Influence of sampling strategies on the monitoring of cyanobacteria in shallow lakes: Lessons from a case study in France

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2 **shallow lakes: lessons from a case study in France**

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9

9 **Abstract**

10 Sampling cyanobacteria in freshwater ecosystems is a crucial aspect of monitoring programs

11 in both basic and applied research. Despite this, few papers have dealt with this aspect, and a

12 high proportion of cyanobacteria monitoring programs are still based on monthly or twice-

13 monthly water sampling, usually performed at a single location. In this study, we conducted

14 high frequency spatial and temporal water sampling in a small eutrophic shallow lake that

15 experiences cyanobacterial blooms every year. We demonstrate that the spatial and temporal

16 aspects of the sampling strategy had a considerable impact on the findings of cyanobacteria

17 monitoring in this lake. In particular, two peaks of *Aphanizomenon flos-aquae* cell

18 abundances were usually not picked up by the various temporal sampling strategies tested. In

19 contrast, sampling once a month was sufficient to provide a good overall estimation of the

20 population dynamics of *Microcystis aeruginosa*. The spatial frequency of sampling was also

21 important, and the choice in the location of the sampling points around the lake was very

22 important if only two or three sampling points were used. When four or five sampling points

23 were used, this reduced the impact of the choice of the location of the sampling points, and

24 allowed to obtain fairly similar results than when six sampling points were used. These

25 findings demonstrate the importance of the sampling strategy in cyanobacteria monitoring,

26 and the fact that it is impossible to propose a single universal sampling strategy that is

27 appropriate for all freshwater ecosystems and also for all cyanobacteria.

28

29 Keywords: sampling strategy, cyanobacteria, spatiotemporal dynamic, *Microcystis*

30 *aeruginosa*, *Aphanizomenon flos-aquae*

31

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

55   reconnaissance to determine the horizontal distribution of cyanobacteria in freshwater

56   ecosystems (Hunter et al., 2009), or spectrofluorometric probes to reveal the vertical

57   distribution of these cyanobacteria in the water column (Leboulanger et al., 2002). Moreover,

58   these spectrofluorometric probes and other sensors have now been integrated into buoys, to

59   provide real-time monitoring of cyanobacteria in freshwater ecosystems (Le Vu et al., in

60   press).

61   However, despite the great potential interest of these tools, their cost will remain

62   prohibitive for their routine use in the foreseeable future, and most of the monitoring

63   programs worldwide for the survey of cyanobacteria will continue to be based on more

64   conventional methods for some years to come. Taking discrete samples of various volumes of

65   water taken from the shoreline of ecosystems is probably the method one most often used in

66   studies. Unfortunately, as a result of spatial and temporal differences in the distribution of

67   cyanobacteria, this approach can often provide a very poor estimation of cyanobacterial

68   abundance and, consequently, of the associated health risk. We therefore need to devise

69   simple sampling strategies for the low cost monitoring of cyanobacteria in shallow lakes. In

70   an attempt to do this, we performed intense spatiotemporal monitoring of cyanobacteria in a

71   shallow lake known to experience cyanobacterial blooms every year.

72

73   2 Materials & Methods

74   **2.1 Study site**

75   This study was performed in a shallow lake named Place (0.08 km^2, 2.5 m max depth,

76   45°43'N, 4°14'E) located in the plain of Forez (Central France), (Fig. 1). This lake is used for

77   extensive fish production and its trophic status is eutrophic to hypereutrophic (OCDE, 1982).

78   *Microcystis aeruginosa* blooms occur every summer.

79

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

104   for this station is given in Supplemental Figure 1, and shows that the two dominant wind

105   directions were NW and SE. The direction of winds blowing from 240° – 60° was classified

106   as NW, and that of winds blowing from 60° – 240° as SE.

107

108   **2.3 Data analysis**

109   The spatial distribution of cyanobacteria in the lake was represented using Surfer (v. 7.0,

110   Golden Software Inc.), and statistical analyses (Wilcoxon test, Spearman correlation) were

111   performed using the R package version 2.10 (R development core Team, 2010).

