Environmental factors controlling colony formation in blooms of the cyanobacteria Microcystis spp. in Lake Taihu, China

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1. Introduction

The cycles of the key macronutrients nitrogen (N) and phosphorus (P) have been massively altered by anthropogenic activities (Elser et al., 2007). Over-enrichment by nitrogen and phosphorus accelerates eutrophication of water bodies and promotes algal blooms, which has been observed in lakes worldwide (Conley et al., 2009a,b; Hautier et al., 2009; Paerl et al., 2011a; Schindler and Hecky, 2009). Cyanobacteria produce toxins and taste and odor compounds which threaten drinking water supply and adversely affect recreational and fishing use of affected systems. Large accumulations of cyanobacteria, surface blooms, can lead to hypoxia and disrupt food webs. Cyanobacteria represent a public health threat and cause undesirable ecological, economic, water resource impacts and related societal problems (Brookes and Carey, 2011; Paerl et al., 2011a). In large eutrophic lakes, such as Lake Taihu, China where blooms are widespread and persistent, there is an increased urgency to deal with this problem.

Many bloom-forming cyanobacterial genera exist as macroscopic colonies that are present in buoyant surface blooms or dispersed throughout the water column. Microcystis is a common and often dominant colonial, bloom-forming genus responsible for harmful (toxic, food-web disrupting, and hypoxia generating) blooms in nutrient-enriched lakes worldwide (Paerl et al., 2011b). The mechanisms underlying how and why Microcystis form colonies remain poorly understood. Microcystis generally exists as single cells, or a few paired cells, in laboratory cultures but occurs in colonies under natural conditions (Reynolds et al., 1981;
2. Experimental procedures

2.1. Experimental design

A series of bioassays were set up with treatments of lake water containing varying N and P concentrations to assess how nutrient additions impacted Microcystis colony size. Thirty liters (30 L) of lake water containing Microcystis colonies was pumped into white plastic buckets (48 buckets, each bucket had a maximum volume of 35 L) on 10 September 2012 and incubated outside at the Taihu Laboratory of Lake Ecosystem Research (TILER) on the shores of Lake Taihu, Wuxi. N and P were added as NaNO₃ and KH₂PO₄. Three nutrient addition treatments (N only, P only and both N&P) manipulated the nutrient concentrations to 2, 4, 8, 16 and 32 times to the ambient lake water (Table 1). The control treatment contained lake water with no nutrient addition. Each treatment was triplicated. Physico-chemical parameters were measured on days 0, 3, 6, 9, 12, 15, 18, between 8:00 am and 9:00 am. Coinciding with the sampling of physico-chemical variables, 0.5 L of water was sampled from each bucket to determine Microcystis colony size, cell concentration, phytoplankton community composition and chlorophyll a. Weather conditions and air temperature were recorded every day. Each bucket was stirred daily at 7:00 and 19:00.

2.2. Measurements

Physico-chemical parameters, including water temperature (WT), dissolved oxygen (DO), pH and electrical conductivity (EC), were measured with a Yellow Springs Instruments (YSI) 6600 multi-sensor probe. 0.5 L water was sampled initially from each bucket to determine biological and chemical parameters, including Microcystis colony size, phytoplankton community composition, and chlorophyll a.

The size of Microcystis colonies was determined using image analysis employing Olympus DP Soft software, with an Olympus BX 51 light microscope and an Olympus DP 71 digital camera, as described by Wilson et al. (2010). To count Microcystis colonies, 1 mL aliquots were counted by microscopy on days 0, 3, 6, 9, 12, 15 and 18 using a wide-mouth pipette. Fifty colonies were randomly chosen from each sample and sized with a calibrated ocular (200× magnification). Colony size was measured as equivalent spherical diameter (ESD) of the colony [ESD = (6/π)p^(1/3)].

Phytoplankton samples were preserved with Lugol’s iodine solution (2% final conc.) and sedimented for 48 h. Cell density was measured with a Sedgewick–Rafter counting chamber under magnification of 200–400×. Phytoplankton species were identified according to Hu et al. (1980). Algal biovolumes were calculated from cell numbers and cell size measurements. Conversion to biomass assumed that 1 mm³ of volume was equivalent to 1 mg of fresh weight biomass. The proportions of Microcystis to total phytoplankton were calculated by Microcystis biovolumes to total algal biovolumes. Chl-α concentrations were determined spectrophotometrically after extraction in 90% hot ethanol (Papista et al., 2002).

2.3. Statistical analysis

Data are presented as means ± SD. Significant differences between control and treated samples were determined by ANOVA with Tukey post hoc test. Statistical analyses were conducted with SPSS17.0.

