Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 32, issue 4, 2022, pp. 162 - 174

TROPHIC RELATIONSHIPS BETWEEN PICOPLANKTON AND ZOOPLANKTON IN LAKE BALATON AND THE SALT LAKES OF KISKUNSÁG

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Abstract: From research on phytoplankton over the last 30 years, it has been shown that phytoplankton of pico size (smaller than 2 µm), from an ecological point of view, are of great importance in both ocean and continental water ecosystems. Studies in this field have shown that heterotrophic nanoflagellates are very important consumers of picoplankton, thus these small autotrophic organisms play a significant role in the so-called microbial loop. The saline lakes of Kiskunság are rich in picophytoplankton and have a high content of suspended inorganic solids. In our experiments, water samples were incubated following exposure to treatments, then quantitative variations in picoplankton abundance were determined. The research results show that the grazing rate of picophytoplankton was lower in Lake Balaton than in saline lakes. The dominance of nanoflagellates in the grazing processes shows a dynamic variation, in some cases, the role of zooplankton being more important.

Keywords: picocyanobacteria, grazing, nanoflagellates, Balaton, saline lakes.

INTRODUCTION

The smallest phytoplankton cell group is the picophytoplankton. These tiny organisms were discovered in the 1960s by Holmes Anderson (1963) and Bailey-Watts et al (1968), and ongoing research to date has clearly shown that picoplankton play an important role in aquatic ecosystems and in global primary production (Craig, 1985; Stockner and Antia, 1986; Stockner, 1988). They contribute 50-70% of annual carbon sequestration in oligotrophic lakes (Caron et al., 1985; Munawar and Fahnenstiel, 1982).

The microbial loop involving picophytoplankton (Azam et al., 1983) plays an important role in the incorporation of dissolved organic carbon into the nutrient chain, because autotrophic picoplankton, in addition to bacteria, also provide a food base for nanoflagellates, which are important in the microbial food web. Thus, in addition to their role in the cycling of organic matter, nanoflagellates are also important regulators of picoplankton abundance. The feeding of nanoflagellates is known in the international literature as "grazing" and has been repeatedly studied in both marine and lacustrine environments.

In Hungary, the study of picoplankton in Lake Balaton and other stagnant waters began in the 1980's, when it became possible to use epifluorescence microscopy (Vörös, 1987, 1989). In Lake Balaton, picocyanobacteria and picoeukaryotes account for a significant proportion of the primary production, but there is no known evidence of consumer pressure of nanoflagellates on picophytoplankton.

Phytoplankton is the collective name for communities of photoautotrophic microorganisms floating in open waters - prokaryotic cyanobacteria and eukaryotic algae - and is one of the most important primary producers of photosynthesis in both marine and freshwater environments (Reynolds, 2006). Algae are photosynthetic organisms with diverse structures, ranging from unicellular to multicellular. Multicellular algae are characterized by filamentous structures, colonies, plates, or tubes. There are also more complex forms with roots, stems, and leaves, although they do not have true roots, leaves, or tissues. Most are aquatic, but some species are found in soil, rocky surfaces, deserts, and extreme habitats. Some are not photosynthetic, but are closely related to photosynthetic species (Kiss, 1998). It is important to note that only some of the aquatic algae are planktonic, many species are found on the surface of sediments (phytobenthos) or on plants and rocks (periphyton).

In the last few years, important results have been published on the unique autotrophic picoplankton communities in saline lakes in Kiskunság (between the Danube and Tisza rivers). Vörös et al (2005) found that the phytoplankton biomass in three saline lakes near the Danube-Tisza River was dominated by picoplankton from spring to fall. During the summer, the abundance of picocyanobacteria exceeded 108 cells ml^{-1}, the highest value reported in the literature. In spring, on the other hand, picoeukkaryotic algae were dominant (Vörös et al., 2008). In addition to these observations, genetic studies by Felföldi et al. (2008) revealed 10 different genotypes of picoalgae from six saline lakes, including several previously unidentified groups.

Nanoflagellates are found in aquatic environments almost everywhere on Earth, from the equator to the poles. They exhibit a very high nutritional diversity, ranging from heterotrophic taxa, which mainly consume bacteria, picocyanobacteria, and pico-algae, to mixotrophic taxa, capable of both heterotrophic and autotrophic feeding (Laybourn-Parry and Parry, 2000). They also form a morphologically diverse group. Their sizes range from 2 to 20 µm.

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The study and mapping of flagellates is still ongoing, and our knowledge of their ecological and physiological properties is continually expanding. Their interactions with bacteria and protozoan groups appear to be clarified, as well as their role in the dissolved organic carbon (DOC) cycle. Their role is particularly important in the transfer of bacterial production to higher trophic levels (Fenchel, 1982; Azam et al., 1983; Berninger et al., 1991).

Quantification of heterotrophic nanoflagellates is a difficult task, as their capture requires care. Their determination is performed by flow cytometry and epifluorescence microscopy (Bloem et al., 1986). Their fragility and loss of fluorescence make their capture and enumeration problematic (Sorokin, 1981; Sherr, 1984).

The degree of grazing of heterotrophic nanoflagellates is being monitored and tracked by various experiments. Adamczewski et al (2010) conducted experiments with dialysis bags in a freshwater lake in Poland, Lake Mikołajski. To determine the extent of swimming, three techniques were used in the experiments: size separation, dilution, and treatment with eukaryotic inhibitors.

On the basis of their results, it was concluded that the blunting effect was visible in the 10 µm mesh size filtered samples, which contained more flexible and smaller nanoflagellates (Wright and Coffin, 1984; Rassoulzadegan and Sheldon, 1986; Kuuppo-Leinikki and Kuosa, 1990). In the samples treated with eukaryotic inhibitors, a mixture of cycloheximide and colchicine acted on the flagella. When larger flagella were filtered out of the sample, bacterial numbers increased and grazing was not strong, whereas when larger flagella were kept in the sample, bacterial numbers decreased and grazing was strong.

In the Carpathian Basin, there is a great chemical diversity of lakes, with a remarkable variety of saline lakes, including soda lakes and NaCl-dominated brackish lakes. The extreme conditions (high turbidity and high salinity) present in these aquatic habitats lead to the predominance of picophytoplankton (Somogyi et al., 2022).

MATERIAL AND METHOD

Sampling sites

Lake Balaton

Lake Balaton is the largest shallow lake in Central Europe. It covers an area of 596 km^2, has an average depth of 3.3 m, its deepest point is 11 m, and it is about 80 km long. It is bordered by the Balaton Mountains in the north, the Zala and Somogy hills in the west and south, and the Mezőföld in the east. The water supply is provided by the Zala River via Kis-Balaton. The main ions present are $Ca^{(2+)}$, $Mg^{(2+)}$ and $HCO3^-$. Conductivity (60-70 mS m^{-1}) and pH (8.3-8.6) are relatively high and constant. The western half of the lake is richer in inorganic nutrients, with eutrophic water and chlorophyll concentrations of 30-60 µg l^{-1}, whereas the Siófok basin in the eastern half is mesotrophic, with chlorophyll concentrations of 10-20 µg l^{-1}. In winter, the lake freezes for 30-60 days. Water samples were taken from the Siófok basin (46°55'19.0"N, 17°55'53.6"E) in July-August 2013.

