Variation within and between species and its impact on cyanobacterial competition: a study of problematic *Raphidiopsis raciborskii* and *Microcystis aeruginosa*

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Abstract

The cyanobacteria *Microcystis* spp. and *Raphidiopsis raciborskii* (basionym *Cylindrospermopsis raciborskii*) are the most problematic harmful freshwater species worldwide. Laboratory culture studies have been conducted by researchers globally to identify the optimal environmental conditions for growth of these species. However, these studies mostly focused on one or two strains, limiting understanding of within-species strain variation, and how this variation contributes to species competition under different environmental conditions. There is also limited knowledge of whether the global laboratory data are sufficient to confidently predict growth patterns of *Microcystis* and *R. raciborskii* in situ. Hence, this thesis aimed to determine the within-species strain variation in growth, and use this information to study how this variation affects species population dynamics.

The laboratory studies I undertook showed that intraspecific variation was greater than interspecific variation based on measurements of growth responses of four *M. aeruginosa* and eight *R. raciborskii* strains (isolated from two adjacent Australian reservoirs) individually to a range of light intensity and temperature conditions. There was greater variation between strains of *R. raciborskii* than *M. aeruginosa* in terms of growth rate, light attenuation coefficient (i.e., self-shading) and cell volume, highlighting the extent of variation in strain responses to environmental conditions. In comparison, *M. aeruginosa* always grew to a higher cell concentration than *R. raciborskii* under the same conditions, indicating the capacity for greater dominance of *M. aeruginosa* over *R. raciborskii* worldwide.

To identify how the strain variability affects population dynamics between *M. aeruginosa* and *R. raciborskii*, pairwise competition between the 12 strains was simulated using a deterministic model, parameterized with my laboratory measurements
of growth and light attenuation for all strains, and run at two temperatures and two light intensities under well-mixed conditions. In total, 17,000 runs were simulated for each pair using a phytoplankton dynamics model incorporated with a Monte Carlo statistical approach, which was to propagate the variability and uncertainty of parameters in the deterministic model. The model outputs showed that cyanobacterial competition outcomes were highly variable, depending on the strains present, as well as the light and temperature conditions. Unlike “competitive exclusion” predicted by previous models, i.e., the most competitive species dominates and drives the other species to extinction, my study found no absolute ‘winner’ under all conditions. There were always strains predicted to co-exist with the dominant strain. This strain co-existence led to uncertainty in the prediction of species competition outcomes, which was due to the substantial variability in growth responses within and between strains. Overall, this study highlights that strain variability may substantially reduce our confidence in predicting phytoplankton competition, as deterministic models are typically based on only one set of parameters for each species or strain.

Improving the ability to predict growth, dominance and dynamics of harmful cyanobacteria is critical to manage and control cyanoHABs. Based on my collation of studies over the last 30 years, Microcystis spp. and R. raciborskii dominate in 78% and 17%, respectively, of freshwater bodies across the globe that are affected by cyanobacterial blooms. I tested the hypothesis that light and temperature are the key drivers of observed differences in dominance by these cyanobacteria on a global scale. I synthesized growth responses of M. aeruginosa (as a proxy for Microcystis spp.) and R. raciborskii from 20 and 16 culture studies, respectively, to predict growth rates (d⁻¹) as a function of light and temperature, including uncertainty in the growth rate predictions of each species. Variation in the dominance of the two species across latitudes in summer was predicted from a population dynamics model. The growth rate
of \textit{R. raciborskii} was predicted to exceed \textit{M. aeruginosa} at temperatures \(\geq 25^\circ\text{C}\) and light intensities \(\geq 150 \, \mu\text{mol photons m}^{-2} \, \text{s}^{-1}\). The population dynamics model predicted that \textit{M. aeruginosa} would outcompete \textit{R. raciborskii} at high latitudes of the temperate zones, but be outcompeted by \textit{R. raciborskii} at tropical and subtropical zones, with coexistence in the lower temperate zones. Field observations, however, indicate widespread dominance of \textit{Microcystis} over \textit{R. raciborskii} irrespective of climatic zones. This is unlikely to be resolved by repeated laboratory studies that test environmental responses in well-mixed cultures with fixed environmental conditions. Instead, the key physiological attributes of colony formation, buoyancy and photoadaptation, and strain-level variation are needed to develop process-based predictive models for cyanobacteria.

