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

Running title: Colony formation of *Microcystis*

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Abstract

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

**Key words**

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

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

The occurrence of *Microcystis* blooms has increased with eutrophication of freshwater systems, and the frequency and severity of blooms is predicted to increase with the increasing temperature and CO$_2$ partial pressure associated with climate change (Paerl et al., 2016; Visser et al., 2016). Initial colony formation of *Microcystis* is a critical step in its bloom development. Blooms of *Microcystis* form in a sequential pattern: cells and small colonies that have overwintered in the sediment rise into the 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. wessenbergii* 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.
Material and methods

Data collection

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

Data analysis

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

The fold-increase of total biomass \( f - B \) was directly calculated from the cell concentration (cell mL\(^{-1}\)), or approximated from dry weight (mg L\(^{-1}\)) or chlorophyll \( a \) concentration (µg L\(^{-1}\)) following

\[
f - B = C_t / C_{t0},
\]

(1)

where \( C_t \) and \( C_{t0} \) are cell concentrations, dry weight or chlorophyll \( a \) concentrations at time \( t \) and the initial time \( t_0 \). If there were no records of cell concentration, dry weight or chlorophyll \( a \) concentration throughout the growth period, then \( f - B \) was derived from the specific growth rate (\( \mu \), day\(^{-1}\)) (Andersen, 2005) as

\[
f - B = e^{\mu \times (t - t_0)}. 
\]

(2)

Fold-increase of cells per colony

Colony size was reported in the literature as colony diameter (D, µm), colony aggregation area (A, µm\(^2\)), particle volume (V, µm\(^3\)), or proportion of different colony size groups (denoted as PS, %).

To calculate the fold-increase of cells per colony \( f - CC \), colony diameter was determined from all available datasets. When calculating from the aggregation area and particle volume, the colony was approximated as a sphere. When calculating from PS, the weighted mean colony diameter was based on the proportion and median colony size in each group. For instance, Wang et al. (2011) set five colony size groups:

\(< 5 \ \mu m, 5-20 \ \mu m, 20-40 \ \mu m, 40-60 \ \mu m \ \text{and} \ > 70 \ \mu m. \ \text{For the three groups in the} \)
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
\[ N = 10^{2.99 \times \log_{10} D - 2.80} \]  
for colonies < 15 μm, N was calculated as
\[ N = \frac{D}{2.59^2} \]
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
\[ f – CC = \frac{N_t}{N_{t0}} \]
where _N_t_ and _N_{t0}_ 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.
Bound-Extracellular Polymeric Substance

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

$$f_{\text{bEPS}} = \frac{\text{bEPS}_t}{\text{bEPS}_c},$$  \hspace{1cm} (6)

where $\text{bEPS}_t$ and $\text{bEPS}_c$ are the amount of bEPS measured at time t under different treatments and control groups. Here, t also indicates the exponential growth phase or beginning of stationary growth phase.

Colony formation mechanism

For the purpose of this study, based on the hypothesis, it was presumed 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. Therefore, the mechanism of colony formation was defined as:

- Cell-division dominates when $f_{\text{B}}/f_{\text{CC}} > 1.$
- Cell-adhesion dominates when $f_{\text{B}}/f_{\text{CC}} < 1.$

Comparison to field studies

The criteria developed defining colony formation by cell-division and cell-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 ($D < 5$ um), and all except 11 used M. aeruginosa. Of the 79
datasets, 48% examined the effects of abiotic factors: low temperature and low light intensities \((n = 28)\), \(\text{Ca}^{2+} \ (n = 5)\), lead \((\text{Pb}^{2+}) \ (n = 3)\) and nutrients (nitrogen and phosphorus, \(n = 1\)); 47% investigated the effects of biotic factors: heterotrophic bacteria \((n = 11)\), microcystins \((\text{MCs}, \ n = 9)\), zooplankton grazing \((n = 8)\), zooplankton filtrate \((n = 7)\) and the presence of the cyanobacterium \textit{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 \(\text{Ca}^{2+}\) concentration with EPS addition \((n = 1)\).

