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Differences in cyanobacterial strain responses to light and temperature reflect species plasticity

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Abstract

Microcystis aeruginosa and *Cylindrospermopsis raciborskii* are two cyanobacterial species that dominate freshwaters globally. Multiple strains of each species with different physiology occur, however, many studies have focused only on one or two strains, limiting our understanding of both strain variation and characterisation of the species. Therefore, in this study we examined the variation in growth and morphology of multiple isolates of both species, isolated from two adjacent Australian reservoirs.

Four *M. aeruginosa* strains (= isolates) (one colony-forming, three single-celled morphology) and eight *C. raciborskii* isolates (five with straight trichomes, three with coiled trichomes) were cultured individually in a factorial designed experiment with four light intensities (L: 10, 30, 50 and 100 μmol photons m^{-2} s^{-1}) and two temperatures (T: 20 and 28^{o}C). The specific growth rate (μ), cell volume, and final cell concentration was measured. The light attenuation coefficient (k_j), a measure of self-shading, was calculated.

The results showed that the intraspecific variation was greater than the interspecific variation. The μ of all isolates of *M. aeruginosa* and *C. raciborskii* ranged from 0.16 to 0.55 d^{-1} and 0.15 to 0.70 d^{-1}, respectively. However, at a specific light and temperature the mean μ of all *M. aeruginosa* isolates and *C. raciborskii* isolates were similar. At the species level, *M. aeruginosa* had higher growth rates at higher light intensity but lower temperature (μ= 0.38 ± 0.04 d^{-1}at L100T20), while straight *C. raciborskii* had higher growth rates at lower light intensity but higher temperature (μ= 0.45 ± 0.12 d^{-1} at L50T28), and coiled *C. raciborskii* had higher growth rates at higher light intensity and higher temperature (μ= 0.37 ± 0.05 d^{-1} at L100T28). The final cell concentration of *M. aeruginosa* was higher than *C. raciborskii*. However, *C. raciborskii* isolates had greater variation in μ, k_j and cell volume than *M. aeruginosa*. k_j varied with light and temperature, and decreased with surface-to-volume ratio within each species. k_j was lower for *M. aeruginosa* compared to *C. raciborskii* as expected

based on cell size, but interestingly, *C. raciborskii* coiled isolates had lower k_j than the straight isolates suggesting lower effect of self-shading.

This study highlights the extent of strain variation to environmental conditions and to species variability.

Key words

Cylindrospermopsis raciborskii; growth rate; light attenuation coefficient (k_j); *Microcystis aeruginosa*

1. Introduction

Microcystis aeruginosa and *Cylindrospermopsis raciborskii* are two of the most harmful blooming cyanobacteria in freshwater ecosystems. *M. aeruginosa* has been recorded as an overwhelmingly dominant species in some of the world's largest lakes, e.g., Lake Taihu (China) (Harke et al., 2016) and Lake Erie (north America) (Bullerjahn et al., 2016), while *C. raciborskii* is increasingly present in bloom proportions in lakes and reservoirs throughout the world (Sinha et al., 2012; Burford et al., 2016). Interestingly, in some subtropical and temperate lakes, these two species have been found to co-occur and/or have successive dominance (Soares et al., 2009). Furthermore, *C. raciborskii* seems to be gradually replacing *M. aeruginosa* in many systems and has become dominant or co-dominant in some tropical reservoirs (Marinho and Huszar, 2002; Moustaka-Gouni et al., 2007).

Previous studies have established that both *M. aeruginosa* and *C. raciborskii* outcompete other cyanobacterial species to be the dominant species in freshwater systems, this is likely a result of their ability to adapt to variable environments (Burford et al., 2016; Harke et al., 2016). *M. aeruginosa* is a single-celled species that forms colonies and blooms on the water

surface. *M. aeruginosa* is positively buoyant and it allows this species to overcome turbulent mixing (Walsby et al., 1995). *C. raciborskii* is a filamentous diazotrophic cyanobacterium, with straight and coiled trichomes. It is a neutrally buoyant species (Kehoe, 2009). There have been a number of reviews highlighting *C. raciborskii's* wide temperature tolerance, low light adaptation, and high efficiency in phosphorus use (Burford and Davis, 2011; Burford et al., 2016).

