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RECRUITMENT OF BENTHIC *MICROCYSTIS* (CYANOPHYCEAE) TO THE WATER COLUMN: INTERNAL BUOYANCY CHANGES OR RESUSPENSION?[1]

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In some lakes, large amounts of the potentially toxic cyanobacterium *Microcystis* overwinter in the sediment. This overwintering population might inoculate the water column in spring and promote the development of dense surface blooms of *Microcystis* during summer. In the Dutch Lake Volkerak, we found photochemically active *Microcystis* colonies in the sediment throughout the year. The most vital colonies originated from shallow sediments within the euphotic zone. We investigated whether recruitment of *Microcystis* colonies from the sediment to the water column was an active process, through production of gas vesicles or respiration of carbohydrate ballast. We calculated net buoyancy, as an indication of relative density, using the amounts and densities of the major cell constituents (carbohydrates, proteins, and gas vesicles). Carbohydrate content of benthic *Microcystis* cells was very low throughout the year. Buoyancy changes of benthic *Microcystis* were mostly a result of changes in gas vesicle volume. Before the summer bloom, net buoyancy and the amount of buoyant colonies in the sediment did not change. Therefore, recruitment of *Microcystis* from the sediment does not seem to be an active process regulated by internal buoyancy changes. Instead, our observations indicate that attachment of sediment particles to colonies plays an important part in the buoyancy state of benthic colonies. Therefore, we suggest that recruitment of *Microcystis* is more likely a passive process resulting from resuspension by wind-induced mixing or bioturbation. Consequently, shallow areas of the lake probably play a more important role in recruitment of benthic *Microcystis* than deep areas.

Key index words: **chl fluorescence; gas vesicles; harmful algal blooms; *Microcystis*; recruitment**

Abbreviations: **Φ_P°, photochemical efficiency of dark-adapted PSII; F_0, baseline fluorescence of PSII; F_m, maximum fluorescence of PSII; F_m', maximum fluorescence of PSII in actinic light; F_s, steady-state fluorescence of PSII in actinic light; PFD, photon flux density; (R)ETR, (relative) electron transport rate in PSII; $RETR_{max}$, maximum relative electron transport rate in PSII; σ_{PSII}, functional absorption cross-section of PSII; Z_{eu}, euphotic depth of the water column**

Compared with many other phytoplankton species of eutrophic waters, cyanobacteria of the genus *Microcystis* have a relatively low specific growth rate (Reynolds 1997, Huisman et al. 1999). Still, these potentially toxic cyanobacteria are able to dominate the phytoplankton completely. There are several possible reasons for the success of *Microcystis* (Hyenstrand et al. 1998, Dokulil and Teubner 2000). One of these reasons is that *Microcystis* cells contain gas vesicles, which make the cells buoyant (Walsby 1994). The buoyancy state of *Microcystis* is regulated by light. In light, excess of photosynthetic energy is stored as carbohydrate ballast, which makes *Microcystis* colonies sink, up to the point where respiration has sufficiently reduced carbohydrate ballast to make *Microcystis* colonies buoyant again (Kromkamp and Mur 1984, Thomas and Walsby 1985, Visser et al. 1995). This buoyancy regulation may give *Microcystis* a competitive advantage, with better access to light than other phytoplankton (Ibelings et al. 1991a,b). Buoyancy also

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prevents sedimentation losses of colonies during summer (Visser et al. 1995).

Another reason for the success of *Microcystis* might be that part of its population overwinters vegetatively in the sediment (Sirenko et al. 1969, Topachevskiy et al. 1969, Imamura 1981, Reynolds et al. 1981, Takamura et al. 1984). In fact, several studies (Takamura et al. 1984, Boström et al. 1989) found that the total amounts of *Microcystis* in sediment can be much higher than the total amounts of *Microcystis* in the water column, even during *Microcystis* blooms. This means that a huge potential inoculum of *Microcystis* can be present in the sediment. Preston et al. (1980) observed that recruitment of *Microcystis* from the sediment to the water column takes place before establishment of the summer bloom in the water column. What is unclear, however, is whether recruitment of *Microcystis* from the sediment is an active process, triggered by internal changes in buoyancy, or whether recruitment is a passive process brought about by resuspension of sedimented *Microcystis* colonies.

Internal changes in buoyancy can be a result of very specific changes in environmental factors. The onset of a *Microcystis* bloom often coincides with enhanced light penetration and anoxia near the sediment (Reynolds 1973, Reynolds et al. 1981, Cáceres and Reynolds 1984, Trimbee and Prepas 1988). Also, increases in temperature have been associated with increases in recruitment of *Microcystis* (Trimbee and Harris 1984). Visser et al. (1995) demonstrated that *Microcystis* sinks in autumn, because of an increase in carbohydrate ballast at reduced temperatures. Once in the sediment, colonies can regain buoyancy because of respiration of carbohydrate ballast or because of gas vesicle synthesis.

Alternatively, if colonies are buried in the sediment, resuspension may reintroduce benthic colonies into the water column. Wind-induced resuspension may be important for recruitment during spring overturn when the water column is unstratified. Resuspension may also be brought about by bioturbation. Ståhl-Delbanco and Hansson (2002), for instance, found increased recruitment rates of *Microcystis* in the presence of benthic macrofauna.

In this study, we investigated *Microcystis* colonies overwintering in the sediment of Lake Volkerak, The Netherlands. In particular, we investigated to what extent *Microcystis* remained photochemically active during overwintering, whether *Microcystis* colonies in the sediment could inoculate the water column by increasing their buoyancy, and whether this inoculation process was restricted to spring.

MATERIALS AND METHODS

Site description and sampling. Lake Volkerak is a freshwater lake situated in southwest Netherlands (Fig. 1). It was created after the damming of the Volkerak estuary in 1987. From 1988 onward, summer blooms of *Microcystis* have gradually increased in intensity. The surface area of Lake Volkerak is 45.7 km^2, with an average depth of 5 m and a maximum depth of 22 m.

We sampled the lake once every 2 weeks from August 1999 to September 2001. Three sampling stations were selected. The water column depths of the sampling stations varied from 2 to 3 m (station A), 9 to 11 m (station B), and 17 to 20 m (station C) due to variations in lake depth within the sampling area. Temperature and oxygen concentration were measured at the water surface of station C and at 1 m above the sediment of stations A, B, and C. Euphotic depth (Z_{eu}) was determined at station C as the depth at which light intensity is 1% of the light intensity at the water surface.

