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Light-dependent germination and subsequent proliferation of N_2-fixing cyanobacteria in a large shallow lake

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Abstract – Cyanobacteria are a worldwide group of photosynthetic prokaryotes that can cause nuisance blooms in eutrophic waters. It is generally accepted that their resting cells, akinetes, play an important role in the dispersal, recruitment, initiation of blooms and survival under unfavourable conditions, therefore information on the germination, distribution and abundance of akinetes in natural sediments is essential for understanding the ecology and bloom dynamics of N_2-fixing cyanobacteria. The present study describes the effect of irradiance on the germination and subsequent growth of N_2-fixing filamentous cyanobacteria developed from natural akinete stock in sediment of Lake Balaton (Hungary) with varying phosphorous supply. The research focuses on the invasive *Cylindrospermopsis raciborskii* and *Aphanizomenon flos-aquae* the most abundant species of this lake. In the experiments, the germination of ten filamentous N_2-fixing cyanobacteria species was observed. The species assemblages of the germinated cyanobacteria populations showed strong light and phosphorus dependence. *Anabaena* and *Anabaenopsis* species became dominant in phosphorous-rich conditions, while in phosphorus-deficient environments *Aphanizomenon* species and *C. raciborskii* dominated. Among the germinated filaments we have detected *Anabaenopsis cunningtonii* and *Anabaena compacta*, which have not been observed in Lake Balaton previously. Our results suggest that among the filamentous heterocytic cyanobacteria of this shallow lake the invasive *C. raciborskii* was the best competitor when phosphorus supply and irradiance were low.

Key words: Akinete / germination / N_2-fixing cyanobacteria / *Cylindrospermopsis raciborskii* / *Aphanizomenon* sp. / light / phosphorous / sediment

Introduction

Cyanobacteria are a worldwide group of photosynthetic prokaryotes that often cause nuisance blooms in eutrophic waters. In Lake Balaton (Hungary), as in many temperate lakes, N_2-fixing cyanobacteria generally form planktonic populations for a period of 1–5 months in a year. In late spring and early summer, the appearance of their filaments is observed in the water column, while in autumn the cyanobacterial blooms collapse and the filaments gradually disappear from the water. Under adverse growth conditions, these species differentiate thick-walled survival cells called akinetes from vegetative cells, which may serve as a resting stage (Herdman, 1987; Sukenik *et al.*, 2007). During differentiation akinetes lose their buoyancy capabilities by increasing cytoplasmic density and gradual regression of gas vacuoles, thus accumulating at the sediment surface awaiting favourable conditions for germination (overwintering).

It is generally accepted that akinetes play an important role in cyanobacteria dispersal, initiation of blooms, and survival under unfavourable conditions; therefore, information on the germination and distribution of akinetes in natural sediments is essential for understanding the ecology and bloom dynamics of N_2-fixing cyanobacteria. Since akinetes are long-lived and also may act as a long-term survival mechanism for a species (Livingstone and Jaworski, 1980), the surface layer of sediment can be seen as an akinete stock of all N_2-fixing species that have differentiated into akinetes during the previous years. At favourable growth conditions, their germination provides a source of inoculum for subsequent populations (Roelofs and Oglesby, 1970; Reynolds, 1972; Gorzó, 1987; Karlsson-Elfgren *et al.*, 2004; Kim *et al.*, 2005; Faithfull and Burns, 2006). These resting propagules may also influence the succession of different species in the plankton community (McQuoid and Hobson, 1995; Rengefors and

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Anderson, 1998) and explain the dominance of some species (Hansson *et al.*, 1994; Head *et al.*, 1998, 1999).

The extent of contribution by akinetes to seasonal growth is likely to vary among species and depend on environmental conditions. Factors that trigger these developmental processes have not been elucidated. Many laboratory-based studies determined the optimum physiological parameters of isolated algal strains and described their physiological properties at the strain level (Yamamoto, 1976; Huber, 1985; van Dok and Hart, 1997; Tsujimura and Okubo, 2003; Kim *et al.*, 2005), but behaviour in an isolated system and the outcome of coexistence at the community level may be different. The aim of this research was to study the effect of light on germination and subsequent proliferation of N_2-fixing cyanobacteria developed from natural akinete stock and on different phosphorous supply. The research focuses on the most abundant N_2-fixing species of Lake Balaton, the invasive *Cylindrospermopsis raciborskii* and *Aphanizomenon flos-aquae*.

