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Mass development of periphyton ciliates in the coastal zone of Southern Baikal in 2019-2020

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ABSTRACT. Changes in biocenoses of the shallow water zone of open Lake Baikal continue. In the autumn of 2019 during scuba dives, we recorded abundant fouling of macrophytes in the littoral zone by ciliates of the subclass Peritrichia, the genus *Vorticella*, at the Listvyanka settlement – Bolshiye Koty settlement section (west coast of Southern Baikal). Despite the fact the *Vorticella* are the permanent component of the Bailak periphyton, such mass development of Peritrichia in the littoral zone of open Baikal has not been previously recorded. Increasing anthropogenic pressure in the littoral zone of the lake can be one of the main causes of this outbreak in the abundance of *Vorticella* cf. *campanula*, the main species of this fouling.

Keywords: ciliate, *Vorticella*, bacteria, Lake Baikal, abundance

1. Introduction

The open littoral zone (i.e. the littoral zone of indigenous Lake Baikal except for warm bays) of Lake Baikal occupies approximately 7% of its area. The landscape of the underwater slope on the western side of Southern Baikal composed of crystalline bedrocks of Archean and Proterozoic complexes has large bottom slopes of 30-35 degrees and a stepped structure with an insignificant layer of loose sediments: rock fragments, pebbles, gravel, grus, and sand (Karabanov et al., 1990). L.A. Izhboldina (1970 and 2007) identified and described five vegetation belts in the open littoral zone, which were located parallel to the coast at different depths: the first *Ulotrix zonata* belt started from the water edge to a depth of 1-1.5 m; the second and third belts predominated by *Tetraspora* and *Draparnaldioides* – to depths of 2-2.5 m and 10-12 m, respectively; the fourth and fifth belts – to depths of 20-35 m and 60-78 m or more. The population of biotopes in the open littoral zone was one of the richest and the most diverse in the lake (Kozhov, 1962). In recent years, the composition of macrophytes and population of these belts have changed significantly. Since 2008, there has been a violation of vertical zonation and massive development of the Spirogyra algae that is untypical for open coasts of Lake Baikal (Kravtsova et al., 2012; 2014). Since 2011, a ubiquitous disease and death of endemic sponges have been recorded (Bormotov, 2011; Khanaev et al., 2018, etc.).

Periphyton serves as one of the important indicators of the ecological state in aquatic ecosystems (Abakumov et al., 1983). Underwater observations that were carried out from October to December 2019 indicated the continuing changes in the composition and distribution of benthic biocenoses in the open littoral zone of the lake, in particular, the periphyton. Thus, ciliates of the genus *Vorticella* have become the main component of abundant fouling of macrophytes. Despite the rich fauna of ciliates (93 species) inhabiting microphytes in the open littoral zone (Gajewskaja, 1933), such a massive development of ciliates has not been previously observed in open Baikal.

Aquatic protozoa are the important intermediate in the transfer of organic matter from the nanoplankton level to higher trophic chains of the water body. In this regard, this abnormal outbreak of the ciliate population may be a signal of significant changes in the picoplankton community of Southern Baikal.

2. Materials and methods

To assess the current state of bottom biocenoses in the open littoral zone of Lake Baikal, underwater observations were carried out at several stations along the west coast of the southern basin of the lake (Fig. 1) from 25 October 2019 to 2 January 2020.

Below, there are the areas and dates of the fieldworks:

