Influence of light, sediment mixing, temperature and duration of the benthic life phase on the benthic recruitment of Microcystis

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The benthic recruitment of Microcystis was assessed in vitro in order (i) to compare the relative influence of the main abiotic factors, and (ii) to investigate the impact of the duration of the benthic life stage. Different benthic populations sampled in a 45 m deep artificial lake, with benthic life phases ranging from a few weeks to almost 3 years, were used to test the impact of three temperatures (4, 7 and 17°C), the absence or presence of light and sediment mixing on Microcystis recruitment. In this study, sediment mixing was the only physical factor found to promote recruitment, indicating that passive resuspension plays a much more important role in the recruitment of Microcystis than light and temperature. Moreover, recruitment occurred from all benthic populations, including one that was nearly 3 years old. No difference in the proportion of recruited cells was observed before and after the usual overwintering period, suggesting that the annual benthic overwintering does not impair the ability of Microcystis to inoculate the water column in the following spring. However, in the oldest population, the proportions of recruited cells were lower under all the experimental conditions tested, indicating a decrease in the ability of older benthic populations of Microcystis to contribute to the recruitment process.

KEYWORDS: benthic recruitment; cyanobacteria; Microcystis; sediment mixing; long-term persistence

INTRODUCTION

Despite efforts to contain and treat nutrient inputs, eutrophication of aquatic ecosystems, and the cyanobacterial proliferations frequently associated with it, is still of major concern in aquatic ecology. The management of these cyanobacterial blooms has health implications if the species proliferating is able to synthesize toxins that endanger human and/or animal health. Control is a complex matter, since most cyanobacteria have an annual development cycle in which planktonic growth and a benthic resting stage alternate. This is the true of the toxic cyanobacterium Microcystis, which is the one most frequently encountered in freshwater ecosystems. After proliferating in the water column, where this potentially toxic cyanobacterium is able to completely dominate the phytoplankton, it is found in abundance...
on the sediment surface. Throughout the winter phase, part of its population overwinters vegetatively in the sediment, retaining its cellular and colonial ultrastructure, some cellular activity and also its microcystin content (Tsujimura et al., 2000; Latour et al., 2004, 2007; Misson et al., 2011).

A part of these viable colonies reinvades the pelagic zone in spring (Reynolds et al., 1981; Brunberg and Blomqvist, 2003; Verspagen et al., 2005), a process known as the benthic recruitment process. Relatively little is known about this process, and the factors determining it remain particularly unclear: recruitment of *Microcystis* from the sediment is regulated by both active processes, triggered by internal changes in buoyancy, and passive processes, depending on sediment mixing. According to the literature, the main factors that trigger recruitment are an increase in temperature and low light intensity reaching the sediment (Reynolds et al., 1981; Trimbee and Harris, 1984; Tsujimura et al., 2000; Brunberg and Blomqvist, 2003; Tan et al., 2008). Anoxia and nutrient inputs are secondary factors which also demonstrate some effect on *Microcystis* recruitment (Caceres and Reynolds, 1984; Stahl-Delbanco et al., 2003; Tan et al., 2008). These environmental parameters, and in particular the temperature increase and the low light level, appear to stimulate the resumption of metabolic activity, so that the colonies regain buoyancy and actively return to the water column. Other authors suggest that a passive process, resulting from the resuspension of sedimented colonies by turbulent mixing or bioturbation, is of particular importance in the recruitment of benthic *Microcystis* (Stahl-Delbanco and Hansson, 2002; Verspagen et al., 2004, 2005). The exact contributions of each of these processes have yet to be determined, and a direct comparison of the main factors thought to trigger the recruitment of *Microcystis* could provide some information.

