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SHORT COMMUNICATION

Benthic overwintering of *Microcystis* colonies under different environmental conditions

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During periods of rest, the meroplanktonic cyanobacterium Microcystis accumulates and survives well on shallow littoral bottoms, where environmental conditions may favour an early start of growth and recruitment in spring.

The cyanobacterium *Microcystis* is a widely distributed organism, which dominates the phytoplankton community in many nutrient-rich lakes. The life cycle includes both pelagic and benthic stages, of which the benthic stage is regarded mainly as a physiological rest, as the poor light conditions restrict photosynthetic activity. However, no morphological differences are found between pelagic and benthic colonies. Investigations of natural populations indicate that *Microcystis* may grow, or at least sustain, under various environmental conditions (Topachevskiy *et al.*, 1969; Reynolds *et al.*, 1981; Moezelaar and Stahl, 1994; Brunberg, 1995), eventually as participants of a microbial consortium developed within the mucilage of the colonies (Steppe *et al.*, 1996; Worm and Søndergaard, 1998; Brunberg, 1999). The benthic survival of *Microcystis* colonies has frequently been classified as 'overwintering' when studied in temperate climate zones (Preston *et al.*, 1980; Fallon and Brock, 1981), due to the regular planktonic development of the population during summer. However, the benthic biomass may substantially exceed the maximum planktonic biomass in eutrophic lakes (Boström *et al.*, 1989), thus indicating that *Microcystis* colonies are able to survive for longer periods and accumulate at the bottom. Another sign of long-term survival is that viable *Microcystis* colonies have been found in substantial numbers at sediment depths corresponding to several years of age (Boström *et al.*, 1989). Long-term laboratory incubations have shown that colonies are able to restart growth even after extended time periods of 'resting' (Reynolds *et al.*, 1981). Benthic colonies occasionally reinvade the water column and serve as an inoculum for the planktonic population that develops during summer (Preston *et al.*, 1980; Reynolds *et al.*, 1981; Trimbee and Harris, 1984). The amount of recruiting colonies depends on several factors, i.e. the number of colonies accumulated at the bottom, how long these survive in the sediments, and the development of environmental conditions favouring recruitment.

The main purpose of this study was to quantify *Microcystis* on littoral versus profundal sediments after the autumn sedimentation and to assess their survival from biomass changes during the winter season, thus estimating the potential for recruitment next summer. We investigated shallow and deep sites, respectively, of Lake Limmaren, Sweden, a naturally eutrophic lake with high pelagic biomasses of cyanobacteria during summer (Pettersson and Lindqvist, 1991). Surface sediments were collected from a shallow bay (1–2 m) and from the deepest area of the lake (6–8 m). *Microcystis* colonies were identified and the numbers of viable cells estimated (Figure 1). There were clear differences between sites in the abundance of *Microcystis* cells during autumn sedimentation, the shallow bay having higher cell numbers than the deep site. The total number of benthic *Microcystis* cells in the 0–1 cm surface sediments of the shallow bay decreased gradually from 10.2×10^9 cells m^{-2} in September to 2.71×10^9 cells m^{-2} in May (Figure 1a). At the deep site, the initial *Microcystis* number was 7.24×10^9

