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Abstract

Sampling cyanobacteria in freshwater ecosystems is a crucial aspect of monitoring programs in both basic and applied research. Despite this, few papers have dealt with this aspect, and a high proportion of cyanobacteria monitoring programs are still based on monthly or twice-monthly water sampling, usually performed at a single location. In this study, we conducted high frequency spatial and temporal water sampling in a small eutrophic shallow lake that experiences cyanobacterial blooms every year. We demonstrate that the spatial and temporal aspects of the sampling strategy had a considerable impact on the findings of cyanobacteria monitoring in this lake. In particular, two peaks of *Aphanizomenon flos-aquae* cell abundances were usually not picked up by the various temporal sampling strategies tested. In contrast, sampling once a month was sufficient to provide a good overall estimation of the population dynamics of *Microcystis aeruginosa*. The spatial frequency of sampling was also important, and the choice in the location of the sampling points around the lake was very important if only two or three sampling points were used. When four or five sampling points were used, this reduced the impact of the choice of the location of the sampling points, and allowed to obtain fairly similar results than when six sampling points were used. These findings demonstrate the importance of the sampling strategy in cyanobacteria monitoring, and the fact that it is impossible to propose a single universal sampling strategy that is appropriate for all freshwater ecosystems and also for all cyanobacteria.

Keywords: sampling strategy, cyanobacteria, spatiotemporal dynamic, *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*

1 Introduction

Due to eutrophication and, to a lesser extent, to climatic changes (Markensten et al., 2010; Paerl and Huisman, 2009) cyanobacterial blooms seem to be increasing in freshwater ecosystems worldwide. These blooms severely disrupt the functioning of these ecosystems and potential water use. Furthermore, many cyanobacterial species are able to produce a variety of toxic metabolites, which can be harmful to both human (Kuiper-Goodman et al., 1999) and animal (Codd et al., 2005) health. For these reasons, numerous attempts have been made in the last 20 years to elucidate the factors that control cyanobacterial blooms and toxin production, and thus to make it possible to evaluate better the health risks associated with bloom events. From all these studies, it is clear that the spatial distribution of cyanobacteria in freshwater ecosystems can display marked horizontal and vertical variations (Porat et al., 2001; Welker et al., 2003). Moreover, by means of a real time PCR analysis of a gene involved in the biosynthesis of microcystins we have shown that considerable fluctuations can also occur in the proportions of potentially microcystin-producing and non-producing cells during the course of *Microcystis aeruginosa* blooms (Briand et al., 2009). Similar results have been found for various different *M. aeruginosa* populations located in the same geographic area (Sabart et al., 2009), which makes it difficult to manage the health risks associated with these events.

All these studies indicate that the sampling strategy used for monitoring cyanobacteria is a critical aspect, both in basic research on cyanobacteria, (e.g. investigation of the factors and processes involved in the development of the blooms), and in applied research, (e.g. implementing monitoring programs of these microorganisms in freshwater ecosystems used to provide drinking water or for recreational activities). In recent years, new tools have been tested with the intention of improving cyanobacterial sampling, for example, remote sensing

reconnaissance to determine the horizontal distribution of cyanobacteria in freshwater ecosystems (Hunter et al., 2009), or spectrofluorometric probes to reveal the vertical distribution of these cyanobacteria in the water column (Leboulanger et al., 2002). Moreover, these spectrofluorometric probes and other sensors have now been integrated into buoys, to provide real-time monitoring of cyanobacteria in freshwater ecosystems (Le Vu et al., in press).

However, despite the great potential interest of these tools, their cost will remain prohibitive for their routine use in the foreseeable future, and most of the monitoring programs worldwide for the survey of cyanobacteria will continue to be based on more conventional methods for some years to come. Taking discrete samples of various volumes of water taken from the shoreline of ecosystems is probably the method one most often used in studies. Unfortunately, as a result of spatial and temporal differences in the distribution of cyanobacteria, this approach can often provide a very poor estimation of cyanobacterial abundance and, consequently, of the associated health risk. We therefore need to devise simple sampling strategies for the low cost monitoring of cyanobacteria in shallow lakes. In an attempt to do this, we performed intense spatiotemporal monitoring of cyanobacteria in a shallow lake known to experience cyanobacterial blooms every year.