Water was sampled from the top 1.5 m of the water column, using a water sampler. Water samples were filtered onto GF filters with a pore size of 1.0 μm and stored at −20° C until chl *a* extraction. In addition, between 2 and 20 L of water was concentrated to 20 mL using a plankton net (mesh size, 30 μm). *Microcystis* colonies concentrated this way were stored at 4° C until analysis on photochemical vitality.

Sediment was sampled at all sampling stations using a box corer (∅ 30 cm, h 50 cm). From the box corer four subsamples were taken with a perspex corer (∅ 4.7 cm, h 30 cm). Cores were divided into the upper 1 cm and the layer below, down to a depth where *Microcystis* colonies were no longer visible, which was between 1 and 10 cm. Subsamples from similar layers were pooled and stored at 8° C until further analysis.

Isolation of colonies from the sediment. Within 24 h after sampling, *Microcystis* colonies were isolated from the sedi-

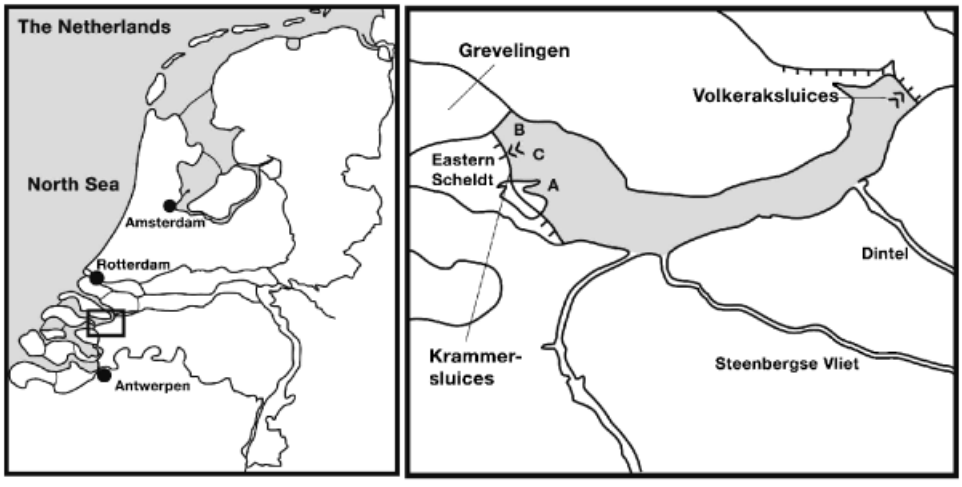

FIG. 1. Lake Volkerak with the locations of the sampling stations A (2–3 m deep), B (9–11 m deep), and C (17–20 m deep).

ment. During isolation, samples were stored on ice. The sediment was homogenized by gently stirring the sediment, but the initial separation of the top 1 cm and the deeper layer was maintained. About 5 to 25 mL of homogenized sediment was suspended in a Percoll mixture (30% Percoll, 70% Ø2 medium described by van Liere and Mur 1978) and centrifuged (1800 rpm, 15 min) to separate *Microcystis* from the sediment. The supernatant obtained after centrifugation was filtered over a fine sieve (mesh size, 25 µm) to trap *Microcystis* colonies. The trapped colonies were suspended in filtered lake water. Two subsamples were taken. One subsample was stored at 4° C in the dark for determinations of photochemical vitality, buoyancy state, and gas vesicle volume. The other subsample was exposed to a pressure of 15 bar to collapse the gas vesicles of *Microcystis*. Two-milliliter subsamples were subsequently transferred to test tubes (in triplicate) and centrifuged (4000 rpm, 5 min). The supernatant was removed and the pellet was stored at –20° C until further analyses of carbohydrate and protein content of the *Microcystis* colonies.

Photochemical efficiency and electron transport rate. Photochemical efficiency of dark-adapted *Microcystis* colonies was determined from August 2000 to September 2001 using a pulse amplitude modulation fluorometer (Phyto-PAM, Heinz Waltz GmbH, Effeltrich, Germany). Cooled samples (4° C) were brought to room temperature. Each measurement was performed after dark adaptation of at least 15 min. Baseline fluorescence (F_0) of PSII was detected by means of a nonactinic pulsed measuring beam with an irradiance of less than 1 µmol photons $\cdot m^{-2} \cdot s^{-1}$. Maximum fluorescence ($F_m$) was induced by a saturating light pulse of 2200 µmol photons $\cdot m^{-2} \cdot s^{-1}$ with a pulse length of 500 ms. Fluorescence measurements were made in triplicate. Because we were specifically interested in the cyanobacterium *Microcystis*, only fluorescence signals from phycocyanin-containing organisms (detected by applying pulses of orange light [620 nm]) were used. Photochemical efficiency of dark-adapted PSII reaction centers (Φ_P°) was calculated as

$$\Phi_P^\circ = (F_m - F_0)/F_m \quad (1)$$

Photosynthetic electron transport rates were measured with the Phyto-PAM at 10 different irradiances in the range of 1–1500 µmol photons $\cdot m^{-2} \cdot s^{-1}$. The 10 irradiances were administered in 10 consecutive 1-min steps. At the end of each 1-min step, a saturating light pulse was given to determine the quantum yield (Φ_P):

$$\Phi_P = (F_m' - F_s)/F_m' \quad (2)$$

Here, F_s is the steady-state fluorescence in actinic light and F_m' is the maximum fluorescence induced by the saturating light pulse after exposure to actinic light. Φ_P can be used to calculate the photosynthetic electron transport rate (ETR) as (Gentry 1989, Kolber and Falkowski 1993):

$$ETR = \Phi_P \times PFD \times \sigma_{PSII} \quad (3)$$

where σ_{PSII} is the functional absorption cross-section of PSII and PFD is the photon flux density. Because we could not measure σ_{PSII} directly, we calculated relative ETR (RETR) as $\Phi_P \times$ PFD. RETR is generally an increasing saturating function of irradiance, comparable with the photosynthesis–irradiance relationship. $RETR_{max}$ was defined as the maximum RETR.

Buoyant colonies and number of colonies. Immediately after isolation of colonies from the sediment, floating and sinking colonies were counted in a Sedgewick-Rafter counting chamber (Pyser-SGI, Edenbridge, UK). At least 100 colonies ($\geq$ 10 cells) or 150 grids were counted. Buoyancy of colonies in the water column was not determined because buoyancy of colonies in the water column is highly variable over the day (Ibelings et al. 1991a).

Chlorophyll. Chl *a* was extracted from lyophilized GF filters using *N*,*N*-dimethylformamide for 2 h at room temperature. Chl *a* was measured photospectrometrically at 647 and 664 nm and calculated according to Porra et al. (1989).