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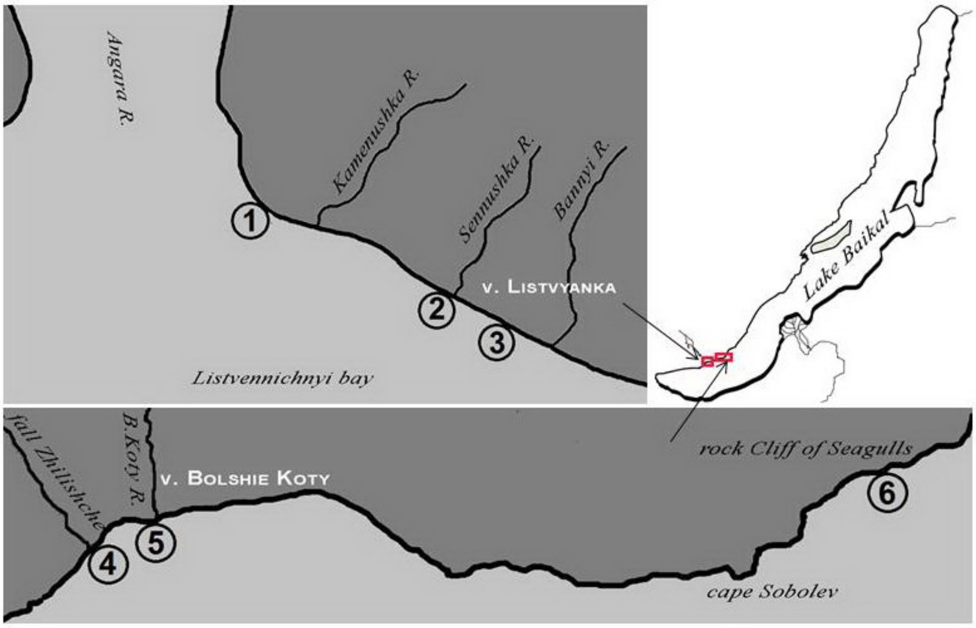

Fig.1. Schematic map of sites of underwater observations and sampling; figures in circles are numbers of stations (sites).

- the Listvyanka settlement, the Angara River source (opposite Baikal Museum), Site 1, the water edge coordinates: N 51°52´03,7´´; E 104°49´52,4´´, dates: 25 and 29 October 2019, and 2 January 2020;
- the Listvyanka settlement (opposite Sennushka Valley), Site 2, the water edge coordinates: N 51°51´51,7´´; E 104°50´37,9´´, dates: 28 October, 1 and 10 November 2019;
- the Listvyanka settlement (between the Sennushka and Banovka valleys), Site 3, the water edge coordinates: N 51°51´46,2´´; E 104°50´51,2´´, 10 November 2019;
- Zhilische Valley (opposite the research station of Limnological Institute SB RAS), Site 4, the water edge coordinates: N 51°53´59,5´´; E 105°03´50,1´´, 4 December 2019;
- the Bolshiye Koty settlement (opposite the Bolshaya Kotinka River), Site 5, the water edge coordinates: N 51°54´11,1´´; E 105°04´19,8´´, 12 November 2019;
- Chyortov Most Rock or Chayachiy Cliff (coast of open Baikal between the Sobolev and Kadilny capes), Site 6, the water edge coordinates N 51°53´54,8´´; E 105°07´50,2´´, 12 November 2019.

The fieldworks were carried out using the autonomous scuba diving equipment. Underwater photo and video recordings were carried out with a Sony A7 photo camera and a GoPro HERO 3+ camera. Samples for quantitative and qualitative analysis were taken from different depths and substrates using a scraper and dive knife. The samples were placed in individual vials and plastic hermetic boxes.

The counting and detection of ciliates were carried out with living material under a LOMO microscope at a magnification of 20^X-40^X under laboratory conditions.

To determine hygiene indicator microorganisms, water samples were inoculated according to the SanPin 2.1.5.980-00 standards and the MUK 4.2.1884-04 guidelines. Total bacterial count and thermotolerant coliform bacteria (TBC and TCB), as well as enterococci, were taken into account. To determine the number of cultured heterotrophic bacteria, 0.5 and 1ml of samples were inoculated on the nutrient media of meat peptone agar (MPA), fish peptone agar/10 (FPA/10) and R2A agar as well as Hissa medium with glucose and incubated at 22°C (room temperature). The cultivation on MPA was carried out at 37°C and on R2A – at 10°C.