Kiskunság salt lakes

The common characteristics of the studied lakes are high salinity, dominated by Na^+ and $HCO3^-$ ions, and alkaline chemistry with pH values between 9-10 (Vörös et al., 2005). For our experiments, water samples were taken from two lakes designated as white-water lakes with high inorganic suspended solids content, lakes Böddi and Zab, and from the brown-water, humic-rich Sós-ér, in July-September 2013.

Sampling

Water samples were taken on the eastern side of Lake Balaton at Tihany, about 400-500 m from the shore. A 6 cm diameter column sampler was used for sampling. The sampling tube was submerged to the bottom, the tap at the bottom of the tube was closed, and the water thus extracted was poured into 1.5 liter sampling containers so that a representative sample could be collected from the entire water column. The sampling tube was emptied repeatedly to obtain a sufficient volume of water for testing. For quantification of bacteria and heterotrophic nanoflagellates, 20 mL per sample was preserved by adding 1 mL of formalin and stored in the refrigerator until microscopic examination could begin.

Brackish lakes were sampled by bucket from the middle of the lake due to their shallow depth. Samples were transported in a cooler bag to the laboratory, where picophytoplankton abundance was determined.

The following experimental treatments were then developed

1. water filtered with a plankton net with a mesh size of 26 µm, in which large zooplankton were targeted to filter out, leaving only heterotrophic nanoflagellates as picoplankton consumers in the sample;
2. water treated with a eukaryotic inhibitor (100 mg/l cycloheximide + 100 mg/l colchicine) was used as a control
3. and a sample of untreated water.

Three replicates of each treatment were performed. Samples were poured into 250 ml Erlenmeyer flasks, 100 ml in each flask. The flasks were sealed with cotton stoppers and covered with aluminum foil to keep them dark. The samples were placed on a shaker (60 rpm) and incubated for 12 h, and the abundance of autotrophic picoplankton was determined at the beginning and end of the experiment.

Microscopic studies

Determination of picophytoplankton abundance

The abundance of picophytoplankton was determined mainly by flow cytometry, but in several cases, cell counts were also performed by epifluorescence microscopy to verify the quantitative data obtained. Depending on the amount of picoplankton, between 0.1 and 5 ml of our samples were filtered. Filtration was performed on a black polycarbonate filter with a pore size of 0.4 µm. The filters were placed on slides embedded in 50% glycerol, and the resulting samples were examined using a Nikon Optiphot 2 epifluorescence microscope

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equipped with a SPOT RT camera. At 1000x magnification, the sample was excited with blue-violet (BV-2A) and green (G-2A) light. Picoeukaryotes exhibited intense red autofluorescence under blue-violet light, whereas their autofluorescence weakened when switched to green light, emitting a bright red due to their chlorophyll-a content. Phycocyanin-pigment-dominant picocyanobacteria exhibit red fluorescence under blue-violet excitation, whereas phycocyanin-dominant picocyanobacteria exhibit yellow autofluorescence. When illuminated with green light, both phycocyanin-dominant and phycocerythrin-dominant picocyanobacteria emit a bright red due to phycobiliproteins, which makes them clearly distinguishable from eukaryotic cells (Figure 1). For each sample, 20 images were taken: 10 randomly selected fields of view were photographed first under blue-violet light and then under green excitation light. Cell autofluorescence was then used to determine the abundance of picoeukaryotes and picocyanobacteria in each image, and their abundance per unit volume was estimated by counting back to the filtered volume (MacIsaac and Stockner, 1993).

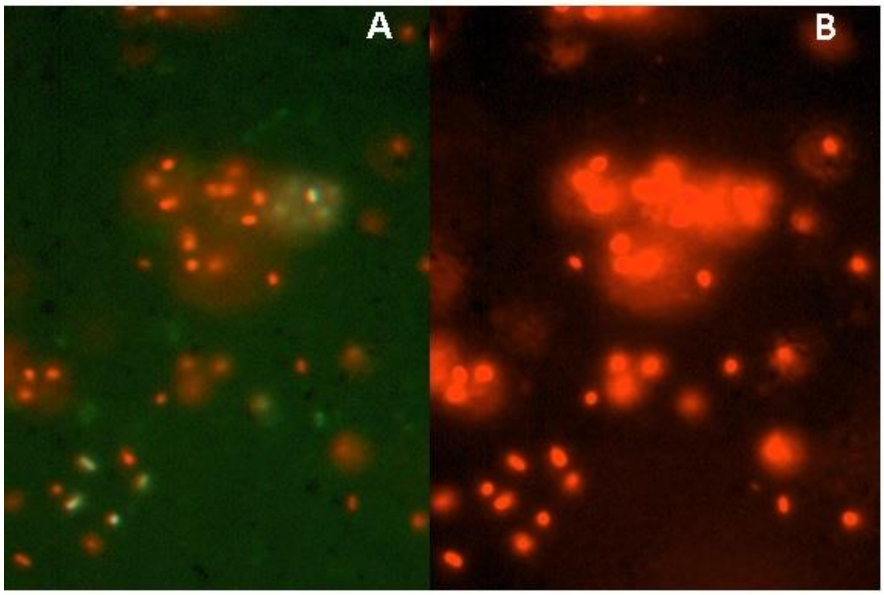

Fig. 1. Epifluorescence microscopy of picocyanobacteria with a predominance of picocianin pigment in a water sample excited by bluish violet (A) and green (B) light. Average cell size 1-2 µm.

Investigation of heterotrophic nanoflagellates

Nanoflagellates were quantified from formalin-fixed samples. Depending on the concentration, 20 µl of proflavin, a fluorescent dye that binds to DNA, was added to 2-5 ml per milliliter of samples, depending on the concentration, left to stand for five minutes, and filtered through a black Nucleopore polycarbonate filter with a pore size of 0.8 µm. The polycarbonate filter was mounted on a slide soaked in immersion oil. The slide was placed under a Nikon Optiphot 2 epifluorescence microscope, and the nanoflagellates were counted in at least 100 fields of view per sample under blue light excitation at 1000x magnification, whose cells emitted green light due to the bound proflavin (Bloem et al., 1986).