Gas vesicle volume. Gas vesicle volume was measured in a capillary compression tube, adapted from Oliver and Walsby (1988). In short, the sample was transferred to the compression chamber of the tube and the position of the meniscus in the capillary was measured. Pressure was slowly applied up to 15 bar to collapse gas vesicles of the colonies and then released again. After stabilization, the position of the meniscus in the capillary was measured again. The gas vesicle volume of the sample is the volume difference between the two meniscus positions. Measurements were carried out in triplicate.

Carbohydrates and proteins. *Microcystis* stores carbohydrates as glycogen. In lyophilized samples, glycogen was hydrolyzed to glucose with 2 M HCl for 1 h at 100° C and subsequently neutralized with 2 M NaOH. The Sigma (510-A) kit (Sigma Diagnostics, St. Louis, MO, USA) was used to determine the amount of glucose in the hydrolyzed samples. Protein was extracted from lyophilized samples by adding 1 M NaOH and incubating the samples for 15 min at 100° C. Protein concentrations were measured using the Folin method of Lowry (Herbert et al. 1971), with BSA as the standard. Measurements were carried out in triplicate.

Net buoyancy. As an indication of cell density, net ballast was calculated according to Oliver and Walsby (1984) as the mass of carbohydrates, proteins, and gas vesicles minus the mass of water they displace. We used a value of 998 kg $\cdot m^{-3}$ for the density of water (20° C), 1330 kg $\cdot m^{-3}$ for the density of carbohydrate, 1550 kg $\cdot m^{-3}$ for the density of protein, and 1.2 kg $\cdot m^{-3}$ for the gas vesicle gas space (Thomas and Walsby 1985). Net ballast values were multiplied by –1, and this was called net buoyancy.

Statistical analysis. We tested whether there were seasonal patterns in the dynamics of the observed parameters. Water temperature changed seasonally; therefore, if the relation of a parameter with water temperature was significant, dynamics were considered seasonal. If data were normally distributed, as tested with the Shapiro-Wilks test, the relation with temperature was calculated using linear regression. If data differed significantly from a normal distribution, even after log-transformation, a nonparametric test of the parameter against water temperature was carried out using Spearman's rank correlation.

We used the generalized linear models repeated measures procedure (Vonesh and Chinchilli 1997) to test for effects of the presence or absence of oxygen on observed dynamics in Φ_P° and $RETR_{max}$. Φ_P° data were log-transformed before analyses to obtain a normal distribution. The data of the deeper layer of site B were left out of the analyses because they contained too many missing values. In all outcomes, we used the Greenhouse-Geisser epsilon to correct the averaged F-test because sphericity (calculated using Mauchly's test) could not be assumed. We chose the Greenhouse-Geisser instead of the Huyn-Feldt epsilon because it is more conservative.

RESULTS

Temperature, oxygen, and light. Temperatures of water and sediment (Fig. 2A) ranged between 1 and 22° C from August 1999 through September 2001. There was no clear temperature stratification within the water column. Hence, water overlying the sediments of the deepest parts of the lake reached temperatures up to 19° C in summer. Oxygen concentrations were high in winter and low in

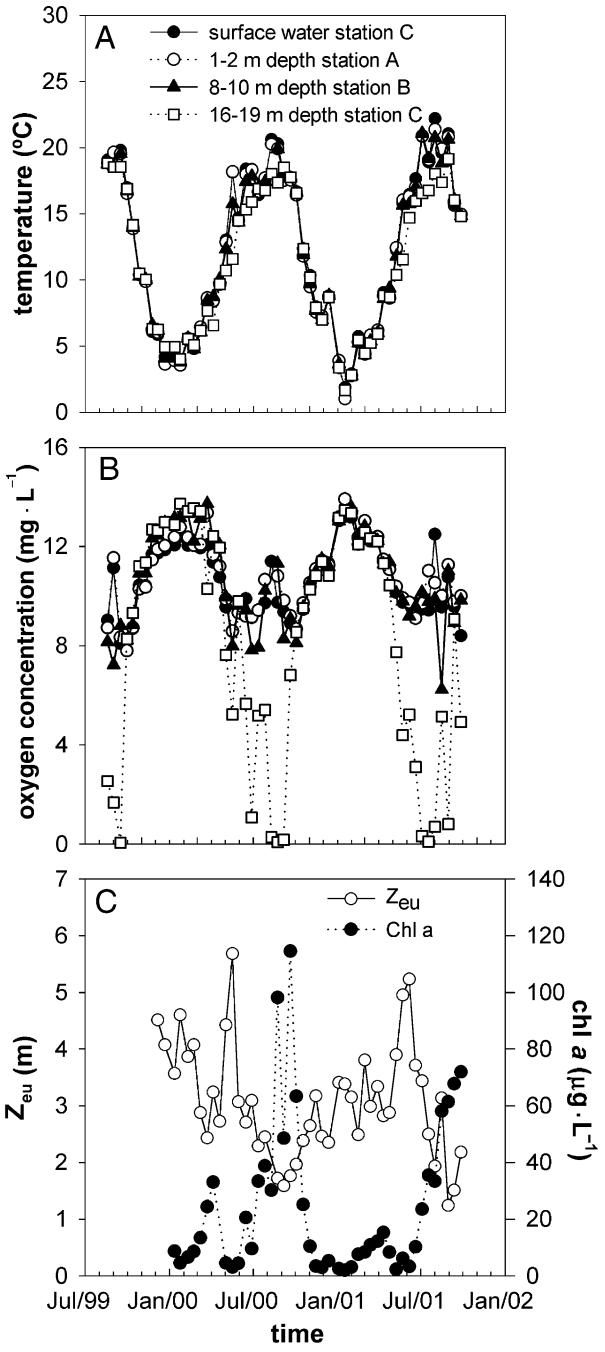

FIG. 2. Seasonal dynamics of (A) temperature of surface water and the water 1 m above sediments located at different depths in the water column (2–3, 9–11, and 17–20 m) in Lake Volkerak, (B) oxygen concentration of surface water and the water 1 m above sediments, and (C) chl *a* concentration of surface water and the euphotic depth (Z_{eu}) at station C.

summer (Fig. 2B). In summer (from June to October) there was a clear vertical gradient in oxygen content, which gradually diminished from a depth of 10–11 m to the bottom sediment (data not shown). Occasionally, water just above the sediment became anoxic. The euphotic depth, Z_{eu}, was quite shallow (Fig. 2C). Z_{eu} was lowest from August to October, during the peak of the *Microcystis* blooms. Z_{eu} was highest during the clear water phase in May. At the shallowest site (sediment at 2–3 m depth), the euphotic depth extended to the lake bottom for most of the year. Sediments at the deeper sites were far below the euphotic zone.