3. Results

3.1. Environmental factors

The weather conditions were cloudy (day 0–2, day 4, day 11, day 17–18), rainy (day 3 and day 12) and sunny (day 5–10, day 13–16). Air temperature ranged from 16 to 28 °C. Water temperature ranged from 18.8 to 22.5 °C. The pH, dissolved oxygen (DO) concentration, dissolved oxygen saturation (DO%), and electrical conductivity (EC) in N plus P additions were greater than those observed in either N additions, or P additions and controls (Table 2).

3.2. N, P and N&P impacts on Microcystis colony size

Microcystis aeruginosa, Microcystis flos-aquae and Microcystis wesenbergii were the main Microcystis species in this study.
Table 2
Range of pH, DO, DO% and EC in various treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>N additions</th>
<th>P additions</th>
<th>N plus P additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.91–9.41</td>
<td>8.79–9.46</td>
<td>8.97–9.54</td>
<td>9.45–11.28</td>
</tr>
<tr>
<td>DO (mg L−1)</td>
<td>8.95–12.24</td>
<td>9.27–14.66</td>
<td>9.08–11.13</td>
<td>9.1–16.94</td>
</tr>
<tr>
<td>DO% (%)</td>
<td>102.9–111.4</td>
<td>105.3–1269</td>
<td>101.1–124.7</td>
<td>101.4–232.3</td>
</tr>
<tr>
<td>EC (μS cm−1)</td>
<td>435–638</td>
<td>439–1211</td>
<td>428–698</td>
<td>488–1346</td>
</tr>
</tbody>
</table>

The pH, dissolved oxygen (DO), dissolved oxygen saturation (DO%), and electrical conductivity (EC) were measured between 8:00 am and 9:00 am on days 0, 3, 6, 9, 12, 15, 18 by a Yellow Springs Instruments (YSI) 6600 multi-sensor sonde.

Microcystis colony sizes from samples of natural lake water ranged from 20 to 1100 μm with an average of 205 ± 21 μm. Colony size decreased in every treatment including the control. P only and high concentration of P plus N additions promoted Microcystis colony size decreased significantly (relative to control). There was no significant \( p > 0.05 \) difference between N addition alone and the control (Fig. 1a). However, Microcystis colony size in only P treatments were significantly smaller than the control after 9 days (Fig. 1b; day 9–12, \( p < 0.05 \) in 2–4× \( p < 0.01 \) in 8–32× and \( p < 0.01 \) after 15 days in all only P addition). Increasing concentration (8–32×) of both N and P relative to lake water (TN > 13.95 mg L−1, TP > 0.74 mg L−1 in buckets) caused a decrease in colony size relative to control and Microcystis existed in the form of single cell and extremely small colonies after 15 days. The 32× addition (TN = 51.15 mg L−1 and TP = 2.706 mg L−1 in buckets) led to a decrease in colony size from day 3 (\( p < 0.05 \)) but 8–16× from day 9 (\( p < 0.01 \)). However, Microcystis colony size in treatments with both N and P additions at concentrations two to four times ambient concentrations were not significantly different from the control (Fig. 1c). The morphology of the colonies also changed over the course of the experiment (Fig. 2). Colony size in treatments 32× P, 32× N&P and control all showed a reduction in size. The characteristic change in colony morphology was from a clathrate form initially to smaller more loosely packed colonies later in the period.

There were no significant correlations \( p > 0.05 \) between environmental factors and Microcystis colony size in all treatments with the exception of pH values which was negatively correlated with colony (Fig. 3).

3.3. N, P and N&P impacts on phytoplankton biomass and dominance of Microcystis

The initial phytoplankton biomass concentration (Chl-a) in lake water was 20.46 ± 0.82 μg L−1. The addition of N alone (2–32× and the) resulted in growth that exceeded the control after 12 days of incubation (Fig. 4a; \( p < 0.05 \)). The biomass of treatments in which only P was added did not differ significantly from controls (Fig. 4b; \( p > 0.05 \)). Biomass in both N&P added treatments was much higher than control and N only additions significantly from day 3 (\( p < 0.05 \)). The treatment with 8 times N&P added (TN = 13.95 mg L−1, TP = 0.74 mg L−1 in buckets) had the highest Chl-a concentration on day 15 (281.6 ± 28.9 μg L−1; Fig. 4c).