Determination of bacterial abundance

From 100-1000 µl of formalin-preserved samples, 20 µl of orange acridine fluorescent dye, which binds to DNA, was added per milliliter to 100-1000 µl and filtered through a black polycarbonate Millipore filter with a pore size of 0.2 µm after five minutes of incubation in the dark. Milli-Q water was added to 1 ml of the sample to make up to 1 ml. The filter was placed on a slide embedded in immersion oil. Bacterial cells were counted using an Optiphot 2 epifluorescence microscope at 1000x magnification. The bacteria exhibit green or orange autofluorescence under blue excitation light. The abundance of bacterioplankton in cells/ml was determined by calculating the average number of cells counted in the 10 images of the sample and applying it to the volume of filtered water. Since the fluorescence of picocianobacteria and picoeukaryotes is also visible in the images, the abundance of picofitoplankton was subtracted from the resulting value (Hobbie et al., 1977).

Flow cytometry

Basic principle

The principle of flow cytometry is that, by introducing the sample to be analyzed into a liquid stream, the cells in the sample are detected one by one, and the optical parameters of each cell are recorded. The cells pass through a beam of excitation light (laser or LED), which scatters part of the light and absorbs the rest. If the cell also contains compounds with fluorescent properties (photosynthetic pigments or fluorescent dyes bound to various cellular components), part of the absorbed light energy is emitted as fluorescence. Both the light scattered by the cells and the light emitted as fluorescence can be detected by the optical channels of the flow cytometer.

The flow cytometer consists of three main systems: a flow system, an optical system, and an electronic system.

The flow system sends the particles toward the light beam. The optical system uses lasers to illuminate the passing particles, and optical filters transmit the resulting light signals to the appropriate detector. The electronic system converts the light signals into electrical signals using a photoelectron multiplier, and the generated signals are processed by a computer.

Testing samples from Lake Balaton and salt lakes

Our measurements were performed using a Partec CyFlow Space Sorter cytometer. The instrument is equipped with two excitation light sources: a 488 nm blue laser with three optical channels (detectors): FL1 536 nm (40 nm bandwidth) - green, FL2 575 nm (26 nm bandwidth) - orange, FL3 675 nm (20 nm bandwidth) - red; and a 638 nm red laser with two optical channels: FL4 675 nm (20 nm bandwidth) - red and far red.

Fluorescence detection is performed through these five channels. In addition, the cytometer has two additional channels: forward scatter (FSC), which detects the intensity of forward scattered light, and side scatter (SSC), which detects side scattered light.

Our samples were tested at a flow rate of 1 µl/s. As sheath fluid, we used Partec Sheath Fluid, a fluid distributed by the manufacturer. Different dominant pigment groups of autotrophic picoplankton were isolated based on Marie et al. (2005). Based on the optical parameters of the cells detected on the FL3 and FL4 channels, the cyanobacteria phycocyanin and phycocerythrin, and piconeucaryotes form well-differentiated groups (point clouds) on an FL3-FL4 dot plot. For saline lakes, due to the high content of suspended inorganic solids and the abundance of picoplankton, the samples were diluted before being loaded into the cytometer. Without dilution, the cells and suspended matter in the concentrated sample may overlap, leading to measurement errors.

The dye used was the green fluorescent nucleic acid dye SYTO9. It binds to nucleic acid and enhances the fluorescence of cells. The dye appears as a stain in live and dead Gram-negative and Gram-positive bacteria. Some important properties of the dye are:

- it is permeable to any cell membrane, including mammalian and bacterial cells;
- high molar absorption, extinction coefficient >50,000 cm^{-1} M^{-1} with a visible absorption maximum;
- extremely low intrinsic fluorescence.

The effect of zooplankton was investigated by modifying the abundance of picoplankton in water samples incubated in different ways. The reproduction rate of picoplankton in the control samples and the grazing rate in the unfiltered and untreated samples were determined:

$$\mu = \frac{lnC(t_2) - lnC(t_1)}{t_2 - t_1}$$

$$g = \frac{lnC(t_1) - lnC(t_2)}{t_2 - t_1}$$

where: µ: reproduction rate (d^{-1})

g: bleaching rate (d^{-1})

C(t): picoplankton abundance at the start of incubation at time t1 and at the end of incubation at time t2 (cells/ml)

The zooplankton grazing rate was obtained by comparing these two measurements. If an increase in abundance was observed in the control samples, the corresponding reproduction rate was added to the grazing rate obtained for the filtered/unfiltered samples. If a decrease was also observed in the control, the grazing rate was reduced by the value of the reproduction rate (hence the negative sign).

RESULTS AND DISCUSSION

A comparison of the abundance values of picocianobacteria determined from samples analyzed by flow cytometry and epifluorescence microscopy shows that the data obtained by the two methods are closely correlated (Figure 2). The Pearson correlation coefficient was 0.952 ($p < 0.0001$) for the salt lakes (Böddi and Zab) and 0.788 ($p < 0.0001$) for Lake Balaton. The relatively lower value obtained for Lake Balaton is due to the greater variance in the number of cells determined by epifluorescence microscopy. This is mainly because the lower absolute and relative abundance of picocianobacteria in Lake Balaton means that the number of cells observed in a microscopic image can vary significantly from one field of view to another. In contrast, the variance of the values obtained with the flow cytometer was lower within a treatment, so this technique provided greater precision for detecting differences between treatments.

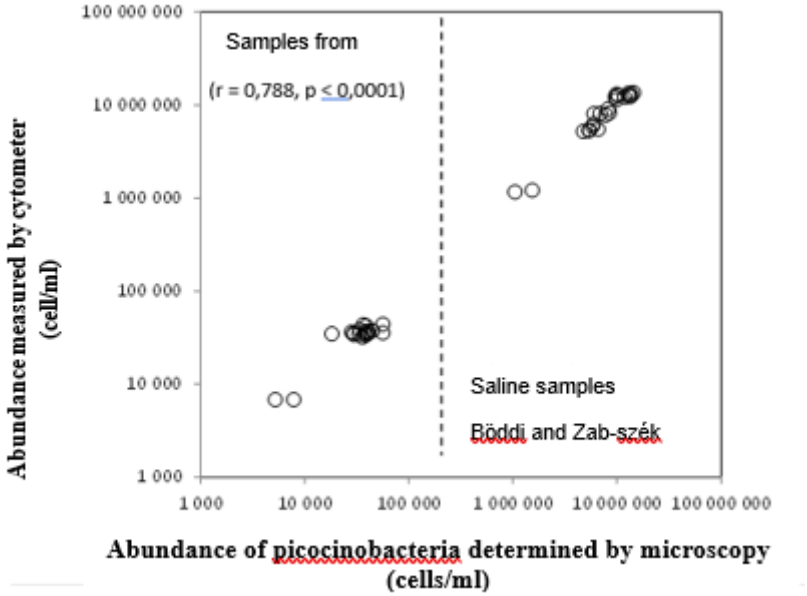

Fig. 2. Effect of zooplankton grazing on autotrophic picoplankton.

