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THE IMPACT OF HORNWORT (*Ceratophyllum demersum* L.) ON CYANOBACTERIAL PHYTOPLANKTON IN HYPETROPHIC CONDITIONS. RESULTS FROM A LABORATORY EXPERIMENT

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Summary. The effect of hornwort (*Ceratophyllum demersum* L.) on natural cyanobacterial assemblages with *Planktothrix agardhii* (Gomont) Anagnostidis et Komarek and *Limnothrix redekei* (Van Goor) Meffert was studied. The research was conducted in the form of an ongoing 21 days laboratory experiment using nine aquariums with a volume of 16 dm^3, planted with various density of hornwort shoots: 5 $g \cdot dm^{-3}$ and 10 $g \cdot dm^{-3}$ (tanks B and C) while aquariums without hornwort were used as a control (A). The concentration of chlorophyll *a*, total phytoplankton biomass, the concentration of dissolved oxygen, total organic carbon, phosphorus as well as pH and electrolytic conductivity were determined every few days. Larger decrease in the concentration of chlorophyll *a* (which was 38.2 ±9.3 $\mu g \cdot dm^{-3}$ at start) was found in aquaria B and C (to 13.3. ±9.6 $\mu g \cdot dm^{-3}$ and to 7.6 ±13.2 $\mu g \cdot dm^{-3}$) in comparison to control aquaria (to 20.3 ±12.5 $\mu g \cdot dm^{-3}$), but this difference was not confirmed statistically ($F = 2.75$, $p = 0.101$, ANOVA). The only parameters that differed significantly between the control and the planted aquarium was the concentration of phosphates (whose value has increased during the experiment), and electrolytic conductivity (which recorded a slight decrease in values).

Key words: *Ceratophyllum demersum*, cyanobacteria, hypertrophic lake

INTRODUCTION

Hypertrophication of any lake usually conducts to the simplifying of its food webs, the domination of cyanobacteria and the loss or severe reduction of submerged macrophytes. Phytoplankton dominated state is stable and maintained by a several buffering mechanisms, such as weakening the phytoplankton grazing by zooplankton [Moss 1998, Jeppesen *et al.* 2000]. In shallow lakes the control of phytoplankton biomass is further modified by macrophytes, especially

submerged [Vakilainen 2005]. The impact of macrophytes on phytoplankton may be direct or indirect [for review see: Van Donk and Van de Bund 2002]. The direct impact can lie on the secretion of substances that inhibit the growth of algae [Mulderij *et al.* 2007], although the efficiency and relevance of this mechanism is still under debate [Gross *et al.* 2007]. Macrophytes may also affect the phytoplankton biomass through the competition for nutrients, light and carbon dioxide [Moss 1998] and also by reducing the resuspension or by increasing sedimentation of the seston [Barko and James 1998] as well as by creating refuges for filter feeding crustaceans grazing on planktonic algae [Timms and Moss 1994]. The knowledge about the relationship between macrophytes and phytoplankton is increasingly being used in the reclamation of lakes and there are known some attempts to use macrophyte planting as a method supporting lakes restoration [Qiu *et al*. 2001]. In hypereutrophic lakes submerged vegetation is represented by a few species capable of growing in low light conditions, such as hornwort (*Ceratophyllum demersum* L.), which has a low light compensation point [Spencer and Wetzel 1993]. It is considered as the species that could limit the development of phytoplankton, both through the mechanism of allelopathy [Jasser 1995, Korner and Nicklisch 2002, Gross *et al.* 2003] and as a result of effective competition for phosphorus [Mjelde and Faafeng 1997], although there are also reports stating the lack of inhibiting action of this plant on planktonic cyanobacteria [Pełechata and Pełechaty 2010]. Data on its role comes from field observations or experimental studies that use unialgal cultures. There is a lack of publication investigating the impact of *Ceratophyllum* on natural phytoplankton communities in the laboratory.

The aim of the study was to examine the reaction of natural cyanobacterial assemblages (derived from a shallow hypertrophic lake) on experimentally increased density of hornwort (derived from the same habitat) in the laboratory. We hypothesized that the presence of hornwort will inhibit the phytoplankton community, changing habitat conditions in experimental tanks.