Material and methods

Sampling sites and sample treatment

The sediment and phytoplankton samples were collected on October 25, 1995 at the centre of the most eutrophic western (Keszthely) basin of Lake Balaton (N46°44.702', E017°15.017) when the temperature of the water column had cooled down to 12 °C gradually and the temperature of the upper layer (2 cm) of the sediment was 13 °C. A water column sampler and column sediment core sampler, as well as a mercury-filled glass thermometer were used during sampling.

Eight sediment cores were collected with sediment core sampler placed at a distance 50 m apart. The head of this sampler contains the changeable sampling tubes made from Plexiglas (500 mm long and 53 mm with inner diameter). After transportation to laboratory in a dark box, the upper 2 cm of each sediment core was removed, pooled and mixed thoroughly. A part of this pooled sample was used to measure the specific density and dry mass content (105 °C) in five replicate subsamples. The specific density of pooled sediment was 1.256 ± 0.026 g.cm^{-3}, the dry mass content was $27.5 \pm 0.11\%$. Another part of this pooled sediment sample was used in germination experiments, while the rest of it was stored in darkness (plastic containers covered in aluminium foil) at 4 °C.

Algae in the water column samples were counted on an inverted microscope. A minimum of 400 units (cells, colonies and filaments) were counted in each sample, giving a counting accuracy, expressed in terms of 95% confidence limits, of $< 10\%$ for the whole phytoplankton (Lund *et al.*, 1958).

Germination experiments

The part of the sediment sample designated for germination experiments was diluted by sterile modified BG-11 medium (see below) and stirred continuously with a magnetic stirrer, while 10 ml of this sediment suspension was pipetted into sterile vials of 25 mL volume and closed with cotton plug. Three vials were prepared per sampling day at each treatment including light and phosphorous. The sediment concentration of the diluted sample was 1.034 mg dry weight sediment.mL^{-1} medium. Dilution and pipetting were carried out in dim light (0.8 μmol.m^{-2}.s^{-1}; tungsten bulb) determined by a spherical quantum microsensor (US-SQS/L, WALZ) in a sterile box located in a dark room.

Besides akinetes, the fresh sediment contains cysts, spores and other resting cells of different algal taxa; therefore, for preferential germination of N_2-fixing, cyanobacteria BG-11 medium (Rippka *et al.*, 1979) was used with the following modifications. Nitrogen content of this medium was decreased almost to zero. $NaNO_3$ was omitted and NH_4Fe(III)citrate was replaced by Fe(III)sulphate, while the iron concentration remained the same as in the original BG-11 medium. The medium was supplemented with 10^{-4} M sodium acetate (Sigma) for stimulating akinete germination (Yamamoto, 1976; Gorzó, 1987), and 10^{-4} M cycloheximide (Sigma) for inhibiting protein biosynthesis in eukaryotic cells (Obrig *et al.*, 1971).

Regarding phosphorus concentration, two experimental variants were prepared. In the phosphorous-rich ("P-enriched") variant, the soluble reactive phosphorus (SRP) concentration was 5600 μg.L^{-1}. In the phosphorous-poor variant ("P-poor"), the sediment was diluted with phosphate-free medium, nevertheless the SRP concentration (190 μg.L^{-1}) was close to the concentration of natural pore water in western basin (Zlinszky, 1987). SRP concentration was determined using the molybdate method (Murphy and Riley, 1962) in triplicates in which the reagent was added to the intact diluted sediment sample used for the germination experiments. The light dependence of akinete germination was determined in the dark (covered with aluminium foils) and at different light conditions (30, 60, 120, 150 and 220 μmol.m^{-2}.s^{-1}) at 23.5 °C temperature. Continuous illumination of the vials was performed by cool white fluorescent tubes (Tungsram F33, 40W).

Based on the results of the preliminary studies, the experiment was terminated on the 15th day, as this allows maximum encystment. Three vials were taken from each treatment on the first day, on the third day and later on every second day until the end of the experiment. The samples were preserved in formaldehyde for microscopic investigation.

Determination of filament abundance

An epifluorescent microscope was used to count the germinated filaments. Samples of known volume were filtered onto black cellulose acetate filters (pore size: 0.45 μm). Filters were placed onto a microscope slide and embedded in 50% glycerol. Germinated filaments were

counted in 40–80 fields at 200 × magnification using green excitation light (Nikon -2A filters set). Total biovolume of the germinated filaments was calculated from the abundances, the length and the diameter of filaments obtained by measuring 15–20 individuals (Hillebrand *et al.*, 1999).

The total biovolumes were tested with a general linear model (GLM; RExcel) also using phosphorus treatment as a categorical variable, with the length of experiment (in days) and light intensity as continuous variables (Baier and Neuwirth, 2007). Normality of error and homogeneity of variance was checked and the necessary transformations applied.