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

The different treatments also affected colony size (Table S1): high \(\text{Ca}^{2+}\) 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).
Colony formation by cell-division

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

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

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

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

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

In this study colony formation by cell-division was defined as occurring when the increase in cells per colony occurred at lower rate than the increase in total biomass. In the cell-division process, cells remain attached after binary fission, and additional production of bEPS surrounds the cells (Xu et al., 2013). Cell-division leads to colonies with regularly arranged cells, however as the colonies continue to increase in size, the arrangement of cells may become irregular (Yang et al., 2008). As colony formation by cell-division requires multiple cell-division events to occur, 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 μm), 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 μm 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
additionally suggests that the mechanism of colony formation needs to be considered in experimental design. This study illustrated that bEPS production differs depending on the mechanism of colony induced and this could affect the response of colonies to other variables tested.

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

Lake studies of *Microcystis* blooms have shown succession of individual species 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 deceasing

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.

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Figure Legends

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

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

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

Figure 4: Comparison of fold-increase in cells per colony ($f - CC$) and fold-increase in total biomass ($f - B$) for M. ichthyoblabe (solid triangles) and M. wesenbergii (open circles) from field populations. The diagonal line represents slope = 1, where colonies
below this line formed by cell-division and colonies above the line formed by cell-adhesion.
### Table S1. Laboratory and field data collected from the published literature, showing details of *Microcystis* colony formation and biotic and abiotic treatments.

<table>
<thead>
<tr>
<th>Environment factor</th>
<th>Treatment</th>
<th>Target species</th>
<th>Strains</th>
<th>Origin</th>
<th>Morphology at start of experiment</th>
<th>Morphology at end of experiment</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Biotic factors</strong></td>
<td></td>
<td><em>M. aeruginosa</em></td>
<td>1450/1</td>
<td>UK</td>
<td>Unicellular</td>
<td>Colony diameter &lt;50 μm</td>
<td>Burkert et al. (2001)</td>
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<tr>
<td>Zooplankton</td>
<td>Grazing</td>
<td><em>Ochromonas</em> sp.</td>
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<td><em>M. aeruginosa</em></td>
<td>FACHB</td>
<td>China</td>
<td>Unicellular</td>
<td>Cells per colony reached 4.1</td>
<td>Yang et al. (2006)</td>
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<td></td>
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<td><em>M. aeruginosa</em></td>
<td>PCC 7820</td>
<td>China</td>
<td>Unicellular</td>
<td>Cells per colony reached 4.6</td>
<td>Yang et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. aeruginosa</em></td>
<td>PCC 7806</td>
<td>China</td>
