1 Review: a meta-analysis comparing cell-division and cell-
2 adhesion in *Microcystis* colony formation

3

4 Running title: Colony formation of *Microcystis*

5

6 Man Xiao^{1, 2, 3}, Anusuya Willis^{2,3}, Michele A Burford^{2,3}, Ming Li^{1*}

7

8 ¹College of Resources and Environment, Northwest A & F University, Yangling
9 712100, PR China

10 ²Australian Rivers Institute, Griffith University, 170 Kessels Rd, Nathan Queensland
11 4111, Australia

12 ³School of Environment, Griffith University, 170 Kessels Rd, Nathan, Queensland
13 4111, Australia

14

15

16 ***Correspondence**

17 Phone: 86+029-87080055

18 Fax: 86+029-87080055

19 Email: lileaf@163.com

27 **Abstract**

28 The freshwater cyanobacterium *Microcystis* is a nuisance species. It forms large
29 blooms on the water surface and overwhelmingly dominates the ecosystem through
30 the formation of colonies from single cells surrounded by mucilage; however, the
31 mechanism of colony formation is poorly understood. Two mechanisms of
32 *Microcystis* colony formation have been proposed: cell-division, where cells remain
33 attached after binary fission; and cell-adhesion, where single cells stick together. This
34 paper examined the published literature on *Microcystis* colony formation to clarify the
35 mechanism of colony formation and its relationship to environmental drivers. This
36 meta-analysis showed that in laboratory experiments, colony formation by cell-
37 division was mainly induced by zooplankton filtrate, high Pb^{2+} concentrations, the
38 presence of the cyanobacterium *Cylindrospermopsis raciborskii*, heterotrophic
39 bacteria, and low temperature and low light intensities. Alternatively, colony
40 formation by cell-adhesion was mainly induced by zooplankton grazing, high Ca^{2+}
41 concentrations, and microcystins. Therefore, colony formation by cell-division
42 appears to be a slower process and to occur under an environmental stress factor,
43 while cell-adhesion occurs more quickly to an environmental threat. Applying the
44 criteria to the different morphospecies of *Microcystis*, found that under natural
45 conditions *M. ichthyoblabe* colonies formed predominantly through cell-division,
46 whereas *M. wesenbergii* colonies formed predominantly through cell-adhesion. This

47 study provides new insights into the mechanisms and environmental drivers of colony
48 formation by *Microcystis*.

49 **Key words**

50 Colonial morphology; colony size; extracellular polymeric substances; harmful algae
51 blooms; morphospecies; growth rate

52 **Introduction**

53 *Microcystis* is a single-celled freshwater cyanobacterium that forms colonies
54 surrounded by mucilage. Colony formation by *Microcystis* is a major feature of its
55 success in freshwater ecosystems. With buoyant colonies, *Microcystis* can form
56 extremely large blooms, covering the water surface of some of the largest lakes on
57 Earth, to an extent visible from space (Bullerjahn et al., 2016; Harke et al., 2016). The
58 colony morphology plays an important role in *Microcystis* ecology, i.e., positive
59 buoyancy particularly of large colonies, enables them to overcome water turbulence
60 (Walsby et al., 1995), reduces zooplankton grazing pressure (Visser et al., 2005),
61 makes them less prone to heavy metals (Wu et al., 2007) and toxic substances, such as
62 linear alkyl-benzene sulfonate (Li et al., 2013a). There has been considerable interest
63 in initiation of colony formation of *Microcystis*, however, the mechanism and
64 environmental drivers of *Microcystis* colony formation are not well understood.

65 The occurrence of *Microcystis* blooms has increased with eutrophication of
66 freshwater systems, and the frequency and severity of blooms is predicted to increase
67 with the increasing temperature and CO₂ partial pressure associated with climate
68 change (Paerl et al., 2016; Visser et al., 2016). Initial colony formation of *Microcystis*
69 is a critical step in its bloom development. Blooms of *Microcystis* form in a sequential
70 pattern: cells and small colonies that have overwintered in the sediment rise into the
71 water column (Reynolds et al., 1981; Cao and Yang, 2010), then, as cell growth

increases small colonies (<100 μm diameter) begin to form, these colonies are positively buoyant and begin to float on the water surface. Along with *Microcystis* growth, larger colonies (>300 μm diameter) form. These large colonies enable them to access optimal light and nutrient environments by vertical migration and sometimes float on the water surface as a thick "scum" (Yamamoto and Nakahara, 2009; Cao and Yang, 2010; Yamamoto et al., 2011). Large *M. aeruginosa* colonies (> 800 cells per colony) have been shown to form when the cells account for over 98% of the total algal biovolume (Yamamoto et al., 2011). Secreted extracellular polymeric substances (EPS) surround the cells and colonies also play a key role in maintaining the integrity of the colonies and buoyancy, and protecting them against environmental factors and grazing stress (Reynolds et al., 1981). The secreted EPS and positive buoyancy of large colonies of *Microcystis* allow them to escape from entrainment in the turbulent flow, and thus increase the duration of blooms (Wu and Kong, 2009; Zhu et al., 2014).

It is suggested that a number of environmental variables play a role in initiating *Microcystis* colony formation, and influencing the size and morphology of the resulting colonies. For example, the presence of grazing zooplankton, such as *Ochromonas* sp., can induce colony formation (Burkert et al., 2001; Jang et al., 2003). This is because the larger size of colonies compared to single cells reduces the ability of zooplankton to graze.

While colony morphology plays an important role in the bloom formation of this species, studies of *Microcystis* colony formation are hindered by the fact that *Microcystis* in cultures typically remain as single cells (Yang et al., 2008; Li et al., 2013c). A range of techniques to induce colony formation in laboratory cultures have been developed based on adjusting biotic or abiotic factors, such as zooplankton predation (Yang et al., 2009), heterotrophic bacteria (Wang et al., 2015), temperature stress (Li et al., 2013c) or chemical additions to the media (Wu et al., 2007; Bi et al., 2016). These techniques have allowed studies with *Microcystis* colonies to be undertaken; however, the mechanisms of initial colony formation and how they are affected by the environmental conditions have remained unclear.

Two mechanisms of *Microcystis* colony formation have been proposed: i) ‘cell-division’, where cells remain attached after binary fission, with the daughter cells enveloped in a layer of secreted EPS preventing their separation (Kessel and Eloff, 1975); or ii) ‘cell-adhesion’, where single cells aggregate with the secretion of adhesive EPS (Yang et al., 2008). Differentiating these two mechanisms has typically been determined by analyzing the arrangements of cells within colonies: cell-division leads to a regular arrangement of cells, while cell-adhesion leads to an arbitrary arrangement of cells. Using cell arrangement can be problematic as it may be species specific. Additionally, species identification in *Microcystis* is also complicated by cryptic morphology, identification of different species by colony morphology, termed

morphospecies, may in fact be genetically the same (Otsuka et al., 2000; Xu et al., 2016b). For example, traditionally *M. viridis* and *M. novacekii* colonies have regularly arranged cells, whereas those in *M. aeruginosa* and *M. wesenbergii* colonies are arbitrarily arranged (Yu et al., 2007), while genetic identification shows that these are not always different species (Otsuka et al., 2001). Additionally, the cell arrangement within a colony can change, with arbitrary arrangement becoming regular when further cell-division events occur (Otsuka et al., 2000; Sun et al., 2015).