Verifying that the akinete stock was representative

The experimental vials contained the natural akinete stock of all N_2-fixing cyanobacteria existing in the lake. From the preliminary results and the literature we made the following assumptions:

- Prior to sediment sampling (end of October) the water cooled down gradually to 12 °C and the temperature of the upper 2 cm of sediment layer was 13 °C at the sampling points. The germination of akinetes is negligible at this temperature in Lake Balaton (Gorzó, 1986).
- Although some reports proved that overwintering populations of vegetative filaments in the plankton may provide the inoculum for the following season's growth (Reynolds, 1975; Jones, 1979; Barbiero and Welch, 1992), at the time of sampling the filaments of N_2-fixing cyanobacteria were not detected in the water and sediment of Lake Balaton, thus the sediment contained all of the newly differentiated akinetes of N_2-fixing cyanobacteria. Filaments that appeared in the experiments originated from the germination of akinetes.
- The low suspended sediment concentration (25 $mg.L^{-1}$) in the water column at the time of sampling means that wave mixing did not affect the assemblage of akinete stock.
- Temperature appears to be one of the most important external factors regulating akinete germination. Every species has a temperature window within which germination is possible; however, this temperature range is species-dependent (Yamamoto, 1976; Rai and Pandey, 1981; Huber, 1985; Fay, 1988; van Dok and Hart, 1997; Tsujimura and Okubo, 2003). In Lake Balaton, Gorzó (1986) studied the temperature dependence of germination of N_2-fixing cyanobacteria. His results showed that the temperature optimum was species-dependent (*Aphanizomenon* spp. 18.4–25.0 °C, *Anabaena spiroides* 18.9–25.0 °C, *Anabaena aphanizomenoides* 19.6–24.0 °C, *C. raciborskii* 22.3–24.0 °C). Our experiments were carried out at 23.5 °C, which is within the optimum temperature range of all N_2-fixing species of Lake Balaton; consequently the chance of germination of all species was the same.

Results

The algal assemblages in the germination experiment

In the water column, N_2-fixing cyanobacteria disappeared on the date of sampling, but one non-fixing filamentous species *Planktolyngbya subtilis* (W. West) Anagnostidis et Komárek (3×10^4 $\mu m^3.mL^{-1}$) was detected. In the sediments collected for germinating experiments (examined with epifluorescent microscopy) two non-fixing filamentous cyanobacteria species (*P. subtilis* 6.4×10^3 $\mu m^3.mL^{-1}$ and *Planktolyngbya circumcreta* (G. S. West) Anagnostidis & Komárek 4.5×10^3 $\mu m^3.mL^{-1}$) were detected, but filaments of N_2-fixing cyanobacteria were absent. In the experiment, ten N_2-fixing cyanobacteria species (*C. raciborskii*/*Wołosz.*/*Seenayya* et Subba Raju; *A. flos-aquae*/*L. Ralfs*; *Aphanizomenon issatchenkoi*/*Ussatzew*/*Proschkina-Lawrenko*; *A. aphanizomenoides*/*Forti*; *A. spiroides*/*Kleb.*; *Anabaena scheremetievi* Elenk; *Anabaena compacta*/*Nygaard*/*Hickel*; *Anabaena circinalis* Rabh; *Anabaenopsis cunningtonii* Taylor; and *Anabaenopsis hungarica* Halász) germinated and proliferated at all irradiances independent of phosphorous supply. The common and dominant N_2-fixing species of Lake Balaton were observed during the experiment, but we also detected the filaments of *A. cunningtonii* Taylor and *A. compacta* (Nygaard) Hickel species, which were not been observed previously in Lake Balaton. Under dark conditions, germination of akinetes was not observed in any variants of the experiment.

Total biovolume of algae germinated at different irradiances and phosphorous supply

The GLM test showed that the length of experiments, light and P treatments on their own significantly determined the variability of the biovolume of germinated filaments with the length of the experiment being the most influential and P treatment showing the less significant influence (Table 1). The interaction between the determinant factors was less pronounced, nevertheless still significant (Table 1). During the experiment, the total biovolume of N_2-fixing filaments increased continuously in both P variants (Fig. 1). Until the 7th day of the experiment, the difference in the total biovolumes was not observed between "P-enriched" and "P-poor" variants except at 220 $\mu mol.m^{-2}.s^{-1}$. Later, the germinating and proliferating N_2-fixers in the "P-enriched" variants outgrew the "P-poor" ones. Moreover, the difference between the total biovolume of the P variants increased gradually with incubation time. Light response curves of the total biovolumes of the algal communities showed distinct light saturation features. In most of the cases, the graphs showed that the total biovolume peaked around 100 $\mu mol.m^{-2}.s^{-1}$ on each day of the experiment (Fig. 1).