<td>Unicellular</td>
<td>Colony diameter reached 180 μm</td>
<td>Yang and Kong</td>
</tr>
<tr>
<td>Zooplankton filtrate</td>
<td>Filtrated lake water</td>
<td><em>M. aeruginosa</em></td>
<td>FACHB</td>
<td>China</td>
<td>Unicellular</td>
<td>Cells per colony reached 1.8; colony diameter &lt; 5 μm</td>
<td>Yang et al. (2005)</td>
</tr>
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<tr>
<td><em>Ochromonas</em> sp.</td>
<td><em>M. aeruginosa</em></td>
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<td>China</td>
<td>Unicellular</td>
<td>Cells per colony reached 1.4</td>
<td>Yang et al. (2009b)</td>
<td></td>
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<tr>
<td><em>Moina macrocopa,</em></td>
<td><em>M. aeruginosa</em></td>
<td>NIES</td>
<td>Japan</td>
<td>Unicellular</td>
<td>Cells per colony reached 1.3; colony diameter &lt; 4 μm</td>
<td>Jang et al. (2003)</td>
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<tr>
<td><em>Daphnia magna</em></td>
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<td><em>Cyanobacteria</em></td>
<td><em>C. raciborskii</em></td>
<td>MIRF-01</td>
<td>China</td>
<td>Unicellular</td>
<td>Cells per colony reached 45</td>
<td>Mello et al. (2012)</td>
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<tr>
<td><em>Microcystins</em></td>
<td>MC-RR (0.25-10 μg L⁻¹)</td>
<td><em>M. wesenbergii</em></td>
<td>DC-M1</td>
<td>China</td>
<td>Colony diameter</td>
<td>Colony diameter reached 580 μm</td>
<td>Gan et al. (2012)</td>
</tr>
<tr>
<td>Species</td>
<td>Morphology</td>
<td>Colony Diameter</td>
<td>Reference</td>
<td></td>
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<tr>
<td><em>M. ichthyoblabe</em> TH-M1</td>
<td>Colonies</td>
<td>&lt;150 µm</td>
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<tr>
<td><em>Microcystis</em> sp. FACHB</td>
<td>Colonies</td>
<td>&lt;150 µm</td>
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<tr>
<td><em>M. aeruginosa</em> FACHB</td>
<td>Unicellular</td>
<td>Colony diameter</td>
<td>Shen et al. (2011)</td>
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<td>1027</td>
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<tr>
<td><em>M. aeruginosa</em> FACHB</td>
<td>Unicellular</td>
<td>Colony diameter</td>
<td>Wang et al. (2016)</td>
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<td>905</td>
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<td><em>M. aeruginosa</em> PCC 7806</td>
<td>Unicellular</td>
<td>Colony diameter</td>
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<tr>
<td><em>M. wesenbergii</em> CH</td>
<td>Unicellular</td>
<td>Colony diameter</td>
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<td>Lake Chaohu,</td>
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<tr>
<td><em>M. wesenbergii</em> TH</td>
<td>Unicellular</td>
<td>Colony diameter</td>
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<td></td>
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<td>Colony diameter</td>
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<tr>
<td>Organism</td>
<td>Location</td>
<td>Morphology</td>
<td>Growth Form</td>
<td>Notes</td>
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<tr>
<td><em>M. flos-aquae</em></td>
<td>Lake Chaohu, China</td>
<td>Unicellular</td>
<td>No colony formation</td>
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<tr>
<td><em>M. viridis</em></td>
<td>Lake Chaohu, China</td>
<td>Unicellular</td>
<td>No colony formation</td>
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<tr>
<td><em>Microcystis sp.</em></td>
<td>Lake Chaohu, China</td>
<td>Unicellular</td>
<td>No colony formation</td>
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</table>