Additionally, both species have multiple strains with different morphologies (Wilson et al., 2000; Wilson et al., 2006). For both species, strains have been shown to vary in their physiological responses under a range of environmental conditions (Pierangelini et al., 2014; Willis et al., 2015). The dominance of co-existing strains have also been found to shift in response to changes with environmental conditions (Van de Waal et al., 2011; Burford et al., 2014; Wang et al., 2015). These variations in strain response may contribute to the highly competitive nature of both species. It is, therefore, critical to understand how much variation exists between strains of these cyanobacterial species in order to predict bloom formation under different environmental conditions.

Changes in light and temperature affect the growth of phytoplankton species (Paerl and Otten, 2013). Light is a key environmental resource for primary production of cyanobacteria, therefore affecting their growth. The photosynthetic ability varies between species (Schwaderer et al., 2011), leading to different optimal light conditions for growth. *M. aeruginosa* and *C. raciborskii* strains have been found to have different light optima (Briand et al., 2004; Wilson et al., 2006). *M. aeruginosa* forms surface "scum" (Reynolds, 2006), and the maximum growth rates of *M. aeruginosa* strains have been recorded at light intensities up to 300 μmol photons m^{-2} s^{-1} (Wicks and Thiel, 1990; Wiedner et al., 2003; Jiang et al., 2008).

In comparison, *C. raciborskii* develops subsurface blooms at depths of 2-3 m in water column (Saker and Griffiths, 2001), or be evenly distributed in the surface mixing layer (O'Brien et al., 2009), and has been shown to have a lower light optima, ~ 50 - 120 μmol photons m^{-2} s^{-1} (Briand et al., 2004; Dyble et al., 2006).

Growth experiments have shown a wide temperature tolerance from 16.5 to 35°C and optima from 24 to 32°C for *M. aeruginosa* strains (Li et al., 2015; Thomas and Litchman, 2016). In comparison, *C. raciborskii* has been shown to be adapted to higher temperatures than *M. aeruginosa*, i.e., with temperature tolerance from 19 to 40°C and optima from 29 to 32°C (Briand et al., 2004; Sinha et al., 2012; Thomas and Litchman, 2016). In field surveys, blooms of *M. aeruginosa* were found at water temperatures from 12 to 30°C (Li et al., 2015), while blooms of *C. raciborskii* typically occurred at >25°C (Saker and Griffiths, 2001; Recknagel et al., 2014).

Temperature also interacts with light to affect cyanobacterial growth rates and biomass accumulation (Yang et al., 2012; Kehoe et al., 2015). Studies have shown an increase in growth rate with an increase of both light and temperature for some strains of both species (Bittencourt-Oliveira et al., 2012; Li et al., 2014). Although it has been shown that light intensity and temperature also interact on growth of the two species, it is unclear how much variation exists between strains of each species.

Most studies on the effect of light on cyanobacterial growth have focused on incident light intensity, however, light availability through the water column is more important for cyanobacterial growth (Kirk, 1994). The availability of light decreases with water depth, this is partly due to shading of algal cells, either created by self-shading or by shading from other

species (Kirk, 1994). Competition for light may occur when faster growing species create a higher biomass that subsequently limits light availability for slower growing species (Passarge et al., 2006). Ultimately self-shading may limit their own growth when the light availability reaches a critical threshold (Huisman and Weissing, 1994). Consequently, when *M. aeruginosa* and *C. raciborskii* co-occur, the accumulation of *M. aeruginosa* on the water surface may reduce the light from penetrating into the deeper layers where *C. raciborskii* occurs. However, because *C. raciborskii* is adapted to low light the effect of shading by *M. aeruginosa* surface blooms may be minimal. Despite the major impact shading may have on the light availability and thus population dynamics of algae in lakes, there has, to our knowledge, been no quantitative studies on self-shading.

In summary, at the species level, both *M. aeruginosa* and *C. raciborskii* have been shown to have wide physiological responses to light and temperature. However, it is unclear how much of this variation is the result of different strains. Therefore, in this study, we compared multiple isolates of *M. aeruginosa* and *C. raciborskii* isolated from two adjacent lakes in Southeast Queensland, Australia, to determine the magnitude of strain versus species variability.