Light intensity together with P supply affected the composition of germinated and proliferated N_2-fixing

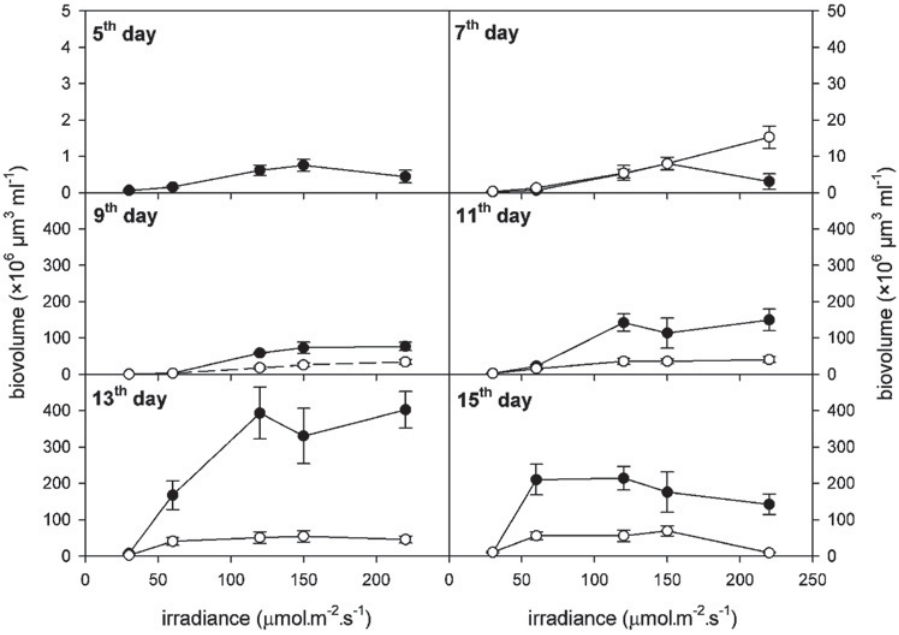

Fig. 1. Changes in algal biovolumes ($\mu m^3.mL^{-1}$) in "P-enriched" (●) and "P-poor" (○) variants during the germination experiment at different irradiances.

Table 1. General linear model (GLM) test on algal biovolumes with phosphorus treatment (P treatment) as categorical variable, length of experiment (day) and light intensity (light) as continuous variables.

	F-value	*P*
Light	24.74	< 0.001
P treatment	16.85	< 0.001
Day	67.19	< 0.001
Light × P treatment	10.69	0.0013
Day × P treatment	29.49	< 0.001

cyanobacterial assemblages (Figs. 2 and 3). In the "P-enriched" variant *C. raciborskii* was dominant (47–56% of the total biovolume) only at the beginning of the experiment at lowest irradiance (30 $\mu mol.m^{-2}.s^{-1}$), but its contribution decreased to 12% towards the end of the experiment. From the 7th day *Anabaena* and *Anabaenopsis* species became dominant independently of irradiance. The contribution of *A. flos-aquae* and *Aphanizomenon issatchenkoi* was insignificant to the algal assemblage in the "P-enriched" variant (Fig. 2).

In "P-poor" variant, *C. raciborskii* was the most successful species at the lowest irradiance (30 $\mu mol.m^{-2}.s^{-1}$) on each day of the experiment. On the 7th day its contribution was 41.8% and reached 60.2% of total biovolume at the end of the experiment. With increasing irradiances *A. issatchenkoi* became dominant, while at the highest irradiances *A. flos-aquae* was the most abundant cyanobacterium at the end of the experiment, its contribution reached 57 and 65% of total biovolume at 120 and 220 $\mu mol.m^{-2}.s^{-1}$ irradiances, respectively (Fig. 3).

The germination and subsequent proliferation of *C. raciborskii* and *A. flos-aquae*

C. raciborskii and *A. flos-aquae* are the most abundant organisms in the phytoplankton communities of Lake Balaton. Phosphorous supply did not affect significantly their germination, but the effect of light on the two species was different. Germination of *C. raciborskii* did not show light dependence. At 30–150 $\mu mol.m^{-2}.s^{-1}$ its germination started after the third day of the experiment and finished by the seventh day. At 220 $\mu mol.m^{-2}.s^{-1}$, *C. raciborskii* filaments were detected on the third day and the number of filaments on the 5th day was nearly equal to the number of filaments on the 7th day at lower irradiances (Fig. 4). The germination of *A. flos-aquae* showed strong light dependence. The first filaments occurred earlier with increasing irradiance. Its filaments appeared at 30 $\mu mol.m^{-2}.s^{-1}$ between the seventh and the ninth day, at 60 $\mu mol.m^{-2}.s^{-1}$ between the fifth and the seventh day, at 120 $\mu mol.m^{-2}.s^{-1}$ between the third and the fifth day. Above 150 $\mu mol.m^{-2}.s^{-1}$ the appearing filaments were shorter than 3 days (Fig. 4). *A. flos-aquae* gradually overgrew *C. raciborskii* with increasing irradiance both in "P-enriched" and "P-poor" variants (Fig. 4).