112

3 Results

113

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

114

115   **species**

116   Two cyanobacterial species, *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*,

117   dominated the phytoplankton community during the summer of 2008. The population

118   dynamics of these two species displayed very contrasting patterns (Fig. 2). The population

119   dynamics of *Microcystis aeruginosa* was characterized by a steady increase in the cell

120   abundance from June to August, apart from a brief dip in the middle of July. The maximum

121   population was reached on August 21 (264,000 cells/mL), and subsequently the cell

122   concentration remained stable until the end of September, and then decreased in October. In

123   contrast, the population dynamics of *Aphanizomenon flos-aquae* were much more chaotic,

124   with the cell abundance reaching two very high and short-lived peaks in July

125   (400,000 cells/mL on July 17, and 560,000 cells/mL on July 23).

126

127   **3.2 Influence of sampling frequency on the estimation of the population dynamics**

128   Our assessment of the changing population dynamics of the two cyanobacteria were obtained

129   using a very frequent high temporal sampling regime (every two days), which would not be

130   practicable in the context of normal monitoring programs. In order to evaluate the impact of

131   the sampling frequency, we simulated weekly, twice-monthly and monthly sampling

132   frequencies to our data set. The results of these simulations are shown in Fig. 3 and 4. From

133   this figure, we can see that changes in *M. aeruginosa* cell abundance over time would have

134   been fairly accurately estimated at all these sampling frequencies. Moreover, for all sampling

135   frequencies, the quality of the estimation of the *M. aeruginosa* population dynamics was not

136   influenced by choice of the first sampling date (Fig. 3). In contrast, the population dynamics

137   of *A. flos-aquae* would have been badly or even very badly estimated by using weekly, twice-

138   monthly and monthly sampling frequencies (Fig. 4). We would only have detected both

139   *A. flos-aquae* peaks in one of the three trials testing the weekly sampling strategy, and we

140   would never have detected these peaks with twice-monthly and monthly sampling

141   frequencies.

142

143   **3.3 Evolution of the horizontal distribution of cyanobacteria in the lake during the**

144   **bloom**

145   As shown in the video (Supplemental Fig. 2), the horizontal distribution of both cyanobacteria

146   displayed marked variations during the course of the study. Moreover, when the spatial

147   distributions of the two species at the same sampling dates were compared, it could be seen

148   that similar or contrasting patterns in the horizontal distribution of *M. aeruginosa* and *A. flos-*

149   *aquae* cells would have been found, depending on the dates chosen (some examples are

150   provided in Fig. 5).

151   In order to obtain a better picture of this spatial variability in the cell concentrations of

152   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

203   BBE torch (A-E, see Fig. 1). As shown in Fig. 9, there was a steady fall in the cyanobacterial

204   biomass at all sampling points during the afternoon and evening, and conversely an increase

205   late at night and in the morning. Moreover, the differences in biomass between the five

206   sampling points were smaller during the night than during the day, as was the standard error

207   (three measurements per sampling point). A multidimensional scaling analysis performed on

208   the same values confirmed these observations, with all the night sampling times being

209   grouped together, whereas the sampling times during the day were much more scattered (Data

210   not shown).

211

4 Discussion

213   As far as we are aware, this is the first attempt to investigate the influence of sampling

214   strategies on the evaluation of spatial and temporal variations in cyanobacterial abundances in

215   shallow lakes, which constitute unstable and complex ecosystems. These lakes are used by

216   humans for numerous activities, including recreational activities and the supply of drinking

217   water, which makes the monitoring of cyanobacteria in such ecosystems of particular

218   importance, especially as part of the evaluation of the health risks linked to cyanobacterial

219   blooms and their toxins. Sampling strategy is also very important in the context of basic

220   studies, because the quality of sampling has a major impact on the quality of the final results.

221   In this study, we found that the sampling frequency required to obtain a good

222   estimation of the temporal evolution of the cyanobacterial abundance depends on the

223   blooming species, *M. aeruginosa* or *A. flos-aquae*. Twice-monthly or monthly sampling

224   provided good results for *M. aeruginosa*, whereas this was not often enough to monitor the

225   chaotic population dynamics of *A. flos-aquae*. These findings are in contradiction with the

226   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

302   in shape and its perimeter small (around 1.3 Km). These findings suggest that for large lakes

303   and also for lakes with a more complex shape, a large number of sampling points would be

304   necessary to obtain a good estimation of the cyanobacterial abundance. Clearly such sampling

305   is time consuming and expensive. One way to reduce these costs would be to collect a large

306   number of samples and then pool equal volumes of these samples in the same flask, before

307   carrying out a single analysis. In this study, as in most of the monitoring programs performed

308   in small lakes, all samples were taken from the shoreline of the lake. This kind of sampling is

309   suitable for small lakes, but it has been shown that for large lakes (Rogalus and Watzin, 2008)

310   shoreline sampling may miss early warning signs of bloom development, and also lead to the

311   overestimation of the concentration of microcystins, when compared to data obtained from

312   offshore samples. For bigger lakes, therefore, the sampling strategy must include offshore

313   samples.