2. Materials and methods

2.1 M. aeruginosa and C. raciborskii isolates

Twelve isolates were used for this study. Four were *M. aeruginosa* isolates: three were single-celled (M2, M3 and M4) and the other one was single-celled with few small colonies (M5). The other eight were *C. raciborskii* isolates: five with straight trichomes (C1, C3, C6, WS01 and WS05) and the other three with coiled trichomes (WC03, WC04 and WC07). All

12 isolates were isolated in 2013 by micromanipulation and/or serial dilution, as described by (Andersen, 2005). All the *M. aeruginosa* isolates and *C. raciborskii* isolates C1, C3 and C6, were isolated from surface water samples from Baroon Pocket Reservoir (26°42′12″S, 152°52′5″E, Southeast Queensland, Australia). The other *C. raciborskii* isolates were isolated from surface water samples of Lake Wivenhoe (27°23′38″S, 152°36′28″E, Southeast Queensland, Australia), a reservoir approximately 40 km southwest of Baroon Pocket reservoir. The water samples were collected by hand at the water depth of 0 to 20 cm. All isolates were maintained in culture flasks with Jaworski Medium (JM, Thompson et al. (1988)), modified by reducing phosphorus content to 25% of the original concentration. This is because studies have shown that the phosphorus concentration in JM media inhibits growth of *C. raciborskii* (Moore et al., 2005; Willis et al., 2015). The cultures were maintained under light conditions of 15 μmol photons m^{-2} s^{-1} on a 12 h: 12 h light: dark cycle using LED lights at a constant temperature of 28^{o}C.

2.2 Growth conditions

Two temperatures (T: 20^{o}C and 28^{o}C) and four light intensities (L: 10, 30, 50 and 100 μmol photons m^{-2} s^{-1}, LED lights and neutral density filters to control light levels) were used for growth experiments. The eight treatments were notated as L10T20, L10T28, L30T20, L30T28, L50T20, L50T28, L100T20 and L100T28. Cultures of all isolates were habituated to each light and temperature treatment for four weeks prior to starting the experiment. Each isolate was cultured in triplicate in 250 mL culture flasks with 225 mL modified JM. The initial cell density of OD750 (optical density at a wavelength of 750 nm) was less than 0.01 to avoid self-shading of the cells, which approximately equated to cell concentrations of 10^5 cells mL^{-1} for *C. raciborskii* and 10^6 cells mL^{-1} for *M. aeruginosa*, respectively. OD750 was measured on 2.5 mL subsamples using a spectrophotometer (Novaspec II, Pharmacia

Biotech). There were high correlations between OD750 and cell counts for all isolates of both species (Table 1). Cultures were gently shaken daily.

2.3 Optical density and growth rate

Subsamples of each culture (2.5 mL) were taken daily for OD750 readings seven hours from the start of light cycle, from Day 0 to Day 5, then on alternate days until cultures reached stationary phase. The specific growth rate (μ, d^{-1}) was calculated during the exponential phase with the first order rate kinetics (Andersen, 2005):

$$\mu\ (d^{-1}) = (\ln (OD750_2/OD750_1))/(t_2-t_1) \quad (1)$$

Where: $OD750_2$ and $OD750_1$ are the OD750 readings at time t_2 and t_1 during the exponential growth phase. Following the measurements, Lugol's iodine solution was added to each subsample (approximately 1% final concentration) to preserve the cells for enumeration.

2.4 Cell size and morphology

The cell diameter of *M. aeruginosa* cells and cell length, cell width and trichome length of *C. raciborskii* isolates were measured at the stationary phase under light microscopy (LEICA ICC50) at ×400 magnification. The cell length and width of *C. raciborskii* were measured from randomly selected cells from each trichome. For each culture, at least 20 measurements were conducted for the cell size. The mean cell length under each treatment was calculated for each *C. raciborskii* isolate. The individual cells of *M. aeruginosa* and *C. raciborskii* were treated as spheres and cylinders respectively, and the cell volume and surface area were calculated following Hillebrand et al. (1999). The surface-to-volume ratio was calculated from these measurements.