cells m^{-2} (Figure 1b). The decline at this site seemed to be concentrated in the first part of the time period studied, and a minimum value of 2.77×10^9 cells m^{-2} was already noted in February, followed by 3.68×10^9 and 3.32×10^9 cells m^{-2} in March and May, respectively. Linear regression of *Microcystis* cell numbers on time was used to calculate the rates of decrease. Differences in rates of decrease were tested by calculating the difference in average cell numbers between the sites on each date, and analysing the obtained 'residuals' for significant changes over time, using linear regression. Calculated over the entire time period studied, there were no significant differences ($P > 0.1$) in the rates of decline of *Microcystis* cell numbers between sites. During the ice-covered winter period, the cell numbers of *Microcystis* were reduced by 30 and 24% in the shallow and deep areas, respectively (calculated from the sampling results on 3 December and 16 March) or by 48 and 40% (calculated between 1 December and 1 April from Figure 1). Looking into species composition, *M. wesenbergii* Kom. was dominating both in the pelagic *Microcystis* biomass during late summer and in the surface sediments during winter, while *M. viridis* (A.Br.) Lemm. and *M. aeruginosa* Kütz. were less abundant. 'Empty' colonies, i.e. mucilage without any cells left, were found in all sediment samples, regardless of site and time of the year. They constituted 70–80% of total colonies at the shallow site and 50–60% of the total colonies counted at the deep site. The number of colonies with viable *Microcystis* cells varied between 8.02 and 2.54×10^7 m^{-2} at the shallow site, and between 5.54 and 2.61×10^7 m^{-2} at the deep site. To what extent the abundance on the bottom is related to the abundance in the pelagic zone is not clear, earlier estimates ranging from a majority of the pelagic population (Boström *et al.*, 1989) to a minor part (Takamura and Yasuno, 1988). The maximum abundance of *Microcystis* cells in plankton during 1998, calculated from integrated samples representing the entire water column, was 24×10^9 cells m^{-3}. Multiplying by the lake volume, 27.3 Mm^3, gives a total maximum of 6.50×10^{17} *Microcystis* cells within the lake. A total sedimentation of this *Microcystis* population, disregarding other possible loss processes within the pelagic environment, would result in a biomass of 111×10^9 cells m^{-2} if equally distributed on the sediments (lake area 5.86 km^2). Comparing with the maximum value of benthic *Microcystis* cells in our measurements, the pelagic abundance was 10 times higher, calculated on an areal basis, despite the fact that the sampling stations were situated at two of the most pronounced accumulation areas of the lake, i.e. in a wind-sheltered bay and in the deepest part of the lake. Losses via the outlet are most likely minimal, due to the long turnover time of the water in Lake Limmaren (~6 years). This indicates that the pelagic summer population of *Microcystis* is severely reduced by loss processes within the water column or by rapid decay and decomposition immediately after sedimentation.

In September 1999, material from a declining bloom of cyanobacteria was collected from the shallow bay of Lake Limmaren and kept within the lake for 1 month in a large container allowing exchange with the atmosphere and adjustment to natural variations in temperature, light, etc. In October, *Microcystis* colonies of the suspension were counted and two different experiments were started. In a field experiment (Figure 2), the *Microcystis* colonies were 'overwintered' in beakers at different sites close to the shoreline, experiencing the natural variations in climate at this site, e.g. autumn rains, ice and snow cover, as well as ice-out and snow melting during spring. We found a very poor survival of the *Microcystis* colonies in general (Figure 2). The vial incubated on land was bright green when collected at the beginning of May, but this colour originated from coccoid green algae, while all *Microcystis* colonies were characterized as non-living. The vials incubated at the shoreline and at 0.7 m water depth showed a survival of 6 and 7%, respectively, of the *Microcystis* cells. Another portion of the collected cyanobacterial bloom from September was stored for 3 months (October–December) at a temperature of 4°C in complete darkness, and then used in a laboratory incubation (Figure 3) where the effects of dark versus light conditions were tested. When the experiment was terminated 4 months later (May 2000), the concentration of dissolved oxygen was well below 1 mg l^{-1} in the dark flasks (average saturation 4%). In the illuminated flasks, the water was supersaturated with dissolved oxygen (average 129%). The cell numbers of *Microcystis* had decreased by 42 and 88%, respectively. Hence, *Microcystis* survived significantly better (Student's *t*-test) in darkness than in light.