For microscopic examination, samples were fixed with formalin (4% final concentration). Species identification of ciliates, algae and cyanobacteria were carried out using an Axio Imager light microscope (Zeiss, Germany) equipped with an HBO 100W mercury lamp and an AxioCam camera according to the key-guides (Komárek and Anagnostidis, 1999; 2005; Pomazkina and Shcherbakova, 2010; Komárek, 2013).

During fluorescent microscopy, algae were observed under blue filter (wavelength 480 nm) by chlorophyll autofluorescence; cyanobacteria were detected by phycobilin autofluorescence under green filter (wavelength 540 nm).

3. Results and discussion

On 25 October 2019 (Listvennichny Bay, the Angara River source, the littoral zone opposite Baikal Museum, Site 1), there were small *Ulotrix zonata* colonies in the near-water edge zone, which consisted of young filaments and numerous spores of these algae. Among filaments, there were various ciliates and rotifers, except for Peritrichia, cyclops and harpacticides. At distance from the water edge, at depths of 3 m and deeper, the Peritrichia colonies occupied various substrates. On rocks, small stones, crustaceous sponges,

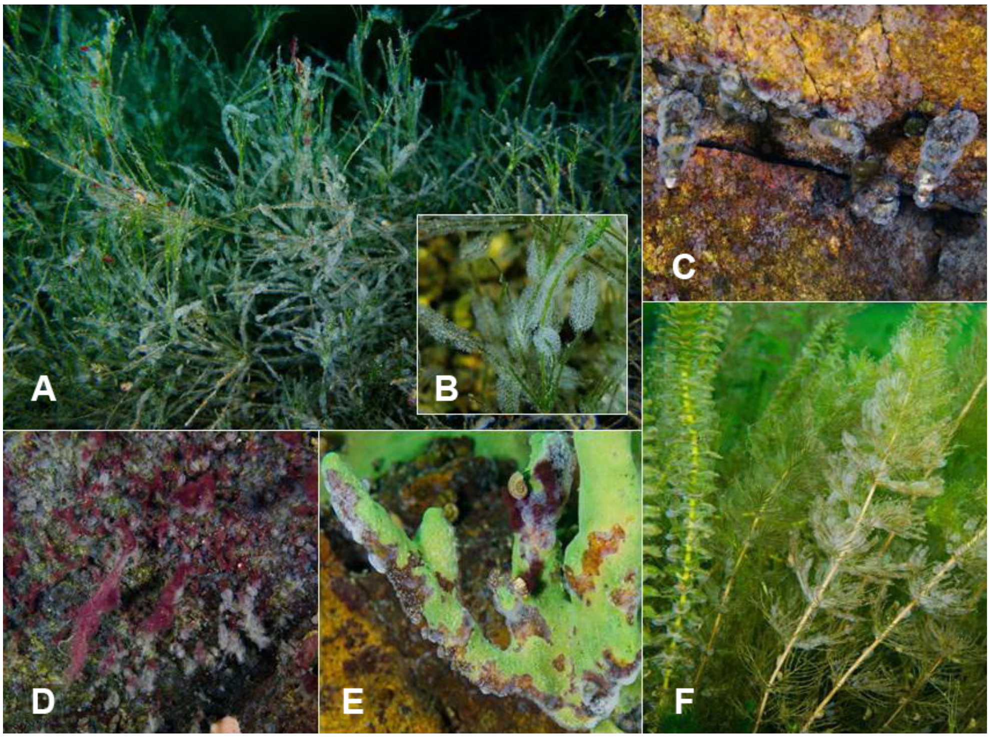

Fig.2. Colonies of *Vorticella* cf. *campanula* on different substrates: A – thickets of charophytes; B – individual enlarged colonies; C - molluscs covered with *Vorticella*; D – cyanobacteria; E – branching sponges *Lubomirskia baikalensis*; F – thickets of hornwort, *Ceratophyllum* sp., and elodea, *Elodea canadensis*.

molluscs, amphipods, etc., Peritrichia were located singly or in small groups (patches), up to 15-20 or 35-50 cells/cm^2. Macrophytes had the most abundant fouling of the *Vorticella* spp. ciliates: the *Myriophyllum* sp. parrot feather, the *Ceratophyllum* sp. hornwort and charophytes (Fig. 2). The poor presence of the *Vorticella* spp. ciliates in the 0-3m zone can be due to intense hydrological impact from surf wave activity.