2 Materials & Methods

2.1 Study site

This study was performed in a shallow lake named Place (0.08 km^2, 2.5 m max depth, 45°43'N, 4°14'E) located in the plain of Forez (Central France), (Fig. 1). This lake is used for extensive fish production and its trophic status is eutrophic to hypereutrophic (OCDE, 1982). *Microcystis aeruginosa* blooms occur every summer.

2.2 Data acquisition

2.2.1 Sampling strategy and cell counting

In order to assess the variations in the horizontal distribution of cyanobacteria in this pond, we monitored six sampling points located around the lake at one meter from the shore (V1-V6; Fig. 1). The water depth in each of these sampling points was around 1 meter. Samples were taken every two days, between 09:00 and 10:00 a.m., from early June 2008 to early October 2008. The first 40-centimeters of the water column were sampled using a watersampler (Uwitech, Austria). This water sample was shacked and then divided into two 1-L bottles, one liter being stored at room temperature with Lugol's iodine solution, and the other at 4°C.

In order to evaluate the vertical distribution of cyanobacteria in the water column, we performed a 22-hour survey (from 4:30 p.m. August 4, 2009 to 2:30 p.m. August 5, 2009), of the variations of cyanobacterial biomass at five sampling points (A-E; Fig. 1) using a BBE Algaetorch (Moldaenke, Germany). This torch is based on the same principle as the BBE spectrofluorometric probe (Beutler et al., 2002), but provides only an estimation of the concentrations of cyanobacteria and total chlorophyll in water. Every hour, the torch was immersed to a depth of 20 centimeters at the five sampling points, and triplicate measurements were performed in each point.

The cyanobacterial cell concentrations were estimated using a Nageotte cell and an optical microscope, as described in Brient et al. (2008). For each rectangular area, we counted at least 400 cells of each cyanobacterial species.

2.2.2 Meteorological data

The speed and direction of wind during our study were obtained from the Metéo France meteorological station at St Etienne-Bouthéon (4°18'E – 45°32'N). The wind direction rose

for this station is given in Supplemental Figure 1, and shows that the two dominant wind directions were NW and SE. The direction of winds blowing from 240° – 60° was classified as NW, and that of winds blowing from 60° – 240° as SE.

2.3 Data analysis

The spatial distribution of cyanobacteria in the lake was represented using Surfer (v. 7.0, Golden Software Inc.), and statistical analyses (Wilcoxon test, Spearman correlation) were performed using the R package version 2.10 (R development core Team, 2010).

3 Results

3.1 Change over time in the population dynamics of the two dominant cyanobacterial species

Two cyanobacterial species, *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*, dominated the phytoplankton community during the summer of 2008. The population dynamics of these two species displayed very contrasting patterns (Fig. 2). The population dynamics of *Microcystis aeruginosa* was characterized by a steady increase in the cell abundance from June to August, apart from a brief dip in the middle of July. The maximum population was reached on August 21 (264,000 cells/mL), and subsequently the cell concentration remained stable until the end of September, and then decreased in October. In contrast, the population dynamics of *Aphanizomenon flos-aquae* were much more chaotic, with the cell abundance reaching two very high and short-lived peaks in July (400,000 cells/mL on July 17, and 560,000 cells/mL on July 23).

3.2 Influence of sampling frequency on the estimation of the population dynamics

Our assessment of the changing population dynamics of the two cyanobacteria were obtained using a very frequent high temporal sampling regime (every two days), which would not be practicable in the context of normal monitoring programs. In order to evaluate the impact of the sampling frequency, we simulated weekly, twice-monthly and monthly sampling frequencies to our data set. The results of these simulations are shown in Fig. 3 and 4. From this figure, we can see that changes in *M. aeruginosa* cell abundance over time would have been fairly accurately estimated at all these sampling frequencies. Moreover, for all sampling frequencies, the quality of the estimation of the *M. aeruginosa* population dynamics was not influenced by choice of the first sampling date (Fig. 3). In contrast, the population dynamics of *A. flos-aquae* would have been badly or even very badly estimated by using weekly, twice-monthly and monthly sampling frequencies (Fig. 4). We would only have detected both *A. flos-aquae* peaks in one of the three trials testing the weekly sampling strategy, and we would never have detected these peaks with twice-monthly and monthly sampling frequencies.

3.3 Evolution of the horizontal distribution of cyanobacteria in the lake during the bloom

As shown in the video (Supplemental Fig. 2), the horizontal distribution of both cyanobacteria displayed marked variations during the course of the study. Moreover, when the spatial distributions of the two species at the same sampling dates were compared, it could be seen that similar or contrasting patterns in the horizontal distribution of *M. aeruginosa* and *A. flos-aquae* cells would have been found, depending on the dates chosen (some examples are provided in Fig. 5).