Microcystis *in the water column*. Chl *a* concentration in the surface water (Fig. 2C) ranged between 2 and 115 $\mu g \cdot L^{-1}$. In March and April there was a spring bloom consisting of diatoms, *Cryptomonas*, and green algae. From July to November the phytoplankton was dominated (>95%) by *Microcystis aeruginosa* (Kützing) Kützing and *M. flos-aqua* (Wittrock) Kirchner. Also, *M. ichtyoblabe* Kützing and *M. viridis* (A. Braun in Rabenhorst) Lemmermann were observed. There was a strong negative correlation between chl *a* and the depth of the euphotic zone (Pearson's correlation, $r = -0.690$, $P < 0.001$, $n = 42$), indicating that dense blooms of *Microcystis* significantly reduced light penetration through the water column.

Microcystis *in the sediment*. Most microphytobenthos (>95%) consisted of *Microcystis* colonies, in concentrations of 100 to almost 200,000 colonies $\cdot mL^{-1}$ of sediment. These consisted of the same four *Microcystis* species that were also observed in the water column. The remaining 5% of the microphytobenthos consisted mainly of diatoms. Many *Microcystis* colonies (both sinking and buoyant) were colonized by low numbers of other organisms, mostly cyanobacterial picoplankton, bacteria, amoebae, and in some cases diatoms. These colonizers were also observed in and on colonies from the water column. Within degenerated colonies we sometimes observed *Aphanocapsum* spp.

Photochemical vitality of Microcystis. Photochemical vitality of *Microcystis* was determined by measuring Φ_P° and $RETR_{max}$. Φ_P° (Fig. 3, A–C) was generally high in summer and autumn (July to December) and low in winter and spring (January to June). There was a sudden peak in Φ_P° in colonies from the water surface at the beginning of March, at a time when there was relatively little *Microcystis* present in the water column. This peak coincided with a bloom of *Cryptomonas* spp., which also contain phycocyanin (Hill and Rowan 1989), and therefore show a fluorescence signal similar to cyanobacteria when exposed to light of 620 nm. The relation between temperature and Φ_P° for *Microcystis* colonies taken from the water surface and from the top centimeter of sediments from the two shallow oxic sites was positive (Table 1). In contrast, this relation was negative for colonies from the deeper layers of the deepest site (17–20 m). This contrast was confirmed by a statistical analysis, which showed that the seasonal dynamics of Φ_P° differed significantly between the two shallow oxic sites (2–3 and 9–11 m) and the deep anoxic site (17–20 m) (Table 2).

Seasonal changes in $RETR_{max}$ (Fig. 3, D–F) were similar to seasonal changes in Φ_P°, but less pronounced

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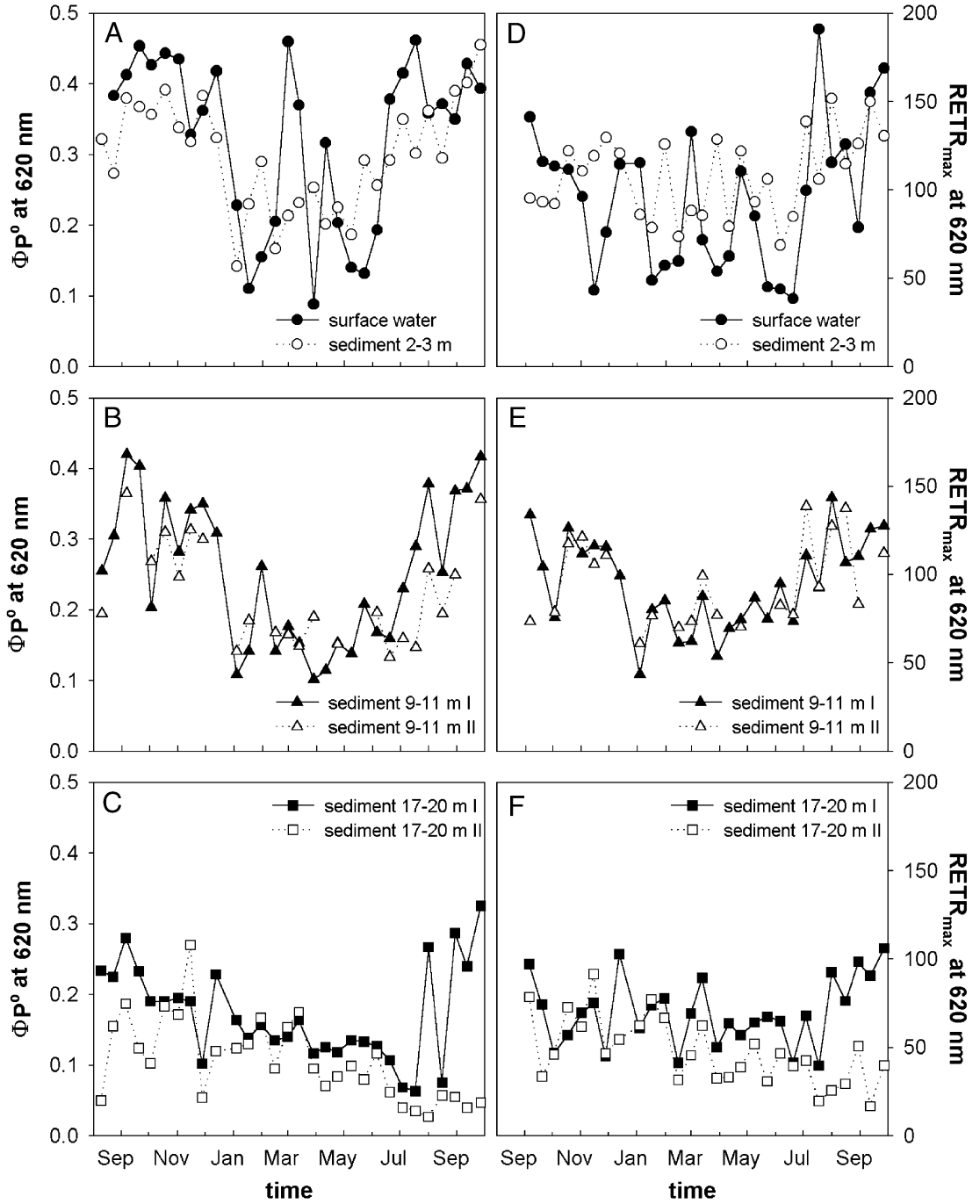

FIG. 3. Seasonal dynamics of photochemical efficiency (Φ_P°) of *Microcystis* colonies from surface water and from sediments located at different water column depths: (A) surface water and sediments of 2–3 m deep, (B) sediments of 9–11 m deep, and (C) sediments of 17–20 m deep. Seasonal dynamics of maximum relative electron transport rate ($RETR_{max}$) of *Microcystis* colonies from surface water and from sediments located at different water column depths: (D) surface water and sediments of 2–3 m deep, (E) sediments of 9–11 m deep, and (F) of sediments of 17–20 m deep. I, colonies isolated from the upper centimeter of sediment; II, colonies isolated from below the upper centimeter of sediment.