The tips of hornwort's branches had the fouling by diatoms of the genera *Cymbella*, *Gomphonema*, *Nitzchia*, and *Cocconeis*. These algae are found together with Peritrichia on pondweed leaves and damaged crustaceous sponges, and small *Cocconeis* densely cover the surface of dying charophytes. *Vorticella* cf. *campanula* dominated on all studied macrophytes (approximately 90%). Its number was from 3-4 x 10^3 cells/cm^2 on pondweed leaves to 7-8.5 x 10^3 cells/cm^2 on hornwort. In contrast, the number of *Vorticella* cf. *campanula* in the experiment and natural conditions of European freshwater bodies was from 4 to 400 cells/cm^2 (Struder-Kypke and Schonborn, 1999; Risse-Buhl and Küsel, 2009). The same situation was observed at Site 2: the Peritrichia colonies occupied various types of substrates, except for the spirogyra fields, which together with *Ulotrix* dominated this area at depths from 3.5 to 15 m.

In three areas of the littoral zone near the Bolshiye Koty settlement (Sites 4, 5 and 6), there were also the *Vorticella* spp. colonies but not in such a scale and abundance as in Listvennichny Bay. Nevertheless, at the site between the estuary of the Kotinka River and the central pier of the Bolshiye Koty settlement, the number of *Vorticella* spp. on the branches of charophytes reached 10 x 10^3 cells/cm^2.

Based on visual estimates, after a week and a half (7 and 10 November 2019), in the littoral zone of Listvennichny Bay (Sites 2 and 3), the total number of the Peritrichia colonies increased at least twofold. The colonies were found everywhere in the depth range from 3.5 to 40 m. The water temperature at that time was 4-5°C, depending on the depth.

On 2 January 2020, at the ice-free site of the lake, near the Angara River source, (Site 1), with the water temperature of 1-2°C, the situation with the *Vorticella* spp. colonies on macrophytes did not change: their abundance remained very high.

Vorticella campanula is a eurythermal species widespread in freshwater bodies. It is an epibiont that prefers to live on algae and aquatic plants but can also inhabit other substrates, including invertebrates (Banina, 1984).

V. campanula is a *β*-mesosaprob, being an indicator of clean or slightly polluted waters with a good oxygen regime. Under favourable conditions, particularly in eutrophic ponds, it can form abundant massive fouling on algae (Banina, 1984; Foissner et al., 1992; Jiang and Shen, 2005).

According to N.S. Gajewskaja (Gajewskaja, 1933), in the 1920s, this species was found in Lake Baikal at temperatures from 3 to 12°C, mainly on the *Ulothrix, Tetraspora* and *Draparnaldioides* algae (i.e. on macrophytes of the first three vegetation belts in the littoral zone) and much less often on gammarids. It was not in the dominant complex at that time. Few available fragmentary data indicate that this species is a permanent component of the Baikal periphyton. Abundant *Vorticella* spp. fouling of the coastal stones was observed in the littoral zone between the Bolshiye

