquarium technology [10]. To do this, a small amount of petroleum jelly was applied with a microscopic needle to the corners of the coverslip. Then, a drop of the test liquid was covered with this slip. In such a micro-reservoir, a drop remains for a long time.

The ciliates were fixed with formol-calcium (according to Baker) and da Fano's fixative; methylene blue, eosin, and iodine solutions were used to identify the common cell morphology. Infraciliature was detected by impregnation with a 0.3 % protargol solution.

The ciliate species frequency index (V) was calculated by the formula:

$$V = \frac{a \times 100}{A} \%$$

where a is the number of samples containing a given species, A is the total number of samples in the reservoir.

The species identified in up to 15 % of hydrobiological samples were classified as solitary ones. Those with a frequency index of 16 to 29 % were related to rare species. Those with a frequency index exceeding 30 % were classified as background ones.

The Ciliata species were identified using identification guides, as well as numerous papers and monographs on individual Ciliata taxa [10, 11]. The taxonomic composition of the ciliofauna is given in the Small and Lynn system with some amendments outlined in the ‘Protists’ monograph [12].

3. Results and Discussion

In the course of the Central City Pond Ciliata community inventory, 43 Ciliata species belonging to 2 subtypes and 11 classes have been identified. The most numerous classes are represented by Spirotrichea (11 species) and Oligohymenophorea (9 species) together making 46.5 % of the total faunistic composition of the reservoir Ciliata population. The largest number of Ciliata species belongs to the Oxytricha and Paramecium genera (3 representatives each). In the Peritrichia subclass, only a single species was recorded, i.e. Vorticella convallaria.

When performing a comparative faunistic analysis of the data obtained earlier on the Middle Amur Region territory [13], we have recorded 20 new species, which emphasizes the insufficient knowledge of the Ciliata population in this region. Similar results were obtained by protistologists for other water reservoirs. Thus, V.V. Zharkov [14] notes that by 1936, 1979, and 2010, in the Volga River basin, 88, 190, and about 512 (among which 50 are parasites) Ciliata species have been found, respectively. The scholar associates the increase in ‘new’ species in the Volga River with poor knowledge of the composition, ecology, and structure of the Ciliata community. I.Kh. Alekperov [15] believes that at the first stages of the study, the main reason for the low protozoa species diversity is the short hydrobiological sampling period. According to the scholar, the total survey time and the number of sampling points play a decisive role.
The ciliate frequency index is given in Table 1.

### Table 1. Frequency Index (V) of Ciliata in the Khabarovsk Central Pond.

| Solitary             | Rare                        | Background                |
|----------------------|-----------------------------|---------------------------|
| Spirostomum ambiguum | Trithigmostoma cucullus    | Paramecium aurelia        |
| Stentor polymorphus  | Litonotus lamella          | Coleps hirtus             |
| Aspidisca cicada     | Oxytricha fallax           | Strombidium viride        |
| Vorticella convallaria | Paramecium caudatum        | Stylonychia mytilus       |
| Holophrya simplex    | Uroleptus piscis           | Uronema marinum           |
| Caenomorpha medusula | Spirostomum teres          |                           |
| Strobilidium caudatum | Loxodes rostrum           |                           |
| Frontonia leucas     | Microthorax sulcatus       |                           |
| Spathidium spathula  | Metopus es                 |                           |
| Prorodon ovum        | Frontonia acuminata        |                           |
| Urocentrum turbo     | Tachysoma pellionellum     |                           |
| Oxytricha minor      | Vorticella convallaria     |                           |
| Paramecium bursaria  | Halteria chlorelligera     |                           |
| Lacrimatoria olor    | Litonotus cygnus           |                           |
| Lembadion lucens     | Oxytricha chlorelligera    |                           |
| Nassula ornata       |                             |                           |
| Euplotoides patella  |                             |                           |
| Colpoda steini       |                             |                           |
| Amphileptus pleurosigma |                 |                           |
| Trimyema compressum  |                             |                           |
| Chaenea teres        |                             |                           |
| Stentor roeseli      |                             |                           |
| Didinium chlorelligerum |                       |                           |

23 15 5

The tabular data analysis indicates that the core of the Central City Pond Ciliata community is represented by solitary species (V is up to 15 %). This group includes 23 representatives, which corresponds to 53.5 % of the total faunistic composition of Ciliata in the reservoir being studied. In the cluster, the rare and background categories include 15 (35 %) and 5 (11.5 %) Ciliata species, respectively.

According to our data, only eurybiontic representatives are first recorded, however, there is a huge number of stenobiont species, the population of which is either very small or associated with the possibility of existence only within certain temperature ranges [16].

Size composition is an important characteristic of Ciliata communities. Linear parameters represent an integrating characteristic of a species determining its ecological niche. The free-living Ciliata size varies within a wide range from 25 to 1,000 µm in length and 20 to 140 µm in width.

Using the methodology adopted [17], several Ciliata size classes have been identified: <40 µm; 40-100 µm; 100-200 µm; > 200 µm (Fig. 1). The first size group is the poorest one, which includes 6 species or 14 % of the entire ciliate fauna identified in the reservoir being studied. The second, third, and fourth class Ciliata shares account for 15 (34.8 %), 14 (32.5 %), and 8 (18.6 %) species, respectively.
Figure 1. Size Spectrum of the Central City Pond Ciliata Population.