MATERIAL AND METHODS

The study was conducted in a laboratory experiment in May 2006 (the beginning of the growing season). Shoots of hornwort (*Ceratophyllum demersum*) were collected from hypertrophic, hardwater and dominated by cyanobacteria lake Syczyńskie (Eastern Poland). Shoots were rinsed under tap water to remove periphyton. Of the sampled material the strongest shoots were selected, which were then stored for a week in a laboratory aquarium. The water with seston (ca. 135 dm^3) was taken from the same lake simultanously and then poured in to nine aquariums with a capacity of 16 dm^3. *Ceratophyllum* shoots were placed in aquariums in variuos density and tanks were denoted by the letters A, B, C, which meant an appropriate density of plants (A – no hornwort, B – 5 $g \cdot dm^{-3}$ of hornwort, C – 10 $g \cdot dm^{-3}$ of hornwort). Each of the three plant densities was rep-

resented by three aquariums acting as experimental replicates. In order to create conditions similar to those found in the lake (water movement, light climate) aquaria were aerated using a standard pump and illuminated (12 h·day^{-1}) using fluorescent lamps with light color similar to daylight (6500 K). The experiment lasted 21 days and tanks were set in laboratory at room temperature (18–20^{o}C). Every few days (1, 3, 5, 8, 11, 15, 18, 21 day) we have made measurements of the basic properties of water. Measurements of temperature and dissolved oxygen concentration was performed using a WTW oxygen meter with oxygen probe OXI EOT. Electrolytic conductivity and pH were measured using a field pH meter (CP-401) and conductivity meter (CC-401) from Elmetron. Samples for laboratory analysis were also collected at every occasions. Water for chlorophyll-*a* determination (ethanol method, ISO 1992) was collected using a plastic bottle of 0.5 dm^3 from a depth of half of the water column of the central part of the aquarium. In the same sample concentration of total organic carbon (spectrophotometrically, UV Pastel SECOM) and – after filtering through a GF/F filter – concentration of phosphates (molybdate method [Hermanowicz *et al.* 1976]) was determined. Samples for microscopic analysis of phytoplankton (0.05 dm^3) were collected at a lower frequency (1, 5, 15 and 21 day) and fixed with Lugol's solution. Phytoplankton total numbers were determined using an inverted Nikon TMS microscope according to Utermohl method [Vollenweider 1969]. Algal biomass was then calculated by comparing them to the appropriate geometric solid [Hillebrand *et al.* 1999]. In order to determine the significance of differences between the control aquaria and tanks with hornwort an ANOVA analysis with Tukey's test was performed using Statistica 6.0 software.

RESULTS

Phytoplankton in all aquaria consisted of a group of filamentous cyanobacteria, dominated by two species: *Planktothrix agardhii* (Gomont) Anagnostidis et Komarek and *Limnothrix redekei* (Van Goor) Meffert. In addition, single cells of green algae and planktonic diatoms have been found in samples.

In all types of aquariums concentration of chlorophyll *a* at the end of the experiment was significantly lower compared to baseline values (Fig. 1). In the control aquarium (A) the chlorophyll *a* values increased in the beginning (mean of 38.2 ±9.3 to 54.1 ±1.6 μg·dm^{-3}) and then fall on consecutive days (mean 7.8 ±7.7 μg·dm^{-3}). From the 11th day of the experiment, the average values of chlorophyll *a* slightly increased again and then fluctuated in the range from 13.5 ±6.9 to 20.3 ±12.5 μg·dm^{-3}. In plant aquariums (B and C) chlorophyll *a* concentrations decreased from the beginning of the experiment, then slightly increased at day 11 and fluctuated afterwards in the range 1.6 ±2.8–13.3. ±9.6 μg·dm^{-3} (aquarium B) and 0.1–7.6 ±13.2 μg·dm^{-3} (aquarium C). Similar trends of changes in the total phytoplankton biomass have been noted. In the control aquarium (A) the value of this parameter decreased during the experiment from 16.6 ±4.0 μg·dm^{-3} on the first