In order to obtain a better picture of this spatial variability in the cell concentrations of the two species, we estimated the coefficients of variation in the mean abundance for each

153 sampling date and for each species from the results obtained at the six sampling points
154 (Fig. 6). These coefficients were usually higher for *A. flos-aquae* than for *M. aeruginosa*
155 (Wilcoxon test, $p=-3.25.10^{-05}$), suggesting that the horizontal distribution of *A. flos-aquae* was
156 more variable. Finally, there was no correlation (Spearman coefficient) between the
157 coefficient of variation and the mean cell abundance for *Aphanizomenon*, and only a weak
158 correlation was found for *Microcystis* (Spearman coefficient, p=0.003 r =-0.4; Supplemental
159 Fig. 3).
160 In order to find out whether wind speed/direction could account for the variations in
161 the horizontal distribution of cyanobacterial cell abundance in the lake, we recorded in a first
162 time, for each species and for each sampling date, the sampling point (out of the six) at which
163 the highest cell abundance was detected. We then constructed a table in which we related
164 these findings to the wind direction and speed in the five hours before the sampling, knowing
165 that only data with wind speed values ≥2.0 m/s were taken into consideration. For *M.*
166 *aeruginosa*, the detection of the highest cell abundances in the southernmost sampling points
167 V2 and V3 were associated with winds blowing from the NW (Table 1), whereas those at the
168 V1 and V4 sampling points were more surprisingly associated with winds from the SE. High
169 cell abundances in the northern most sampling points V5 and V6 were equally associated with
170 winds from NW and SE. For *A. flos aquae*, the results were more complicated, and no
171 obvious link could be seen between the direction of the wind and the distribution of the
172 cyanobacteria (Table 1). The same analyses were performed by taking into account the wind
173 data one and two days before sampling (instead 5-10 hours before sampling), but no obvious
174 relationship was detected (data not shown).
175
176 **3.4 Influence of the number of sampling points on the estimated cyanobacterial cell**
177 **abundances in the lake**

178 The cyanobacterial cell abundances in the shallow lake were estimated by calculating the
179 average value for the six sampling points (see Fig. 1). In order to determine the number of
180 sampling points required to obtain a good estimation of cyanobacterial cell abundances in the
181 lake, we compared the estimations of cell abundance based on using samples from just one,
182 two, three, four or five sampling points with that based on all six. To do this, we calculated
183 the correlation coefficients (Spearman) between the estimations based on the six sampling
184 points and those based on one to five sampling points for each species (Fig. 7). We considered
185 all possible combinations of points, and the results are classified in the figure on the basis of
186 increasing order of r values within each combination of groups. For both species, we found
187 that the estimations of cell abundances based on only one or two sampling points were
188 generally rather badly correlated with those obtained using all six sampling points. On the
189 other hand, it appeared that good correlations (around or > 0.9) were obtained when at least
190 three sampling points were used, but also that the variations due to the choice of the sampling
191 points was still considerable when only three sampling points were used.
192 In order to find out which combinations of sampling points provided the best results
193 when only two or three sampling points were used, we classified all the possible combinations
194 of points. To do this, we added the rank of each combination of sampling points obtained for
195 the two species (*M. aeruginosa* and *A. flos-aquae*). From Figure 8, we can see that the best
196 estimations obtained using only two or three sampling points were provided by combinations
197 in which the sampling points used were on the shore opposite to the prevailing wind direction
198 over the lake.
199
200 **3.5 Diel variations in the subsurface cyanobacterial biomass in the lake**
201 Finally, we carried out a 24-hour estimation of the variations in the total cyanobacterial
202 biomass in the subsurface water (20 cm depth) of the lake, at five sampling points using the

BBE torch (A-E, see Fig. 1). As shown in Fig. 9, there was a steady fall in the cyanobacterial biomass at all sampling points during the afternoon and evening, and conversely an increase late at night and in the morning. Moreover, the differences in biomass between the five sampling points were smaller during the night than during the day, as was the standard error (three measurements per sampling point). A multidimensional scaling analysis performed on the same values confirmed these observations, with all the night sampling times being grouped together, whereas the sampling times during the day were much more scattered (Data not shown).