Thus, the average size species play a leading role in the biological production of the ciliate community. Despite the relatively high density and reproduction rate, small Ciliata cannot give a serious growth of biomass due to their small size, and large individuals are too few and reproduce slowly. The data obtained are consistent with the results of other studies [18-19].

However, according to studies by V. Feussner [20], the average freshwater Ciliata length is 161 µm, which exceeds this parameter determined for the Central City Pond Ciliata (145 µm) and the ciliofauna of small rivers in Khabarovsk and its surroundings [21] by more than 15 and 30 µm, respectively. In our opinion, the Khabarovsk Ciliata size is affected by serious anthropogenic pressure (location near enterprises, residential areas, etc.).

When analyzing the data on the Ciliata confinement to different ecological zones of the reservoir (Fig. 2), we were guided by the below assumptions:
- usually, at a shallow water reservoir depth, the preferred habitat zones are conditionally divided that is reflected, e.g., in the occurrence of benthic species in plankton samples,
- all species recorded in several biotope ecological zones have been classified as ‘transitional’ ones.

Figure 2. Ecotopic Distribution of the Khabarovsk Central Pond Ciliofauna.
Thus, the Ciliata community core is represented by ‘transitional’ species living in two or three ecotopes. This category accounts for 21 Ciliata species, which is 49 % of the total faunistic diversity of the Central Pond Ciliata. The dominance of the ‘transitional’ zone in the biotope and the poor species diversity of truly periphytic (7 %) and planktonic (16 %) species are associated with the insignificant depths of the reservoirs being studied and, consequently, the lack of clear boundaries between individual zones.

Such an abundance of eurytopic species in water reservoirs has earlier been discussed by the authors, however, there is still no consensus on the reasons for such a distribution. Thus, I.V. Burkovsky [18] notes that the vertical Ciliata distribution is determined by the temperature regime and the abundance of food, with the temperature factor playing the dominant role. I.Kh. Alekperov [22] indicates a more even Ciliata distribution in shallow water, which is explained by more uniform heating of the water column, however, this is a secondary indicator, and the dominant ones are the gas regime and, especially, the trophic factor.

4. Conclusion
In the course of the Ciliata species diversity inventory, 43 species belonging to 2 subtypes and 11 classes have been identified, the most numerous of which are Spirotrichea and Oligohymenophorea, which in total make up the Ciliata community core of the ciliocenosis being studied.

The overwhelming majority of ciliate species (53 %) are solitary ones, and the background group is represented by Coleps hirtus, Strombidium viride, Stylonychia mytilus complex, Paramecium caudatum, and Uronema marinum. When analyzing the linear cell parameters, it has been found that the average size (≤ 200 μm) species predominate. This is because small individuals cannot give a serious growth of biomass and large ones (> 200 μm) are too few and reproduce slowly.

The ecotopic Ciliata distribution indicates the dominance of the ‘transitional’ ecological group, i.e. most species do not have a definitely preferred zone in the reservoir, which, in our opinion, is due to the shallow depth of the Central City Pond.

5. References
[1] Kolkwitz R, Marsson M 1909 Intern. Rev. Gesamten Hydrobiologie 2 126-152
[2] Sladecek V 1973 Ergebnisse der Limnologie Archiv Hydrobiologie 7 218
[3] Makrushin A 1974 Biological analysis of water quality (Leningrad: Science) p 59
[4] Foissner W, Berger H, Zechmeister-Boltensen S 2003 Oesterreichesche gesellschaft fur bodenbiologie 125
[5] Nikitina L 1997 Soil infusoria of the Middle Amur region (Khabarovsk: KhGPU) p 102
[6] Song W, Wilbert N 2000 Polar biology 3 212-222
[7] Tribun M 2012 Ecological features of the ciliofauna of small rivers in the vicinity of Khabarovsk p 154
[8] Zhukov A 2012 Ecological and biological characteristics and indicative value of the ciliofauna of the treatment facilities in Khabarovsk p 150
[9] Tribune M 2010 Notes of the Grodekov Museum pp 35-37
[10] Berger H, Foissner W 2003 Biologische Methoden der Gewässeranalysen 17 160 p
[11] Foissner W, Berger H 1996 Freshwater Biology 35 375-482
[12] Protists: Manual of Zoology 2007 (St. Petersburg: Science) 2 p 1144
[13] Nikitina L, Prikhodko A, Zhukov A, Tribun M 2011 Ciliofauna of natural and technogenic ecosystems of the Middle Amur Region (Khabarovsk: FVGUPS) p 160
[14] Zharkov In 2011 Ecology of free-living simplest terrestrial and aquatic ecosystems 26-27
[15] Alekperov I 2011 Ecology of free-living simplest terrestrial and aquatic ecosystems 6-7
[16] Tribune M 2012 Scientific and technical problems of transport, industry and education 2 309-313
[17] Bykova Since 2005 Fauna and ecology of ciliates of small reservoirs of Samarskaya Luka and Saratov reservoir since 207
[18] Burkovsky I 1984 Ecology of free-living ciliates (Moscow: Moscow State University) from 208
[19] Obolkina L 2003 Planktonic ciliates of Lake Baikal: ecology, taxonomy p188
[20] Foissner W, Berger H 1999 Informationsberichte des Bayer Landsamtes fur Wasserwirtschaft 3
    793
[21] Tribun M, Nikitina L 2014 Bulletin of the Amur State University 1 126-127
[22] Alekperov I 2012 Free-living ciliates of Azerbaijan (Baku: Elm) from 520