This thesis has provided important new findings on the contribution of strains to cyanobacterial species variation and the effect of physical conditions to cyanobacterial blooms. It has also highlighted challenges that hinder our understanding of cyanobacterial global distribution and dominance including: failure to resolve within-species strain variability which reduces the predictive ability of deterministic models; failure to induce physiological characteristics of species in laboratory experiments, e.g., colony morphology in \textit{Microcystis} which affects buoyancy regulation; introduced inaccuracy from inconsistent laboratory techniques across studies; failure to mimic realistic physical conditions such as light fluctuations and high light levels; and a variable degree of deviation between strains under culture conditions, etc. This thesis concludes with the need for laboratory studies that accurately reflect field conditions, including understanding of buoyancy, colony and interactions with turbulent mixing in formation of \textit{Microcystis} blooms, to better align with global distribution of cyanoHABs.
This work has not previously been submitted for a degree or diploma in any university.
To the best of my knowledge and belief, the thesis contains no material previously
published or written by another person where due reference is made in the thesis itself.

(signed)

Man Xiao

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Included in this thesis are papers in Chapters 2, 3 and 4, which are co-authored with other researchers. My contribution to each co-authored paper is outlined at the front of the relevant chapter. The bibliographic details/status for these papers including all authors are:

Chapter 2:


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Supervisor: Prof. Michele Burford
Chapter 1 General Introduction

1 General Introduction

1.1 Background of cyanoHABs

Cyanobacteria are oxygen-producing microorganisms, arising on earth over 3 billion years ago (Schopf, 2000). Cyanobacteria played a pivotal role in shaping the oxygenic atmosphere and are now found in habitats from the tropics to the poles. However, in more recent times, cyanobacterial harmful algal blooms (cyanoHABs) have proliferated worldwide in many aquatic systems as a result of eutrophication, and these blooms are exacerbated by the increased temperature and carbon dioxide (CO₂) associated with climate change (Briand et al., 2009; Antunes et al., 2015; Harke et al., 2016; Visser et al., 2016b).

CyanoHABs cause a chain of serious environmental problems, e.g. production of unpleasant odours, reduction of water clarity and consumption of dissolved oxygen during decomposition (Qin et al., 2010). Additionally, some cyanobacterial species are potentially toxic, producing cyanotoxins, and pose severe health risks to humans and other mammals (Codd et al., 2005; Rastogi et al., 2014). Blooms of these toxic species cause substantial economic costs due to the requirement for intensive water treatment, decreased tourism and recreation revenue, and lowered property values (Dodds et al., 2009; Hamilton et al., 2013).

Cyanobacterial growth is affected by a combination of biological, chemical and physical processes. The net growth of cyanobacteria can be affected by the species’ cell physiology, grazing rates, nutrient concentrations, as well as physical factors such as temperature, light and turbulent mixing (Reynolds, 2006). Excessive nutrients are believed to be a key driver in the increase in frequency and magnitude of cyanoHABs (Paerl et al., 2001; Conley et al., 2009). Many researchers have investigated increasing
nutrient availability and it is well established that increasing nutrients lead to increasing cyanobacterial growth and biomass. However, cyanoHABs also occur in oligotrophic lakes, which can typically be explained by the competitive abilities of certain cyanobacteria scavenging low phosphate concentrations, their ability to fix nitrogen and store nutrients (Burford et al., 2017). Physical factors also affect cyanobacterial growth and biomass (Carey et al., 2012), however, much uncertainty remains in the interactive effects of physical factors on cell physiology in relation to bloom formation.

Cyanobacteria are able to dominate in a wide range of physical conditions, which suggests a species plasticity. Increasingly, one of the reasons we see the wide range of cyanobacterial responses to environmental conditions is because populations of cyanobacterial (and other algal) species contain multiple co-occurring strains (Lakeman et al., 2009). These strains vary in their physiological responses to environmental changes (Wilson et al., 2006b; Willis et al., 2015), toxin cell quota (Wilson et al., 2006a; Willis et al., 2015) and genomes (Willis et al., 2018). Yet, the variability in the physiological responses to different environmental conditions between and within species remains unclear.