4 Discussion

As far as we are aware, this is the first attempt to investigate the influence of sampling strategies on the evaluation of spatial and temporal variations in cyanobacterial abundances in shallow lakes, which constitute unstable and complex ecosystems. These lakes are used by humans for numerous activities, including recreational activities and the supply of drinking water, which makes the monitoring of cyanobacteria in such ecosystems of particular importance, especially as part of the evaluation of the health risks linked to cyanobacterial blooms and their toxins. Sampling strategy is also very important in the context of basic studies, because the quality of sampling has a major impact on the quality of the final results.

In this study, we found that the sampling frequency required to obtain a good estimation of the temporal evolution of the cyanobacterial abundance depends on the blooming species, *M. aeruginosa* or *A. flos-aquae*. Twice-monthly or monthly sampling provided good results for *M. aeruginosa*, whereas this was not often enough to monitor the chaotic population dynamics of *A. flos-aquae*. These findings are in contradiction with the recommendations of Codd et al. (1999), who proposed weekly or a twice-monthly sampling

227 for species that do not form scum (*A. flos-aquae* for example), and more frequent sampling
228 for scum-forming species (such as *M. aeruginosa*), because they can display more rapid
229 changes in concentration. On the other hand, in agreement with these authors, our findings
230 also demonstrate that a reactive approach to cyanobacterial sampling is called for, and that
231 appropriate monitoring programs must be devised for each ecosystem based on what is known
232 about how these systems function. It is clear that sampling only once or twice a month can
233 lead to a very considerable under-estimation of cyanobacterial concentrations, and thus of the
234 health risks associated with the bloom. As a result, a weekly sampling frequency seems to be
235 required for cyanobacteria in small freshwater ecosystems.

236 Our data on the variability of the spatial distribution of cyanobacteria in the lake
237 indicate that at least three sampling points were needed to obtain a good estimation of the
238 abundance, based on a comparison with estimations based on six sampling points. It appeared
239 also that if only three sampling points are used, the choice of the location of these sampling
240 points is very important for the quality of the estimation. The most reliable results were
241 obtained using sampling points located on the opposite side of the lake shore to the main axis
242 of the wind direction, and that adding more sampling points reduces the impact of the choice
243 of the location of the sampling points. Such horizontal variability in the distribution of
244 cyanobacteria has been previously documented for many ecosystems, and also for many
245 cyanobacterial species. For example, in a recent study, Briand et al. (2009) showed that the
246 spatial distribution of *M. aeruginosa* in a large freshwater reservoir on a given date could vary
247 from 7.10^3 cells/mL to 2.10^8 cells/mL, depending on the location of the sampling points in the
248 reservoir. Many factors and processes can influence the horizontal distribution of
249 cyanobacteria in a freshwater ecosystem. Among them, wind and surface currents seem to
250 have the greatest impact. For example, the distribution of *Microcystis* spp. in lake Taihu (see
251 the review paper of Qin et al., 2010) and in Lake Ontario (Hotto et al., 2007) is clearly

252 influenced by both winds and currents. Similarly, Moreno-Ostos et al. (2009) have shown that
253 in a Spanish reservoir currents have a marked effect on the distribution of cyanobacteria, and
254 more globally on the phytoplankton community. In this study, we found that the horizontal
255 distribution of *M. aeruginosa* in the lake was influenced more by wind direction than that of
256 *A. flos-aquae*. This could be explained by the fact that *M. aeruginosa* colonies are located at
257 the surface of the lake at the end of the night, and thus are more subjected to the influence of
258 the wind than *A. flos-aquae* filaments, which are distributed over the entire water column. We
259 found also that two sampling points in the lake (V5 and V6) were less influenced by wind
260 direction that the others. This could be explained by the fact that these two sampling points
261 are protected from the influence of winds blowing from the NW by an embankment located in
262 the North part of the lake. Finally, we also demonstrated that in such a small lake, the impact
263 of wind occurred at the scale of a few hours, in contrast to the previous findings of Welker et
264 al. (2003) showing that the distribution of cyanobacteria was influenced by winds that had
265 been blowing one or two days earlier.
266 In addition to this variability in their horizontal distribution; the vertical distribution of
267 cyanobacteria was also variable. Indeed, during the 24 h for which we used the BBE Torch to
268 monitor the concentrations of cyanobacteria, we found that they were lower in the subsurface
269 layer early at night than during the day. The greatest variations in biomass were recorded
270 during the daytime, both at the scale of one sampling point when the three measurements
271 were compared, and at the scale of the five sampling points monitored during this study.
272 These findings also suggest that several sampling points are necessary to obtain an accurate
273 assessment of the cyanobacterial biomass and that integrated sampling of the first meter of the
274 water column reduces the variability in the estimation of the biomass due to the position of
275 cyanobacteria in the water column. This finding is consistent with data reported by Ahn et al.
276 (2008) showing that an integrated method was the most appropriate sampling method for