Total phytoplankton biovolume was 36.6 ± 3.9 mg L−1 and the Microcystis proportion was 30.89 ± 1.5% initially. The 2× N addition had no significant \( p > 0.05 \) effect on the proportion of Microcystis but 4–32 times N additions was higher than control on day 12 (Fig. 5a; \( p < 0.05 \)). There was no significant difference between the P addition treatment and the control (Fig. 5b; \( p < 0.05 \)). The proportion of Microcystis declined until day 6 in the N&P addition treatment but then steadily increased (Fig. 5c).

4. Discussion

Interestingly, the additions of P and high concentration of P plus N resulted in Microcystis colonies smaller than the control, which is in contrast with the N only treatments and treatments with low concentration of P plus N (Fig. 1). Larger colonies persisted in treatments with N addition alone and low concentrations of N + P. In the high concentration N + P treatments the colonial form was almost entirely absent. The observed change in colony size could be a combined effect of both aggregation and disaggregation of existing colonies, and the production of new colonies. Such as filaments of Anabaena circinalis aggregated under low light conditions, effectively increasing their size and buoyancy (Brookes et al., 1999).

Colonial formation is a trade-off between nutrient uptake, diffusion of gases into and throughout the colony, light requirements, predator avoidance, buoyancy regulation and the ability to vertically migrate rapidly enough to exploit resource (e.g., nutrient) availability in the water column, which are often.
Vertically separated (Ganf and Oliver, 1982). Resource availability impacts upon these aspects of growth, polysaccharide accumulation and colony formation. N and P are essential elements for the synthesis of nucleic acids, ATP or proteins, which are necessary for cell division and growth (Conley et al., 2009b). Extracellular polysaccharides (EPS), comprised of C, H and O, are the main constituents of the colonial Microcystis sheaths associated with cell aggregates (Plude et al., 1991). Both soluble carbohydrates and total carbohydrates have been shown to be significantly higher in cells and sheaths of colonial Microcystis than in disaggregated cells (Zhang et al., 2007). When there is an adequate supply of N and P (more than 7.75–13.95 TN mg L⁻¹ and 0.41–0.74 TP mg L⁻¹ in this study) for cell division and growth, the total phytoplankton biomass is able to increase until a resource, such as light, becomes limiting (Van de Waal et al., 2009). Under these nutrient replete conditions photosynthetic products are transformed to proteins, nucleic acids and ATP for cell proliferation and growth and normal activities. Excess accumulation of EPS is less in these environments and there is a reduced need for Microcystis to form colonies which limiting growth (Li et al., 2013). In contrast, Microcystis may produce more EPS when nutrient supply is inadequate. N or P starvation has often been described as a condition that enhances EPS synthesis (De Philippis et al., 2001; Huang et al., 2007; Otero and Vincenzini, 2003), and has been shown to lead to carbohydrate accumulation within cells (Brookes and Ganf, 2001). Low P availability led to an increase in EPS production in many cases (De Philippis et al., 2001; Huang et al., 2007; Nicolaus et al., 1999; Roux, 1996). However, the relationship between phosphorus availability and the production of EPS is not straightforward (Grillo and Gibson, 1979), and there are examples where an increase in P concentration had little effect on the amount of exopolymers (Pereira et al., 2009). While a mechanism leading to a reduction in

**Fig. 2.** The morphology of colonial Microcystis in buckets added 32 times P, 32 times N&P treatment and control in day 0, 3, 9 and 18. All colonies were common and chosen randomly, bar = 20 μm.
colony size has not been identified in the present study, it is plausible a change in colonial EPS is implicated. N availability may also affect EPS production and several authors have observed an increase in EPS synthesis with increasing N concentrations (Otero and Vincenzini, 2003; Suresh Kumar et al., 2007). The amount of polymer produced varied according to the N source supplied (Philippis and Vincenzini, 1998). The bioassays revealed N limitation (Fig. 4a), and N limitation has also been observed in Lake Taihu during Microcystis bloom (Xu et al., 2010). Interestingly although growth rate varied between the control treatments and the N addition treatments, there was no difference in colony size.

However, colony size decreased steadily with time in all treatments, including in the control treatment. This may have been due to CO₂ limitation, even in the controls, because there was growth on the controls and pH went up (Talling, 1976). The pH also negatively correlated with the size of the colonies, especially in N plus P additions (Fig. 3). Carbon limitation may limit photosynthetic production of extracellular polysaccharides to form colonial Microcystis sheaths. The preference for CO₂ over HCO₃⁻ and CO₃²⁻ as a photosynthetic carbon source but there is little free CO₂ in water when pH exceeds 10 (Paerl and Ustach, 1982). In addition, it is extremely difficult for cells inside of large Microcystis colonies to

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Fig. 3. The relationship between pH value and colonies size of virous treatment bucket. There is no significant ($p > 0.05$) relationships between pH and colonies size in control ($n = 21$), P additions ($n = 105$) and N additions ($n = 105$). In contrary, clear linear relationship in N plus P additions ($n = 105$).