**Abiotic factors**

<table>
<thead>
<tr>
<th>Light Intensity</th>
<th>Organism</th>
<th>Location</th>
<th>Morphology</th>
<th>sEPS Yield Reached</th>
<th>References</th>
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<tbody>
<tr>
<td>25 μmol m⁻² s⁻¹</td>
<td><em>M. aeruginosa</em></td>
<td>FACHB 942, China</td>
<td>Unicellular, 2-3 μm</td>
<td>0.03 μg mg⁻¹ DW d⁻¹</td>
<td>Wu and Song (2008)</td>
</tr>
<tr>
<td></td>
<td><em>M. aeruginosa</em></td>
<td>PCC 7806, The Netherland</td>
<td>unicellular, 2-3 μm</td>
<td>0.03 μg mg⁻¹ DW d⁻¹</td>
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<tr>
<td></td>
<td><em>M. aeruginosa</em></td>
<td>NSW 924, Australia</td>
<td>unicellular, 2-3 μm</td>
<td>0.05 μg mg⁻¹ DW d⁻¹</td>
<td></td>
</tr>
<tr>
<td><strong>M. aeruginosa</strong></td>
<td>FACHB</td>
<td>Location</td>
<td>Description</td>
<td>sEPS Yield</td>
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<tr>
<td>905</td>
<td>Dianchi, China</td>
<td>unicellular, 2-3 μm</td>
<td>0.04 μg mg⁻¹ DW d⁻¹</td>
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<tr>
<td>975</td>
<td>Wudalianchi, China</td>
<td>Small colonies, average colony diameter: 37.5–50 μm</td>
<td>0.13 μg mg⁻¹ DW d⁻¹</td>
<td></td>
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<tr>
<td>910</td>
<td>Xinyan, China</td>
<td>Small colonies, colony diameter: 25-50 μm</td>
<td>0.23 μg mg⁻¹ DW d⁻¹</td>
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<tr>
<td>938</td>
<td>Tuanshang, China</td>
<td>Large colonies, colonies diameter &gt; 650 μm</td>
<td>0.33 μg mg⁻¹ DW d⁻¹</td>
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<tr>
<td>907</td>
<td>Dianchi, China</td>
<td>Intermedium colonies, colony diameter: 62.5 – 100 μm</td>
<td>0.23 μg mg⁻¹ DW d⁻¹</td>
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<tr>
<td>909</td>
<td>Bao’anhu, China</td>
<td>Large colonies, colony diameter &gt; 205 μm</td>
<td>0.23 μg mg⁻¹ DW d⁻¹</td>
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</tr>
<tr>
<td>Light</td>
<td>10 μmol m$^{-2}$ s$^{-1}$</td>
<td><em>M. aeruginosa</em></td>
<td>FACHB 469</td>
<td>China</td>
<td>Unicellular</td>
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<tr>
<td>Temperature</td>
<td>15°C</td>
<td><em>M. aeruginosa</em></td>
<td>FACHB 469</td>
<td>China</td>
<td>Unicellular</td>
</tr>
<tr>
<td>Light and temperature</td>
<td>10 μmol m$^{-2}$ s$^{-1}$</td>
<td><em>M. aeruginosa</em></td>
<td>FACHB 469</td>
<td>China</td>
<td>Unicellular</td>
</tr>
<tr>
<td></td>
<td>and 15°C</td>
<td><em>M. wesenbergii</em></td>
<td>FACHB 908</td>
<td>China</td>
<td>Unicellular</td>
</tr>
<tr>
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<td><em>M. viridis</em></td>
<td>FACHB 979</td>
<td>China</td>
<td>Unicellular</td>
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<tr>
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<td><em>M. ichthyoblabe</em></td>
<td>FACHB 1294</td>
<td>China</td>
<td>Unicellular</td>
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<tr>
<td></td>
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<td><em>M. flos-aquae</em></td>
<td>FACHB 1272</td>
<td>China</td>
<td>Unicellular</td>
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<tr>
<td>Ca$^{2+}$</td>
<td>100 mg L$^{-1}$</td>
<td><em>M. aeruginosa</em></td>
<td>XW01 Xuanwu</td>
<td>Small colonies, colony</td>
<td>Colony diameter: 130 μm</td>
</tr>
<tr>
<td>Concentration</td>
<td>Species</td>
<td>Reference</td>
<td>Colony Diameter</td>
<td>Additional Information</td>
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</tr>
<tr>
<td>20, 50, 100 mg L(^{-1})</td>
<td><em>M. aeruginosa</em> FACHB 469 China</td>
<td>Unicellular</td>
<td>Colony diameter reached &gt; 75 μm, averaged at 32, 37 and 51 μm</td>
<td>Zhao et al. (2011)</td>
<td></td>
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<tr>
<td>1000 mg L(^{-1})</td>
<td><em>M. aeruginosa</em> UTEX LB 2061 USA</td>
<td>Unicellular</td>
<td>Cells per colony: 120</td>
<td>Sato et al. (2016)</td>
<td></td>
</tr>
<tr>
<td>10, 20, 40 mg L(^{-1})</td>
<td><em>M. aeruginosa</em> FACHB China</td>
<td>Unicellular</td>
<td>Colony diameter: 20 μm</td>
<td>Bi et al. (2013)</td>
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<tr>
<td>N=1.98 mg L(^{-1}) and P=0.65 mg L(^{-1})</td>
<td><em>M. aeruginosa</em> PCC 7806 China</td>
<td>Unicellular</td>
<td>The maximum colony diameter reached 275 μm</td>
<td>Yang and Kong (2013)</td>
<td></td>
</tr>
<tr>
<td>N=1.59, 3.20, 6.39, 12.79, 25.58, 51.16 mg</td>
<td><em>Microcystis</em> spp. Lake, Taihu, China</td>
<td>Colonies, average in 205 ± 21 mm and 99, 102, 103, 106, 108 and 111 μm</td>
<td>Colony diameter decreased to</td>
<td>Ma et al. (2014)</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Colony diameter decreased to</td>
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<tr>
<td>0.08, 0.17, 0.34, 0.68, 1.35, 2.68 mg L(^{-1})</td>
<td>112, 65, 62, 58, 50, 43 and 39 µm</td>
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</table>