It has also been shown that benthic *Microcystis* can survive for several years in the sediment, located a few centimeters beneath the sediment surface, without an annual return to the planktonic phase, but nevertheless preserving all they need to return to growth subsequently (Latour et al., 2007). Even though *Microcystis* is able to survive in the sediment for longer periods than the annual resting stage of a few months, we may wonder whether it is still able to take part in the recruitment process after spending several years trapped in the sediment. Indeed, artificial reservoirs such as the Grangent reservoir (Massif Central, France) are exposed to major floods and/or anthropogenic management of the water level that could promote sediment resuspension and that of cyanobacteria buried for several years in the first few centimeters of sediment. From the perspective of water management in such ecosystems, it seems to be essential to consider the potential recruitment of both newly sedimented *Microcystis* populations and populations that have been sedimented for several years.

As the recruitment process constitutes an important phase that influences the quantity and quality of the planktonic cyanobacterial inoculum, and thus contributes to the pelagic bloom, we conducted an experimental study under controlled conditions to find out more about what is decisive in the *Microcystis* recruitment. The aims of our work were therefore (i) to investigate and compare in the same experiment the relative influences of the main factors thought to drive *Microcystis* benthic recruitment, namely temperature, light level and sediment mixing, (ii) to find out whether a prolonged phase of benthic life could affect the recruitment rate. We therefore compared the recruitment of two benthic populations of *Microcystis* sampled in the Grangent reservoir in sediment layers of different ages: a newly sedimented population, the same population at the end of the overwintering phase, and a third population which had sedimented almost 3 years ago.

**MATERIAL AND METHODS**

**Study site and sampling**

The Grangent reservoir, an artificial lake which was dammed in 1957, is located at an altitude of 420 m in the south-east of the Massif Central in France, ~15 km west of Saint-Étienne (45°27’N, 4°15’E). The lake, which is confined to the narrow valley of the upstream Loire River, provides a major water reserve (57.4 × 10⁶ m³). It measures 21 km in length, has a maximum depth of 50 m near the dam wall and a surface area of 3.65 km². Its main functions are power generation and irrigation of the Forez plain. Stream inflows are mainly restricted to winter, with an annual average flow of 41 m³ s⁻¹. A great deal of tourism has also developed, including the Saint-Victor-sur-Loire nautical center.

Two sampling campaigns were carried out in the deepest part of the Grangent reservoir (45 m depth): (i) the first in November 2005, when three 50 cm sediment cores were collected, and the first 2 cm corresponding to the surface layer were sampled and used to investigate the recently sedimented cyanobacterial population of the year (within the last 3 months), (ii) the second in May 2006, when three 2 m long sediment cores were collected. The dating of the sediment, previously performed using radioactive isotopes (Latour et al., 2007), allowed us to collect two *Microcystis* benthic populations by sampling sedimentary layers containing *Microcystis*.
colonies originating from the blooms of either 2003 (that had remained in the sediment for almost three years) or 2005 (preserved in the sediment over just one winter). During both campaigns, the cores were brought back to the lab, opened immediately and the sediment samples were stored at 4°C in the dark for a few days, until the experiment began.

Fifty liters of surface water was also sampled, filtered on 1.2 µm glass fiber filters (GF/C filters; Millipore, Billerica, MA, USA) and then stored at 4°C in darkness until processed.

**Experimental settings and in vitro sampling**

Two experiments were conducted.

The first concerned the benthic population originating from the bloom of 2005, which was sampled in November 2005. The first 2 cm of the surface layers of each core, containing benthic *Microcystis*, was pooled and homogenized before distributing into glass containers. Fifteen milliliters of this homogenized sediment was placed in 40 mL glass containers (cylinders, basal area of 6.2 cm²), and 20 mL of filtered lake water was added. These experimental devices were exposed in triplicate to three different temperatures [4°C to represent the temperature found in spring at the bottom of the epilimnion], with or without light [10 µE m⁻² s⁻¹], which corresponds to low light intensity observed in the littoral area of the Grangent reservoir and which is similar to the light conditions used in other recruitment experiments [Stahl-Delbanco and Hansson, 2002; Rengefors et al., 2004; Schöne et al., 2010], and with or without mechanical mixing of the sediment (once a day for 1 min, by hand). The experiment lasted 8 days, and water samples were collected from each container on days 1, 3, 6 and 8. At each sampling date and for each container, 18 mL of the supernatant water was removed, leaving the bottom 2 mL so as to collect only buoyant *Microcystis* that had just been recruited. This also allowed us to exclude possible accidental sediment mixing during sampling. The containers were then refilled with 18 mL of filtered lake water, which was added very carefully to avoid mixing the sediment when this was not appropriate. Mechanical mixing (when appropriate) was performed by hand, immediately after changing the water by up-ending each container several times until all the sediment was suspended in the water.