(Tables 1 and 2). There was a significant positive relation between $RETR_{max}$ and temperature for the upper centimeter of sediments at a depth of 9–11 m but a significant negative relation for the deeper layers of sediments at 17–20 m depth (Table 1). $RETR_{max}$ of colonies from the water surface and from the sediments of the shallow site were highly variable, especially in winter and spring. Average Φ_P° and $RETR_{max}$ of benthic colonies decreased with increasing water column depth (Fig. 3).

TABLE 1. Relation and correlation of photochemical efficiency (Φ_P°), maximum relative electron transport rate ($RETR_{max}$), and the percentage of buoyant colonies versus temperature

(Cor)related parameters	Site	r	n	P
Temperature, log Φ_P°	Surface water	0.448[a]	28	<0.05
	2–3 m	0.586[a]	28	<0.01
	9–11 m I	0.509[a]	28	<0.01
	9–11 m II	0.214[a]	22	N.S.
	17–20 m I	0.093[a]	28	N.S.
	17–20 m II	−0.532[a]	28	<0.001
Temperature, $RETR_{max}$	Surface water	0.358[a]	28	N.S.
	2–3 m	0.293[a]	28	N.S.
	9–11 m I	0.543[a]	28	<0.01
	9–11 m II	0.419[a]	22	N.S.
	17–20 m I	0.180[a]	28	N.S.
	17–20 m II	–0.410[a]	28	<0.05
Temperature, % buoyant colonies	2–3 m	0.533[b]	54	<0.001
	9–11 m I	0.517[b]	52	<0.001
	9–11 m II	0.035[b]	28	N.S.
	17–20 m I	0.366[b]	54	<0.01
	17–20 m II	–0.020[b]	40	N.S.

I, colonies isolated from the upper centimeter of sediment; II, colonies isolated from below the upper centimeter of sediment; N.S., not significant.

[a]Linear regression.

[b]Spearman's rank correlation.

Buoyancy of benthic colonies. During microscopical counting, we observed that before isolation of the colonies, sediment particles were attached to *Microcystis* colonies. After isolation in Percoll, most of these sediment particles had detached from the colonies. Compared with buoyant colonies, sinking colonies appeared degenerated, and often detritus was attached to sinking colonies. Yet, most sinking colonies had not lost their gas vesicles.

The percentage of colonies that were buoyant after isolation decreased gradually during winter and spring (Fig. 4). In July, there was a sharp increase, from 10% to 90%, in the percentage of buoyant colonies. The percentage of buoyant colonies remained high during late summer and autumn. This pattern was similar in almost all sampled sediments. There was a positive correlation with temperature (Table 1) in the top centimeter of sediment of all sampled sites but not in deeper layers of the sediments. Seasonal dynamics in the percentage of buoyant colonies were remarkably similar in the years 1999–2000 and 2000–2001 (Fig. 5; Spearman's rank correlation, $\rho = 0.701$, $P < 0.01$, $n = 103$).

In the two relatively shallow oxic sites (2–3 and 9–11 m), the number of buoyant colonies per milliliter of sediment was high in summer, dropped quite quickly in autumn to low levels in winter and spring, and then increased rapidly in July again (Fig. 6, A and B). At the deepest site (17–20 m), the number of buoyant colonies increased from summer to winter, dropped rapidly in spring, and gradually increased again in July (Fig. 6C). Therefore, whereas the percentage of buoyant colonies in the sediment was independent of water column depth (Fig. 4), the absolute number of buoyant colonies per milliliter of sediment increased with increasing water column depth (Fig. 6).

Figure 7 shows the contributions of carbohydrate ballast, gas vesicle volume, and protein to the net buoyancy of *Microcystis* colonies isolated from the top layer of the sediment. Results from the deeper layers are similar to results from the top layers. The contribution of protein to net buoyancy was almost seven times higher than the contribution of carbohydrate ballast. However, ballast due to carbohydrates and protein was not enough to offset buoyancy provided by gas vesicles, except for three occasions in June and July at the shallowest site. No clear seasonal dynamics in net buoyancy were observed. The carbohydrate content and gas vesicle volume of *Microcystis* colonies were positively correlated (Pearson's correlation, $r = 0.875$, $P < 0.01$, $n = 124$).

DISCUSSION

Microcystis colonies that overwinter in the sediment can act as an inoculum that initiates *Microcystis* blooms in lakes (Preston et al. 1980, Brunberg and Blomqvist 2003). However, overwintering benthic populations can only act as an inoculum if they remain vital and if they are able to leave the sediment. There are two possible mechanisms of recruitment: passive resuspen-

TABLE 2. Effect of time and oxygen availability on the dynamics of photochemical efficiency (Φ_P°) and maximum relative electron transport rate ($RETR_{max}$)

Measured parameter	Factor	G-G ε	df	MS	F value	P
Log Φ_P°	Time	0.076	2.121	0.934	3.389	N.S.
	Time × oxygen availability	0.076	2.121	0.802	2.929	N.S.
	Oxygen availability		1	4.305	13.33	<0.05
$RETR_{max}$	Time	0.076	2.047	14,216	2.148	N.S.
	Time × oxygen availability	0.076	2.047	10,236	1.547	N.S.
	Oxygen availability	—	1	55,341	16.18	<0.05

The test is based on the generalized linear models repeated measures (GLM-RM) procedure. Oxygen availability was defined as a binominal present-absent variable, where oxygen was present at the two relatively shallow sites (2–3 and 9–11 m) but absent at the deep site (17–20 m). The Greenhouse-Geisser epsilon (G-G ε) is a correction factor that is used in GLM-RM to adjust the degrees of freedom (df). As a result, df is not an integer value.