Therefore, to clarify the process of initial *Microcystis* colony formation and the effect of environmental drivers on the mechanism of colony formation, the published literature was reviewed and a meta-analysis was performed comparing colony formation and EPS production under a variety of environmental drivers. For the purpose of this study, a hypothesis was made that colony formation due to cell-division is the dominant process if the increase of cells per colony is slower than the increase of total biomass, and conversely, colony formation due to cell-adhesion is the dominant process when the increase of cells per colony is faster than the increase of total biomass.

128 **Material and methods**

129 *Data collection*

130 Published literature was obtained from ISI Web of Science under the search
131 terms '*Microcystis*' and 'colony formation', with further manual selection of research
132 focused on *Microcystis* colony formation in the presence of biotic and abiotic factors.
133 Data were taken directly from the published literature where available, otherwise data
134 were extracted from the graphs by ScanIt (AmsterCHEM, Almería, Spain)
135 (Supplementary Tables S1, S2). Some of the publications have investigated more than
136 one factor on colony formation, and all the mentioned factors have been extracted for
137 analysis in this study.

138 *Data analysis*

139 To differentiate between colony formation dominated by cell-division or cell-
140 adhesion, the increase of cells per colony was compared to the increase of total
141 biomass. From each literature source the increase factor, i.e., fold-increase of total
142 biomass ($f - B$) and fold-increase of cells per colony ($f - CC$) were calculated during
143 the growth experiments. Calculating the fold-increase allowed comparison of data
144 with different units across all literature. The fold-increase of bound-EPS ($f - bEPS$)
145 was also calculated, and this was to compare between different treatments with
146 controls.

147 *Fold-increase of total biomass*

148 The fold-increase of total biomass ($f - B$) was directly calculated from the cell
 149 concentration (cell mL⁻¹), or approximated from dry weight (mg L⁻¹) or chlorophyll *a*
 150 concentration (µg L⁻¹) following

$$151 \quad f - B = C_t / C_{t_0}, \quad (1)$$

152 where C_t and C_{t_0} are cell concentrations, dry weight or chlorophyll *a* concentrations at
 153 time t and the initial time t_0 . If there were no records of cell concentration, dry weight
 154 or chlorophyll *a* concentration throughout the growth period, then $f - B$ was derived
 155 from the specific growth rate (μ , day⁻¹) (Andersen, 2005) as

$$156 \quad f - B = e^{\mu \times (t - t_0)}. \quad (2)$$

157 *Fold-increase of cells per colony*

158 Colony size was reported in the literature as colony diameter (D , µm), colony
 159 aggregation area (A , µm²), particle volume (V , µm³), or proportion of different colony
 160 size groups (denoted as PS, %).

161 To calculate the fold-increase of cells per colony ($f - CC$), colony diameter was
 162 determined from all available datasets. When calculating from the aggregation area
 163 and particle volume, the colony was approximated as a sphere. When calculating from
 164 PS, the weighted mean colony diameter was based on the proportion and median
 165 colony size in each group. For instance, Wang et al. (2011) set five colony size groups:
 166 < 5 µm, 5-20 µm, 20-40 µm, 40-60 µm and > 70 µm. For the three groups in the

middle, the median diameters were 12.5 µm, 30 µm and 50 µm, respectively. For the groups of < 5 µm and > 70 µm, median diameters were deduced from morphological photographs and the size range of the colonies provided in the literature.

Cells per colony (N) was then calculated from the colony diameter as below: for colonies > 15 µm, N was calculated following Reynolds and Jaworski (1978) as

$$N = 10^{2.99 \times \log_{10} D - 2.80}; \quad (3)$$

for colonies < 15 µm, N was calculated as

$$N = \frac{D}{2.59}; \quad (4)$$

where D is the colony diameter (µm), 2.99 and 2.80 were constants, 2.59 was the mean diameter of *M. aeruginosa* single cells calculated from the range of 2.38 to 2.81 µm given by Reynolds (2006).

The fold-increase of cells per colony (f – CC) was calculated as

$$f - CC = N_t / N_{t_0}, \quad (5)$$

where N_t and N_{t_0} are the values of cells per colony at time t and initial time t_0 .

To calculate f-B and f – CC, time t was selected during the exponential growth phase or steady-state phase of the growth. t_0 was selected as the beginning of the experiment. These selections were made under all biotic and abiotic conditions, except for the zooplankton grazing treatments. This is because zooplankton grazing reduces the total cell biomass during the first couple of experimental days, thus t_0 was selected as the time of minimum biomass in the zooplankton grazing experiments.

187 *Bound-Extracellular Polymeric Substance*

188 Bound-extracellular polymeric substance (bEPS) was variously described as
 189 bEPS, EPS and soluble carbohydrate in the literature. The fold-increase in bEPS (
 190 $f - \text{bEPS}$) was calculated between different treatments and the control groups as

$$191 \quad f - \text{bEPS} = \text{bEPS}_t / \text{bEPS}_c, \quad (6)$$

192 where bEPS_t and bEPS_c are the amount of bEPS measured at time t under different
 193 treatments and control groups. Here, t also indicates the exponential growth phase or
 194 beginning of stationary growth phase.

195 *Colony formation mechanism*

196 For the purpose of this study, based on the hypothesis, it was presumed that
 197 colony formation due to cell-division is the dominant process if the increase of cells
 198 per colony is slower than the increase of total biomass, and conversely, colony
 199 formation due to cell-adhesion is the dominant process when the increase of cells per
 200 colony is faster than the increase of total biomass. Therefore, the mechanism of
 201 colony formation was defined as:

202 Cell-division dominates when $f - B / f - CC > 1$.

203 Cell-adhesion dominates when $f - B / f - CC < 1$.

204 *Comparison to field studies*

205 The criteria developed defining colony formation by cell-division and cell-
 206 division was applied to field studies. Colony size and cell concentration of

Microcystis were analysed based on field sampling in Lake Taihu, China (31°14'N, 120°8'E) from April 2011 to December 2013 (Zhu et al., 2014; Zhu et al., 2015; Zhu et al., 2016). The two indicators $f - B$ and $f - CC$ were calculated for two species *M. ichthyoblabe* and *M. wesenbergii* that occurred during the growth period, and the ratio of $f - B / f - CC$ was used to determine the dominant mechanism of colony formation for both species.