The autotrophic picoplankton in Lake Balaton is dominated by picocianobacteria during the summer (Vörös et al., 2009). This was also the case for the samples collected for our experiments. On the dotpots of the blue laser red detector (FL3) and the red laser red detector (FL4), picocianobacteria dominated by the pigments phycocyanin and phycoerythrin, and large eukaryotic algae formed well-defined groups. Cyanobacteria with phycocyanin, due to their pigment composition, exhibit stronger fluorescence under red laser excitation (FL4 channel) compared to blue excitation (FL3). Thus, their group is always in the upper right half of the FL3-FL4 diagram compared to the other two groups. Epifluorescence microscopy also revealed colonies of picochlorella in the samples, which, due to their size, are probably unaffected or only negligibly affected by zooplankton grazing. Quantifying these colonies by microscopy is problematic, whereas they can be well differentiated from unicellular forms by flow cytometry and their higher FSC (forward scatter) values (Figure 3).

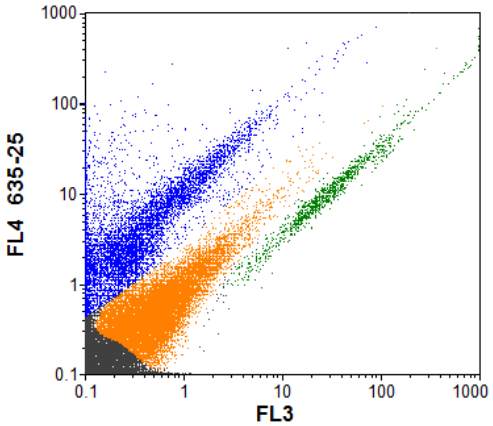

Fig. 3. Cytometric diagram of a sample from Lake Balaton (Tihany). Optical parameters of detected cells – FL3 channel: blue (488 nm) laser excitation, red detector (675 nm); FL4 channel: red (638 nm) laser excitation, red detector (675 nm). Pcy: cyanobacteria with phycocyanin pigment dominance; Pe: cyanobacteria with phycoerythrin pigment dominance; Euk: eukaryotic algae.

Among the grazing tests, the first sample (July 30) at the beginning of the experiment had an almost identical number of cells in the three treatments. After 12 hours, the untreated sample had a cell count of 17,467 cells/ml, the filtered sample had a cell count of 17,877.5 cells/ml, and the control had a cell count of 18,993 cells/ml (Figure 4).

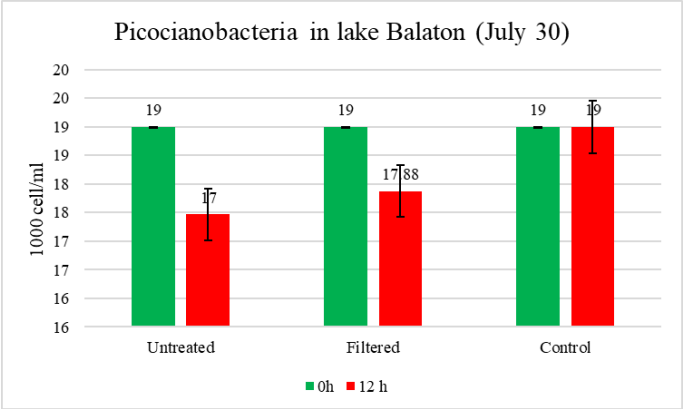

Fig. 4. Changes in the abundance of unicellular picocianobacteria (phycocytes and phycocyanins) in Lake Balaton during the incubation experiment on July 30.

The sample from August 7 yielded a much higher number of cells at the beginning of the experiment. The untreated sample yielded 87,125 cells/ml, the filtered sample yielded 85,460 cells/ml, and the control sample yielded 87,738.333 cells/ml. After 12 hours of incubation, this number changed as follows, increasing significantly in all three samples: 114,981.67 cells/ml in the untreated sample, 108,685 cells/ml in the filtered sample, and 113,696.67 cells/ml in the control sample (Figure 5).

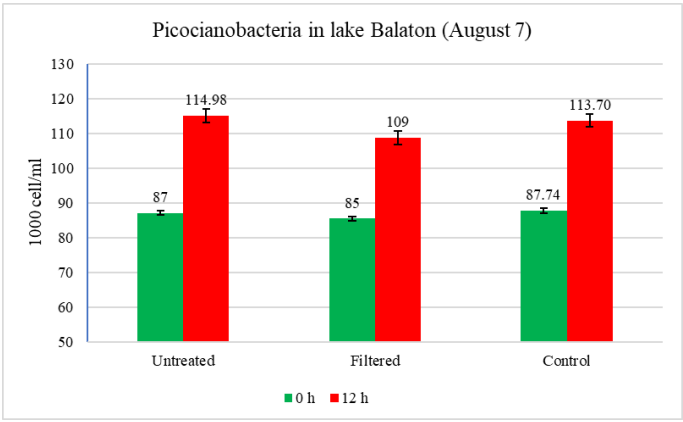

Fig. 5. Changes in the abundance of unicellular picocyanobacteria (phycocytes and phycocyanins) in Lake Balaton during the incubation experiment on August 7.

The number of cells at the start of the experiment, on August 13, was 83,313.33 cells/ml in the untreated sample, 85,825 cells/ml in the filtered sample, and 87,056.67 cells/ml in the control sample. After 12 hours, the number of cells increased significantly in the control sample to 104,530 cells/ml, 85,547.5 cells/ml in the untreated sample, and 86,187.5 cells/ml in the filtered sample. The zooplankton grazing effect was most pronounced at this point, with an increase in pico cyanobacteria in the control samples treated with eukaryotic inhibitors, while no significant change was observed in the untreated and filtered samples. Similar bleaching rates obtained for untreated and filtered samples suggest that the regulation of picocyanobacteria was dominated by heterotrophic nanoflagellates, with abundances exceeding those obtained in the two previous experiments (Figure 6).

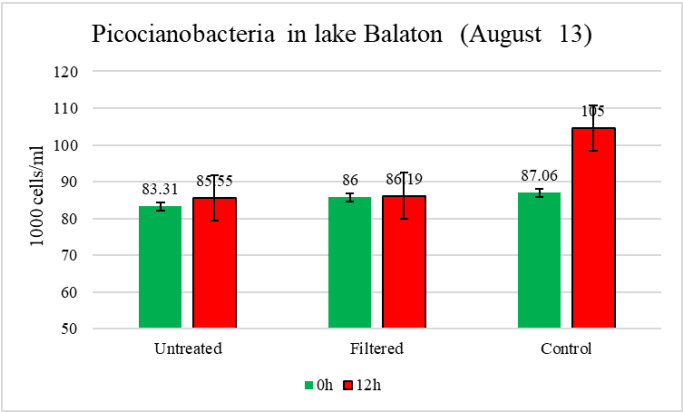

Fig. 6. Changes in the abundance of unicellular picocianobacteria (phycocytes and phycocyanins) in Lake Balaton during the incubation experiment on August 13.