In addition, cyanobacteria are the microorganisms with relatively short generation times, hence, the capacity for ‘acclimation’ or rapid evolution in a short-term, as well as genetic evolution and adaptation in a long-term, could be significant (Lakeman et al., 2009). Strains of the same species isolated from the field will unavoidably deviate in their environmental responses over multiple generations (Lakeman et al., 2009). Therefore, in addition to species plasticity, rapid evolution and adaptation might also confound predictions of cyanobacterial responses to different environmental conditions.
The cyanobacterial species, *Microcystis* spp. and *Raphidiopsis raciborskii* (basionym *Cylindrospermopsis raciborskii*) are of particular concern in freshwater ecosystems due to their high dominance and toxin production (Antunes et al., 2015; Harke et al., 2016). *Microcystis* is a unicellular microorganism, but forms colonies which are positively buoyant and able to disentrain from turbulent mixing and form surface sums (Fig. 1-1A). *Raphidiopsis raciborskii* is filamentous, neutrally buoyant, and forms invisible blooms in water deeper layers (Fig. 1-1B). *Microcystis* and *R. raciborskii* have been found to dominate singularly, successively or simultaneously within the same waterbody (McGregor and Fabbro, 2000; Yamamoto et al., 2011; Zhu et al., 2016). Furthermore, *R. raciborskii* seems to be gradually replacing *Microcystis* to become the dominant or co-dominant species in some tropical reservoirs (Marinho and Huszar, 2002). Recent reviews have highlighted the physiological characteristics of these species that have contributed to their global spread (Burford et al., 2016; Xiao et al., 2018). Hence, predicting which species will dominate in a given location in a changing environment is critical for water management. This thesis is, therefore, based upon increasing our understanding of these nuisance species and their population dynamics when co-existing.

### 1.2 Physical factors

Temperature, light and turbulent mixing are the three main physical factors that affect cyanobacterial growth and biomass accumulation. Turbulent mixing also affects the cell growth by affecting the light availability and stratification of the water column. Certain conditions may favour cyanobacteria over other phytoplankton, or favour one cyanobacterial species over the other (Carey et al., 2012).
Fig. 1-1: Surface ‘scum’ of *Microcystis* (A) and invisible bloom of *R. raciborskii* (B). Photos were taken by Michele A. Burford.

1.2.1 Temperature

Temperature affects cyanobacterial bloom formation directly through metabolic processes, such as photosynthesis, respiration, growth, and reproduction (Ibelings et al., 2011). Most cyanobacteria achieve their maximal growth rate at optimal temperatures ranging from 25 to 35°C in cultures (Reynolds, 2006). In natural systems, warming temperatures have led to longer and earlier bloom periods (Dale et al., 2006) and geographical expansion of blooms (Padisák, 1997; Paerl and Paul, 2012), which indicate that cyanobacteria favour higher temperatures (Paerl and Huisman, 2008; Kosten et al., 2012).

Warmer temperatures also cause a number of indirect effects, including increased stability and stratification of the water column (Dale et al., 2006), which is likely to favour cyanobacteria capable of efficient buoyancy-regulation. Thermal stratification occurs when water is calm, i.e. when the energy of mixing, which is primarily provided by wind, is not sufficient to overcome the thermal energy introduced by sunlight (Boehrer and Schultze, 2008). The upper, well-lit and well-mixed layer can have very small temperature variability, termed ‘surface mixed layer’ (SML). Mixing is generally
restricted to this layer, inhibiting replenishment of nutrients from the bottom layers upward and oxygen from this layer downwards (Vant, 1987). Therefore, under thermal stratification, cyanobacteria that can regulate their buoyancy gain a competitive advantage over species that cannot by rising to the upper water column for higher light availability, or to deeper water column for higher nutrient availability (Reynolds et al., 2002).

1.2.2 Light intensity

Light is the key energy source for photosynthesis and therefore growth of cyanobacteria (Zohary et al., 2010; Falkowski and Raven, 2013). ‘Light’ refers to the visible wavelengths (\( \lambda \approx 400 – 700 \text{ nm} \)) of the totality of electromagnetic radiation emanating from the sun, which band accounts for almost half the solar energy reaching Earth. Cyanobacteria generally have low light adaptation compared to other phytoplankton species. Schwaderer et al. (2011) conducted growth experiments of 10 species and collected literature data of 63 species, including diatoms, green algae and cyanobacteria, and found that cyanobacteria achieved the highest initial slope of the growth-irradiance curve (0.04 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \text{ d}^{-1} \)) amongst all species, while the light for optimal growth was only half the value of the green algae Chlorophyceae. The light for optimal growth also varied between cyanobacterial species, ranging from 30 to 100 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) (Schwaderer et al., 2011) and in some cases can be up to 400 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) (Wiedner et al., 2003).

Light also interacts with temperature in affecting cyanobacterial photosynthetic processes and growth rate, and the magnitude of this interaction is species dependent. Kehoe et al. (2015) exposed field phytoplankton samples dominated by R. raciborskii to a range of temperatures (20 – 32°C with an interval of 4°C), and found that 32°C
resulted in the highest level of photoinhibition. Yet, Briand et al. (2008) found no significant difference in growth between 10 and 20°C for two *Planktothrix agardhii* strains.