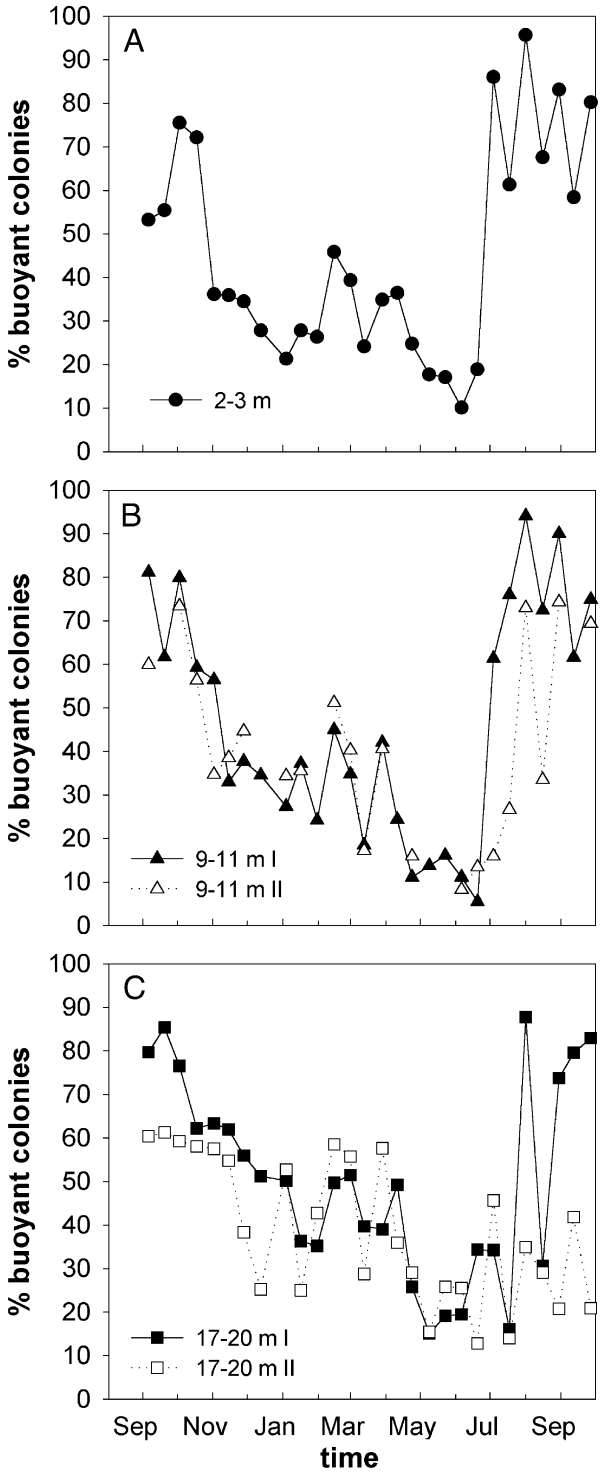

FIG. 4. Seasonal dynamics of the percentage of buoyant *Microcystis* colonies isolated in the period September 2000 to September 2001 from sediments located at different water column depths: (A) 2–3 m, (B) 9–11 m, and (C) 17–20 m. I, colonies isolated from the upper centimeter of sediment; II, colonies isolated from below the upper centimeter of sediment.

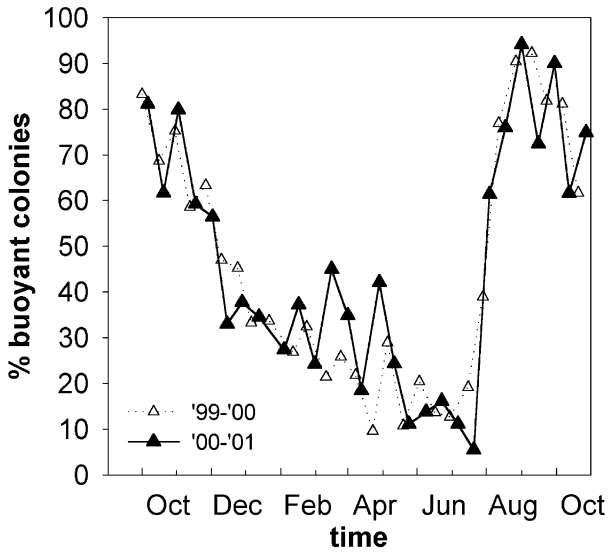

FIG. 5. Comparison of the seasonal dynamics in the years 1999–2000 and the years 2000–2001 of the percentage of buoyant *Microcystis* colonies in the upper centimeter of sediments located at a depth of 9–11 m.

sion of benthic *Microcystis* colonies and active decrease in the density of *Microcystis* cells, resulting in buoyant propagules. In this study we investigated whether this second mechanism might result in the inoculation of the water column with *Microcystis*.

Vitality of overwintering Microcystis. Throughout the year, there were photochemically viable *Microcystis* colonies in the water column and sediments of Lake Volkerak. It was unclear whether this vital water population could only exist during winter because of continuous recruitment from the sediment or whether conditions in the water column allowed a small part of the population to overwinter in the water column.

The average vitality of benthic *Microcystis* populations, as measured by both photochemical efficiency and maximum relative electron transport rate, decreased with increasing depth of the water column. This coincided with a reduced light and oxygen availability in sediments at deeper parts of the lake. Colonies on the sediment surface at less than 5 m depth still received some light, whereas sediment surfaces beyond 5 m depth were virtually in the dark. Furthermore, sediment surfaces at less than 11 m depth were still aerobic and had a brown color, whereas sediment surfaces at more than 11 m depth were anaerobic and black. Several studies showed that prolonged periods of darkness, for several months, may lead to degradation of light-harvesting complexes and photosynthetic reaction centers in phytoplankton and macroalgae (Murphy and Cowles 1997, Lüder et al. 2002). *Microcystis* is able to survive under anoxic conditions by fermentation (Moezelaar and Stal 1994, 1997). However, cyanobacterial photosynthesis is generally sensitive to the high sulfide concentrations often found in anaerobic sediments, because sulfide reacts with metals in cytochromes and haemoproteins (Oren et al. 1979, Cohen et al. 1986,

