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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 °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 (L100T20), while straight *C. raciborskii* had higher growth rates at lower light intensity but higher temperature (L50T28), and coiled *C. raciborskii* had higher growth rates at higher light intensity and higher temperature (L100T28). The final cell concentrations of *M. aeruginosa* were 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.

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).

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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 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).

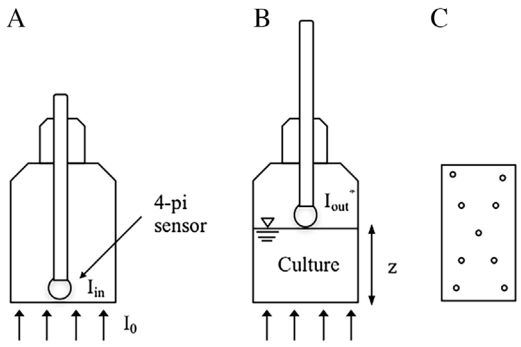

Fig. 1. Method used to measure light attenuation coefficient (k_j). (A) Initial light intensity I_0 illuminated from the bottom of the flasks by the LED light table, and the incident light intensity I_{in} for culture growth measured on Day 0; (B) Critical light intensity I_{out}^* at stationary phase over the culture depth z (cm); (C) Areal view of placement of nine measurements for I_{in} and I_{out}^* on the bottom inside the flask and at the culture surface.

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 0–20 cm. All isolates were maintained in culture flasks with Jaworski Medium (JM, Thompson et al. (1988)), modified by