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Cytometric and microscopic results showed that in the summer of 2013, cyanobacteria dominated by phycocyanin pigment were present in Zab-szék, in addition to the typical cyanobacteria dominated by phycocyanin pigment (Figure 7). This phytoplankton composition is not typical for this time of year, as summer phytoplankton in our saline lakes is usually characterized by an absolute dominance of pico-cyanobacteria (Somogyi et al., 2009). On July 24, the abundance of pico-cyanobacteria was relatively low, around 55,000 cells/ml, but at the time of the August experiments, it had already exceeded 8,000,000 cells/ml. This abundance remained of the same order of magnitude until the September sampling (5,000,000 cells/ml). Since colchicine and cycloheximide, used in our experiments, inhibit the function of eukaryotic organisms, their effect extends to picoeukaryotes in the control treatment, and therefore, grazing pressure on picoeukaryotes cannot be investigated by this method. Given this, only changes in the abundance of pico cyanobacteria were investigated in our incubation experiments.

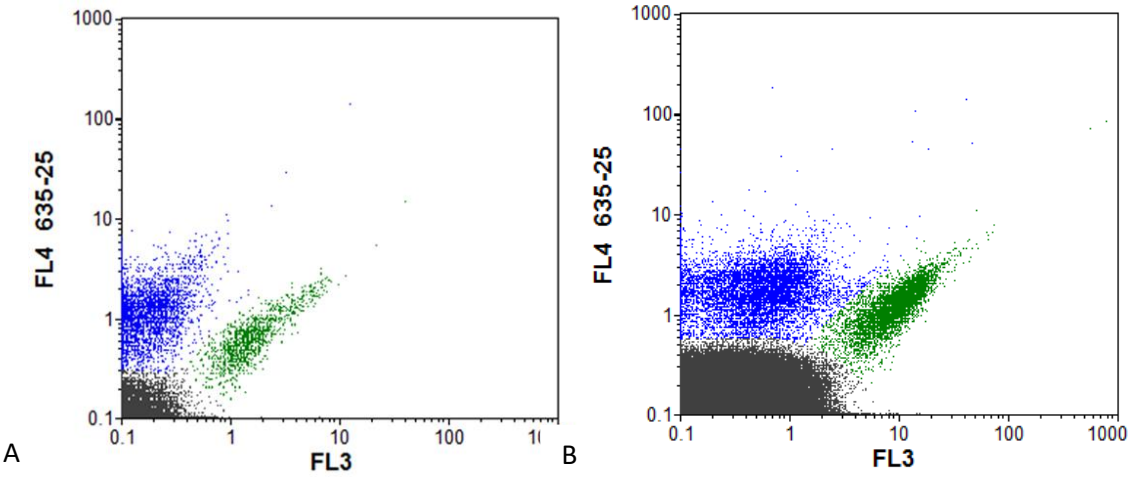

Fig. 7. A, Cytometric dot plot of the sample collected on August 15, 2013, from Zab-szék (250x dilution). Optical parameters of detected cells - FL3 channel: blue (488 nm) laser excitation, red detector (675 nm); FL4 channel: red (638 nm) laser excitation, red detector (675 nm). Pcy: cyanobacteria dominated by picocianin pigment; PEuk: picoeukaryotes.
B. Cytometric dot plot of the sample collected on September 24, 2013, from Zab-szék (200x dilution). Optical parameters of detected cells - FL3 channel: blue (488 nm) laser excitation, red detector (675 nm); FL4 channel: red (638 nm) laser excitation, red detector (675 nm). Pcy: cyanobacteria dominated by picocianin pigment; PEuk: picoeukaryotes.

The phytoplankton composition in Böddi-szék was similar to that in Zab-szék, with picoeukaryotic algae present alongside phycocyanic cyanobacteria (Figure 8). Thanks to control treatments with eukaryotic inhibitors, we also monitored the change in the abundance of pico cyanobacteria in this lake, which went from 160,000 cells/ml in July to approximately 13,000,000 cells/ml in August.

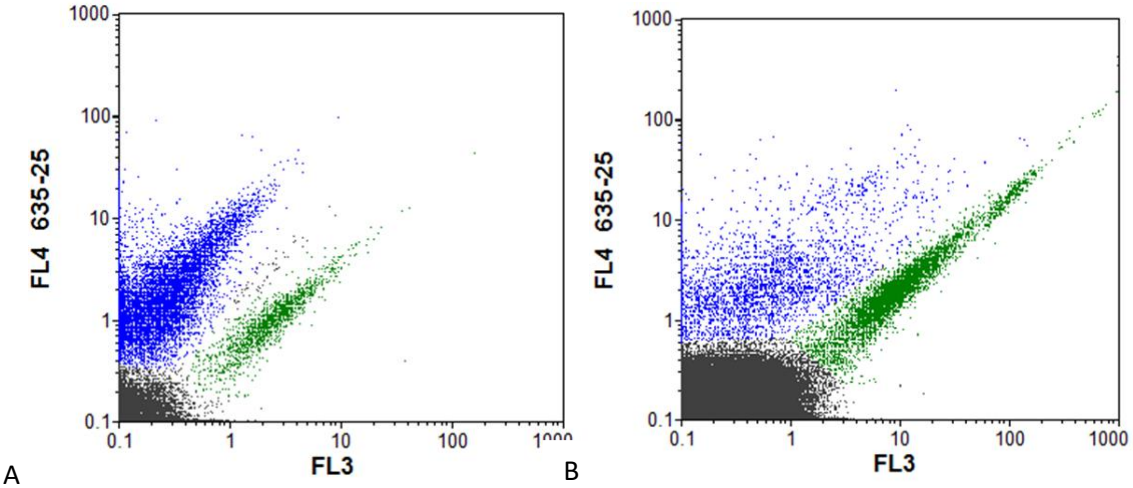

Fig. 8. A. Cytometric dot plot of the sample collected on August 15, 2013, in Böddi-szék (200x dilution). Parameters of detected cells - FL3 channel: red detector of a blue excitation laser (488 nm) (675 nm); FL4 channel: red detector of a red excitation laser (638 nm) (675 nm). Pcy: cyanobacteria dominated by picocianin pigment; PEuk: picoeukaryotes
B. Cytometric dot plot of the sample collected on September 24, 2013, at Böddi-szék (100x dilution). Optical parameters of detected cells - FL3 channel: red detector of a blue excitation laser (488 nm) (675 nm); FL4 channel: red detector (638 nm) of a red excitation laser (675 nm). Pcy: cyanobacteria dominated by picocianin pigment; PEuk: picoeukaryotes

The first grazing experiment was conducted on July 24. Samples were taken from two salt lakes, Böddi and Zab-szék. At the beginning of the experiment, the same number of cells was counted in the untreated and control samples from Böddi-szek, 160,850 cells/ml, and the same was observed in the untreated and control samples from Zab-szék, 55,300 cells/ml were counted in the sample at the beginning. After 12 hours, the number of cells in the untreated sample from Böddi-szék was 106,383.3 cells/ml, while in the control, it was 134,266 cells/ml. In the samples from Zab-szék, the cell count after 12 hours was as follows: 13,700 cells/ml in the untreated sample and 33,166.67 cells/ml in the control sample (Figure 9).