Competition for light occurs when multiple species co-occur. Resource ratio theory (the R* rule) attempts to predict which species will become dominant as the result of competition for resources. When multiple species are competing for a single limiting resource, then whichever species can survive at the lowest equilibrium resource level, i.e., the R*, will outcompete other species. Huisman and Weissing (1994) found that in a well-mixed and constant environment, where there is an ample supply of nutrients but light is the only limiting resource, cyanobacterial species with the lowest ‘critical’ light requirements (I*) will be the superior competitor. The critical light intensity of a species corresponds to the light intensity of well-mixed water column at which this species can just grow. Hence, the superior competitor for light may create a higher biomass that subsequently limits light availability for the inferior competitor. Similarly, in oligotrophic ecosystems where there is an ample supply of light while nutrient is the only limiting resource, competition theory predicted that species with the lowest ‘critical’ nutrient requirements will be the superior nutrient competitor (Tilman, 1982).

In comparison, in the ecosystems where light and nutrient supplies are not limiting, three contrasting hypotheses have been put forward based on the abilities of species to compete for light and nutrients. The first theory describes no trade-off between the competitive abilities for light and nutrients, and in this situation one species will completely win over the other, resulting in competitive exclusion (Grime, 1973). The second hypothesis describes when there is a trade-off, i.e., if the growth of one species is limited by light and the other species limited by nutrients, stable co-existence may exist when the competition gradually shifts from competition for nutrients to
competition for light as conditions change (Tilman, 1982). Passarge et al. (2006) tested this hypothesis using a model and laboratory competition experiments, and found that the trade-offs between competitive abilities for light and phosphorus were rare, and competition for light and phosphorus led to competitive exclusion. The third hypothesis also has a trade-off, but one species is the superior competitor for light and the other is a superior competitor for nutrients. When these species co-occur, the superior competitor for light takes advantage of the available light and causes light limitation, while the superior competitor for nutrients consumes more nutrients and causes nutrient limitation. This leads to an alternating dominance between the two species, and the winner depends on the initial light and nutrient conditions.

Most studies on the effect of light on cyanobacterial growth have focused on incident light intensity and in the case of laboratory studies, constant light intensities. However, underwater light availability is actually what drives cyanobacterial biomass accumulation (Kirk, 1994). The underwater light field depends on the incident irradiance, the solar angle, the degree and type of cloud cover, resulting in strong diurnal and seasonal variability. The availability of light also decreases through the water column approximately as an exponential function, described by the Beer-Lambert law. The decreasing light availability is also attenuated by the medium, dissolved substances and suspended particles, as well as shelf-shading of the growing phytoplankton biomass (Kirk, 1994).

1.2.3 Turbulent mixing

Turbulent mixing in water is caused by hydrodynamic drivers, including wind-induced waves, currents and physical mixing processes (Wüest and Lorke, 2003; Reynolds, 2006; Qin, 2008). Turbulent mixing directly affects the physiology of cyanobacteria. Mixing accelerates the nutrient transport into the cells (Lohrenz et al., 1999; Maar et al.,
2002) as small-scale turbulence can disrupt the cell surface boundary layer (Berdalet and Estrada, 2005). Mixing also affects cell aggregation and colony formation, potentially negatively affecting cell growth or bloom formation. Under low mixing intensity (turbulent dissipation rates: $\varepsilon < 10^{-5} \text{ m}^2 \text{s}^{-3}$), increased cell aggregation and colony formation have been measured in species such as *Phaeocystis globosa* (Schapira et al., 2006); however, when $\varepsilon$ increased from $10^{-10} \text{ m}^2 \text{s}^{-3}$ to $10^{-4} \text{ m}^2 \text{s}^{-3}$, large *Microcystis* colonies disaggregated (O’Brien et al., 2004; Regel et al., 2004).

Experiments in an oscillating grid tank showed that when the turbulent mixing rate exceeded 2.93 m s$^{-1}$, metabolic activity and viability of *M. aeruginosa* cells decreased (Regel et al., 2004).