175 ***2.5 Cell enumeration***

176 Cell enumeration of all cultures was conducted at stationary phase. *M. aeruginosa* cells were
177 counted with a Sedgewick Rafter counter under light microscopy (LEICA ICC50) at ×400
178 magnification. Small colonies of *M. aeruginosa* isolate M5 were dissociated by a boiling
179 pretreatment method (Joung et al., 2006) prior to enumeration. For *C. raciborskii*, trichome
180 counts were conducted under light microscopy (LEICA ICC50) at ×100 magnification. The
181 cell concentrations (w, cells mL^{-1}) of *C. raciborskii* were calculated from counts of the
182 trichome concentration (trichomes mL^{-1}), trichome length and the average cell length with the
183 equation:
184 Cell concentration (w, cells mL^{-1}) = trichome concentration × (Trichome length / average cell
185 length) (2)
186

187 ***2.6 Light attenuation coefficient of individual cells***

188 Light intensity was measured with a 4-pi sensor (LI-1400, Li-Cor Biosciences) at nine evenly
189 distributed locations inside the flasks and on the culture surface (Fig. 2C). I_0 was the initial
190 light intensity for the growth experiment (L10, L30, L50 and L100) illuminated from the
191 bottom of the culture flasks. I_{in} was measured on Day 0 on the bottom inside the flasks (Fig.
192 2A). I_{out}^* was measured on the culture surface at the stationary phase (Fig. 2B).
193 Light attenuation coefficient (k_j) was calculated for all cultures at stationary phase, using the
194 following equation (Huisman et al., 2002):
195 k_j (cells $(mL)^{-1})^{-1}$ m^{-1}) =log (I_{in} / I_{out}^*) /w /z (3)
196 Where: I_{in} is the incident light intensity for culture to grow, I_{out}^* is the critical light intensity
197 through the culture depth z, w is the cell concentration.
198

2.6 Statistical analyses

Differences in μ, k_j and cell volume under different light and temperature treatments were tested with two-way ANOVA. Differences in μ between isolates across the different light and temperature treatments were tested with three-way ANOVA, for each of the three groups. Leneve's test was used to test the homogeneity of variance, and Shapiro-Wilk test to test the normal distribution of residuals. Data were Boxcox transformed to better meet the assumptions of homogeneity of variances and the normally distributed residuals (Zuur et al., 2010). All ANOVA were followed by a Tukey post-hoc test (Day and Quinn, 1989). All statistical analyses were carried out with R software (www.r-project.org). Significant levels were recorded at $p < 0.05$.

To compare the variation of μ between all isolates, the deviation of μ from the species mean for each isolate was calculated as following: first, within each species, the mean growth rate of all isolates were calculated irrespective of the light or temperature; second, the difference of growth rate of each isolate from the species mean was calculated.

The interspecific and intraspecific variation of growth rate was also evaluated by calculating the standard error. The interspecific variation was calculated from all results for all isolates irrespective of treatment, while intraspecific variation was calculated from the results for isolates within each species under all treatments.

3. Results

3.1 Growth rate

The 12 isolates had a range of growth rates under the same light and temperature conditions, and each isolate had different growth rates under each light and temperature condition (Fig.

2). The μ of all isolates of *M. aeruginosa* and *C. raciborskii* ranged from 0.16 to 0.55 d^{-1} and 0.15 to 0.70 d^{-1}, respectively (Fig. 2, Supplementary Fig. 1). In general, *C. raciborskii* isolates had greater variation in growth rate than *M. aeruginosa* isolates, and straight *C. raciborskii* isolates had greater variation than the coiled ones. Of the straight *C. raciborskii* isolates, C3 had the largest variation, ranging from 0.22 ± 0.01 d^{-1} at L10T28 to 0.64 ± 0.50 d^{-1} at L50T28 (Figs. 2, 3). The *M. aeruginosa* isolate M3 had the smallest variation, ranging from 0.26 ± 0.03 to 0.37 ± 0.01 d^{-1} at L10T20 and L50T28, respectively (Fig. 2, 3). The mean μ of all *M. aeruginosa* isolates, all *C. raciborskii* isolates under a specific light and temperature were similar, for example, 0.36, 0.40 d^{-1} respectively under L100T28 (Fig. 4).