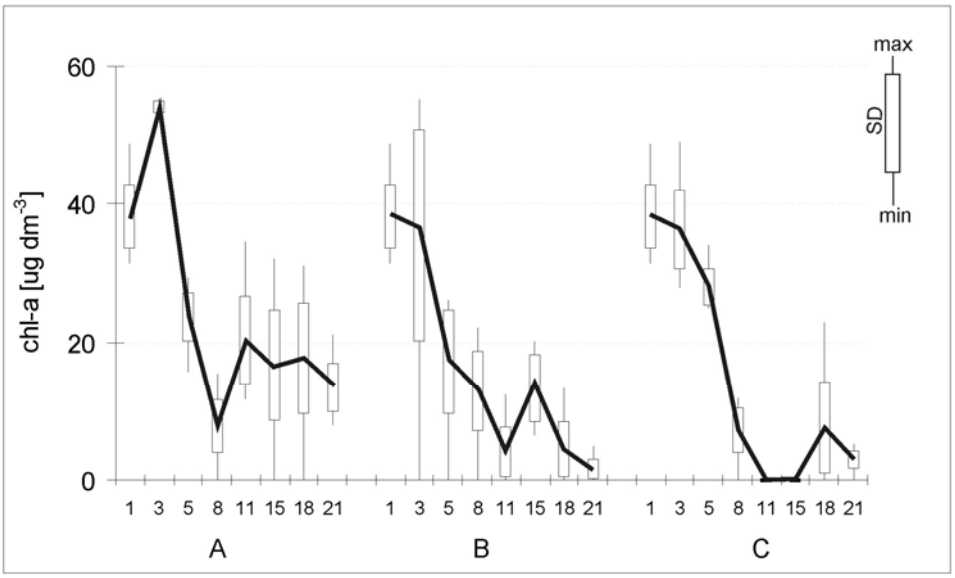

Fig. 1. Values of chlorophyll *a* concentration in aquaria during the experiment: 1, 3, 5... – consecutive days of the experiment; A – control aquaria; B – aquaria with hornwort density of 5 g dm^{-3}; C – aquaria with hornwort density of 10 g dm^{-3}; SD = standard deviation, N = 3; solid line shows changes of mean values

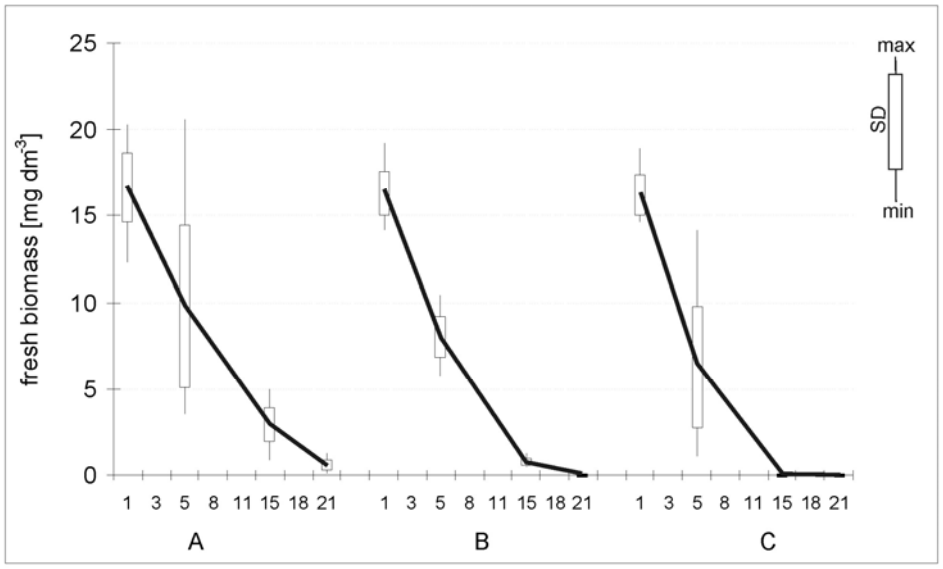

Fig. 2. Total biomass of phytoplankton in aquaria during the experiment (symbols as in Fig. 1)