Discussion

Appearance of new N_2-fixing species in Lake Balaton

Environmental changes might allow new, competitive or hidden species to invade and out-compete original inhabitant species. In Lake Balaton, new N_2-fixing species

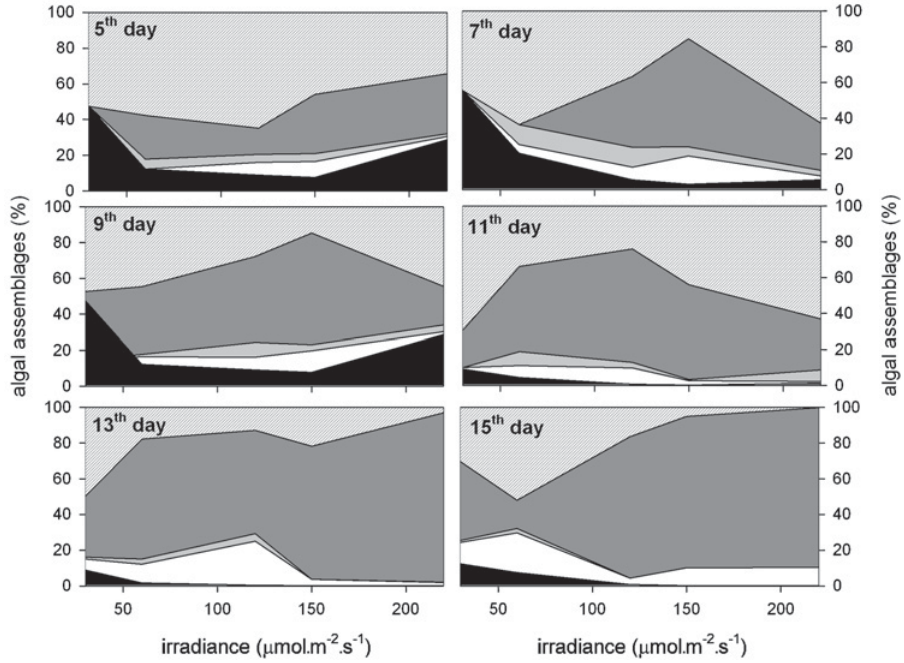

Fig. 2. Changes of algal assemblages in "P-enriched" experimental variant during the germination experiment (black: *Cylindrospermopsis raciborskii*; white: *Aphanizomenon flos-aquae*; light grey: *Aphanizomenon issatchenkoi*; dark grey: *Anabaena aphanizomenoides*; striped: *Anabaena* except *A. aphanizomenoides* and *Anabaenopsis* species).