The standard error of the growth rates was always lower when comparing all *M. aeruginosa* to all *C. raciborskii* isolates, than when comparing isolates within in each species. There was a 75% probability that *M. aeruginosa* had a lower standard error than *C. raciborskii* (Table 2).

Light and temperature both significantly ($p < 0.05$) affected growth rate of each isolate (Fig. 2, Table 3), but the optimal light and temperature for each isolate varied (Fig. 2). M2, C6, WS05 and WC03 had the highest growth rate at L100T28; M4, WS01 and WC04 had the highest growth rate at L100T20; M3, M5, C1 and C3 had the highest growth rate at L50T28; while WC07 grew the fastest at L30T28 (Fig. 2). When combining all isolates for both species, they had different optimal light and temperature conditions for maximum growth, i.e., *M. aeruginosa* was 0.38 ± 0.04 d^{-1} at L100T20, while straight *C. raciborskii* was 0.45 ± 0.12 d^{-1} at L50T28 and coiled *C. raciborskii* was 0.37 ± 0.05 d^{-1} at L100T28 (Fig. 4).

3.2 Culture cell volume at stationary phase

All isolates had different cell volumes under different treatments but *C. raciborskii* isolates were more variable than *M. aeruginosa* (Fig. 5). The straight *C. raciborskii* isolate, WS01, varied the most between treatments, and its maximum cell volume, 82.38 μm^3 $cell^{-1}$, was 4.3-fold higher than the smallest. *C. raciborskii* isolate C6 varied the least between treatments compared with other *C. raciborskii* isolates. Its maximum cell volume was approximately 2.4-fold higher than the smallest value. In comparison, *M aeruginosa* M4 was the most variable between treatments of the *M. aeruginosa* isolates, and its maximum cell volume was 1.9-fold higher than its smallest volume. *M aeruginosa* M3 varied the least compared to all of the studied isolates, and its maximum cell volume was 1.6-fold higher than the smallest volume.

3.3 Cell concentration at stationary phase

The time to reach the stationary phase depended on light intensity, temperature and the isolates. The shortest time to reach stationary phase was approximately 2 weeks, and the maximum was 7 weeks.

Cell concentration at stationary phase of all *M. aeruginosa* and *C. raciborskii* isolates under all treatments were compare with cell volume. Cultures with the highest cell concentration at stationary phase had the lowest cell volume (Adjusted R^2 =0.73, p <0.001, Fig. 6 (A)). Compared with *C. raciborskii*, the cell concentrations of *M. aeruginosa* isolates were more similar, except for that of M3, which had the lowest values of around 1.10×10^6 cells mL^{-1} under L100T20. In general, *M. aeruginosa* isolates had higher cell concentrations than *C. raciborskii* under most of the treatments, specifically, L30T20, L50T20, L50T28 and L100T28 (Fig. 6 (B)). Some isolates of *C. raciborskii* had comparable cell concentrations to

M. aeruginosa depended on the isolate and treatments, e.g. straight isolate WS05 under L10T28 (Fig. 6 (B)).

3.4 Light attenuation coefficient

The light attenuation coefficient (k_j) decreased with increasing surface-to-volume ratio for both *M. aeruginosa* and *C. raciborskii* cells under all treatments (Fig. 7). The slope of the decrease was lower for coiled *C. raciborskii* than the straight ones. *C. raciborskii* isolates had a higher k_j which was also more variable, 4.43×10^{-7} to 1.31×10^{-5} (cells $(mL)^{-1})^{-1}$ m^{-1}), compared to *M. aeruginosa* isolates, 5.2×10^{-7} to 2.2×10^{-6} (cells $(mL)^{-1})^{-1}$ m^{-1}). *C. raciborskii* isolates had a similar range of surface-to-volume ratios with *M. aeruginosa*, 1.67 to 2.14 μm^{-1} and 1.19 to 2.43 μm^{-1}, respectively.