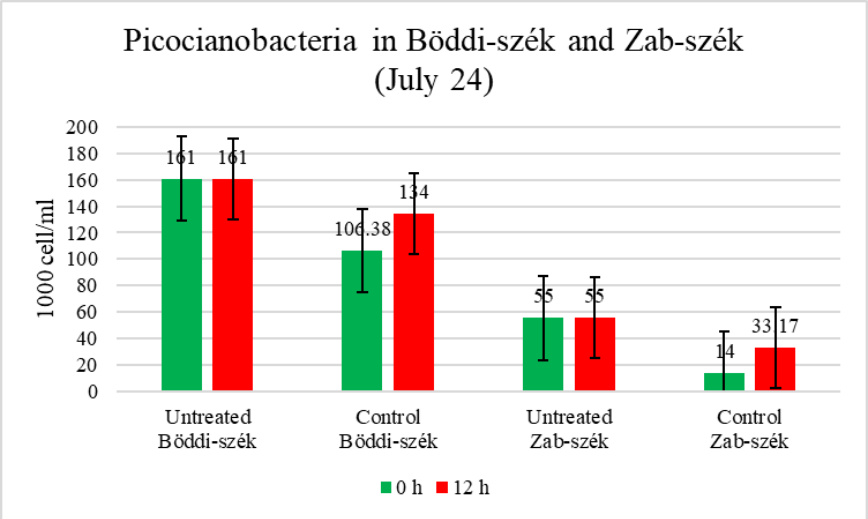

Fig. 9. Changes in the abundance of phycocyanin-containing pico cyanobacteria in Böddi-szék and Zab-szék during the incubation experiment on July 24.

The second sampling was carried out on August 15, also in Böddi and Zab-szék. In the three samples from Böddi-szék, the following values were obtained before the start of the experiment: untreated sample 13,306,000 cells/ml, filtered sample 13,314,000 cells/ml, and control sample 13,532,000 cells/ml. After 12 hours, the number of cells in the three samples changed as follows: untreated sample 10,871,000 cells/ml, filtered sample 12,763,333 cells/ml, control sample 12,243,000 cells/ml.

At the beginning of the experiment, the samples from Zab-szék had a lower number of picocianobacteria cells than the samples from Böddi-szék. The following cell counts were obtained: 8,223,250 cells/ml in the untreated sample, 9,136,250 cells/ml in the filtered sample, and 8,978,500 cells/ml in the control sample. After 12 hours of incubation, the number of cells in the untreated sample and in the filtered sample decreased significantly. The number of cells in the untreated sample was 5,778,833 cells/ml, and in the filtered sample, it was 5,390,333 cells/ml. The number of cells in the control sample also decreased, but to a lesser extent. In this sample, the number of cells was 7,992,250 cells/ml after incubation (Figure 10).

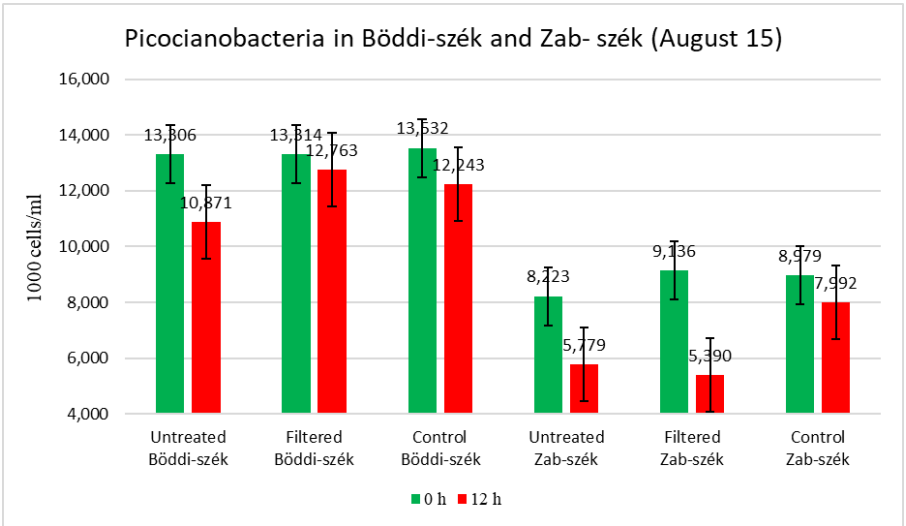

Fig. 10. Changes in the abundance of phycocyanin-containing pico cyanobacteria in Böddi-szék and Zab-szék during the incubation experiment on August 15.

The third sampling took place on August 23, when samples were taken from Böddi-szék and Sós-ér. In both Böddi-szék and Sós-ér, the abundance of picocyanobacteria was extremely low. This is a

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common phenomenon in Sós-ér, which is rich in humus, where autotrophic picoplankton is not dominant, and filamentous cyanobacteria (Anabeana sp.) were abundant at the time of the study. In Böddi-szék, the decrease in pico-cyanobacteria abundance was caused by a specific phenomenon. Hot weather without precipitation led to a significant drop in water level, with the water depth being extremely low, approximately 5 cm. At this depth, the water in the lakes is not agitated or is agitated only by very strong winds, which causes the sedimentation of suspended solids and a drastic reduction in autotrophic picoplankton. At the same time, large eukaryotic algae (mainly diatoms) appeared, which are now in a more favorable state. Their dominance was also evident in the cytometric dot plot. To investigate the grazing pressure on picoplankton in the collected samples, in the waters of Böddi-szék and Sós-ér (Tihany algae strain collection, strain 9808), a phycocyanin cyanobacterium strain isolated from saline lakes was added, and changes in the abundance of the added strain were investigated (Figure 11).