<table>
<thead>
<tr>
<th>Nitrogen and phosphorus</th>
<th>Colony diameter decreased to</th>
</tr>
</thead>
<tbody>
<tr>
<td>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}), and P=0.34 mg L(^{-1}), N=12.79 mg L(^{-1}), and P=0.68 mg L(^{-1}), N=51.16 mg L(^{-1}), and</td>
<td>118, 85, 26, 0.43 and 9.43 µm</td>
</tr>
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</table>
P=2.68 mg L\(^{-1}\)

**Combined biotic and abiotic factors**

<table>
<thead>
<tr>
<th>Zooplankton/foods</th>
<th>(Ochromonas) sp.</th>
<th>(M. aeruginosa)</th>
<th>FACHB-927</th>
<th>China</th>
<th>Unicellular</th>
<th>The maximum cells per colony reached 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>gazing and nutrients</td>
<td>with modified BG-11 medium (N=10%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>(Ca^{2+}) and EPS</th>
<th>(1000) mg L(^{-1})</th>
<th>(M. aeruginosa)</th>
<th>UTEX LB</th>
<th>USA</th>
<th>Unicellular</th>
<th>Cells per colony: 209</th>
</tr>
</thead>
<tbody>
<tr>
<td>and 20 mg L(^{-1})</td>
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</table>

Wang et al. (2010)

Sato et al. (2016)

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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 \(D_{50}(D_{90})\): 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.

<table>
<thead>
<tr>
<th>Environmental factors</th>
<th>Target species</th>
<th>Origin</th>
<th>Maximum colony size</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td><em>M. wesenbergii</em></td>
<td>Hirosawa-no-ike Pond, Japan</td>
<td>Cells per colony reached 700</td>
<td>Yamamoto et al. (2011)</td>
</tr>
<tr>
<td></td>
<td><em>Microcystis</em> spp.</td>
<td>Lake Taihu, China</td>
<td>Colony diameter: $D_{50} = 100 \mu m$ in spring; Colony diameter: $D_{50} = 500 \mu m$ in summer</td>
<td>Zhu et al. (2016)</td>
</tr>
<tr>
<td>Light and Nutrients</td>
<td><em>M. ichthyoblabe</em></td>
<td>Lake Taihu, China</td>
<td>Colony diameter: $D_{50} = 300 \mu m$</td>
<td>Zhu et al. (2015); Zhu et al. (2014)</td>
</tr>
<tr>
<td></td>
<td><em>M. wesenbergii</em></td>
<td>Lake Taihu, China</td>
<td>Colony diameter: $D_{50} = 700 \mu m$, $D_{90} = 950 \mu m$</td>
<td>al. (2014)</td>
</tr>
</tbody>
</table>
Fold-increase of cells per colony vs. Fold-increase of bPES production.

- Cell-adhesion: $y = 25.12x - 36.10$
- Cell-division: Adjusted $R^2 = 0.74$, $n = 25$

$p < 0.001$
The graph illustrates the relationship between the proportion of cells forming a colony by division and the growth rate. The equation for the fitted curve is:

\[ y = 141.43e^{-5.56x} \]

with an adjusted R\(^2\) of 0.72 and n = 27, indicating a significant correlation at p < 0.001.

The data points are categorized as follows:
- Heterotrophic bacteria (△)
- Microcystins (◇)
- Zooplankton filtrate (×)
- Pb\(^{2+}\) (■)
- Temperature & light (▲)
- Grazing & nutrients (□)