Table 1. Basic chemical characteristics of water in aquaria during experiment

	A (control)							
	1	3	5	8	11	15	18	21
pH	8.00 ±0.00	8.37 ±0.1	8.47 ±0.1	8.37 ±0.0	8.43 ±0.1	8.43 ±0.1	8.37 ±0.1	8.37 ±0.1
EC	528.7 ±0.6	523.7 ±0.6	499.0 ±8.0	523.3 ±1.1	520.7 ±0.6	509.0 ±4.4	506.7 ±7.0	505.0 ±9.5
O_2	8.3 ±0.1	8.0 ±0.5	7.6 ±0.1	7.9 ±0.5	8.4 ±0.1	8.4 ±0.2	9.0 ±0.3	8.5 ±0.1
TOC	8.3 ±0.1	6.7 ±0.4	7.2 ±0.1	7.1 ±0.1	8.5 ±0.2	7.3 ±0.0	6.8 ±0.1	6.7 ±0.0

	B (5 $g.dm^{-3}$ of hornwort)							
	1	3	5	8	11	15	18	21
pH	8.00 ±0.0	8.42 ±0.0	8.50 ±0.1	8.47 ±0.1	8.37 ±0.1	8.47 ±0.1	8.47 ±0.1	8.47 ±0.1
EC	532.0 ±6.1	518.0 ±2.0	515.0 ±3.5	508.3 ±6.7	502.3 ±11.4	496.7 ±16.6	491.7 ±21.9	488.7 ±28.2
O_2	9.4 ±0.1	8.4 ±0.3	8.1 ±0.2	8.3 ±0.4	8.6 ±0.1	8.6 ±0.2	9.1 ±0.1	8.4 ±0.3
TOC	8.1 ±0.1	6.9 ±0.1	6.7 ±0.2	6.8 ±0.2	7.9 ±0.3	7.0 ±0.1	6.9 ±0.1	6.9 ±0.1

	C (10 $g.dm^{-3}$ of hornwort)							
	1	3	5	8	11	15	18	21
pH	8.00 ±0.0	8.51 ±0.0	8.40 ±0.1	8.43 ±0.1	8.47 ±0.1	8.60 ±0.1	8.50 ±0.1	8.43 ±0.1
EC	528.0 ±1.0	511.7 ±2.3	524.3 ±1.1	483.3 ±15.0	470.7 ±20.5	474.0 ±15.6	467.3 ±12.7	456.3 ±28.6
O_2	9.5 ±0.1	7.8 ±0.3	7.6 ±0.4	8.1 ±0.1	8.0 ±0.7	8.2 ±0.4	8.8 ±0.3	8.7 ±0.7
TOC	8.1 ±0.1	7.6 ±1.5	6.3 ±0.0	6.2 ±0.1	7.7 ±0.1	7.2 ±0.3	6.9 ±0.1	6.9 ±0.1

1,3, 5... – consecutive days of experiment; EC = electrolytic conductivity, $\mu S \cdot cm^{-1}$; O_2 = oxygen concentration, $mg \cdot dm^{-3}$; TOC = total organic carbon, $mg \cdot dm^{-3}$

day to 0.6 ±0.3 $\mu g \cdot dm^{-3}$ on the last day. Aquariums with plants (B and C) showed a more pronounced decline in the value of this parameter: from the 15th day of the experiment the values were close to 0.1 $mg \cdot dm^{-3}$.

Despite finding some differences between the mean values of chlorophyll *a* concentrations in control aquariums (A) and experimental (B and C together) ANOVA results did not confirm the significance of these differences ($F = 2.75$, $p = 0.101$).

During the experiment, changes in the basic properties of water have been seen. The pH of water in all tanks ranged from 8.0 ±0.1 to 8.6 ±0.1, but there were no major changes in this parameter throughout the experiment (Tab. 1). A similar trend was found for the concentration of oxygen and total organic carbon (Tab. 1). Electrolytic conductivity decreased during the experiment in all ty-