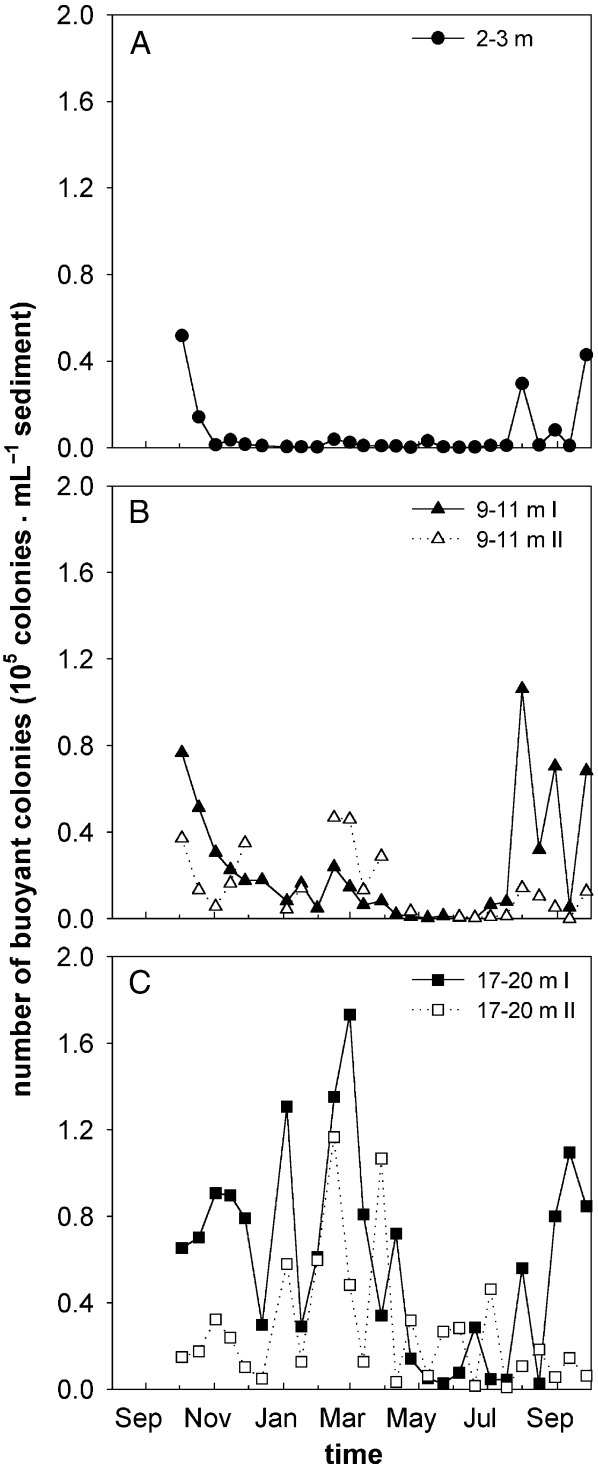

FIG. 6. The number of buoyant colonies per milliliter of sediment in sediments located at different water column depths: (A) 2–3 m, (B) 9–11 m deep, and (C) 17–20 m. I, colonies isolated from the upper centimeter of sediment; II, colonies isolated from below the upper centimeter of sediment.

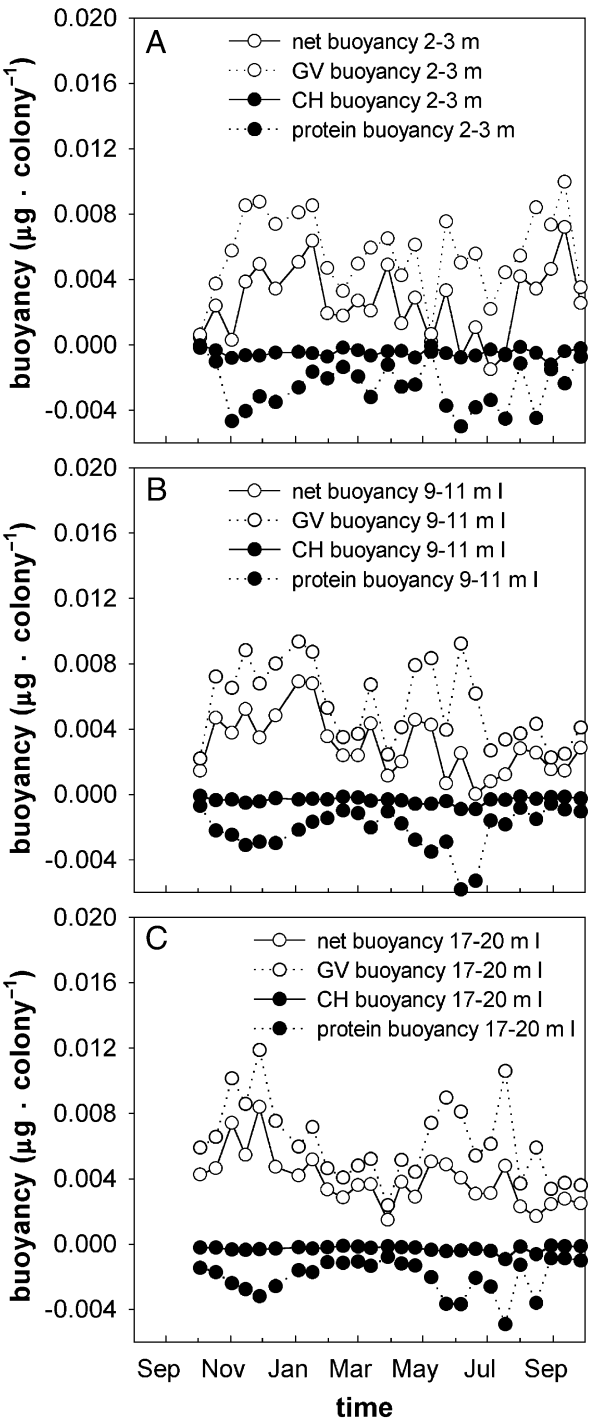

FIG. 7. Net buoyancy and the buoyancy obtained by gas vesicles (GV), carbohydrates (CH), and proteins (in µg · colony^{-1}) of *Microcystis* colonies isolated from the upper centimeter of sediments located at different water column depths: (A) 2–3 m, (B) 9–11 m, and (C) 17–20 m. Net buoyancy is calculated as the sum of gas vesicle buoyancy, carbohydrate buoyancy, and protein buoyancy.

Terjung et al. 1996). Therefore, the reduced photochemical vitality of *Microcystis* colonies found at sediment surfaces of deeper parts of the lake is likely to be a result of a damaged photosynthetic apparatus due to prolonged periods of darkness and/or anoxic conditions.

Photochemical vitality of benthic *Microcystis* colonies was lowest during spring, before the onset of the *Microcystis* bloom. Yet, we managed to grow *Microcystis* colonies in the laboratory, even when they were obtained from the sampling sites with lowest vitality. Therefore, throughout the year, benthic *Microcystis* populations were photochemically active and sufficiently vital to serve as an inoculum for the initiation of a *Microcystis* bloom in the lake. Colonies in the shallowest sediments of the lake were still within the euphotic zone and had the highest photochemical vitality. Hence, colonies from the shallow sediments seem physiologically better adapted to inoculate the water column than colonies from deeper sites.