Statistical analysis

The effect of bEPS on colony formation, and the relationship between colony size and the cell growth were both analyzed with generalized linear models, at a statistical significance of $p < 0.05$. Shapiro-Wilk test was used to test the normal distribution of residuals before conducting the generalized linear models. Data were Boxcox transformed to better meet the assumptions of the normally distributed residuals (Zuur et al., 2010). All calculations were performed with EXCEL and R software (www.r-project.org/).

Results

Colony formation by cell-division or cell-adhesion – laboratory studies

Seventy-nine datasets (treatments from 21 publications (Table S1)) were identified from the literature on the induction of colonies from *Microcystis* single cells or smaller colonies ($D < 5 \mu m$), and all except 11 used *M. aeruginosa*. Of the 79

datasets, 48% examined the effects of abiotic factors: low temperature and low light intensities ($n = 28$), Ca^{2+} ($n = 5$), lead (Pb^{2+}) ($n = 3$) and nutrients (nitrogen and phosphorus, $n = 1$); 47% investigated the effects of biotic factors: heterotrophic bacteria ($n = 11$), microcystins (MCs, $n = 9$), zooplankton grazing ($n = 8$), zooplankton filtrate ($n = 7$) and the presence of the cyanobacterium *Cylindrospermopsis raciborskii* ($n = 3$). The remaining studies (5%) considered the combined effects of biotic and abiotic factors, namely, zooplankton grazing with low nutrient concentrations ($n = 3$), and high Ca^{2+} concentration with EPS addition ($n = 1$).

Colony formation by cell-division dominated with the treatments of zooplankton filtrate, high Pb^{2+} concentrations, *C. raciborskii*, heterotrophic bacteria, and low temperature combined with low light intensities. Colony formation by cell-adhesion dominated with the treatments of zooplankton, high Ca^{2+} concentration combined with EPS, microcystin addition, and nutrient addition combined with zooplankton (Fig. 1).

The different treatments also affected colony size (Table S1): high Ca^{2+} concentrations, nutrient addition combined with zooplankton grazing treatments resulted in relatively large colonies ($> 100 \mu\text{m}$); and the largest colony was approximately $300 \mu\text{m}$ across. The remaining factors induced smaller colonies ($< 100 \mu\text{m}$). One study (Ma et al., 2014) found that adding nutrients resulted in the disaggregation of colonies (Table S1).

247 *Colony formation by cell-division*

248 Colony formation by cell-division had a positive linear regression with the
 249 increase of bEPS ($p < 0.001$, $n = 25$). When the fold-increase of bEPS reached 6, the
 250 f – CC by cell-division reached 100 (Fig. 2). There was no significant correlation
 251 between colony formation by cell-adhesion and bEPS (Fig. 2).

252 When comparing all the treatments that induced colony formation by cell-
 253 division, the proportion of cells that form colonies decreased exponentially as the
 254 growth rate increased ($p < 0.001$, $n = 27$). This showed that when the growth rate
 255 increased from 0.2 to 0.5 day⁻¹, the cells forming colonies by cell-division decreased
 256 by around 50% (Fig. 3).

257 *Colony formation by cell-division or cell-adhesion – Field studies*

258 Seasonal variation in growth and colony formation of two *Microcystis* species, *M.*
 259 *ichthyoblabe* and *M. wesenbergii* from Lake Taihu, China were analyzed from four
 260 datasets (Table S2). Comparison of f – B and f – CC showed that colony formation of
 261 *M. ichthyoblabe* occurred via cell-division, while colony formation of *M. wesenbergii*
 262 was predominantly via cell-adhesion (62%) but occasionally by cell-division (38%)
 263 (Fig. 4).

264

265 Discussion

266 This study showed that initial colony formation from *Microcystis* single cells and
267 small colonies could be induced either predominately by cell-division or cell-adhesion,
268 and the mechanism used was dependent on the biotic or abiotic factors present.
269 Colony formation by cell-division dominated in the presence of: zooplankton filtrate,
270 high Pb^{2+} concentrations, the cyanobacterium *C. raciborskii*, heterotrophic bacteria,
271 and combined effect of low temperature and low light intensities. In contrast, colony
272 formation by cell-adhesion was the dominant process in the presence of zooplankton
273 grazing, high Ca^{2+} concentrations and the addition of microcystins. Colony formation
274 by cell-adhesion is a faster process, suggesting a response to an immediate threat,
275 while colony formation via cell-division is slower and occurs in response to an
276 environmental stress.

277 In this study colony formation by cell-division was defined as occurring when
278 the increase in cells per colony occurred at lower rate than the increase in total
279 biomass. In the cell-division process, cells remain attached after binary fission, and
280 additional production of bEPS surrounds the cells (Xu et al., 2013). Cell-division
281 leads to colonies with regularly arranged cells, however as the colonies continue to
282 increase in size, the arrangement of cells may become irregular (Yang et al., 2008).
283 As colony formation by cell-division requires multiple cell-division events to occur,
284 this process is slower than colony formation by cell-adhesion. Consistent with a

previous study, the growth rate was inversely correlated with colony formation, showing that with the highest growth rates colonies did not form by cell-division (Li et al., 2013c). This correlation suggests that in laboratory cultures under optimal conditions with high growth rates, colonies are unlikely to form.

Colony formation by cell-adhesion occurred when single cells in culture adhered to each other, defined as occurring when the increase in cells per colony was greater than the increase in total biomass. This process can occur more quickly than cell-division, and assumes an adhesive bEPS can quickly be employed or secreted by the single cells to allow adhesion to each other. Cell-division is likely still occurring in colonies formed by cell-adhesion, however the process would be slower and the dominant mechanism is cell-adhesion. As there was no correlation found between bEPS production and colony formation by cell-adhesion, this suggests that the bEPS coating the single cells contains adhesive polymers that can be activated. This activation of adhesion suggests communication between cells, with a mechanism which, as yet, has not been identified.

Extracellular polymeric substances (EPS) are an important component in *Microcystis* colony formation (Yang et al., 2008; Li et al., 2013c), with bEPS surrounding the cells and soluble EPS secreted into the surrounding media (Yang et al., 2008). Although in laboratory cultures production of bEPS only correlated with colonies formed by cell-division, in field samples colony size has been found to

correlate with the amount of bEPS, suggesting that additional bEPS secretion may occur once colonies have formed (Li et al., 2013c; Xu et al., 2016a). Addition of bEPS to laboratory cultures could induce colony formation (Wu and Song, 2008; Yang et al., 2008; Li et al., 2013c), but soluble EPS did not (Sato et al., 2016), reflecting the differing roles of the different forms of EPS (Pereira et al., 2009). A study of the composition of EPS of *Microcystis* (Forni et al., 1997) showed a carbohydrate composition similar to that of the adhesive EPS of diatoms, a eukaryotic microalgae, where rhamnose, fucose and xylose are common components (Willis et al., 2013). Additional changes in composition also affect the adhesive qualities of the EPS, for example increase of the uronic acid content has been found to lead to increased adhesion strength (Verspagen et al., 2006). This suggests that changes in EPS composition could be stimulated under different environmental drivers and lead to more cell-adhesion. The evidence that Ca^{2+} ions also promote colony formation by cell-adhesion (Wang et al., 2011; Sato et al., 2016), also suggests a similar adhesive mechanism to that found in diatom EPS (Chiovitti et al., 2008).