Summarizing the field sampling results, we could not find any significant differences between survival of *Microcystis* on deep versus shallow bottoms. Higher cell numbers were found in the shallow bay both in September and in May, although the difference was not very large in May. From this, we conclude that there are at least equal amounts of *Microcystis* surviving at the shallow and the deep site; thus, both areas may provide important inocula for planktonic populations. A seasonal study in Lake Biwa, Japan (Tsujimura *et al.*, 2000), also found that *Microcystis* could survive on shallow sediments, although the decline during winter was considerable. In deeper parts (70–90 m) of the lake, where they found maximum abundance of colonies, no decline in abundance was recorded. However, they concluded that *Microcystis* on these deeper sites were trapped in conditions unfavourable for reinvasion of the water column and that shallow sites are also important for inoculation of *Microcystis* in Lake Biwa.

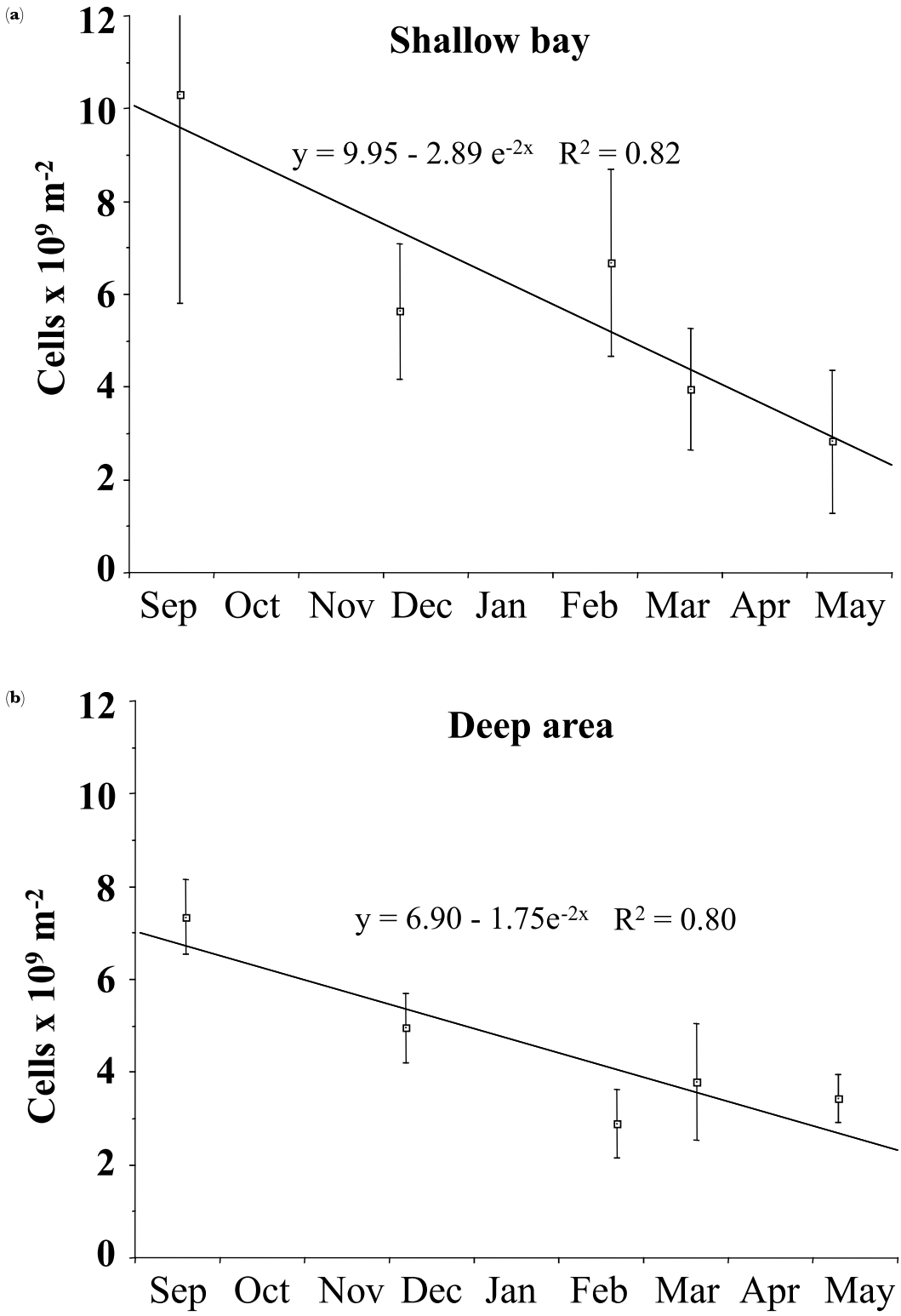

Fig. 1. Biomass of *Microcystis* in surface sediments (0–1 cm) of Lake Limmaren during 1998–1999 in (**a**) a shallow bay and (**b**) a deep area. Sediments were taken with a Willner core sampler from a boat in September (n = 3), from ice in December (n = 10), February (n = 5), March (n = 10) and from a boat in May (n = 10). The surface layer (0–1 cm) was diluted and preserved with 4% formaldehyde in water/tap water (1/1), sedimented for at least 4 h in phytoplankton chambers, and counted in an inverted light and autofluorescence microscope (Leica 090-131.002).