Turbulent mixing also affects vertical distribution of phytoplankton cells. Studies have found that when mixing induced by wind surpassed a threshold velocity of 3 – 4 m s$^{-1}$, *M. aeruginosa* surface blooms disappeared and the population dispersed into deeper layers (Ha et al., 2000; Cao et al., 2006). In contrast, mixing could maintain taxa without the ability to regulate their buoyancy in the water column (Huisman et al., 2002a; Peeters et al., 2007; Visser et al., 2016a). For marine ecosystems, changes in turbulent mixing are often accompanied by the shift of dominance of dinoflagellates at weak turbulent mixing to dominance of diatoms at intense turbulent mixing (Irigoien et al., 2000). In lakes, intensified mixing has led to species replacements from buoyant cyanobacteria towards green algae and diatoms (Visser et al., 1996a) or from more buoyant *Microcystis* to less buoyant *R. raciborskii* (Antenucci et al., 2005). In laboratory cultures and modelling studies, *Microcystis* has been shown to dominate at a low turbulent diffusivity, whereas sinking diatoms and green algae dominate at a high turbulent diffusivity (Huisman and Weissing, 1994; Huisman et al., 2004).
Due to mixing, phytoplankton community is constantly mixed over the underwater light gradient and periodically circulated between light levels less than 1% of incident light, and surface waters where they are photoinhibited. In stratified lakes with limited mixing, through to lakes with moderate mixing, the buoyancy of some cyanobacterial species plays an important role in maximizing light interception and hence growth, by disentraining from mixing and floating up to the surface mixed layers. Brookes and Ganf (2001) found that the short-term buoyancy response to light, where carbohydrate is accumulated and respired, is nested within a longer-term response that is a function of both the cyanobacterium’s nutrient and light history, and the effect these resources have on gas vesicle production and cell metabolism.

The underwater gradient and dynamic nature of light results in different growth responses between phytoplankton species (Litchman and Klausmeier, 2001). Phytoplankton can only grow in the euphotic zone (typically down to 1% of incident light intensity). The total rate of photosynthesis increases as mixing decreases because the average amount of accessible photosynthetically active radiation (PAR) increases (Kirk, 1994; Diehl et al., 2002). However, the capability of coping with the variability of the underwater light intensity and the light history is likely to affect species growth and competition more substantially than constant light exposure. Ibelings et al. (1994) found that the cyanobacterium M. aeruginosa was more susceptible to fluctuating light than a green alga Scenedesmus sp.

Turbulent mixing also causes horizontal transport of cyanobacteria (Reynolds, 2006). Lake Biwa (Japan) has been dominated by Microcystis blooms since 1997, especially offshore. This is surprising as the nutrient concentrations are too low for Microcystis growth. The offshore occurrence of Microcystis was found to be the result of large-scale horizontal transport of cells that have grown in nearshore high-nutrient waters, and
accumulated in downwelling as the result of a wind-induced gyre (Ishikawa et al., 2002). A similar process was investigated in the Meiliang Bay of Lake Taihu (China), where the most frequent and severe Microcystis surface blooms have been observed, compared to the rest of Lake Taihu in the last two decades (Duan et al., 2014). These surface scums of Microcystis accumulating at Meiliang Bay were found to be partly from existing Microcystis driven from the main body of the lake by the current (Wu et al., 2010).

1.3 Cell buoyancy regulation

Cyanobacteria possess a wide range of unique physiological traits, leading to a high level of adaptation to changing environments (Litchman et al., 2010; Mantzouki et al., 2016). When mixed into deeper layers, one of the most important strategies for species capable of buoyancy regulation is to optimize light and nutrient availability by migrating through the water column (Ganf and Oliver, 1982). This migration is highly affected by the interaction of turbulent mixing (Aparicio Medrano et al., 2013), the cell’s buoyancy regulation (Reynolds, 1987), as well as cell morphology and size (Wu and Kong, 2009).

Cyanobacterial species have different strategies regarding their degree of buoyancy. Microcystis and Dolichospermum, have been recognised as positively buoyant species, and can migrate 12-m vertically to gain access to nutrients at the water bottom and to light at the water surface in a 30 – 34 m deep lake, taking advantages of available nutrients and light (Ganf and Oliver, 1982). Microcystis has higher floatation velocities than any other cyanobacterial species, with a maximum recorded of 10.08 m h⁻¹ from a single colony with the diameter of 1200 µm (Li et al., 2016a), leading to higher light acquisition for cell growth. Dolichospermum sp. has lower flotation velocities than
Microcystis, ranging from 0.01 – 1.00 m h\(^{-1}\) in natural populations (Bormans and Condie, 1997; Brookes et al., 1999). Two other widely distributed cyanobacteria, Planktothrix rubescens and R. raciborskii, maintain neutral buoyancy with relatively low flotation velocities of nearly zero (Walsby, 2005; Walsby and Holland, 2006; Kehoe, 2009).