The meta-analysis in this study demonstrated the two mechanisms of initial colony formation and the differentiation of mechanisms by threat or stress to the cells. The immediate threat imposed by grazing caused colony formation by cell-adhesion, as colonies are more difficult to consume by zooplankton (Yang et al., 2006; Yang et al., 2008). This appears as a direct response to an immediate threat. Interestingly,

zooplankton filtrate did not have the same effect (Yang et al., 2005; Yang and Li, 2007), which led to colonies forming by cell-division. This difference in response further suggests communication between *Microcystis* cells arises during active grazing by zooplankton, which resulted in the increase of adhesiveness of the EPS. The addition of microcystins also caused colony formation by cell-adhesion, this indicates that the release of this molecule, possibly through cell-lysis or active exportation, potentially acts in this communication (Gan et al., 2012). Once *Microcystis* colonies reach a certain size ($> 50 \mu\text{m}$), their microcystin production increases and correlates with a further increase in colony size (Wang et al., 2013).

The mechanism of colony formation, either dominated by cell-division or cell-adhesion, may result in colonies with different responses to environmental stimuli. In laboratory set-up, *Microcystis* cultures grow fast under optimal conditions and exist predominantly as single cells, supporting the idea that the role of colonies is to serve as protection from environmental stressors. Additionally the size and morphology of *Microcystis* colonies induced in laboratory cultures differs from field populations, with laboratory colonies smaller and with more uniform size and shape (Xu et al., 2016a), although colonies as large as $275 \mu\text{m}$ have been reported (Yang and Kong, 2013). As a result, it has been difficult to compare results from single cell cultures and field studies. Therefore, laboratory studies with *Microcystis* colonies that were formed under relevant conditions would be ideal for use in further research. This study

345 additionally suggests that the mechanism of colony formation needs to be considered
346 in experimental design. This study illustrated that bEPS production differs depending
347 on the mechanism of colony induced and this could affect the response of colonies to
348 other variables tested.

349 In field populations, *Microcystis* blooms consist predominately of colonies, with
350 colony formation by cell-division the accepted mechanism (Yang et al., 2008; Xu et
351 al., 2016a). The analysis of the field data of *M. ichthyoblabe* and *M. wesenbergii*
352 suggests the mechanism may be species and/or condition specific (Yamamoto and
353 Nakahara, 2009; Cao and Yang, 2010). *M. ichthyoblabe* colonies, which were found
354 to arise from cell-division, form gradually and increase in size throughout spring
355 (Yamamoto and Nakahara, 2009; Cao and Yang, 2010). Scanning electron
356 microscopy images show a featureless slimy layer, with bEPS around individual cells
357 (Kessel and Eloff, 1975). In comparison, *M. wesenbergii* colonies were larger, ~200
358 μm in diameter, with loosely arranged cells, likely arising from cell-adhesion (Li et al.,
359 2013b; Zhu et al., 2015). Further colony morphology changes occur as cells actively
360 rearrange themselves within the colonies (Mulling et al., 2014). The differences in
361 colony morphology of these two *Microcystis* morphospecies suggest they would
362 respond differently to environmental stimuli.

363 Lake studies of *Microcystis* blooms have shown succession of individual species
364 with different colonial morphologies. From June to November, lakes such as Lakes

Taihu and Chaohu in China, and Lakes Suwa, Biwa, Hirosawa-no-ike in Japan, have successive dominance of *M. ichthyoblabe*, *M. wesenbergii* and *M. aeruginosa* (Park et al., 1993; Ozawa et al., 2005; Yamamoto and Nakahara, 2009; Jia et al., 2011; Li et al., 2013b; Zhu et al., 2015; Zhu et al., 2016). In the early period of the blooms, *M. ichthyoblabe* dominates with smaller colonies (<100 μm), the results in this study suggested that these colonies form by cell-division during spring and early summer. Subsequently, *M. wesenbergii* and *M. aeruginosa* dominate with colonies formed by cell-adhesion, with average colony diameter of 400 μm . The two morphospecies are present until autumn (Li et al., 2013b; Zhu et al., 2015). These colonies arise quickly suggesting formation by cell-adhesion (Zhu et al., 2015). Field studies comparing environmental variables to colony formation show that generally colony size decreases with increasing temperature and nutrient concentrations (Zhu et al., 2016), potentially a result of increased growth rate.

In this meta-analysis, a direct method was developed to determine the mechanism of initial colony formation for *Microcystis*. In investigating the criteria of colony formation, the two mechanisms are assumed to be distinct; however, it is likely that both occur simultaneously, with the criteria developed in this study describing the dominant mechanism used during initial colony formation. There are a number of factors that may have introduced bias into the analysis. Interpretation of data from published literature presented in different formats, such as cell number, dry

weight or chlorophyll a , can introduce errors. Using the fold-increase for each factor reduced the error that otherwise occurs due to conversion of units. The growth period was also standardized and discounted studies of cells in stationary phase. Estimating the number of cells per colony also requires careful interpretation, because cell size was found to vary under different environmental factors such as temperature, light intensity and nutrient supply (Reynolds, 2006). The size of *Microcystis* colonies with their surrounding mucilage are also difficult to estimate, especially when colonies exhibit variable morphologies. Consequently, the number of cells per colony is easily underestimated; therefore, the calculations from this study were compared with the reported results whenever possible.

By conducting meta-analysis of multiple literature, the mechanism of initial colony formation for *Microcystis* was determined based on our criteria. Nevertheless, due to the depth and accuracy of literature on issues of colony formation varies among different authors, the systematic analysis and related inference could be further improved. There are several future directions for research on colony formation of *Microcystis*: (1) To find out the trigger factors inducing colony formation of *Microcystis* via cell-adhesion in both laboratory experiment and field investigation; (2) To confirm the effects of previously reported biotic and abiotic factors on inducing colony formation by field-based mesocosm studies and field investigation; (3) To induce single *Microcystis* cells to form colonies that have typical colonial morphology

as in the field; (4) To find out the environmental factors that lead to decreasing *Microcystis* colony size and prevent *Microcystis* colony formation; (5) To establish feasible method disaggregating *Microcystis* colonies in lakes and reservoirs to control *Microcystis* blooms.

Conclusions

In this meta-analysis, different methods used for inducing colony formation from single cell cultures were compared and a practical method was demonstrated to determine possible mechanisms of initial colony formation. The results showed that two mechanisms, cell-division and cell-adhesion, can be induced either under environmental stress or immediate threat, respectively. These conclusions suggest that the formation of colonies in natural environments occurs for the protection of the cells, potentially, at the expense of rapid growth.