3.5 Interaction of temperature and light

Cell volume and k_j of *M. aeruginosa* isolates at stationary phase significantly decreased from T20 to T28 at all light intensities (Fig. 8). Coiled *C. racibor*skii isolates also had significantly lower cell volumes and k_j at T20 compared with T28 at all light intensities, except for the cell volume under L50. Straight *C. raciborskii* isolates had significantly lower cell volume at T20 than T28 under L10 and L30, while there was no significant difference under higher light intensities, i.e. L50 and L100. Straight *C. raciborskii* isolates also had significantly lower k^j at T20 than T28 under L10.

4. Discussion

This study demonstrated that *M. aeruginosa* and *C. raciborskii* isolates from two adjacent reservoirs had greater intraspecific variation than interspecific variation in growth rate, under the different light and temperature conditions tested. The high intraspecific variation suggests

297 that the occurrence of multiple isolates, i.e. strains, in a population is a key driver of species
298 adaptation to various environmental conditions.
299
300 Previous studies have identified variation in strain responses to environmental variables. For
301 example, (Sandrini et al., 2014; 2016) investigated CO_2-concentrating gene sequences of 20
302 *M. aeruginosa* strains, and conducted growth experiments with some strains; the authors
303 showed strains currently exist that have a selective advantage under higher-CO_2 conditions,
304 which will allow a rapid response to a higher-CO_2 world. Higher CO_2 levels have been
305 shown to shift populations of *M. aeruginosa* strains from toxic to non-toxic ones (Van de
306 Waal et al., 2011; Sandrini et al., 2016). In other examples, decreasing water transparency
307 and phosphorus concentrations have also been shown to promote the shift from toxic to non-
308 toxic strains of *M. aeruginosa* (Kardinaal et al., 2007; Wang et al., 2015). Similarly, multiple
309 co-occurring strains of *C. raciborskii* have been described (Willis et al., 2016), and the
310 proportion of toxic and non-toxic *C. raciborskii* strains has also been shown to shift with
311 phosphorus availability (Burford et al., 2014). These studies demonstrate how intraspecific
312 strain variations of the two species result in adaptation to changing environmental conditions,
313 with the result that species appear to have high plasticity.
314
315 The greater intraspecific variation compared to the interspecific variation also illustrates the
316 importance of considering the occurrence of multiple strains when comparing species. In
317 particular, competition models, which are used to predict the competition between different
318 functional phytoplankton species (Huisman et al., 2006), can be improved by understanding
319 the intraspecific rather than only the interspecific effects.
320

4.1 Interaction of light and temperature for cell growth

The mean growth rate of all *M. aeruginosa* isolates and all *C. raciborskii* isolates increased with increasing light and temperature, which was consistent with previous studies (Bittencourt-Oliveira et al., 2012; Li et al., 2014). However, the optimal light and temperature for the maximum growth for the two species was different, i.e., L100T20 for *M. aeruginosa*, interestingly, L50T28 for straight *C. raciborskii* and L100T28 for coiled *C. raciborskii*. Because of the significant difference in optimal growth conditions for the straight and coiled *C. raciborskii* isolates they were grouped separately. Interestingly, this study also found that the optimal light and temperature conditions for maximum growth rate varied for each isolate. There were no trends when comparing the optimal light and temperature to growth rate, for example L100T20 for WS01 to L30T28 for WC07 within *C. raciborskii*. The extent of variation also differed between isolates, for example the growth rate of C3 changed 3.3-fold with the different light and temperature treatments, while M3 varied by 1.6-fold. Therefore, the wide range of temperature and light optima reported for both species, may be a result of different strains used in the studies (Reynolds, 2006; Burford et al., 2016). The difference in optima for maximum growth rate of different strains demonstrates the importance of considering the intraspecific variation within one species.