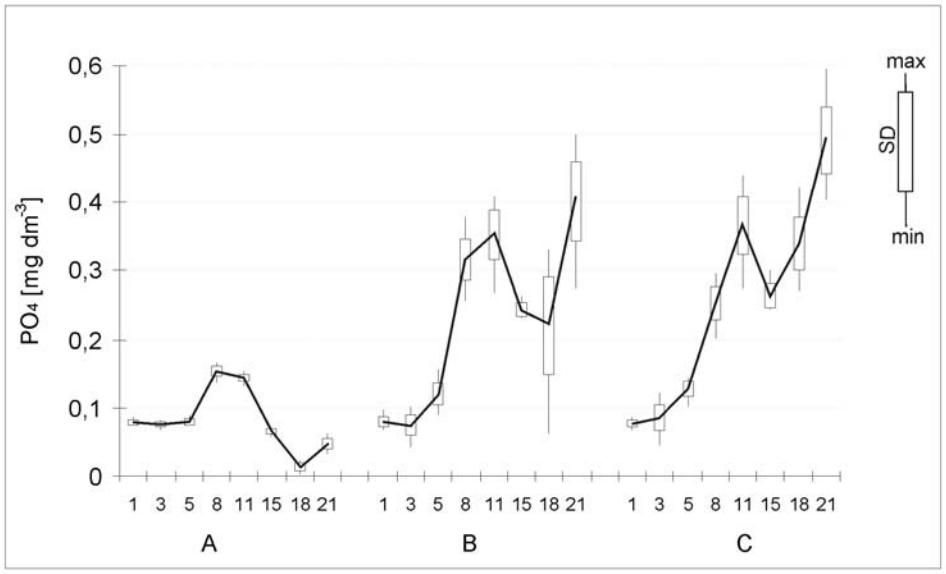

Fig. 3. Values of phosphates concentration in aquaria during the experiment: 1, 3, 5... – consecutive days of the experiment; A – control aquaria; B – aquaria with hornwort density of 5 g dm^{-3}; C – aquaria with hornwort density of 10 g dm^{-3}; SD – standard deviation, N = 3; solid line shows changes of mean values

pes of aquariums. The smallest decrease was observed in the control aquarium (A): from 528.7 ±0.6 to 505.0 ±9.5 $\mu S \cdot cm^{-1}$. In aquariums with plants decrease of the values was higher and amounted in aquaria B: from 532.0 ±6.1 to 488 ±28.2 $\mu S \cdot cm^{-1}$ and aquaria C: from 528.0 ±1.0 to 456 ±28.6 $\mu S \cdot cm^{-1}$ (Tab. 1). Comparing the differences between the values of this parameter in the control tanks and planted aquariums one can find their statistical significance ($F = 8.8$, $p < 0.005$, ANOVA). The concentrations of phosphates were found to have oscillatory changes of their values, but in planted aquariums concentrations were significantly higher at the end of the experiment, whereas in the control aquaria we have recorded lower values (Fig. 3). In all types of tanks there was also similar decrease in phosphates concentration between 11^{th} and 18^{th} (or 15^{th}) days of experiment followed by their growth in the last days. It was also found that differences between the concentration of phosphates in planted aquariums and control tanks were statistically significant ($F = 28.0$, $p < 0.001$, ANOVA).

DISCUSSION

The experiment studied the reaction of natural phytoplankton assemblages from hypertrophic lake on experimentally increased density of hornwort. We hypothesized that the presence of plants will inhibit the growth of cyanobacterial phytoplankton by changing habitat conditions. Reducing the population of cya-

nobacteria was observed in all types of aquariums, which could be caused by nutrient limitation, as there were no addition of the culture medium to any tanks [Korner and Nicklisch 2002]. It can be seen that the decrease in biomass was most intense in the tanks with the largest hornwort densities. However, although the mean values of chlorophyll *a* concentrations were lower in planted tanks, which could indicate the positive verification of the hypothesis, statistical analysis did not confirm the significance of differences between controls and planted aquariums. This makes the interpretation of results rather limited. The trends observed in the average values are, however, consistent with studies of other authors. Jasser [1995] concluded inhibiting effect of hornwort on cyanobacteria both in field and laboratory experiments, indicating the form of chemical action through the secretion of inhibitory substances. Similarly, the inhibitory effect of *Ceratophyllum* was stated by Nicklisch and Korner [2002] examining cyanobacterial unialgal cultures in laboratory experiments (*Planktothrix aghardii* and *Limnothrix redekei* amongs others). Interestingly, they found no decrease in the concentration of chlorophyll *a* (although the biomass was reduced), which is explicable in that the cells of cyanobacteria in the declining populations produced more chlorophyll *a* per cell. This may explain the phenomenon found in our experiment, when the algae biomass decrease was more pronounced than the decrease in the concentration of chlorophyll *a*. Laboratory tests performed by Gross *et al.* [2003] showed, in turn, the inhibitory effect of extracts from the hornwort on two species of cyanobacteria (of the genus *Anabaena*), but the authors emphasize that their results are not straightforward applicable to the conditions found in natural habitats.