Acknowledgments

ML received funding from the National Natural Science Foundation of China (Grant Nos. 51409216). MX received funding from a Griffith University Postgraduate International Scholarship and Griffith University School of Environment. MX and AW received funding from the Australian Research Council (ARC: linkage project LP130100311).

425

426 **References**

427 Andersen, R.A., 2005. Algal culturing techniques. Academic press, London, UK.

428 Bi, X., Yan, R., Li, F., Dai, W., Jiao, K., Zhou, Q., Liu, Q., 2016. Sequestration and
429 distribution characteristics of Cd (II) by *Microcystis aeruginosa* and its role in
430 colony formation. BioMed research international 2016.

431 Bullerjahn, G.S., McKay, R.M., Davis, T.W., Baker, D.B., Boyer, G.L., D'Anglada,
432 L.V., Doucette, G.J., Ho, J.C., Irwin, E.G., Kling, C.L., 2016. Global solutions
433 to regional problems: collecting global expertise to address the problem of
434 harmful cyanobacterial blooms. A Lake Erie case study. Harmful Algae 54,
435 223-238.

436 Cao, H., Yang, Z., 2010. Variation in colony size of *Microcystis aeruginosa* in a
437 eutrophic Lake during recruitment and bloom formation. J. Freshw. Ecol.
438 25(3), 331-335.

439 Chiovitti, A., Heraud, P., Dugdale, T.M., Hodson, O.M., Curtain, R.C., Dagastine,
440 R.R., Wood, B.R., Wetherbee, R., 2008. Divalent cations stabilize the
441 aggregation of sulfated glycoproteins in the adhesive nanofibers of the
442 biofouling diatom *Toxarium undulatum*. Soft Matter 4(4), 811-820.

- 443 Forni, C., Telo', F.R., Caiola, M.G., 1997. Comparative analysis of the
444 polysaccharides produced by different species of *Microcystis* (Chroococcales,
445 Cyanophyta). *Phycologia* 36(3), 181-185.
- 446 Gan, N., Xiao, Y., Zhu, L., Wu, Z., Liu, J., Hu, C., Song, L., 2012. The role of
447 microcystins in maintaining colonies of bloom - forming *Microcystis* spp.
448 *Environ. Microbiol.* 14(3), 730-742.
- 449 Ganf, G., Oliver, R., 1982. Vertical separation of light and available nutrients as a
450 factor causing replacement of green algae by blue-green algae in the plankton
451 of a stratified lake. *Journal of Ecology* 70(3), 829-844.
- 452 Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A.,
453 Paerl, H.W., 2016. A review of the global ecology, genomics, and
454 biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae* 54,
455 4-20.
- 456 Jia, X., Shi, D., Shi, M., Li, R., Song, L., Fang, H., Yu, G., Li, X., Du, G., 2011.
457 Formation of cyanobacterial blooms in Lake Chaohu and the photosynthesis of
458 dominant species hypothesis. *Shengtai Xuebao/Acta Ecologica Sinica* 31(11),
459 2968-2977.
- 460 Kessel, M., Eloff, J.N., 1975. The ultrastructure and development of the colonial
461 sheath of *Microcystis marginata*. *Arch. Microbiol.* 106(3), 209-214.

- 462 Li, M., Zhu, W., Dai, X., Li, X., 2013a. Effects of linear alkylbenzene sulfonate on
463 extracellular polysaccharide content and cells per particle of *Microcystis*
464 *aeruginosa* and *Scenedesmus obliquus*. Fresen. Environ. Bull. 22(4 B), 1189-
465 1194.
- 466 Li, M., Zhu, W., Gao, L., Huang, J., Li, L., 2013b. Seasonal variations of
467 morphospecies composition and colony size of *Microcystis* in a shallow
468 hypertrophic lake (Lake Taihu, China). Fresen. Environ. Bull. 22(12), 3474-
469 3483.
- 470 Li, M., Zhu, W., Gao, L., Lu, L., 2013c. Changes in extracellular polysaccharide
471 content and morphology of *Microcystis aeruginosa* at different specific
472 growth rates. J. Appl. Phycol. 25(4), 1023-1030.
- 473 Ma, J., Brookes, J.D., Qin, B., Paerl, H.W., Gao, G., Wu, P., Zhang, W., Deng, J., Zhu,
474 G., Zhang, Y., 2014. Environmental factors controlling colony formation in
475 blooms of the cyanobacteria *Microcystis* spp. in Lake Taihu, China. Harmful
476 Algae 31, 136-142.
- 477 Mulling, B., Wood, S., Hamilton, D., 2014. Intra-colony motility of *Microcystis*
478 *wesenbergii* cells. N.Z. J. Bot. 52(1), 153-159.

- 479 Otsuka, S., Suda, S., Li, R., Matsumoto, S., Watanabe, M.M., 2000. Morphological
480 variability of colonies of *Microcystis* morphospecies in culture. J. Gen. Appl.
481 Microbiol. 46(1), 39-50.
- 482 Otsuka, S., Suda, S., Shibata, S., Oyaizu, H., Matsumoto, S., Watanabe, M.M., 2001.
483 A proposal for the unification of five species of the cyanobacterial genus
484 *Microcystis* Kützing ex Lemmermann 1907 under the rules of the
485 Bacteriological Code. International Journal of Systematic and Evolutionary
486 Microbiology 51(3), 873-879.
- 487 Ozawa, K., Fujioka, H., Muranaka, M., Yokoyama, A., Katagami, Y., Homma, T.,
488 Ishikawa, K., Tsujimura, S., Kumagai, M., Watanabe, M.F., 2005. Spatial
489 distribution and temporal variation of *Microcystis* species composition and
490 microcystin concentration in Lake Biwa. Environ. Toxicol. 20(3), 270-276.
- 491 Paerl, H.W., Gardner, W.S., Havens, K.E., Joyner, A.R., McCarthy, M.J., Newell,
492 S.E., Qin, B., Scott, J.T., 2016. Mitigating cyanobacterial harmful algal
493 blooms in aquatic ecosystems impacted by climate change and anthropogenic
494 nutrients. Harmful Algae 54, 213-222.
- 495 Park, H.D., Watanabe, M.F., Harada, K.I., Suzuki, M., Hayashi, H., Okino, T., 1993.
496 Seasonal variations of *Microcystis* species and toxic heptapeptide microcystins
497 in Lake Suwa. Environ. Toxicol. Water Qual. 8(4), 425-435.