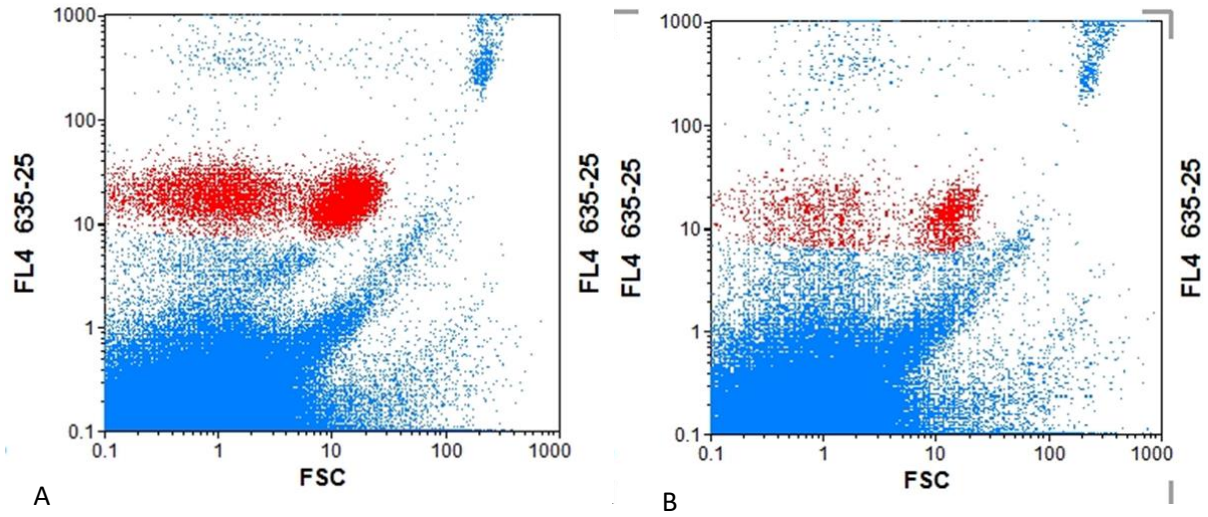

Fig. 11. Cytometric dot plot of the sample taken from Sós-ér on September 24, 2013 (100x dilution) at the beginning (A) and end (B) of incubation. Optical parameters of the detected cells - FL3 channel: red detector (675 nm) of a blue excitation laser (488 nm); FL4 channel: red detector (675 nm) of a red excitation laser (638 nm). The picocianobacterium 9808 strain added to the sample is indicated in red.

The number of cells obtained at the beginning of the experiment in Böddi-szék was as follows: 2,480,750 cells/ml in the untreated sample, 2,516,917 cells/ml in the filtered sample, and 2,578,250 cells/ml in the control sample. After 12 hours of incubation, the number of cells decreased in the untreated sample, 2,330,667 cells/ml, while it increased in the other two samples. The filtered sample had 2,572,917 cells/ml, and the control sample had 2,748,875 cells/ml.

In the case of Sós-ér, before the start of the experiment, the following cell counts were obtained: 2,128,083 cells/ml in the untreated sample, 2,338,333 cells/ml in the filtered sample, and 2,148,250 cells/ml in the control sample. After 12 hours of incubation, a significant decrease in the number of picocianobacteria was observed in the untreated sample. The cell count was 691,583 cells/ml. In the filtered sample, the result was almost similar to the initial result of 2,327,917 cells/ml. A decrease was also observed in the control sample, where the cell count was 1,887,250 cells/ml (Figure 12). The drastic decrease in abundance observed in the untreated samples clearly indicates that nanoflagellates were not involved in picoplankton grazing. Optical microscope examination of the samples showed that the saline was dominated by a large community of roundworms (Rotatoria), which are capable of efficiently ingesting autotrophic picoplankton through their filtering activity, while the filamentous cyanobacterium Anabaena sp. is probably not affected by its size.

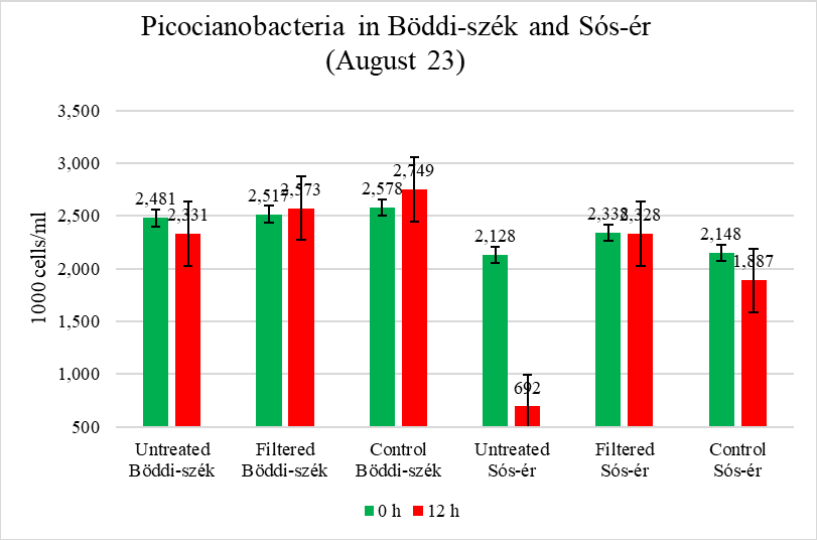

Fig. 12. Changes in the abundance of phycocyanin-containing pico cyanobacteria in Böddi-szék and Zab-szék during the incubation experiment on August 23.

The fourth sampling took place on September 24, also from two salt lakes, Böddi-szék and Zab-szék. In Böddi-szék, before incubation, the cell count was 964,000 cells/ml in the untreated sample, 952,500 cells/ml in the filtered sample, and 984,500 cells/ml in the control sample. After 12 hours of incubation, the cell count was 894,833.3 cells/ml in the untreated sample, 931,000 cells/ml in the filtered sample, and 894,666.7 cells/ml in the control sample (Figure 13).

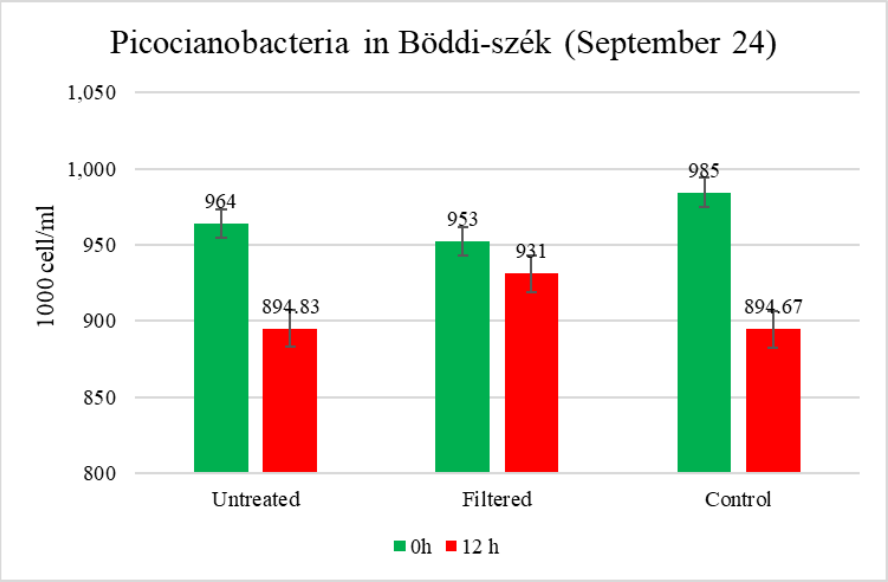

Fig. 13. Changes in the abundance of phycocyanin-containing picocianobacteria in Böddi-szék during the incubation experiment on September 24.