The entrainment of phytoplankton cells within turbulent eddies is a function of the floatation/sinking velocity of the phytoplankton and the velocity of the turbulent eddies that transport cells (Humphries, 1982). The higher floatation velocities of Microcystis compared to any other freshwater cyanobacterial species (Xiao et al., 2018) allows this species to disentrain more easily from turbulent mixing to float up towards the water surface thereby form blooms.

### 1.4 Cell morphology and size

Cyanobacteria are the oldest unicellular organisms, and have gradually evolved into multicellular forms including filaments or colonies in the Early Proterozoic (Carroll, 2001). Species cover a wide range of sizes, from a few microns of small single cells, up to a few centimetres of multi-cell filaments and large colonies (Whitton and Potts, 2000). Cell morphology also differs within species (Whitton, 2012). For example, R. raciborskii forms straight, coiled through to sigmoid shaped trichomes (Padisák, 1997), while Microcystis spp. exist as single cells or (more rarely) as paired cells in laboratory cultures, but form colonies under natural conditions (Xiao et al., 2017c).

Cell morphology and size has major effects on cyanobacterial physiological responses to environmental factors (Reynolds, 2006), such as the ability to utilize light and nutrients (Sunda and Huntsman, 1997; Litchman, 2007), buoyancy regulation (Booker and Walsby, 1979), and the ability to disentrain from turbulent mixing (Wu and Kong,
Small filamentous cells, due to a higher surface-to-volume ratio compared to larger colonial cells, have more efficient acquisition of limiting nutrients and light (Reynolds, 2006). In contrast, colonial morphology protects inner-colony cells from photoinhibition under high irradiance (Wu et al., 2011). Larger colonies also attain faster flotation/sinking rates compared to smaller cells and filaments, given by the Stokes’ law. Padisák et al. (2003) modelled the sinking rate of different phytoplankton morphologies and found that increased length/width ratio resulted in slower sinking rates for filaments. Moreover, Holland (2010) concluded that sinking rates of filaments had a curvilinear decrease with the sinking orientation from vertical to horizontal.

1.5 Modelling phytoplankton population dynamics

Cyanobacterial population dynamics have mainly been predicted with process-based models, which incorporate physical, chemical and biological variables through a combination of algebraic and differential equations (Huisman et al., 2004; Huisman et al., 2006). These models can be used to explore cyanobacterial growth and species competition under a range of environmental scenarios.

Current phytoplankton models have modeled species competition for light, especially when cells are circulated into variable light conditions under turbulent mixing (Huisman et al., 2004; Passarge et al., 2006; Marinho et al., 2013). For example, Huisman et al. (1999) simulated the competition for light between three groups of species, which differ in light utilization, i.e., a ‘super species’ that has higher productivity in all levels of light intensity; a ‘sun species’ that has high productivity at high light levels but lower productivity under low light levels; and a ‘shade species’ that prefers a lower light intensity. The authors predicted that super species always outcompeted sun and shade species, while the shade species dominated under complete mixing but lost under
incomplete mixing. Huisman et al. (2004) predicted that positively buoyant *Microcystis* dominated at low turbulent diffusivity, whereas sinking diatoms and green algae dominated at high turbulent diffusivity, when they competed for light under different mixing intensities. However, when mixing surpassed the intensity threshold and water depth was deeper than the light-depth threshold, both the positively buoyant and sinking species went extinct because of insufficient light to maintain their growth.

Current phytoplankton models, including the above competition models, mostly predict competitive exclusion – where the most effective competitor dominates the resource and drives all the other species in the system to extinction. In reality, a single millilitre of water may contain dozens of co-existing phytoplankton species, which is widely known as ‘the paradox of the plankton’ (Hutchinson, 1961). Historically, studies on species co-existence have sought to explain biodiversity using two different perspectives. On the one hand, the classical niche-based view, emphasizing that phenotypic differences among species will reduce interspecific competition and thereby allow co-existence. On the other hand, the neutral view, suggesting that species are competitively equivalent and hence, diversity is driven by stochasticity and dispersal. Recently, community ecologists have looked at ways to reconcile niche-based and neutral mechanisms, providing a comprehensive perspective for the stable co-existence of species. Chesson (2000) proposed that species co-existence could be a balance between stabilizing and equalizing forces. The stabilizing forces are niche-based differences to limit conspecifics’ growth more than individuals from other species, and to maintain diversity via niche differentiation. The equalizing forces are to minimize fitness differences between species and to drive the best-adapted species to exclude others within a particular ecological niche.
Upon the mismatch of phytoplankton diversity between current model prediction and field observation, this thesis aims to answer the following questions: what is missing from the current phytoplankton models to predict species co-existence rather than competitive exclusion? How can we improve the current models to better understand phytoplankton dynamics under a limited range of resources?