Fig. 4. Chl-a concentration dynamic in buckets added N (a), P (b) and N&P (c). Controls are same in A–C.

Fig. 5. Microcystis proportion (bio volume) dynamic in buckets added N (a), P (b) and N&P (c). Controls are same in A–C.
obtain CO₂ under high pH condition (Gavis and Ferguson, 1975). Thus, at high pH levels which were promoted by N&P additions, there would be an advantage to existing as individual cells as opposed to colonies in order to minimize constraints on CO₂ diffusion.

Grazing also plays a role in colony formation (Fulton and Paerl, 1987; Yang et al., 2008). Watson (Watson et al., 1992) suggested that below concentrations of 8–10 μg L⁻¹ TP, total biomass is dominated by edible alg(e) (<35–50 μm). Above this range, there is a transition zone where both edible and inedible (>35–50 μm) alg(e) are similar in relative abundance. With increasing concentrations to approximately 30 μg L⁻¹, the inedible alg(e) rapidly become more dominant, until at TP > 50 μg L⁻¹ the phytoplankton biomass consists almost entirely of this larger size fraction. In our experiments, colony size of Microcystis in 2 times N&P addition always and 4 times (TN = 7.75 mg L⁻¹ and TP = 0.41 mg L⁻¹ in buckets) addition were slightly higher than the control in previous 9 days (Fig. 1c). The nutritional value of colonies may vary with size, with large colonies being able to store more nutrients thereby preventing predation and countering other potential negative environmental factors effectively (Cyr and Curtis, 1999; Kearns and Hunter, 2001; Paerl, 1996; Yamamoto et al., 2011). Since colony size strongly influences buoyancy and vertical migration (Kromkamp and Walsby, 1990), the vertical distribution of colonies can be affected by colony size and large Microcystis colonies typically float to water surface, while small colonies (less than 36 μm) and single-cell Microcystis showed a nearly uniform vertical distribution over depth (Wu and Kong, 2009).

Bacteria may also affect the colony formation. Shen et al. (2011) have shown that axenic cultures of Microcystis isolated from Lake Taihu became colonial when exposed to bacteria isolated from Lake Taihu. Heterotrophic bacteria also enhanced the aggregation of cultures of Microcystis aeruginosa, most likely through stimulation of through mucilage production by M. aeruginosa (Shen et al., 2011). This is supported by previous research, which shows extensive mucilage buildups on the surface of Microcystis cells in naturally occurring blooms (Gao and Yang, 2008; Tien et al., 2002).

Microcystis colony formation may mirror microcystin production. Microcystis populations are known to be comprised many different strains, from toxic and non-toxic with regard to microcystin production (Davis et al., 2009), and this is also the case in Lake Taihu (Otten et al., 2012). Possibly, high TP and TN concentration induce the selection of strains that are not able to form colonies and low P and TN inhibit the selection of Microcystis strains able to form colonies. Li et al. (2013) have also suggested that under stressful conditions (low light intensities and temperatures), Microcystis aeruginosa formed small colonies and exhibited low specific growth rates but standard culture conditions yielded single or paired cells with high specific growth rates.

It has been proposed that large colonial alg(e) lose advantage when they compete with small colonies or single cell alg(e) which can more effectively take up nutrients at low concentrations, and have less self shading during photosynthesis (Wilson et al., 2010). Bacterial miniaturization has been shown to occur during periods of stress to increase the surface to volume ratio of cells (Morita, 1975). However, if nutrients or light are in short supply the ability to move through a water column and scavange resources may be advantageous and this is enhanced as colony size increases and buoyancy can be controlled. The velocity of movement through the water is a function of colony size as velocity is proportional to the square of the colony radius (Reynolds, 1984).

Colony formation is a complex process influenced by a range of environmental factors. This research has shown that nutrient availability affected colony size, and this was particularly evident in the case of P availability. However, exactly what the cyanobacteria are responding to, nutrients or elevated pH, remains unclear. In nature, the populations are likely to be comprised of cells/colonies at different ages with different metabolic activities (Brookes et al., 2000). This heterogeneity is likely to lead to different growth rates, different ability to acquire resources or production of polysaccharides, which will also affect colony size, buoyancy and consequently, bloom formation.

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