The sample from Zab-szék was tested in the same way, with three treatments. Before incubation, the cell count was 5,048,000 cells/ml in the untreated sample, 5,117,000 cells/ml in the filtered sample, and 5,063,000 cells/ml in the control sample. After 12 hours of incubation, the cell count in the untreated sample was significantly reduced to 4,031,000 cells/ml. The filtered sample had a cell count of 4,930,000 cells/ml, while the control had 4,966,333 cells/ml (Figure 14).

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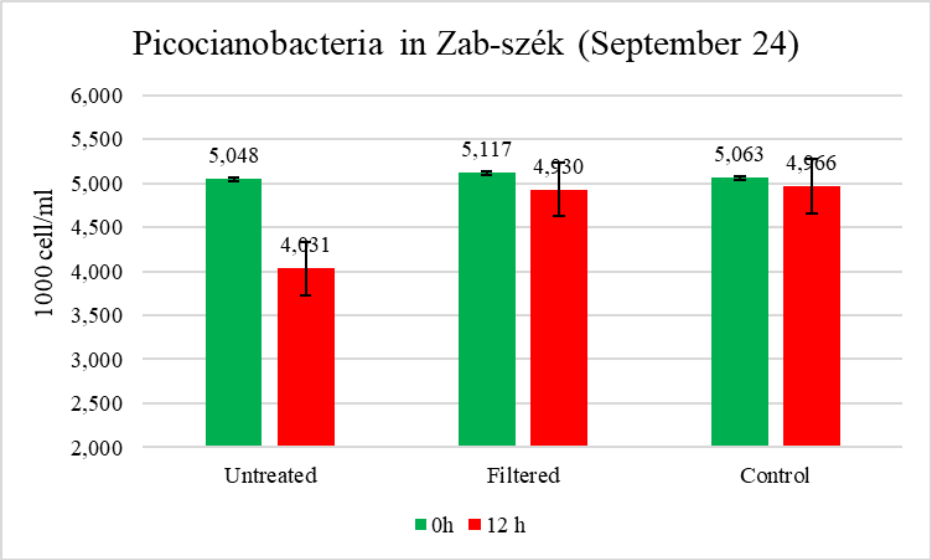

Fig. 14. Changes in the abundance of phycocyanin-containing pico cyanobacteria in Zab-szék during the incubation experiment on September 24.

The results of the research showed that, in the three lakes, in Lake Balaton, the total zooplankton grazing rate was 0.23 d^{-1} on July 30, 0 on August 7, and 0.29 d^{-1} on August 13. The zooplankton grazing rate for zooplankton smaller than 26 µm was 0.11 d^{-1} on July 30, 0.4 d^{-1} on August 7, and 0.31 d^{-1} on August 13.

The total grazing rate of zooplankton in Böddi-szék was 0.4 d^{-1} on July 24, 0.21 d^{-1} on August 15, 0.26 d^{-1} on August 23, and 0.19 d^{-1} on September 24. The grazing rate of zooplankton smaller than 26 µm was not recorded on July 24, 0 on August 15, 0.08 d^{-1} on August 23, and 0.06 d^{-1} on September 24.

The total grazing rate of zooplankton in Zab-szék was 1.78 d^{-1} on July 24, 0.48 d^{-1} on August 15, and 0.41 d^{-1} on September 24. The grazing rate of zooplankton smaller than 26 µm was not recorded on July 24, 0.82 d^{-1} on August 15, and 0.04 d^{-1} on September 24.

For Sós-ér, we have data from August 23, the total zooplankton grazing rate was 2.24 d^{-1}, the grazing rate of zooplankton smaller than 26 µm was 0 d^{-1} (Table 1).

Based on the grazing rates determined from the experiments, the grazing pressure on picofitoplankton in Lake Balaton was often lower than in saline lakes, despite the higher suspended sediment content in the latter. The effect of zooplankton was most intense in Sós-ér, rich in humus, which was not due to nanoflagellates, but to the community of roundworms observed in the sample. The dominance of nanoflagellates in terms of grazing varied dynamically, with large zooplankton playing a more important role in some cases. Further detailed experimental work is needed to elucidate the reasons for this variability and to clarify the effect of floating material on the efficiency of swimmers.

Table 1.

Grazing rates of total organisms and zooplankton smaller than 26 µm for autotrophic picoplankton and abundance of heterotrophic nanoflagellates in shallow lakes studied in July-August 2013

Place	Date	Total zooplankton grazing rate (d^{-1})	<26 m Grazing rate of zooplankton (d^{-1})	Heterotrophic nanoflagellate abundance (cell/ml)
Balaton	07.30.	0,23	0,11	367
	08.07.	0,00	0,04	891
	08.13.	0,29	0,31	1415
Böddi-szék	07.24.	0,47	-	3493
	08.15.	0,21	0,00	545
	08.23.	0,26	0,08	1247
	09.24.	0,19	0,06	no data
Zab-szék	07.24.	1,78	-	10218
	08.15.	0,48	0,82	9853
	09.24.	0,41	0,04	no data
Sós-ér	08.23.	2,24	0,00	no data

CONCLUSIONS

1. Grazing was detectable in Lake Balaton. It was low (0-0.31 d^{-1}), probably due to the low abundance of nanoflagellates, zooplankton.

2. The total grazing rate of zooplankton exceeded that of nanoflagellates in several cases, for example, in Sós-ér, Zab-szék. This suggests that when investigating top-down control of picoplankton, it is not sufficient to consider only nanoflagellates, but also larger zooplankton (ciliates, nanoflagellates), but this would require more detailed studies and the involvement of other experts.

3. The largest fished area was in Sós-ér, where the amount of suspended inorganic solids was the lowest. When Balaton was compared to Böddi-szék and Zab-szék, the results were not so clear. Due to the high amount of suspended solids, we assumed that the two salt lakes would have a lower degree of grazing, but in several cases, we obtained lower values in Lake Balaton. The degree of grazing in all three water bodies showed high variability, which would require a more detailed series of experiments to understand the trophic relationship between picoplankton and zooplankton.

AUTHORS CONTRIBUTIONS

I would like to thank Dr. Károly Pálffy for his help during my summer internships and in writing my thesis, Dr. Judit Papp for her guidance during the writing of my thesis, and Balázs Németh from the Ecological Research Center of the Hungarian Academy of Sciences for his help in collecting samples. I am also grateful to Dr. Lajos Vörös, scientific advisor (MTA Ecological Research Center, Tihany) for the useful experience I gained during my work there.

CONFLICT OF INTEREST

The authors have no competing financial, professional, or personal interests from other parties.

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