1.6 Characteristics of *Microcystis* spp. and *R. raciborskii*

1.6.1 *Microcystis* spp.

*Microcystis* exists as single cells or (more rarely) as paired cells in laboratory cultures (Yang et al., 2008; Li et al., 2013), but forms large colonies and blooms on the water surface. It blooms in some of the largest lakes on earth, such as Lake Erie (North America) and Lake Taihu (China) (Bullerjahn et al., 2016; Zhu et al., 2016; Levy, 2017). Many *Microcystis* strains produce microcystins (MCs), which are cyclic heptapeptides that are potent liver toxins (Carmichael, 2001). These blooms affect drinking-water supply systems (Guo, 2007; Paerl et al., 2014).

More than 50 *Microcystis* morphospecies have been recognised according to variations in colony form, mucilage structure, cell diameter, cell arrangement within a colony, ratio of the pigments phycocyanin and phycoerythrin, and the life cycle (absence/presence of certain stages, identification of atypical-dormant stages, limits of morphological variability) (Komárek and Komárková, 2002). The most commonly observed variants of *Microcystis* are *M. aeruginosa* (Kützing) Kützing, *M. flos-aquae* (Wittrock) Kirchner, *M. ichthyoblabe* Kützing, *M. novacekii* (Komárek) Compére, and *M. wessenbergii* (Komárek) Komárek (Fig. 1-2A – E), and their colonial morphology differs from each other. *Microcystis aeruginosa* colonies are normally irregularly shaped, elongated or lobed with irregular-shaped holes, and the cells are arbitrarily
arranged inside the colonies. *Microcystis ichthyoblabe* colonies are normally soft, sponge-like, and have a homogeneous distribution of cells inside the colony. *Microcystis novacekii* colonies are normally small and firm, not lobed, and have tightly aggregated cells. *Microcystis wesenbergii* colonies are normally spherical, elongated, and lobed with a visible margin that is filled with mucilage, and have cells irregularly arranged inside the colonies. Identifying these morphospecies is challenging due to morphological, biochemical and genetic differences, and classification at species level is contentious and continually being refined (Otsuka et al., 1999; Otsuka et al., 2001; Komárek and Komárková, 2002; Otten and Paerl, 2011; Xu et al., 2016b).

The global success of *Microcystis* has been attributed, in part, to the formation of colonies (Yamamoto et al., 2011; Xiao et al., 2018), which are buoyant and provide faster flotation velocities than any other freshwater cyanobacteria and unicellular cells of *Microcystis*. Even though the effect of colonial *Microcystis* on light extinction is less than a population of the same number of unicellular cells (known as the sieve or package effect, Kirk (1994)), the higher floatation velocities of colonies help *Microcystis* achieve dominance in eutrophic lakes: their large surface biomass shades light and thereby inhibits the growth of other species including green algae and diatoms in deeper water layers. Li et al. (2016a) also determined that colonies of various morphospecies differed in flotation velocity: *M. ichthyoblabe* tended to have a higher velocity than *M. aeruginosa* and *M. wesenbergii* for the same colony size.
Colony formation also provides *Microcystis* with many other ecological advantages compared to single cells. These include the ability to adapt to varying light which is possibly due to the higher photosynthetic pigment content (Wu and Song, 2008; Wu et al., 2011; Zhang et al., 2011), and to low nutrient availability which is partly due to higher affinity for phosphorus and inorganic carbon (Shen and Song, 2007; Wang et al., 2014; Li et al., 2016b). *Microcystis* colonies are also better protected from chemical stressors such as copper sulphate (Wu et al., 2007), lead (Bi et al., 2013), and zooplankton grazing (Burkert et al., 2001; Yang et al., 2006; Yang et al., 2008; Yang and Kong, 2012), possibly due to the higher activities of antioxidative enzymes in colonies compared to single cells. However, all these benefits are responses to environmental stresses, with an associated cost, which is the reduction in specific growth rates of colonial *Microcystis* compared to single cells (Xiao et al., 2017c).
Previous culture studies have found that *Microcystis* can grow at light intensities of up to 400 µmol photon m$^{-2}$ s$^{-1}$ (Wiedner et al., 2003), however, it was also found it is more susceptible to high and fluctuating light (up to 1100 µmol photon m$^{-2}$ s$^{-1}$) compared to a eukaryotic competitor, the green alga *Scenedesmus* sp. (Ibelings et al., 1994).