314   Different programs worldwide are testing alternatives to water sampling for the

315   monitoring of cyanobacteria in freshwater ecosystems. Two main approaches have been

316   investigated. The first one is based on the use of remote sensing, which has long been in use

317   in marine ecosystems (see for example Bracher et al., 2009). In freshwater ecosystems, the

318   paper of Hunter et al. (2008) has shown the potential of high resolution images for the

319   assessment of the spatial distribution of *M. aeruginosa* in a shallow eutrophic lake. However,

320   the cost of these images and the impact of meteorological conditions are limiting factors for

321   envisaging the use of this tool in routine cyanobacteria monitoring programs. One alternative,

322   lower-cost solution could be based, in the future, on the use of drones to take aerial

323   photographs of freshwater ecosystems, but these tools are still in development. Moreover,

324   they will be only useful for cyanobacterial species that live in the surface water of lakes.

325   The second way of monitoring of cyanobacteria without sampling the water being

326   investigated is the use of buoys equipped with a variety of sensors, including, for example, a

327 submersible spectrofluorometer to quantify the biomass of the cyanobacteria. This kind of

328 tool permits the real-time monitoring of phytoplankton, including cyanobacteria, as shown for

329 example in the paper of Le Vu et al. (in press). The two obstacles to their use in routine

330 cyanobacteria monitoring programs are the high price of these systems, and the fact that they

331 only provide estimations for one sampling point. Despite this, the possible use of such buoys,

332 combined with the spatial monitoring of cyanobacteria by water sampling looks very

333 promising for surveying cyanobacteria in freshwater ecosystems.

334 5 Conclusion

335 The sampling of cyanobacteria in freshwater ecosystems is a hot topic, in particular in the

336 context of programs for surveying these toxic microorganisms in ecosystems used for the

337 production of drinking water or for recreational activities. Paradoxically, fewer studies deal

338 with the impact of sampling strategies on the estimation of cyanobacterial cell abundances in

339 freshwater ecosystems. In this study, we demonstrate that the choice of sampling strategy can

340 lead to very different estimations of the cell abundances of two blooming species in a shallow

341 lake and also that, depending on the cyanobacterial species involved, different sampling

342 strategies are required to obtain a good estimation of their population dynamics. All these

343 findings suggested that monthly or twice-monthly sampling strategies at just one sampling

344 point do not allow to provide an accurate estimation of cyanobacterial abundances, and thus

345 of the health risks associated with the presence of toxic species in aquatic ecosystems.

346 Moreover, although promising new technologies are being developed for monitoring

347 freshwater cyanobacteria, their cost and some other drawbacks mean that at present they

348 cannot replace water sampling, which will remain the basis of most of these monitoring

349 programs for the foreseeable future.

350

351

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443

444

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

468 Fig. 6: Change over time in the coefficients of variation of the mean cell abundances of

469 *Microcystis aeruginosa* (black triangle) and *Aphanizomenon flos-aquae* (white square)

470 estimated at all six sampling points.

471

472 Fig. 7: Spearman correlation values between *Microcystis aeruginosa* (top) and

473 *Aphanizomenon flos aquae* (bottom) cell abundances estimated from the mean values for all

474 six sampling point values, and those estimated from only one, two, three, four or five of these

475 six sampling points.

476

477 Fig. 8: Location of the sampling points providing the best (left) and worst (right) estimations

478 of cyanobacterial cell abundances, compared to estimations based on six sampling points. We

479 give the combinations for two (top) and three (bottom) sampling points. The polar plot shows

480 the direction of the maximum daily wind speed during the study. The different line types

481 permit to distinguish the two best or the two worst combinations of sampling points, using

482 two or three sampling points.

483

484 Fig. 9: Cyanobacterial biomass in the subsurface water of the lake over a 24-hour period at

485 five sampling points (♦ point A, ■ point B, ▲ point C, × point D, and ◊ point E). The error

486 bars indicate the standard deviation.

487

488

489 Supplemental Figure 1. Distribution of the wind directions at the St-Etienne-Bouthéon

490 meteorological station during this study (June, 13 to October, 10, 2008). The curve and the

491 bars indicate respectively the mean speed and the occurrence per hour of the wind in each

492 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)

500