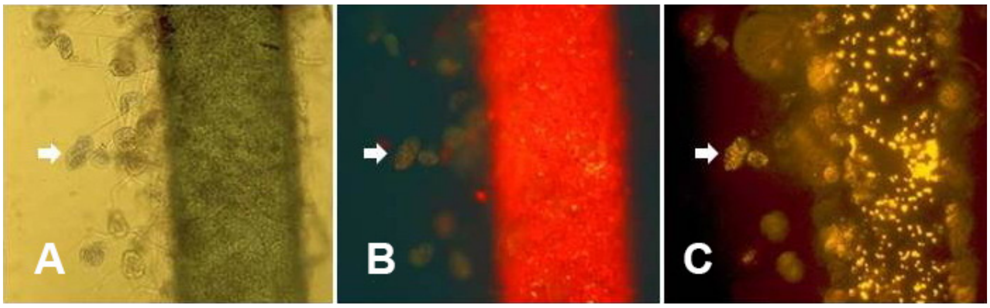

Fig.3. Ciliates of the genus *Vorticella* and diatoms of the genus *Cocconeis* on the surface of charophytes. A – light microscopy, B – epifluorescence microscopy, chlorophyll autofluorescence, C – epifluorescence microscopy, cyanobacterial phycobilin autofluorescence. Arrows indicate ciliates containing picocyanobacteria inside the body.

Koty settlement and Chyornaya Valley during the survey in September 1988. In March 2013, *Vorticella campanula* comprised the fouling of stones in the near-water edge littoral zone close to Site 1 (the Angara River source).

Bacteria is the main food for *Vorticella campanula*, but it can also consume small algae and detritus (Struder-Kypke and Schonborn, 1999; Risse-Buhl and Küsel, 2009).

To confirm this, during the examination of the preparations using epifluorescence microscopy (wavelength 540 nm), we detected numerous picocyanobacteria in the bodies of ciliates (Fig. 3), which most likely served as food for them. Based on shape, dimensions (diameter less than 3 μm) and division type, we determined these picocyanobacteria as species of the genera *Synechococcus* C. Nägeli and *Cyanobium* R. Rippka & G. Cohen-Bazire (Synechococcales). Due to the difficulty in their identification at the microscopy level, the members of these genera are often combined into the joint *Synechococcus/Cyanobium* cluster. *Synechococcus/Cyanobium* dominate in abundance among phototrophic microorganisms in the water column of Lake Baikal in all seasons of the year, being the main component of autotrophic picoplankton (up to 97%) (Belykh and Sorokovikova, 2003; Belykh et al., 2006).

In biofilms formed on macrophytes, we detected filamentous and coccoid morphotypes of cyanobacteria belonging to the orders Synechococcales и Chroococcales. Among them, members of the planktonic cluster *Synechococcus/Cyanobium* prevailed. Other species of cyanobacteria are also often found: *Chamaesiphon fuscus* (Rostafinski), *Leptolyngbya margaritata* (Kufferath), *Pseudanabaena galeata* Böcher (Synechococcales) and *Aphanocapsa parasitica* (Kützing) (Chroococcales).

The intake rate of bacteria by a single *Vorticella* spp. is 38-1078 bacteria/hour (Königs and Cleven, 2007), thereby the estimated number of bacteria that are the food base for the detected number of ciliates (3-10 x 10^3 cells/cm^2) ranges from 0.11 to 10.78 x 10^6 cells/ml. At the same time, it should be taken into account that ciliates are not the only consumers of bacteria. Hence, their real number should be significantly higher.

High total abundance of picocyanobacteria and heterotrophic bacterioplankton defined as TBC is typical of Lake Baikal in the autumn, especially in September and October (Belykh and Sorokovikova, 2003; Belykh et al., 2006; Maksimov and Schetinina, 2009). Thus, in September 2019, TBC in the pelagic zone, 2 km from the Listvyanka settlement, was 2.4 million cells/ml in the 0-5 m layer; picocyanobacteria – 221 thou. cells/ml. Regular observations of the seasonal and interannual dynamics of the picocyanobacteria abundance revealed their maximum concentration in August, for example, in the pelagic zone of Southern Baikal, up to 3 million cells/ml, as well as the minimum concentration in February and December, up to 8-14 thou. cells/ml (Belykh and Sorokovikova, 2003; Belykh et al., 2007). In the autumn, the abundance of picocyanobacteria decreases. The abundance of picocyanobacteria in the upper 50-m layer was 223 thou. cells/ml in September and 179 thou. cells/ml in October, but in November, during autumn homothermy, it was 49 thou. cells/ml (Belykh et al., 2006).

Therefore, the estimated abundance of bacterioplankton, which explains the number of the detected ciliates, several times exceeded that typical of Lake Baikal.