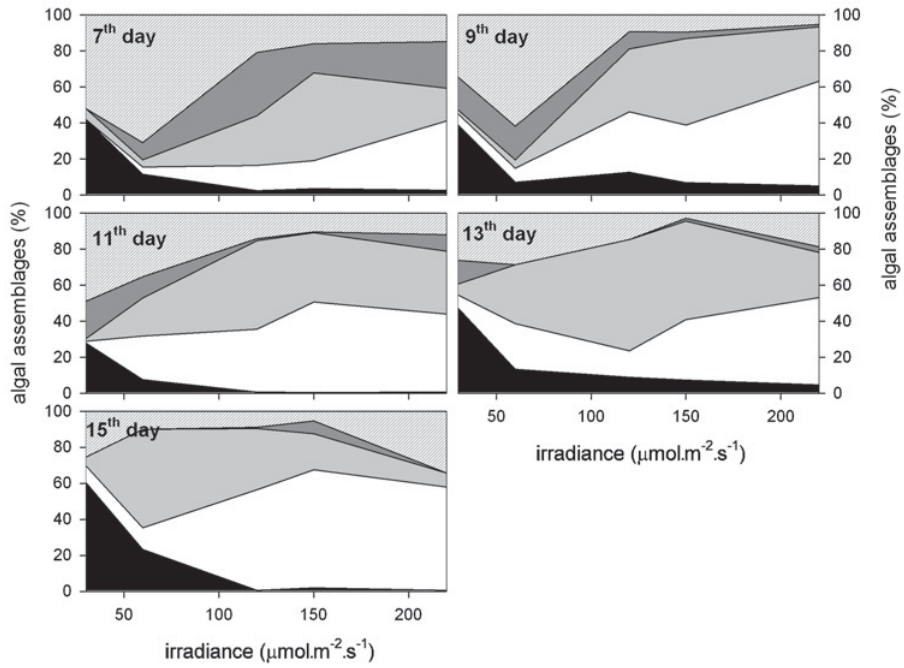

Fig. 3. Changes of algal assemblages in "P-poor" experimental variant during the germination experiment (black: *Cylindrospermopsis raciborskii*; white: *Aphanizomenon flos-aquae*; light grey: *Aphanizomenon issatchenkoi*; dark grey: *Anabaena aphanizomenoides*; striped: *Anabaena* except *A. aphanizomenoides* and *Anabaenopsis* species).

appeared in parallel with eutrophication including: *A. aphanizomenoides, A. issatschenkoi, Anabeanopsis elenkii, C. raciborskii and Rhapidiopsis mediterranea* (H.-Bartha, 1974; Tamás, 1974; Hegewald *et al.*, 1975; Oláh *et al.*, 1981; Padisák and Reynolds, 1998). In the 1980s only *Anabaena contorta* was observed as a new species (Uherkovich and Lantos, 1987). During the 1990s *A. circinalis and Aphanizomenon hungaricus* were also recorded in the algal community of the lake (Padisák and Reynolds, 1998). In the present investigation, ten species germinated including the common and dominant N_2-fixing species of Lake Balaton and we also detected

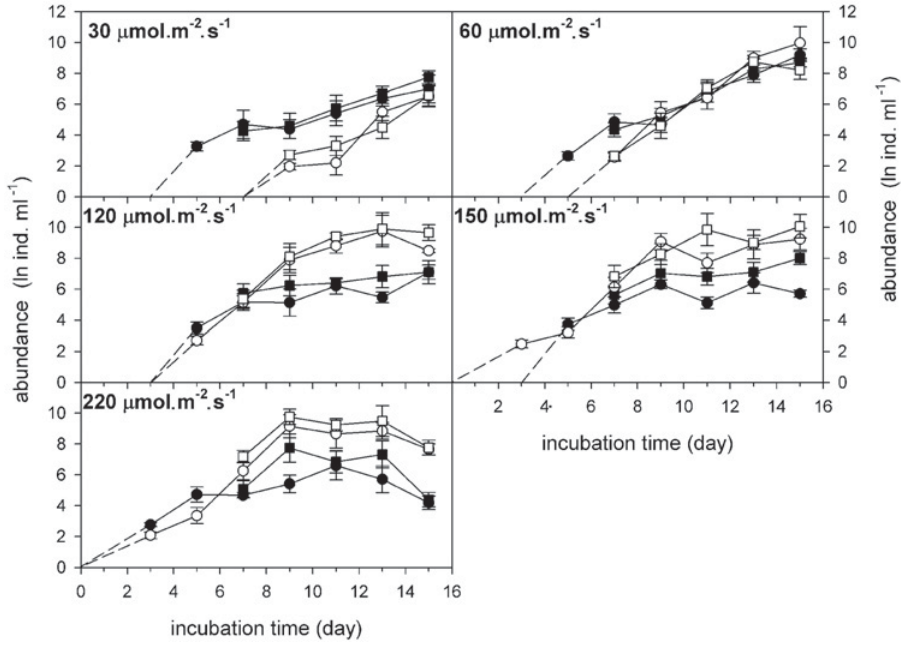

Fig. 4. Germination and subsequent proliferation of *Cylindrospermopsis raciborskii* (closed symbols) and *Aphanizomenon flos-aquae* (open symbols) in "P-enriched" (circle symbols) and "P-poor" (square symbols) variants at different irradiance.

the filaments of *A. cunningtonii* and *A. compacta*, species which had not been observed in Lake Balaton previously. These results call attention to the fact that the process observed over recent decades resulting in the appearance of new species has not come to an end.