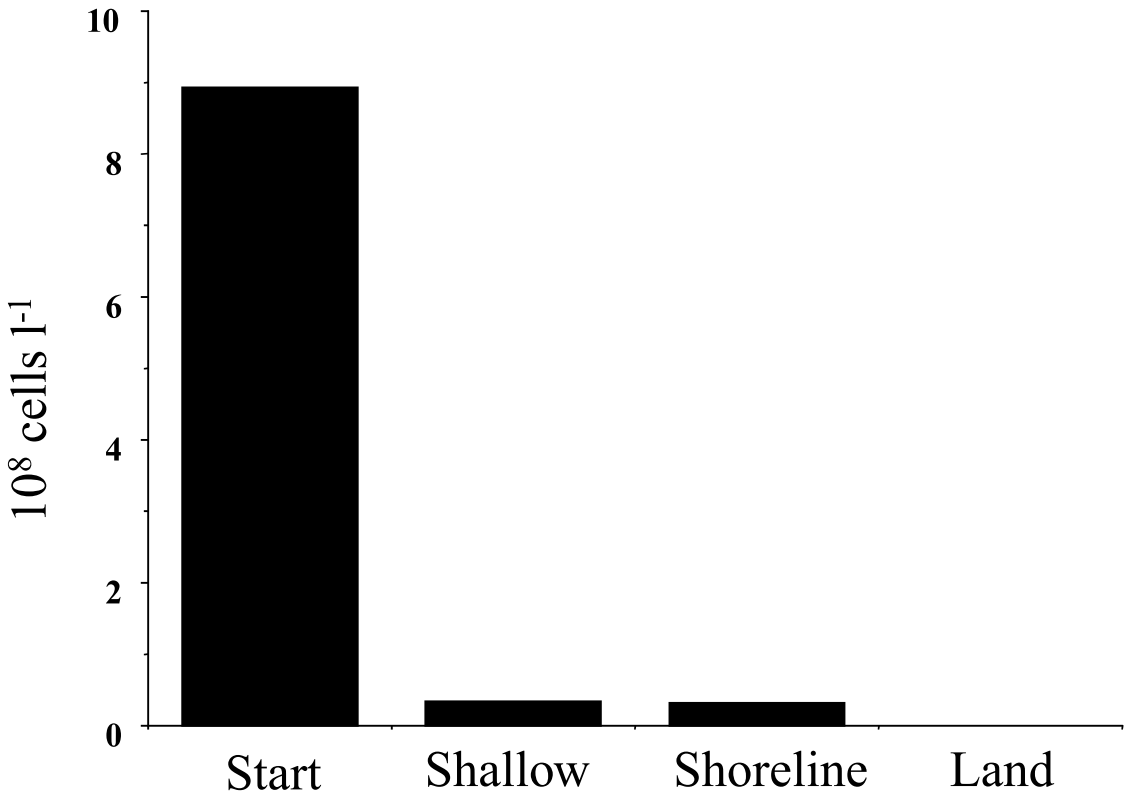

Fig. 2. Survival of *Microcystis* placed in various environmental conditions over the winter season. Subsamples (150 ml) from a declining bloom of cyanobacteria were placed in transparent beakers covered with a 40 μm net and placed (1) at the bottom of the lake at a water depth of 0.7 m, (2) at the shoreline (water depth 5 cm) and (3) on land 10 m from the shoreline (2 m above lake water level). The beaker placed on land had nets at both the top and bottom, in order to allow the sample to follow the natural changes between dry and wet conditions. At the start and termination of the experiment, subsamples were preserved with Lugol's solution and *Microcystis* colonies were counted.

The aim of our study, to investigate the survival of benthic *Microcystis*, originated from our efforts to assess the conditions precedent for reinvasion of colonies as inoculum for planktonic growth next summer. Thus, we were looking for net survival, including all loss processes. One objection against this would be that redistribution of colonies between different parts of the lake might occur. In order to cover the longest possible period free from resuspension events, sampling was performed from the very first ice-cover (3 December) and as close to ice-out as possible (16 March). Another undertaking to minimize errors due to heterogeneous distribution was the large number of replicates (n = 10 at most), taken randomly within the two sampling areas. The largest heterogeneity, both in time and space, was