The results from this study show that temperature drives the growth variability of the multiple isolates in response to light. Both the cell volume and k_j decreased with the increasing temperature under the same light intensity. Under the higher temperature, the kj was lower, this indicates greater access to light and leads to higher cell concentrations (Sivonen, 1990). Cells also became smaller under higher temperature, which was consistent with previous studies (Reynolds, 1997). Under lower temperatures, the cells were larger with a lower cell concentration compared to the higher temperature conditions. In comparison,

346 there was no trend between strains with increasing light intensity under the same temperature.
347 This illustrates that the isolates have greater physiological variability in their response to
348 temperature than light.
349
350 ***4.2 Higher species plasticity of C. raciborskii* than *M. aeruginosa***
351 *Cylindrospermopsis raciborskii* had greater variation between isolates compared to *M.*
352 *aeruginosa* in all measurements; i.e. μ, k_j and cell volume. A recent review of *C. raciborskii*
353 has identified it as a highly plastic species based on its range of physiological responses to
354 light, temperature and inorganic carbon, as well as large differences in toxin cell quota
355 (Burford et al., 2016). The plasticity of the species appears to be a result of multiple co-
356 occurring strains. For example, a recent study of 24 *C. raciborskii* isolates, i.e., 17 straight
357 and 7 coiled isolated from a single surface water sample, showed that each isolate exhibited
358 differences in growth rate (from 0.10 to 0.21 day^{-1}), toxin cell quota (from 90.9 to 278.9 fg
359 cylindrospermopsin $cell^{-1}$) and cell volume (from 32.5 to 262.9 μm^3 $cell^{-1}$) (Willis et al.,
360 2016). While another ten *C. raciborskii* strains isolated from a range of temperate and
361 tropical lakes were also found to have wide light tolerance, from 30 to 400 μmol photons m^{-2}
362 s^{-1}, suggesting even greater species plasticity (Briand et al., 2004).
363
364 Our study found straight *C. raciborskii* isolates had more variation than the coiled ones in μ,
365 k_j and cell volume, suggesting possible divergence into sub-species. The μ and cell volume of
366 straight isolates varied by 4.6 and 6.6-fold, while the coiled only by 2.4 and 3.1-fold,
367 respectively. The coiled isolates were also less affected by self-shading than the straight
368 isolates, indicating greater access to light at lower light intensities. Previous studies have
369 found that coiled strains of *C. raciborskii* grew slightly faster than straight ones under low
370 light levels of 10 μmol photons m^{-2} s^{-1} (Pierangelini et al., 2014), which was consistent with

371 our results under L10T20 but not with those under L10T28. These differences between the
372 straight and coiled isolates may reflect the typical occurrence of coiled strains at lower depths
373 in the water column compared to the straight strains (Bittencourt-Oliveira et al., 2011).
374
375 At the stationary phase, **M. aeruginosa** cultures had higher cell concentrations than *C.*
376 *raciborskii* under nearly all of the treatments. The higher cell concentration reached by *M.*
377 *aeruginosa* may help explain how *M. aeruginosa* dominates in lentic waterbodies from small
378 ponds to very large lakes across the world (Visser et al., 2005; Zhu et al., 2016). Yamamoto
379 et al. (2011) investigated 84 eutrophic to hypereutrophic ponds in northern Taiwan and found
380 that *M. aeruginosa* could dominate the phytoplankton community with high cell densities,
381 and when cells accounted for over 98% of the total biovolume very large colonies (> 800
382 cells per colony) formed. Therefore, for *M. aeruginosa*, reaching a high cell concentration
383 and thereafter forming colonies are the prerequisites for this species to dominate over other
384 species. Conversely, *C. raciborskii* does not dominate the phytoplankton as completely as *M.*
385 *aeruginosa* in terms of abundance, but was more likely to have high frequency of occurrence
386 (Yamamoto et al., 2011; Burford et al., 2016).
387
388 The higher cell concentration of *M. aeruginosa* in this study compared with *C. raciborskii*
389 may be explained by the lower self-shading compared to *C. raciborskii* isolates. Consistent
390 with our study, Baker and Lavelle (1984) also found that smaller cells had less scattering of
391 light. As is found with picophytoplankton, smaller cells have pigment molecules that are
392 more effective at absorbing photons (Raven et al., 2005), which means a higher light
393 availability for smaller cells. Comparing the two species, individual cells of *C. raciborskii*
394 were, on average, over 5-fold larger than *M. aeruginosa*, which suggests that *M. aeruginosa*
395 has higher light availability enabling higher cell concentrations.