- 498 Pereira, S., Zille, A., Micheletti, E., Moradas-Ferreira, P., De Philippis, R., Tamagnini,
499 P., 2009. Complexity of cyanobacterial exopolysaccharides: composition,
500 structures, inducing factors and putative genes involved in their biosynthesis
501 and assembly. FEMS Microbiol. Rev. 33(5), 917-941.
- 502 Reynolds, C., Jaworski, G., 1978. Enumeration of natural *Microcystis* populations.
503 Brit. Phycol. J. 13(3), 269-277.
- 504 Reynolds, C., Jaworski, G., Cmiech, H., Leedale, G., 1981. On the annual cycle of the
505 blue-green alga *Microcystis aeruginosa* Kütz. emend. Elenkin. Philosophical
506 Transactions of the Royal Society of London. Series B, Biological Sciences,
507 419-477.
- 508 Reynolds, C.S., 2006. Ecology of Phytoplankton. Cambridge University Press,
509 Cambridge, UK.
- 510 Sato, M., Amano, Y., Machida, M., Imazeki, F., 2016. Colony formation of highly
511 dispersed *Microcystis aeruginosa* by controlling extracellular polysaccharides
512 and calcium ion concentrations in aquatic solution. Limnology 18(1), 111-119.
- 513 Sun, Q., Zhu, W., Li, M., Tan, X., 2015. Morphological changes of *Microcystis*
514 *aeruginosa* colonies in culture. J. Limnol. 75(1), 14-23.

- 515 Verspagen, J.M., Visser, P.M., Huisman, J., 2006. Aggregation with clay causes
516 sedimentation of the buoyant cyanobacteria *Microcystis* spp. *Aquat. Microb.*
517 *Ecol.* 44(2), 165-174.
- 518 Visser, P.M., Ibelings, B.W., Mur, L.R., Walsby, A.E., 2005. The ecophysiology of
519 the harmful cyanobacterium *Microcystis*, *Harmful Cyanobacteria*. Springer, pp.
520 109-142.
- 521 Visser, P.M., Verspagen, J.M., Sandrini, G., Stal, L.J., Matthijs, H.C., Davis, T.W.,
522 Paerl, H.W., Huisman, J., 2016. How rising CO₂ and global warming may
523 stimulate harmful cyanobacterial blooms. *Harmful Algae* 54, 145-159.
- 524 Walsby, A.E., Hayes, P.K., Boje, R., 1995. The gas vesicles, buoyancy and vertical
525 distribution of cyanobacteria in the Baltic Sea. *Eur. J. Phycol.* 30(2), 87-94.
- 526 Wang, W., Shen, H., Shi, P., Chen, J., Ni, L., Xie, P., 2015. Experimental evidence
527 for the role of heterotrophic bacteria in the formation of *Microcystis* colonies.
528 *J. Appl. Phycol.* 28(2), 1111-1123.
- 529 Wang, X., Sun, M., Xie, M., Liu, M., Luo, L., Li, P., Kong, F., 2013. Differences in
530 microcystin production and genotype composition among *Microcystis* colonies
531 of different sizes in Lake Taihu. *Water Res.* 47(15), 5659-5669.

- 532 Wang, Y., Zhao, J., Li, J., Li, S., Zhang, L., Wu, M., 2011. Effects of calcium levels
533 on colonial aggregation and buoyancy of *Microcystis aeruginosa*. Curr.
534 Microbiol. 62(2), 679-683.
- 535 Willis, A., Chiovitti, A., Dugdale, T.M., Wetherbee, R., 2013. Characterization of the
536 extracellular matrix of *Phaeodactylum tricornutum* (Bacillariophyceae):
537 structure, composition, and adhesive characteristics. J. Phycol. 49(5), 937-949.
- 538 Wu, X., Kong, F., 2009. Effects of light and wind speed on the vertical distribution of
539 *Microcystis aeruginosa* colonies of different sizes during a summer bloom. Int.
540 Rev. Hydrobiol. 94(3), 258-266.
- 541 Wu, Z., Gan, N., Huang, Q., Song, L., 2007. Response of *Microcystis* to copper
542 stress-Do phenotypes of *Microcystis* make a difference in stress tolerance?
543 Environ. Pollut. 147(2), 324-330.
- 544 Wu, Z., Song, L., 2008. Physiological comparison between colonial and unicellular
545 forms of *Microcystis aeruginosa* Kütz.(Cyanobacteria). Phycologia 47(1), 98-
546 104.
- 547 Xu, F., Zhu, W., Xiao, M., Li, M., 2016a. Interspecific variation in extracellular
548 polysaccharide content and colony formation of *Microcystis* spp. cultured
549 under different light intensities and temperatures. J. Appl. Phycol. 28(3),
550 1533-1541.

- 551 Xu, H., Cai, H., Yu, G., Jiang, H., 2013. Insights into extracellular polymeric
552 substances of cyanobacterium *Microcystis aeruginosa* using fractionation
553 procedure and parallel factor analysis. Water Res. 47(6), 2005-2014.
- 554 Xu, S., Sun, Q., Zhou, X., Tan, X., Xiao, M., Zhu, W., Li, M., 2016b. Polysaccharide
555 biosynthesis-related genes explain phenotype-genotype correlation of
556 *Microcystis* colonies in Meiliang Bay of Lake Taihu, China. Sci. Rep. 6.
- 557 Yamamoto, Y., Nakahara, H., 2009. Seasonal variations in the morphology of bloom-
558 forming cyanobacteria in a eutrophic pond. Limnology 10(3), 185-193.
- 559 Yamamoto, Y., Shiah, F.-K., Chen, Y.-L., 2011. Importance of large colony
560 formation in bloom-forming cyanobacteria to dominate in eutrophic ponds.
561 Ann. Limnol-Int. J. Lim. 47, 167-173.
- 562 Yang, Z., Kong, F., 2013. Abiotic factors in colony formation: effects of nutrition and
563 light on extracellular polysaccharide production and cell aggregates of
564 *Microcystis aeruginosa*. Chin. J. Oceanol. Limnol. 31(4), 796-802.
- 565 Yang, Z., Kong, F., Shi, X., 2005. Effects of filtered lake water on colony formation
566 and growth rate in *Microcystis aeruginosa* of different physiological phases. J.
567 Freshw. Ecol. 20(3), 425-429.

- 568 Yang, Z., Kong, F., Shi, X., Cao, H., 2006. Morphological response of *Microcystis*
569 *aeruginosa* to grazing by different sorts of zooplankton. *Hydrobiologia* 563(1),
570 225-230.
- 571 Yang, Z., Kong, F., Shi, X., Zhang, M., Xing, P., Cao, H., 2008. Changes in the
572 morphology and polysaccharide content of *Microcystis aeruginosa*
573 (cyanobacteria) during flagellate grazing. *J. Phycol.* 44(3), 716-720.
- 574 Yang, Z., Kong, F., Yang, Z., Zhang, M., Yu, Y., Qian, S., 2009. Benefits and costs of
575 the grazer-induced colony formation in *Microcystis aeruginosa*. *Ann. Limnol-*
576 *Int. J. Lim.* 45(03), 203-208.
- 577 Yang, Z., Li, J.-J., 2007. Effects of *Daphnia*-associated infochemicals on the
578 morphology and growth of *Scenedesmus obliquus* and *Microcystis aeruginosa*.
579 *J. Freshw. Ecol.* 22(2), 249-253.
- 580 Yu, G.-L., Song, L.-R., Li, R.-H., 2007. Taxonomic notes on water bloom forming
581 *Microcystis species* (Cyanophyta) from China-an example from samples of the
582 Dianchi lake. *Acta. Phytotax. Sin.* 45(5), 727–741. (Chinese with English
583 Abstract).
- 584 Zhu, W., Li, M., Dai, X., Xiao, M., 2015. Differences in vertical distribution of
585 *Microcystis* morphospecies composition in a shallow hypertrophic lake (Lake
586 Taihu, China). *Environ. Earth. Sci.* 73(9), 5721-5730.