The increase in photochemical efficiency in the pelagic *Microcystis* population in June marked the onset of the *Microcystis* bloom. This was followed a few weeks later by an increased photochemical efficiency of the benthic population. The timing of this increased photochemical efficiency in the sediment did not match improved physical conditions (increasing light and oxygen availability) on the sediment. Oxygen availability at the sediment surface decreased during summer. Improved light conditions during summer would only affect shallower parts of the lake, whereas increases in benthic photochemical efficiency occurred throughout the lake. However, the increased photochemical efficiency on the sediment surface was accompanied by an increased total amount of *Microcystis* in the top layer of sediment (data not shown). We therefore conclude that the increased vitality of benthic *Microcystis* in late summer was probably not a result of improved photochemical physiology but was more likely the result of sedimentation of fresh *Microcystis* colonies from the newly established water population.

Buoyancy of overwintering Microcystis. Many *Microcystis* colonies isolated from the sediment were buoyant. Percentages of buoyant colonies increased rapidly in summer and then gradually decreased during autumn, winter, and spring. Tsujimura et al. (2000) found similar seasonal dynamics in Lake Biwa. However, these authors attributed the sudden increase in buoyancy from July onward to the utilization of carbohydrates for the production of gas vesicles, stimulated by an increasing temperature (Thomas and Walsby 1986). Our results do not support this hypothesis. In the sediment of Lake Volkerak we did not find any indication that carbohydrate content and gas vesicle volume showed a negative correlation. Instead, carbohydrate content and gas vesicle volume showed a highly significant positive correlation.

To determine whether changes in gas vesicle volume and carbohydrate ballast could explain changes in buoyancy of benthic colonies, we calculated net buoyancy as an indication of cell density (Oliver and Walsby 1984). This measure includes carbohydrates, proteins, and gas vesicles. Other cell constituents, such as lipids, are not accounted for because carbohydrates, proteins, and gas vesicles are the major cell constituents determining the internal buoyancy of *Microcystis* cells. The carbohydrate content of pelagic *Microcystis* colonies is generally in the range of 0.1–1.5 carbohydrates per protein (Ibelings et al. 1991b, Visser et al. 1995). In contrast, we found that benthic *Microcystis* colonies in Lake Volkerak had a considerably lower carbohydrate content, in the range of 0.05–0.25 carbohydrates per protein. The gas vesicle volume in the benthic colonies (0.2–1.3 $nL \cdot \mu g^{-1}$ protein) was in a similar range, perhaps slightly lower, than the gas vesicle volume measured in pelagic colonies (0.9–1.2 $nL \cdot \mu g^{-1}$ protein) (Visser et al. 1995). Therefore, in the benthic colonies of Lake Volkerak, buoyancy provided by gas vesicles was higher than ballast provided by carbohydrates and protein together. This is in line with findings of Reynolds et al. (1981), who examined the ultrastructure of overwintering benthic *Microcystis* colonies and noted that the number of glycogen granules (carbohydrates) was low, whereas every benthic cell still contained gas vesicles.

The question remains how benthic colonies can enter the water column. Changes in internal buoyancy seem an unlikely mechanism. The carbohydrate content in benthic *Microcystis* is so low that a further decrease cannot bring about changes in buoyancy. Neither was there a substantial increase in gas vesicle volume in spring. Therefore, recruitment of *Microcystis* colonies was probably not brought about by changes in carbohydrate content and gas vesicle volume.

The other possible recruitment mechanism is resuspension of sediment. It is known that cyanobacteria can sink because of attached ballast material, like clay particles and detritus (Avnimelech et al. 1982, Oliver et al. 1985, Atkins et al. 2001). This mechanism may play a role in Lake Volkerak. Both photochemical vitality and the number of buoyant *Microcystis* colonies in the sediment increased rather quickly in July. These buoyant vital colonies appeared on top of the sediment and only floated after sediment particles had been removed from them, causing us to conclude that these colonies had sedimented recently from the newly established *Microcystis* bloom in the water column. Similarly, Fallon and Brock (1979) and Reynolds and Wiseman (1982) found the highest sedimentation rates during the blooming period of *Microcystis* in the water column. Reynolds and Wiseman (1982) also reported that the colonies they found in the sediment during this period looked healthier than those collected at other times and that they were similar in size and structure to those present in the water column at that time. In contrast, Oliver et al. (1985), Takamura and Yasuno (1988), and Visser et al. (1995) only found high sedimentation rates at the decline of the blooming period (September through November). Such differences in sedimentation patterns of *Microcystis* colonies during summer in different lakes could be a result of differences in the concentration and type of suspended material (Søballe and Threlkeld 1988).

We observed that attached sediment could be removed from benthic *Microcystis* colonies by gentle

washing or resuspension of the colonies in water, a phenomenon that was also observed by Oliver et al. (1985). Intense mixing of the water column may be sufficient to both resuspend the sediment (including benthic *Microcystis*) and remove attached sediment particles, so buoyant colonies can enter the water column again. Similarly, bioturbation by macrofauna may also result in recruitment of benthic *Microcystis* (Ståhl-Delbanco and Hansson 2002). This makes it likely that recruitment is mostly coming from shallow frequently resuspended parts of the lake. Frequent resuspension in shallow parts of the lake would explain why sediments of the shallow parts of Lake Volkerak contain a lower number of colonies per milliliter of sediment than sediments in the deep parts of the lake. It also matches recent finding of Brunberg and Blomqvist (2003), who found a higher recruitment of benthic *Microcystis* in shallow areas of Lake Limmaren than in deeper areas of that lake.

In conclusion, throughout the year there were buoyant photochemically vital *Microcystis* colonies in the sediments of Lake Volkerak, with the most vital colonies surviving in the shallow sediments of the lake. In contrast to pelagic colonies, carbohydrate content did not play a role in buoyancy changes of benthic colonies. Carbohydrate content was so low that it is even questionable whether carbohydrates could act as an energy reserve for overwintering colonies. The buoyancy state of benthic *Microcystis* colonies was mostly a result of gas vesicle volume and attached sediment. We did not observe major internal buoyancy changes in benthic colonies. Recruitment therefore is probably not a result of internal buoyancy changes but most likely a result of resuspension because resuspension can remove attached sediment particles. This suggests that recruitment of benthic *Microcystis* colonies into the water column probably originates from the frequently resuspended shallow parts of the lake and is most intense during storms and periods of active bioturbation.

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