found for *M. wesenbergii*. This more dynamic pattern of distribution implies that these colonies might be close to positive buoyancy and thus more susceptible to resuspension, bioturbation and other processes redistributing the sediment particles, and perhaps also more easily transported back to the water column. The data published from Lake Biwa (Tsujimura *et al.*, 2000) also show that *M. wesenbergii* is more focused to surface sediments than *M. aeruginosa*. However, the authors do not comment whether this may be attributed to differences in buoyancy or decomposition rate.

According to our field experiment, survival of *Microcystis* in very shallow, shoreline and terrestrial environments during winter is very poor. Cyanobacteria are well known from various extreme environments, surviving below-zero temperatures as well as desiccation (Vincent, 2000). The cyanobacterium *Gloeotrichia echinulata* has been found to survive and start growing after overwintering at the shore of the nearby Lake Erken (Forsell, 1998). Earlier investigations have shown that pelagic *Microcystis* populations sampled at different times during the season may have different abilities to meet changes in environmental conditions (Fallon and Brock, 1981; Brunberg, 1995). Accordingly, our experiment was not started until mid-October, in order to keep conditions as close as possible to the natural autumn development. Despite this, *Microcystis* seemed to be less tolerant than *Gloeotrichia* to the harsh winter conditions on the shoreline. The lack of