The second experiment was conducted using the samples collected in May 2006. Two benthic populations of *Microcystis* originating from the blooms of 2005 (the same population as in the first experiment, but which had spent a further 6 months in the sediment) and of 2003 were collected, pooled by year and homogenized before being distributed into glass containers. The procedure described above for the first experiment was used again. However, the highest temperature of 17°C was not used here. The experiment lasted 14 days, and the sampling days were as follows: days 1, 2, 5, 7, 9, 12 and 14.

**Cyanobacterial abundance and recruitment rate**

Every supernatant containing recruited cyanobacteria was fixed in 1% glutaraldehyde. Rapid disruption (<1 min) of the colonial structure was produced by low-power ultrasonic vibration (20 kHz) [Reynolds and Jaworski, 1978; Latour et al., 2007]. Isolated cells were then counted in triplicate under a microscope (Carl Zeiss, Oberkochen, Germany) at ×400 magnification using a Thoma counting chamber (Dominique Dutscher SAS, Brumath, France). The basal area of the microcosm and the time that had elapsed since the last sampling were then used to calculate a recruitment rate per day and per square meter.

Since the quantity of cells recruited depends on the initial abundance of benthic cells and in order to compare recruitment between the different populations, we calculated the percentage of the initial benthic stock that had been recruited over the whole experiment, for each benthic population. To do this, the initial benthic cyanobacteria were isolated from the sediments using the method of Verspagen et al. (Verspagen et al., 2004). Four milliliters of sediment was diluted 10-fold, and silica solution was added [30% Ludox TM50 (Sigma-Aldrich, St Louis, MO, USA), 70% diluted sediments]. The mixture was then centrifuged (centrifuge 5804 R; Eppendorf, Hamburg, Germany) at 400 g for 20 min. One milliliter of every supernatant containing cyanobacteria was sonicated to dissociate the colonies, as previously described. Isolated cells were then counted under a microscope (Carl Zeiss, Oberkochen, Germany) at ×400 magnification with a Thoma counting chamber (Dominique Dutscher SAS, Brumath, France).

**Statistical analysis**

All the statistical analyses were performed with XLStat 2010 (2009.4.05 version, Addinsoft, Paris, France). The level of significance was set at 5%. Differences between the experimental conditions over time were tested by
RESULTS

Recruitment of newly sedimented Microcystis

During the first experiment, the monitoring of the recruitment rate revealed variations depending on both the time and the conditions tested. Indeed, it appeared that for all the conditions tested, the recruitment rate was highest at the first sampling date (Fig. 1). Thereafter, the recruitment rate was very low under all conditions. In spite of their similar recruitment kinetics, statistical analysis highlighted significant differences between the different conditions. Indeed, recruitment was significantly higher under mixing conditions on the first day of the experiment, with average recruitment rates of $7.3 \times 10^6$ cells day$^{-1}$ m$^{-2}$ with mixing, compared with $2.0 \times 10^6$ cells day$^{-1}$ m$^{-2}$ without mixing (repeated measures ANOVA, Tukey’s post hoc test, $P < 0.0001$). When the experiment as a whole is considered, the mean proportion of recruited cells was 4.1% with mixing compared to 1.4% without mixing (Table I).