Our research also showed that the presence of *Ceratophyllum* contributed significantly to the water parameters such as phosphates concentration and electrolytic conductivity. What is interesting in aquariums with plants, the concentration of phosphates at the end of the experiment were several times higher compared to the initial state, which was not observed in control. These results were inconsistent with the expectation presupposes that plants will help to reduce the concentration of this parameter according to its ability to quick phosphorus uptake [Lombardo and Cooke 2003]. These results also stand in contradiction to the prevalent concept that hornwort can effectively inhibit the growth of phytoplankton through competition for phosphorus [Mjelde and Faafeng 1997, Van Donk and Van de Bund 2002]. Interestingly, fluctuations in the concentration of phosphates (increase – decrease – increase) were opposite to changes in chlorophyll *a* concentrations (decrease – increase – decrease). This suggests that an additional pool of phosphates could be secreted from dying and sedimenting cyanobacterial cells. The presence of macrophytes could therefore affect phytoplankton not by competition for phosphorus, but by forcing an increased sedimentation of this formation [Barko and James 1998]. The significant decrease in water conductivity values observed in aquariums with plants can only be explained by processes of intensive biogenic decalcification associated with photosynthesis of dense macrophyte shoots. Although there are no data on

occurence of this phenomenon in *Ceratophyllum demersum*, it is described in some other cases like dense *Chara* beds in lakes [Kufel and Kufel 2002].

CONCLUSIONS

1. According to the hypothesis, the presence of hornwort gave some inhibitory effect on cyanobacterial community, although there was no statistical significance of this process.

2. Abiotic factors that have changed under the influence of plants presence were: the concentration of phosphates and electrolytic conductivity.

3. Mechanisms that caused the decline in the biomass of cyanobacteria could be connected with increased sedimentation and not with competition for phosphorus.

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WPŁYW ROGATKA SZTYWNEGO (*Ceratophyllum demersum* L.) NA FITOPLANKTON SINICOWY W WARUNKACH HIPERTROFII. WYNIKI EKSPERYMENTU LABORATORYJNEGO

Streszczenie. Badano wpływ rogatka sztywnego (*Ceratophyllum demersum* L.) na naturalne zbiorowiska fitoplanktonu sinicowego, w których dominowały *Planktothrix agardhii* (Gomont) Anagnostidis et Komarek oraz *Limnothrix redekei* (Van Goor) Meffert. Badania przeprowadzono w formie trwającego 21 dni eksperymentu laboratoryjnego z użyciem 9 akwariów o objętości 16 dm^3, w których umieszczono pędy rogatka w zagęszczeniu odpowiednio 5 $g \cdot dm^{-3}$ i 10 $g \cdot dm^{-3}$ (akwaria B i C). Jako zbiorniki kontrolne (A) służyły akwaria bez rogatka. Zarówno pędy rogatka, jak i woda wraz z fitoplanktonem pochodziły z tego samego hipertroficznego Jeziora Syczyńskie. W kolejnych dniach eksperymentu oznaczano koncentrację chlorofilu *a*, biomasę ogólną fitoplanktonu, koncentrację tlenu rozpuszczonego, ogólnego węgla organicznego, fosforanów, a także odczyn i przewodnictwo elektrolityczne wody. Większy spadek wartości koncentracji chlorofilu *a,* która w pierwszym dniu wynosiła 38,2 ±9,3 $\mu g \cdot dm^{-3}$, stwierdzono w akwariach B i C (odpowiednio do 13,3 ±9,6 oraz do 7.6 ±13,2 $\mu g \cdot dm^{-3}$) w stosunku do akwariów kontrolnych (do 20,3 ±12,5 $\mu g \cdot dm^{-3}$), jednak różnica ta nie została potwierdzona statystycznie ($F = 2,75$, $p = 0,101$, ANOVA). Jedynymi parametrami, które istotnie różniły się w akwariach z roślinami i kontrolnymi była koncentracja fosforanów (wartości wzrosły w czasie eksperymentu) oraz przewodnictwo elektrolityczne (zanotowano lekki spadek wartości).

Słowa kluczowe: *Ceratophyllum demersum*, cyjanobakterie, jezioro hipertroficzne