277 *Oscillatoria* and *Microcystis* blooms. The causes of these variations in the position of
278 cyanobacteria in the water column have been studied for different species. Several papers
279 (Porat et al., 2001; Rabouille and Salençon, 2005; Rabouille et al., 2005; Visser et al., 2005;
280 Walsby, 1994) have shown that migrations of cyanobacteria in the water column are probably
281 due to the dynamics of the carbon-reserve metabolism, and are strongly influenced by light,
282 temperature, and water mixing.

283 From all these findings, guidelines should be proposed for the monitoring of
284 cyanobacteria in shallow lakes Codd et al. (1999) propose that the choice of sampling
285 frequency and the choice of the number and location of the sampling sites should depend on
286 the purpose of monitoring. For example, sampling near public bathing sites was
287 recommended in freshwater ecosystems used for recreational activities. However, this
288 strategy might generate data relevant only to the immediate vicinity of the bathing area, which
289 do not reflect the global distribution of cyanobacteria in the lake. This is especially true when
290 this distribution is very varied, and could make it difficult to prevent or manage blooms. On
291 the basis of our findings, we proposed a different sampling strategy, which does not depend
292 on the purpose of the monitoring. In order to minimize the cost of the cyanobacteria survey,
293 twice-monthly sampling could be the norm for monitoring, but only if it is complemented by
294 regular visual surveys. Changes in the appearance of the water (e.g. its color) between two
295 successive dates would lead to an immediate increase in the sampling frequency. If it is not
296 possible to carry out this visual survey, only a weekly sampling strategy can ensure that a
297 sporadic cyanobacterial bloom is not missed. With regard to the number of sampling points,
298 we found that at least three sampling points were necessary to obtain an accurate assessment
299 of the cyanobacterial biomass (based on comparison with six sampling points). However,
300 even when three sampling points were used, we found that the choice of the location of the
301 sampling points was also very important (Fig. 8), even though the lake was fairly rectangular

in shape and its perimeter small (around 1.3 Km). These findings suggest that for large lakes and also for lakes with a more complex shape, a large number of sampling points would be necessary to obtain a good estimation of the cyanobacterial abundance. Clearly such sampling is time consuming and expensive. One way to reduce these costs would be to collect a large number of samples and then pool equal volumes of these samples in the same flask, before carrying out a single analysis. In this study, as in most of the monitoring programs performed in small lakes, all samples were taken from the shoreline of the lake. This kind of sampling is suitable for small lakes, but it has been shown that for large lakes (Rogalus and Watzin, 2008) shoreline sampling may miss early warning signs of bloom development, and also lead to the overestimation of the concentration of microcystins, when compared to data obtained from offshore samples. For bigger lakes, therefore, the sampling strategy must include offshore samples.

Different programs worldwide are testing alternatives to water sampling for the monitoring of cyanobacteria in freshwater ecosystems. Two main approaches have been investigated. The first one is based on the use of remote sensing, which has long been in use in marine ecosystems (see for example Bracher et al., 2009). In freshwater ecosystems, the paper of Hunter et al. (2008) has shown the potential of high resolution images for the assessment of the spatial distribution of *M. aeruginosa* in a shallow eutrophic lake. However, the cost of these images and the impact of meteorological conditions are limiting factors for envisaging the use of this tool in routine cyanobacteria monitoring programs. One alternative, lower-cost solution could be based, in the future, on the use of drones to take aerial photographs of freshwater ecosystems, but these tools are still in development. Moreover, they will be only useful for cyanobacterial species that live in the surface water of lakes.

The second way of monitoring of cyanobacteria without sampling the water being investigated is the use of buoys equipped with a variety of sensors, including, for example, a

submersible spectrofluorometer to quantify the biomass of the cyanobacteria. This kind of tool permits the real-time monitoring of phytoplankton, including cyanobacteria, as shown for example in the paper of Le Vu et al. (in press). The two obstacles to their use in routine cyanobacteria monitoring programs are the high price of these systems, and the fact that they only provide estimations for one sampling point. Despite this, the possible use of such buoys, combined with the spatial monitoring of cyanobacteria by water sampling looks very promising for surveying cyanobacteria in freshwater ecosystems.