396

397 ***4.3 Species competition in waterbodies***

398 The co-occurrence of *M. aeruginosa* and *C. raciborskii* in freshwater systems suggests
399 resource competition between these species may exist. All the *M. aeruginosa* isolates and *C.*
400 *raciborskii* isolates C1, C3 and C6 were isolated from the same reservoir and co-occurred
401 naturally. By comparing the results of these isolates, we can examine what our results mean
402 in terms of species competition in waterbodies.

403

404 For example, a comparison of a scenario where light competition occurs: using an incident
405 light intensity of 1000 μmol photons m^{-2} s^{-1} at the water surface, background turbidity with
406 depth (light attenuation coefficient of the water column) of 0.2 m^{-1}, and mean k_j of *M.*
407 *aeruginosa* and *C. raciborskii* cells of 10^{-6} and 5×10^{-6} $(cells\ mL^{-1})^{-1}$ m^{-1}, respectively. We
408 can then calculate the light intensity that penetrates the water column based on the Lambert
409 beer's law (Kirk, 1994).

410

411 Hypothetically, a *M. aeruginosa* surface bloom which occurs over a depth of 0.01 m, with a
412 cell concentration of 10^8 cell mL^{-1}. Light intensity beneath the top 0.01 m would decrease to
413 367 μmol photons m^{-2} s^{-1}, and then to 246 μmol photons m^{-2} s^{-1} at the 2 m depth. Under these
414 conditions, *C. raciborskii* in the water column up to 2 m depth still has sufficient light to
415 grow and the two species could co-occur. However, if the *M. aeruginosa* cell concentration
416 increased to 10^9 cells mL^{-1}-at the top 0.01 m, as occurred in lake Taihu (Zhu et al., 2014),
417 then the light intensity would decrease dramatically to nearly 0 just beneath the 'scum', and
418 *C. raciborskii* would be unable to grow (Supplementary Fig. 2A). This indicates how *M.*
419 *aeruginosa*, with high cell concentrations, can dominate freshwater ecosystems.

420

This first scenario does not consider temperature, if we hypothesize a situation where the temperature is optimal for *C. raciborskii*, the growth rate of cells within the water column would influence the competition. With the same initial light intensity of 1000 μmol photons m^{-2} s^{-1} at the water surface, and background turbidity of the water column of 0.2 m^{-1}, but an initial *C. raciborskii* cell concentration of 10^5 cells mL^{-1} across the first 5 m depth, the light intensity would decrease to 30 μmol photons m^{-2} s^{-1} at 5 m depth. This is not sufficient for *M. aeruginosa* to grow and therefore it would not reach the cell concentration at the water surface that would block the light and reduce the growth of *C. raciborskii* (Supplementary Fig. 2B). This situation illustrates how *C. raciborskii* could also dominate a freshwater system.

Competition studies between *M. aeruginosa* and *C. raciborskii* have found that the outcome was strain dependent (Marinho et al., 2013). However, another competition experiment between two *M. aeruginosa* strains and two *Planktothrix agardhii* strains has shown that *M. aeruginosa* strains always dominated irrespective of strains when light intensity was below 40 μmol photons m^{-2} s^{-1}, even though all the strains had similar growth rates in monoculture (Torres et al., 2015), suggesting an additional factor favoring *M. aeruginosa* dominance. Therefore, competition between cyanobacterial species needs further investigation relating to species and strain variation under variable environments.

5. Conclusions

In conclusion, this study found that the intraspecific variation in μ, kj and cell volume was greater than the interspecific variation between *M. aeruginosa* and *C. raciborskii* in response to a range of light and temperature conditions. Light attenuation coefficient decreased with changes in surface-to-volume ratio within both species, with coiled isolates having a lower

rate of decrease than the straight isolates, showing that smaller cells have less self-shading. In general, *M. aeruginosa* reached higher cell concentrations with nearly all of the treatments compared with *C. raciborskii*. However, *C. raciborskii* isolates showed greater variation indicating a wider tolerance to environmental variables. Therefore, we propose that high frequency of occurrence of *C. raciborskii* is due to high species plasticity as a result of variation between strains, while *M. aeruginosa* dominates by high cell concentrations leading to shading of other species.

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