- 587 Zhu, W., Li, M., Luo, Y., Dai, X., Guo, L., Xiao, M., Huang, J., Tan, X., 2014.
588 Vertical distribution of *Microcystis* colony size in Lake Taihu: Its role in algal
589 blooms. J. Great Lakes Res. 40(4), 949-955.
- 590 Zhu, W., Zhou, X., Chen, H., Gao, L., Xiao, M., Li, M., 2016. High nutrient
591 concentration and temperature alleviated formation of large colonies of
592 *Microcystis*: Evidence from field investigations and laboratory experiments.
593 Water Res. 101, 167-175.
- 594 Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid
595 common statistical problems. Methods Ecol. Evol. 1(1), 3-14.
- 596

597 **Figure Legends**

598 Figure 1: Comparison of fold-increase in cells per colony (f – CC) with fold-increase
 599 in total biomass (f – B) of *Microcystis* cultures. Different environmental factors in the
 600 experiments are designated by different symbols in the legend. The diagonal line
 601 represents slope = 1, where colonies below this line formed by cell-division, and
 602 colonies above the line formed by cell-adhesion.

603 Figure 2: Comparison of the fold increase in the amount of bEPS in colonies (f –
 604 bEPS), compared to the fold-increase in cells per colony (f – CC), with solid
 605 triangles showing colonies formed by cell-division and open circles showing colonies
 606 formed by cell-adhesion.

607 Figure 3: Comparison of the proportion of cells that form colonies by cell-division in
 608 *Microcystis* cultures with cell growth rate (day^{-1}). The culture treatments are shown
 609 by the different symbols as designated in the label. The presented data were from all
 610 studies where cell-division occurred. The proportion of cells that form colonies within
 611 the total culture (single cells + colonies) were compared to the measured growth rates
 612 of the cultures.

613 Figure 4: Comparison of fold-increase in cells per colony (f – CC) and fold-increase in
 614 total biomass (f – B) for *M. ichthyoblabe* (solid triangles) and *M. wesenbergii* (open
 615 circles) from field populations. The diagonal line represents slope = 1, where colonies

616 below this line formed by cell-division and colonies above the line formed by cell-
617 adhesion.

618 **Supplemental Information**

619 Table S1. Laboratory and field data collected from the published literature, showing details of *Microcystis* colony formation and biotic
620 and abiotic treatments.

Environment l factor	Treatment	Target species	Strains	Origin	Morphology at start of experiment	Morphology at end of experiment	Reference
Biotic factors							
Zooplankton grazing	<i>Ochromonas</i> sp.	<i>M. aeruginosa</i>	1450/1	UK	Unicellular	Colony diameter <50 µm	Burkert et al. (2001)
		<i>M. aeruginosa</i>	FACHB	China	Unicellular	Cells per colony reached 4.1	Yang et al. (2006)
		<i>M. aeruginosa</i>	PCC 7820	China	Unicellular	Cells per colony reached 4.6	Yang et al. (2008)
		<i>M. aeruginosa</i>	PCC 7806	China	Unicellular	Colony diameter reached 180 µm	Yang and Kong

							(2012)
Zooplankton filtrate	Filtrated lake water	<i>M. aeruginosa</i>	FACHB	China	Unicellular	Cells per colony reached 1.8; colony diameter < 5 µm	Yang et al. (2005)
	<i>Ochromonas</i> sp.	<i>M. aeruginosa</i>	PCC 7806	China	Unicellular	Cells per colony reached 1.4	Yang et al. (2009b)
	<i>Moina macrocopa</i> , <i>Daphnia magna</i>	<i>M. aeruginosa</i>	NIES strains 44, 87, 88 and 99	Japan	Unicellular	Cells per colony reached 1.3; colony diameter < 4 µm	Jang et al. (2003)
Cyanobacteria	<i>C. raciborskii</i>	<i>M. aeruginosa</i>	MIRF-01	China	Unicellular	Cells per colony reached 45	Mello et al. (2012)
Microcystins	MC-RR (0.25-10 µg L ⁻¹)	<i>M. wesenbergii</i>	DC-M1	China	Colony diameter reached 200- 300 µm	Colony diameter reached 580 µm	Gan et al. (2012)

		<i>M. ichthyoblabe</i>	TH-M1	China	Colonies diameter <150 µm	Colony diameter reached 525 µm	
		<i>Microcystis</i> sp.	FACHB 1027	China	Colonies diameter <150 µm	Colony diameter reached 285 µm	
Heterotrophic bacteria	Attached to the colonial <i>Microcystis</i> from lake water	<i>M. aeruginosa</i>	FACHB	China	Unicellular	Colony diameter reached 150 µm	Shen et al. (2011)
	Attached to the colonial <i>Microcystis</i> from lake water	<i>M. aeruginosa</i>	FACHB 905	China	Unicellular	Colony diameter reached 50 µm	Wang et al. (2016)
		<i>M. aeruginosa</i>	PCC 7806	China	Unicellular	Colony diameter reached 50 µm	
		<i>M. wesenbergii</i>	CH	Lake Chaohu, China	Unicellular	Colony diameter reached 50 µm	
		<i>M. wesenbergii</i>	TH	Lake Chaohu,	Unicellular	Colony diameter reached 50 µm	

China							
		<i>M. flos-aquae</i>		Lake Chaohu,	Unicellular	No colony formation	
China							
		<i>M. viridis</i>		Lake Chaohu,	Unicellular	No colony formation	
China							
		<i>Microcystis</i> sp.		Lake Chaohu,	Unicellular	No colony formation	
China							
Abiotic factors							
Light	25 $\mu\text{mol m}^{-2} \text{s}^{-1}$	<i>M. aeruginosa</i>	FACHB	Dianchi,	Unicellular, 2-3 μm	sEPS yield reached 0.03 μg	Wu and
			942	China		$\text{mg}^{-1} \text{DW d}^{-1}$	Song
		<i>M. aeruginosa</i>	PCC 7806	The	unicellular, 2-3 μm	sEPS yield reached 0.03 μg	(2008)
				Netherland		$\text{mg}^{-1} \text{DW d}^{-1}$	
				s			
		<i>M. aeruginosa</i>	NSW 924	Australia	unicellular, 2-3 μm	sEPS yield reached 0.05 μg	
						$\text{mg}^{-1} \text{DW d}^{-1}$	