Concerning the other parameters tested, i.e. temperature and the presence or absence of light, we recorded very similar proportions of cells recruited during the whole experiment, ranging from 2.3 to 3.3%, without any significant differences (Table I).

Recruitment of Microcystis which had spent one winter in the sediment

The results of this experiment were very similar to those of the first one. Both experiments dealt with the same benthic population, but after different durations of benthic life. The percentage of benthic cells recruited was very similar (Mann–Whitney test, $P = 0.3$), as shown in Table I. The second experiment revealed a similar kinetic pattern to that observed for the same benthic population just after it had sedimented (in the first experiment described above). A maximum recruitment rate was measured on the first day, followed by very low recruitment rates until the end of the experiment (Fig. 2). Similarly, sediment mixing was the only condition that significantly promoted benthic recruitment, whatever the light or temperature conditions, and for both the maximum recruitment rate on the first day (Fig. 2; repeated measures ANOVA, Tukey’s post hoc test, $P = 0.016$) and the total proportion of benthic cells recruited during the entire experiment (Table I; ANOVA, Tukey’s post hoc test, $P < 0.0001$). Moreover, the percentages of recruited cells recorded with or without mixing were in the same range in both experiments (Table I). The other factors, namely the temperature and the presence or absence of light, did not seem to impact significantly on either the recruitment rates or the proportion of benthic cells recruited during the entire experiment.

### Table I: Initial abundance and proportions of cells recruited during the overall experiments for the different benthic populations investigated

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Newly sedimented</th>
<th>After one winter</th>
<th>After 3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benthic initial cells (cells mL$^{-1}$)</td>
<td>$1.02 \times 10^6$</td>
<td>$1.10 \times 10^6$</td>
<td>$4.95 \times 10^5$</td>
</tr>
<tr>
<td>With mixing (%)</td>
<td>$0.52 \pm 0.19**$</td>
<td>$1.04 \pm 0.18*$</td>
<td>$0.14 \pm 0.10*$</td>
</tr>
<tr>
<td>Without mixing (%)</td>
<td>$0.17 \pm 0.08$</td>
<td>$0.29 \pm 0.25$</td>
<td>$0.04 \pm 0.04$</td>
</tr>
<tr>
<td>Light (%)</td>
<td>$0.41 \pm 0.28$</td>
<td>$0.75 \pm 0.51$</td>
<td>$0.10 \pm 0.13$</td>
</tr>
<tr>
<td>Dark (%)</td>
<td>$0.29 \pm 0.16$</td>
<td>$0.58 \pm 0.36$</td>
<td>$0.07 \pm 0.03$</td>
</tr>
<tr>
<td>4°C (%)</td>
<td>$0.41 \pm 0.31$</td>
<td>$0.77 \pm 0.37$</td>
<td>$0.11 \pm 0.11$</td>
</tr>
<tr>
<td>7°C (%)</td>
<td>$0.34 \pm 0.12$</td>
<td>$0.56 \pm 0.50$</td>
<td>$0.06 \pm 0.07$</td>
</tr>
<tr>
<td>17°C (%)</td>
<td>$0.29 \pm 0.23$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean (%)</td>
<td>$0.35 \pm 0.23$</td>
<td>$0.67 \pm 0.44$</td>
<td>$0.09 \pm 0.09$</td>
</tr>
</tbody>
</table>

Mean values (± SD) of microcosms replicates. Asterisks represent a significant difference between the values corresponding to the different conditions for one parameter within a benthic population (* for $P$-value <0.01, ** for $P$-value <0.001).
Recruitment of Microcystis which had spent almost 3 years in the sediment

We observed very low recruitment rates from the oldest benthic population, with a quite different kinetic pattern. Indeed, higher values were once more measured on the first day of the experiment (with an average recruitment of $3.5 \times 10^7$ cells day$^{-1}$ m$^{-2}$) followed by very low recruitment rates, but on the last day, high values in the same range as on the first day were recorded (Fig. 2). We found that the average percentage of benthic cells recruited during the whole experiment from this oldest population was significantly lower (Mann–Whitney test, $P = 0.001$), with only 1.2%, which is 3.5 times lower than in the population originating from the bloom of 2005 sampled after it had overwintered. Similarly to what had been observed in the two previous experiments, sediment mixing appeared to be the only condition that significantly promoted recruitment, independent of temperature and light (Table I).