Sediment of lakes can be seen as a "resting seed bank" and its role was undoubtedly crucial during the fast invasion of *C. raciborskii* in Lake Balaton. Their filaments were first observed on the western part of Lake Balaton in 1978 (Oláh *et al.*, 1981), but one year later in the summer of 1979, its biomass was twice as high as the native *A. flos-aquae* in the most eutrophic basin of lake (Vörös *et al.*, 1983). Three years later (in 1982) this cyanobacterium became a dominant species in the entire (597 km^2) lake (Vörös *et al.*, 1983; Gorzó and Kiss, 1984, 1985; Padisák *et al.*, 1984). During the last large bloom (in 1994, when the biomass of phytoplankton reached 36 $mg.L^{-1}$) the whole water body of the lake was almost a pure culture of *C. raciborskii*. Its biomass contributed to more than 90% of total phytoplankton (Présing *et al.*, 1996). Similarly, in Lake Kinneret, *C. raciborskii* invaded in 1998 and has formed major summer blooms since 2003. A particularly dense population developed in 2005, which comprised 85% of total phytoplankton biomass (Zohary and Shlichter, 2009; Alster *et al.*, 2010).

The effect of phosphorous concentration on germination and subsequent proliferation

The akinete as a survival cell has a sufficient P pool for germination and the first division but not for subsequent growth (Talpasayi, 1962; Reddy, 1983; van Dok and Hart, 1997). In our experiment, the differences in the total biovolumes of N_2-fixing cyanobacteria were not significant between "P-enriched" and "P-poor" variants until the 7th day. Later, the algae in the "P-enriched" variants outgrew the "P-poor" ones. Similar results have been observed by Gorzó (1986) who showed that akinete originating from Lake Balaton germinated in 100% on the 7th day of experiment at 23.5 °C on 2000 lux (*ca.* 24 $\mu mol.m^{-2}.s^{-1}$ in the case of white fluorescent lamp) irradiance. His results showed that the amount of phosphorous (0–400 $mg.L^{-1}$ PO_4–P) had no influence on the germination of N_2-fixing cyanobacteria (including *C. raciborskii* and *A. flos-aquae*). A study on *Anabaena dolinum* and *Fischerella mucicola* also agreed with this statement (Kaushik *et al.*, 1971). In contrast phosphorous supply enhanced germination of *Anabaena vaginicola* (Rai and Pandey, 1981), *Anabaena variabilis* and *Nostoc linckia* (Reddy, 1983), *Anabaena fertilissima* (Reddy, 1984) and *A. circinalis* (Fay, 1988; van Dok and Hart, 1997). Owing to the different methodological approaches, it is difficult to determine the effect of phosphorous concentration on akinete germination from the literature (van Dok and Hart, 1997). In our case, fresh sediment of Lake Balaton was used in the experiments, the P content of which can affect akinete germination. In the "P-poor" variant soluble reactive phosphorous concentration was 190 $\mu g.L^{-1}$, although the true concentration of biologically available phosphorous was not known. Van Dok and Hart (1997) reported that 200 $\mu g.L^{-1}$ of available phosphorous (as K_2HPO_4) increased the germination of *A. circinalis* in isolated akinete cultures by 12% (after 24 h) and 50% (after 48 h) as compared to the P-free variant.

P supply also changed the species composition of the assemblages of N_2-fixing cyanobacteria. In the "P-enriched" variant *Anabaena* and *Anabaenopsis* species

became dominant independently of the light intensity, while in the "P-poor" variant *C. raciborskii*, *A. flos-aquae* and *A. issatchenkoi* gradually outgrew the *Anabaena* and *Anabaenopsis* species. These results mimicked the natural situation well, since *C. raciborskii*, *A. flos-aquae* and *A. issatchenkoi* are the dominant species in summer in Lake Balaton, where P is the main growth limiting factor of planktonic algae (Herodek *et al.*, 1988).