<i>M. aeruginosa</i>	FACHB 905	Dianchi, China	unicellular, 2-3 μm	sEPS yield reached 0.04 μg $\text{mg}^{-1} \text{DW d}^{-1}$
<i>M. aeruginosa</i>	FACHB 975	Wudalianc hi, China	Small colonies, average colony diameter: 37.5–50 μm	sEPS yield reached 0.13 μg $\text{mg}^{-1} \text{DW d}^{-1}$
<i>M. aeruginosa</i>	FACHB 910	Xinyan, China	Small colonies, colony diameter: 25-50 μm	sEPS yield: 0.23 $\mu\text{g mg}^{-1} \text{DW}$ d^{-1}
<i>M. aeruginosa</i>	FACHB 938	Tuanshang , China	Large colonies, colonies diameter > 650 μm	sEPS yield: 0.33 $\mu\text{g mg}^{-1} \text{DW}$ d^{-1}
<i>M. aeruginosa</i>	FACHB 907	Dianchi, China	Intermedium colonies, colony diameter :62.5 -100 μm	sEPS yield: 0.23 $\mu\text{g mg}^{-1} \text{DW}$ d^{-1}
<i>M. aeruginosa</i>	FACHB 909	Bao'anhu, China	Large colonies, colony diameter > 205 μm	sEPS yield: 0.23 $\mu\text{g mg}^{-1} \text{DW}$ d^{-1}

Light	10 $\mu\text{mol m}^{-2} \text{s}^{-1}$	<i>M. aeruginosa</i>	FACHB 469	China	Unicellular	Colony diameter: $D_{50}=40 \mu\text{m}$, $D_{90}=100 \mu\text{m}$	Li et al. (2013c)
Temperature	15°C	<i>M. aeruginosa</i>	FACHB 469	China	Unicellular	Colony diameter: $D_{50}=40 \mu\text{m}$, $D_{90}=100 \mu\text{m}$	Li et al. (2013c)
Light and temperature	10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 15°C	<i>M. aeruginosa</i>	FACHB 469	China	Unicellular	Colony diameter: $D_{50}=18 \mu\text{m}$	Xu et al. (2016a)
		<i>M. wesenbergii</i>	FACHB 908	China	Unicellular	Colony diameter: $D_{50}=15 \mu\text{m}$	
		<i>M. viridis</i>	FACHB 979	China	Unicellular	Colony diameter: $D_{50}=7 \mu\text{m}$	
		<i>M. ichthyoblabe</i>	FACHB 1294	China	Unicellular	Colony diameter: $D_{50}=20 \mu\text{m}$	
		<i>M. flos-aquae</i>	FACHB 1272	China	Unicellular	Colony diameter: $D_{50}=22 \mu\text{m}$	
Ca^{2+}	100 mg L^{-1}	<i>M. aeruginosa</i>	XW01	Xuanwu	Small colonies, colony	Colony diameter: 130 μm	Wang et al.

				Lake, China	diameter < 25 µm		(2011)
	20, 50, 100 mg L ⁻¹	<i>M. aeruginosa</i>	FACHB 469	China	Unicellular	Colony diameter reached > 75 µm, averaged at 32, 37 and 51 µm	Zhao et al. (2011)
	1000 mg L ⁻¹	<i>M. aeruginosa</i>	UTEX LB 2061	USA	Unicellular	Cells per colony: 120	Sato et al. (2016)
Pb ²⁺	10, 20, 40 mg L ⁻¹	<i>M. aeruginosa</i>	FACHB	China	Unicellular	Colony diameter: 20 µm	Bi et al. (2013)
Nitrogen and phosphorus	N=1.98 mg L ⁻¹ and P=0.65 mg L ⁻¹	<i>M. aeruginosa</i>	PCC 7806	China	Unicellular	The maximum colony diameter reached 275 µm	Yang and Kong (2013)
Nitrogen	N=1.59, 3.20, 6.39, 12.79, 25.58, 51.16 mg	<i>Microcystis</i> spp.		Lake Taihu, China	Colonies, average in 205 ± 21 mm	Colony diameter decreased to 99, 102, 103, 106, 108 and 111 µm	Ma et al. (2014)

	L ⁻¹	
Phosphorus	P=0.08, 0.17, 0.34, 0.68, 1.35, 2.68 mg L ⁻¹	Colony diameter decreased to 112, 65, 62, 58, 50, 43 and 39 µm
Nitrogen and phosphorus	N=1.59 mg L ⁻¹ and P=0.08 mg L ⁻¹ ; N=3.20 mg L ⁻¹ and P=0.17 mg L ⁻¹ ; N=6.39 mg L ⁻¹ and P=0.34mg L ⁻¹ ; N=12.79 mg L ⁻¹ and P=0.68 mg L ⁻¹ ; N=51.16 mg L ⁻¹ and	Colony diameter decreased to 118, 85, 26, 0.43 and 9.43 µm

P=2.68 mg L⁻¹

Combined biotic and abiotic factors

Zooplankton	<i>Ochromonas</i> sp.	<i>M. aeruginosa</i>	FACHB-	China	Unicellular	The maximum cells per colony	Wang et al.
gazing and	with modified		927			reached 6	(2010)
nutrients	BG-11 medium						
	(N=10%)						
Ca ²⁺ and EPS	1000 mg L ⁻¹	<i>M. aeruginosa</i>	UTEX LB	USA	Unicellular	Cells per colony: 209	Sato et al.
	and 20 mg L ⁻¹		2061				(2016)

621 **FACHB: Freshwater Algae Culture Collection at the Institute of Hydrobiology, China.

622 NIES: National Institute for Environmental Studies, Japan.

623 PCC: strain passport.

624 D₅₀ (D₉₀): 50% (90%) of the total mass of the colonies is below this size.

625

Table S2. Field data collected from the published literature showing details of colonies formation for *Microcystis* spp.

Environmental factors	Target species	Origin	Maximum colony size	reference
Temperature	<i>M. wesenbergii</i>	Hirosawa-no-ike Pond, Janpan	Cells per colony reached 700	Yamamoto et al. (2011)
	<i>Microcystis</i> spp.	Lake Taihu, China	Colony diameter: D ₅₀ = 100 µm in spring; Colony diameter: D ₅₀ = 500 µm in summer	Zhu et al. (2016)
	<i>M. ichthyoblabe</i>	Lake Taihu, China	Colony diameter: D ₅₀ =300 µm	Zhu et al. (2015); Zhu et
Nutrients	<i>M. wesenbergii</i>		Colony diameter: D ₅₀ =700 µm, D ₉₀ =950 µm	al. (2014)