**DISCUSSION**

In this study, we investigated the recruitment process of *Microcystis* under laboratory conditions, for the first time, testing the relative influence of temperature, light and resuspension of sediment in the same experiment. Another original aspect of our work was that we looked at the effect of these abiotic parameters on benthic *Microcystis* populations of different ages, i.e. which had been trapped in the sediment for periods ranging from a few weeks to nearly 3 years. Previous studies of the recruitment process dealt with cyanobacteria located in the first few centimeters just below the sediment surface, which corresponded only to the bloom of the current year (Tsujimura et al., 2000; Verspagen et al., 2004, 2005; Ihle et al., 2005; Schöne et al., 2010). The factors involved in the recruitment of populations of different ages had never previously been compared, and our data could provide interesting information about the mechanisms regulating this process. Knowing that prolonged survival in the sediments induces a loss of viability (Latour et al., 2007), our approach allowed us to find out whether recruitment is mainly triggered by passive mechanisms, as suggested by Verspagen et al. (Verspagen et al., 2004), whether it is mainly triggered by active processes or whether it results from a combination of both active and passive mechanisms.

**Influence of residence time on the benthic recruitment of Microcystis**

The first finding of our study is that benthic *Microcystis* colonies buried in the sediment for almost 3 years are still able to contribute to the recruitment process. Although the recruitment rate of this oldest population was low, it does confirm the long-term resistance of these organisms confronted by unfavorable conditions (darkness, low

![Fig. 2. Recruitment dynamics observed at different temperatures, and with light or in darkness, in a population that had survived one winter in the sediment (A and B), or a population that had survived for 2.5 years in the sediment (C and D). (A and C) With sediment mixing. (B and D) Without sediment mixing. Values are mean (+ SD) recruitment rates of microcosm replicates.](http://plankt.oxfordjournals.org/)}
temperature and anoxic conditions), as previously suggested by the work of Latour et al. (Latour et al., 2007). This finding is of particular interest for the management of water resources, and suggests that further consideration should be given to limiting the resuspension of sediment containing overwintering cyanobacteria, so as to avoid promoting their inoculation of the water column. This long-term survival provides a certain ecological plasticity to *Microcystis*, allowing it to adapt to varied or changing environments. This ability could explain its occurrence and persistence in many freshwater systems.

Comparison of the proportions of cells recruited from the youngest population before and after its first overwintering period did not reveal any difference. This seems to indicate that the ability of *Microcystis* to inoculate the water column is well conserved over a period of a few months, corresponding to the benthic overwintering phase of its life cycle, without any loss of recruitment. On the other hand, the population trapped in the sediment for almost 3 years contained a far lower proportion of recruited cells, indicating that its ability to participate in the benthic recruitment process was reduced. The origin of this difference remains to be determined, but the decrease in viability after increasing durations of benthic life recorded by Latour et al. (Latour et al., 2007) might explain our observations.