5 Conclusion

The sampling of cyanobacteria in freshwater ecosystems is a hot topic, in particular in the context of programs for surveying these toxic microorganisms in ecosystems used for the production of drinking water or for recreational activities. Paradoxically, fewer studies deal with the impact of sampling strategies on the estimation of cyanobacterial cell abundances in freshwater ecosystems. In this study, we demonstrate that the choice of sampling strategy can lead to very different estimations of the cell abundances of two blooming species in a shallow lake and also that, depending on the cyanobacterial species involved, different sampling strategies are required to obtain a good estimation of their population dynamics. All these findings suggested that monthly or twice-monthly sampling strategies at just one sampling point do not allow to provide an accurate estimation of cyanobacterial abundances, and thus of the health risks associated with the presence of toxic species in aquatic ecosystems. Moreover, although promising new technologies are being developed for monitoring freshwater cyanobacteria, their cost and some other drawbacks mean that at present they cannot replace water sampling, which will remain the basis of most of these monitoring programs for the foreseeable future.

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444 Table 1: Relationship between wind direction and high cell abundance recorded for
445 *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* at the different sampling points. We
446
447 Fig. 1: Geographical location of the study site in France (left), and of the sampling points in
448 the lake (right)
449
450 Fig. 2: Changes over time of the concentrations of *Microcystis aeruginosa* (top) and
451 *Aphanizomenon flos-aquae* (bottom). These concentrations were estimated by calculating the
452 average cell count for the six samples at each date. The error bars indicate the standard
453 deviation.
454
455 Fig. 3: Simulation of the change over time of *Microcystis aeruginosa* cell concentrations
456 found using a weekly (top), twice-monthly (middle) or monthly sampling frequency (bottom),
457 with lags for the first sampling day of zero days (⁻), 2 days (--) and 4 days (….) comparing to
458 our first sampling day. The gray curve corresponds to the reference data.
459
460 Fig. 4: Simulation of the change over time of the biomass of *Aphanizomenon flos-aquae*
461 found using a weekly (top), twice-monthly (middle), or monthly sampling frequency
462 (bottom), and with lags for the first sampling day of zero days (⁻), 2 days (--) and 4 days (….)
463 comparing to our first sampling day. The gray curve corresponds to the reference data.
464
465 Fig. 5: Spatial distribution of two cyanobacteria, *Microcystis aeruginosa* and *Aphanizomenon*
466 *flos-aquae*, in the lake at four sampling dates (July, 9, 17 & 23; August, 8)
467

Fig. 6: Change over time in the coefficients of variation of the mean cell abundances of *Microcystis aeruginosa* (black triangle) and *Aphanizomenon flos-aquae* (white square) estimated at all six sampling points.

Fig. 7: Spearman correlation values between *Microcystis aeruginosa* (top) and *Aphanizomenon flos aquae* (bottom) cell abundances estimated from the mean values for all six sampling point values, and those estimated from only one, two, three, four or five of these six sampling points.

Fig. 8: Location of the sampling points providing the best (left) and worst (right) estimations of cyanobacterial cell abundances, compared to estimations based on six sampling points. We give the combinations for two (top) and three (bottom) sampling points. The polar plot shows the direction of the maximum daily wind speed during the study. The different line types permit to distinguish the two best or the two worst combinations of sampling points, using two or three sampling points.

Fig. 9: Cyanobacterial biomass in the subsurface water of the lake over a 24-hour period at five sampling points (♦ point A, ■ point B, ▲ point C, × point D, and ◊ point E). The error bars indicate the standard deviation.

Supplemental Figure 1. Distribution of the wind directions at the St-Etienne-Bouthéon meteorological station during this study (June, 13 to October, 10, 2008). The curve and the bars indicate respectively the mean speed and the occurrence per hour of the wind in each direction.

493

494 Supplemental Fig. 2. Evolution of the spatio-temporal distribution of *Microcystis aeruginosa*
495 (left) and *Aphanizomenon flos aquae* (right) in the lake during our study (the scale is the same
496 than in Fig. 5).

497

498 Supplemental Fig. 3. Relationship between the cell concentration and the coefficient of
499 variation for *Microcystis aeruginosa* (top) and *Aphanizomenon flos-aquae* (bottom)

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