Unlike other attached and free-living forms of ciliates, *Vorticella campanula* has a positive significant correlation with the water temperature, chemical oxygen demand (COD), ammoniacal nitrogen and total phosphorus (Li et al., 2017). Due to the high anthropogenic pressure and the absence of treatment facilities in the Listvyanka and Bolshiye Koty settlements, there are signs of eutrophication and changes in underwater landscapes associated with the areas of these settlements (Kravtsova et al., 2012; Timoshkin et al., 2012).

The number of organotrophic (saprophytic bacteria) microorganisms (the FPA/10 medium) that serve as indicators of the presence of readily biodegradable organic matter was high both in the water (1296 CFU/ml) and scrapings (1360 CFU/ml) (Fig. 4). The identified indicators significantly exceeded the values previously obtained for Lake Baikal. Based on the systematic observations, during 1996, the number of saprophytic bacteria from the 0-10 m layer of the pelagic zone varied from a minimum of 15 ± 2 CFU/ml in February to a maximum of 350 ± 38 in August; in September, October and November, it decreased to 157 ± 17 CFU/ml, 69 ± 8 CFU/ml and 29 ± 3 CFU/ml,

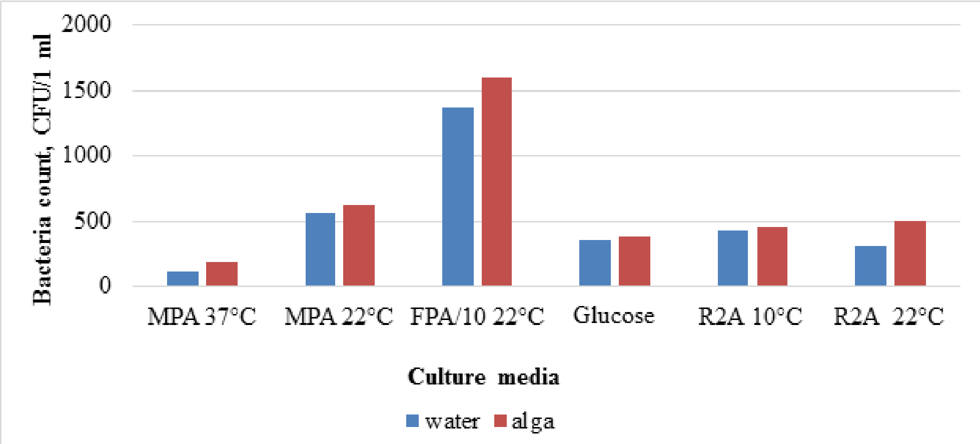

Fig.4. The number of bacteria isolated from water and scrapings from macrophytes during cultivation on different media at different temperatures.

respectively (Schetinina, 2003). Relatively recently, in October-November 2006-2008, the number of saprophytic bacteria was 352 CFU/ml (Parfenova et al., 2009). At the same time, in the analysed water samples and scrapings from macrophytes, we have not detected hygiene indicator microorganisms (TBC, TCB and enterococci), which evidences the absence of faecal pollution and indicates another source of dissolved organic matter.

In general, we can assume that the detected mass development of ciliates was due to the intensive development of bacterioplankton resulted from an increased influx of nutrients caused by the destruction of an abnormally large amount of dead filamentous algae and cyanobacterial mats developing in this zone of Lake Baikal.

4. Conclusion

From the second half of October 2019 to January 2020, we recorded an abnormally high abundance of the *Vorticella* cf. *campanula* ciliates in the littoral zone of Lake Baikal adjacent to the Listvyanka and Bolshiye Koty settlements. Taking into account the changes in the nutrition of ciliates, we can assume that an increase in the number of bacterioplankton, as the main source of their food, has resulted from an increase in nutrients caused by the destruction of organic matter of filamentous algae and cyanobacteria that abundantly develop in these areas during summer. This, in turn, is associated with increasing anthropogenic pressure on these sites of the littoral zone of Lake Baikal.

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