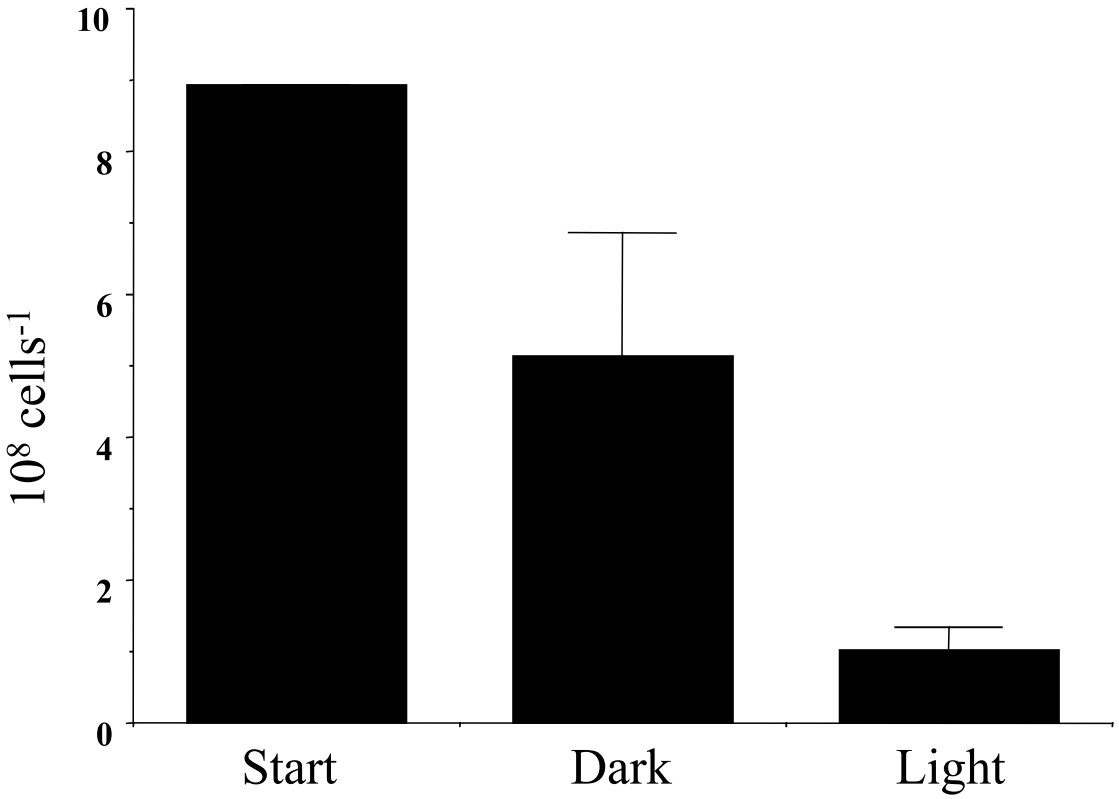

Fig. 3. Long-term survival of *Microcystis* in a laboratory experiment with incubation during dark versus light conditions. The figure shows averages from two sets of five bottles, filled with 250 ml each of a cyanobacterial suspension, closed and kept at 10°C for 4 months. One set (five bottles) was kept in darkness and the other in a 12 h/12 h dark/light cycle (30 μE m^{-2} s^{-1}). On 8 May, concentrations of dissolved oxygen were measured and samples for counting of *Microcystis* were preserved with Lugol's solution.

a morphologically distinguishable resting stage may be one reason for this difference in survival.

In the laboratory incubations, light exposure led to supersaturated oxygen conditions and a very poor survival of *Microcystis*. Dark conditions and low concentrations of dissolved oxygen led to enhanced survival. Applied to natural conditions, this might seem as a disadvantage for *Microcystis* colonies settling in the shallow areas of the lake. However, as long as the lake is covered with ice and snow, the light availability is also severely restricted in shallow areas. Low oxygen conditions are probably also prevailing, even in the uppermost parts of the sediments, as both the water movements and photosynthetic activity are cut off by the ice and snow cover. When spring comes, a shallow position may instead be advantageous for initiation of growth and recruitment, as these areas are reached earlier by enhanced temperature and light penetration, which together with anoxic conditions have been identified as important for triggering growth of benthic *Microcystis* colonies (Cáceres and Reynolds, 1984). The 1% level for light penetration in Lake Limmaren is fairly constant at 3 m depth during the summer period (June–September; A. K. Brunberg, unpublished). The bottom area shallower than 3 m constitutes 25% of the total lake area. Assuming that our shallow sampling site is representative for these bottoms, the total amount of *Microcystis* in the 0–1 cm surface sediments in spring would be 3.75×10^{13} colonies or 4.42×10^{15} cells. This is probably an overestimation, as the sampling station was situated in a wind-sheltered bay, promoting accumulation of sedimenting particles. Nevertheless, substantial amounts of *Microcystis* colonies may be settled, and also survive, at shallow sediments. Owing to the cold climate with ice-covered lakes in northern countries, the otherwise unexpected combination (Trimbee and Harris, 1984) of anoxia during winter, followed by favourable light conditions and enhanced temperature in spring, may occur in shallow areas. Hence, although the most extreme conditions close to the shoreline may be less favourable, settling of *Microcystis* in shallow areas should not be disregarded in studies of benthic overwintering and population dynamics of these meroplanktonic cyanobacteria.

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