**Relative influence of the three abiotic factors tested on the benthic recruitment of Microcystis**

In this study, the highest recruitment rates were obtained under the same experimental conditions whatever the age of the population. Indeed, our results clearly showed that resuspension of sediment was the main abiotic factor enhancing recruitment. Sediment resuspension might allow *Microcystis* buried in the first few centimeters of sediment to be recruited, whereas in the absence of sediment mixing, only *Microcystis* lying on the sediment surface can participate in the benthic recruitment. This could partly explain why the recruitment of *Microcystis* is significantly promoted by sediment resuspension. Moreover, the work of Ihle et al. (Ihle et al., 2005) also supports the idea that sediment resuspension could influence the recruitment process, although these authors suggest that this occurred indirectly. What they suggested is that wind-induced resuspension of sediment could displace *Microcystis* colonies from deep to littoral areas, thus bringing them into contact with abiotic factors that are directly involved in recruitment, namely higher temperatures and moderate light conditions. In the same way, Schöne et al. (Schöne et al., 2010) have shown that light and sediment resuspension have cumulative effects in promoting the recruitment of *Microcystis*. These authors concluded that sediment mixing facilitates *Microcystis* recruitment rather than triggering it. However, when parameters potentially involved in recruitment are disentangled and tested separately, as in our experiment, it appears that sediment resuspension could in fact promote the recruitment of benthic *Microcystis*, without any concomitant increase in light or temperature, as has also been suggested by other authors (Stahl-Delbano and Hansson, 2002; Verspagen et al., 2004; Misson et al., 2011). Under natural conditions, sediment mixing could correspond to wind-induced resuspension during the spring overturn when the water column is unstratified, and/or to resuspension brought about by bioturbation (Stahl-Delbano et al., 2003; Rengefors et al., 2004; Verspagen et al., 2004). Indeed, the resuspension of sediment can lead to the detachment of sediment particles, thus increasing the buoyancy of benthic *Microcystis* colonies, and triggering their recruitment (Verspagen et al., 2004).

On the other hand, in all the populations tested in our experiment, light and higher temperature did not demonstrate any significant effect on recruitment, in contrast to other studies of the benthic recruitment of *Microcystis* (Tsujimura et al., 2000; Rengefors et al., 2004; Ihle et al., 2005; Verspagen et al., 2005; Schöne et al., 2010). These factors were previously thought to improve both the benthic survival and the benthic recruitment of *Microcystis*, and, as a consequence, to make this benthic recruitment more important in shallow littoral zones, where the light illuminates the sediment and where the temperature can be quite high (Brunberg and Blomqvist, 2002, 2003; Rengefors et al., 2004). Our results suggest that the factors involved in the recruitment of *Microcystis* sedimanted in deep zones (45 m in this experiment) may differ from those involved in shallow zones.

Finally, higher light levels and above all of increased temperature were previously thought to be responsible for the resumption of the metabolic activity of the benthic cells in spring, resulting in an active lightening of the cells allowing them to migrate back into the water column (Reynolds et al., 1981; Trimbee and Harris, 1984; Visser et al., 1995; Rengefors et al., 2004). The fact that none of these parameters influenced the recruitment of *Microcystis* in our study tends to negate the possibility that active processes are involved in *Microcystis* recruitment. On the other hand, if *Microcystis* benthic recruitment were a simple response to passive resuspension mechanisms, we would not have observed any differences between populations of different ages, or any recruitment without sediment mixing. Thus, from this study, it appears that the recruitment process is
greatly enhanced by passive resuspension of sediment, but that it also relies on an unidentified metabolic process. *Microcystis* benthic recruitment may, therefore, result from a combination of both active and passive processes, rather than of only one of them, as previously suggested (Visser et al., 1995; Verspagen et al., 2004).

**CONCLUSION**

In short, benthic populations of *Microcystis* settled in a deep zone of the Grangent reservoir are able to participate to the recruitment process after a wide range of benthic life durations. While an overwintering lasting a few months did not seem to affect this recruitment ability, a benthic life phase lasting several years resulted in this process. In this experiment, sediment resuspension. On the basis of these results, we propose that the benthic recruitment of *Microcystis* in deep and dark zones is triggered, firstly, by passive mechanisms such as sediment mixing and, secondly, by active mechanisms related to the physiological state of the cells. Until now, the benthic recruitment of *Microcystis* was thought to take place mainly in littoral areas. This study shows that recruitment can also take place in deep zones, even from old populations, and this implies that we need to reconsider the benthic recruitment process in deep areas.

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**REFERENCES**