The effect of light on germination and subsequent proliferation

Light is one of the essential factors for germination. In the present study, germination of akinetes was not observed in the dark (Fig. 1). Several reports support the uniformity of this phenomenon (Yamamoto, 1976; Braune, 1979; Pandey and Talpasayi, 1981; Huber, 1985; Gorzó, 1987; van Dok and Hart, 1997; Baker and Belifemine, 2000; Tsujimura and Okubo, 2003). The presence or absence of light is crucial and not a simple question from the point of view of the recruitment. In autumn, the differentiated akinetes sink to the sediment surface where light is absent or low depending on numerous factors (decreasing insolation, water depth, suspended solids, algal biomass and dissolved organic materials). Akinetes do not germinate in darkness except for the phenomena of fructose-induced dark germination (Neely-Fisher *et al.*, 1989). For successful germination and inoculation, akinetes have to adapt to very low irradiances or have to wait for mixing to the upper water layers. Sukenik *et al.* (2007) observed that the light saturation constant of photosynthesis (I_k) of akinetes in filaments of akinete-induced *Aphanizomenon ovalisporum* cultures decreased to a quarter compared with the I_k value of vegetative cells from exponentially grown cultures. Theoretically, there should be a threshold of light intensity needed for the initialization of germination; however, in the literature, there is no consensus about the light signal. Generally, authors use aluminium foil to create darkness, but light conditions during the sample preparations were not described in detail. In the present study, sample preparation was carried out at 0.8 $\mu mol.m^{-2}.s^{-1}$ (light source: tungsten bulb; sensor type: spherical sensor) in a dark room. Lack of the germinated filaments in the dark condition proved that during the sample preparation (*ca.* 1 h) this irradiance was not sufficient for the germination of akinetes collected from Lake Balaton. Whereas, during the sample preparation Karlsson-Elfgren *et al.* (2004) a dark room lamp was used (*ca.* 650 nm red light, 0.05 $\mu mol.m^{-2}.s^{-1}$), but very low biovolume of *Gloeotrichia echinulata* was observed in the dark treatments. The authors did not address the question whether this observation was due to experimental conditions or this dim light was sufficient to start germination. Low-level germination of *A. flos-aquae* occurring in the dark might also be a consequence of light exposure during microscopic observations (Kim *et al.*, 2005). Braune (1979) showed that the lower limit for the light stimulation of *Anabaena* was only 0.1–0.3 $W.m^{-2}$ (*ca.* 0.47–1.28 $\mu mol.m^{-2}.s^{-1}$, light source: halogen lamp). Huber (1985) reported that the akinetes of *Nodularia* failed to germinate in the dark, however, as little as 0.5 $\mu mol.m^{-2}.s^{-1}$ irradiance (light source: cool-white fluorescent tube) allowed significant germination (29% and 56% at 5 days in two trials). It should be noted that this feature may be species-dependent and these observations are likely to be strongly affected by the duration of irradiation.

Besides the fact that the presence of light is an essential initial factor of germination, its intensity determines the velocity of germination and recruitment. It is a very important feature especially in shallow lakes since the favourable condition for germination and subsequent growth to reach a bloom is time-limited. Our results showed that the germination of *A. flos-aquae* became more faster with increasing irradiance. Gorzó (1987) reported that at l00 lux (*ca.* 1.2 $\mu mol.m^{-2}.s^{-1}$) 5–8% of the akinetes, at 500 lux (*ca.* 6 $\mu mol.m^{-2}.s^{-1}$) 100% of the akinetes germinated during 3 weeks, while above 2000 lux (*ca.* 24 $\mu mol.m^{-2}.s^{-1}$) the germination time was only 1 week using a white fluorescent light source. Yamamoto (1976) also showed a decrease in the lag time of germination of *Anabaena cylindrica* with increasing irradiance. In contrast, van Dok and Hart (1997) found that there was no significant difference between germination of *A. circinalis* at 15, 30 or 50 $\mu mol.m^{-2}.s^{-1}$.

Light is a crucial environmental factor that can also determine the assemblages of the algal community. During recruitment and subsequent proliferation, the most competitive species outgrow others under given environmental conditions. Results of the present investigation showed that light influenced the appearance and proliferation of the most important N_2-fixing species in Lake Balaton. At the lowest irradiance (30 $\mu mol.m^{-2}.s^{-1}$), *C. raciborskii* was the most successful N_2-fixer in the experiment, however, at higher irradiances *A. flos-aquae* outgrew *C. raciborskii* and became the most abundant cyanobacterium at the end of the germination experiment.

Our research provides information on the germination and subsequent growth of bloom forming cyanobacteria and contributes to understanding the natural events leading to the establishment of a new algal community. There is no doubt that the algal mass productions are determined by external and internal nutrient loading; however, main physical environmental factors, namely temperature and underwater light conditions markedly affect the assemblages of the algal community including seasonal dynamics of species and succession. Temperature is one of the main factors that determine the timing of the germination and appearance of species, but differences in the light requirement of species can also serve as an explanation for phytoplankton succession. In Lake Balaton, the assemblages of algal community show a characteristic seasonal pattern from year to year. In the period characterised by low temperature and high light intensities (end of spring and beginning of summer) *A. flos-aquae* appears and becomes dominant first. After this,

appearance of the other heterocitic species was observed regularly (*Anabaena* spp. and *A. issatschenkoi*) in parallel with increasing self-shading effect in the water body. The hegemony of N_2-fixing cyanobacteria always closed with the dominance of *C. raciborskii* at the end of summer or the beginning of autumn (Gorzó and Kiss, 1985; Kiss, 1998). Regarding the results of algal assemblages in a low phosphorus environment (like Lake Balaton) the lower light requirements of *C. raciborskii* have an advantage over not only *A. flos-aquae* but also on other N_2-fixing cyanobacteria species under low light conditions.

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