*Microcystis aeruginosa* has been shown to have a wide temperature tolerance from 16.5 to 35°C, with optima from 24 to 32°C amongst culture studies (Li et al., 2015; Thomas and Litchman, 2016). In the field, *Microcystis* blooms were found at water temperatures from 12 to 30°C (Li et al., 2015). Despite the reputation of cyanobacteria as ‘blooms like it hot’ (Paerl and Huisman, 2008), Huisman et al. (2018) recently reviewed that cyanobacteria as superior than eukaryotic phytoplankton under warmer temperatures is possibly due to that the growth rate of cyanobacteria increases faster with increasing temperature, instead of a higher growth rate.

### 1.6.2 Raphidiopsis raciborskii

*Raphidiopsis raciborskii* is a diazotrophic, filamentous cyanobacterium. It was first identified as a harmful species after a toxic bloom event occurred in 1979 at Solomon Dam, Palm Island, Australia (Hawkins et al., 1985). Since then *R. raciborskii* strains have been found to produce a suite of highly potent toxins, the hepatotoxic alkaloids called cylindrospermopsins (CYN) (Ohtani et al., 1992; Wood and Stirling, 2003), the neurotoxic anatoxin-a and saxitoxin (Molica et al., 2005), and possibly some still uncharacterized cyanotoxins (Saker et al., 2003). Toxin production varies among geographic locations (Burford et al., 2016).

*Raphidiopsis raciborskii* was initially identified as a tropical/subtropical species (Padisák, 1997), however, distribution beyond these regions has been recorded from temperate zones such as continental Europe (Fastner et al., 2007), East Asia (Lei et al., 2014), New Zealand (Ryan et al., 2003; Wood and Stirling, 2003) and Uruguay (Vidal
and Kruk, 2008). Some researchers consider it to be an invasive species, increasing in distribution with a warmer climate (Padisák, 1997; Sinha et al., 2012; Antunes et al., 2015), while Wood et al. (2014) attributed the recent widespread of *R. raciborskii* in New Zealand to a change in phytoplankton population composition with the collapse of extensive macrophytes, increased water turbidity and nutrient availability. However, the spread of *R. raciborskii* throughout the world is consistent with a wide temperature tolerance of 19 – 35°C as found in laboratory cultures (Briand et al., 2004; Thomas and Litchman, 2016). *Raphidiopsis raciborskii* typically blooms in summer months in temperate and subtropical areas, and all year round in the tropics (Briand et al., 2004; Chonudomkul et al., 2004).

*Raphidiopsis raciborskii* blooms in the euphotic zone, with a sub-surface peak in abundance at 2 – 3 m depth (Saker and Griffiths, 2001), or can be distributed in the surface mixed layer (SML) (O’Brien et al., 2009). Aligning with its vertical distribution in the water column, *R. raciborskii* was found to grow at a wide range of light intensities ranging from 50 to 150 µmol photon m$^{-2}$ s$^{-1}$ and as high as 348 µmol photon m$^{-2}$ s$^{-1}$ amongst strains isolated from Australia, Europe, Asia and South America and Africa (Briand et al., 2004; Dyble et al., 2006; Carneiro et al., 2009; Holland et al., 2012). *Raphidiopsis raciborskii* can also adapt to much lower light, ~10 µmol photons m$^{-2}$ s$^{-1}$ (Bonilla et al., 2012; Pierangelini et al., 2015; Willis et al., 2015). Maximum cell concentrations have been measured when ratios of SML depth and euphotic layer depth ($z_m : z_{eu}$) were greater than one (McGregor and Fabbro, 2000; Saker and Griffiths, 2000). The optically deep mixing has been reported to increase the dominance of *R. raciborskii* (Burford and O'Donohue, 2006), which was due to its increased primary productivity when circulated through the euphotic zone after dark-acclimation, compared to high light exposure (O'Brien et al., 2009).
The global success of *R. raciborskii* has also been attributed, in part, to its high level of flexibility with respect to nutrient use: *R. raciborskii* appears to be more competitive when phosphorus and nitrogen availability is low and/or variable (Burford et al., 2017). *Raphidiopsis raciborskii* has been shown to have a high affinity and storage capacity for dissolved inorganic phosphorus (Isvánovics et al., 2000; Willis et al., 2017). It also utilizes organic phosphorus (Bai et al., 2014). *Raphidiopsis raciborskii* can fix atmospheric dinitrogen, which only provides enough N to support relatively low growth under N-limited conditions (Willis et al., 2016a). The growth rate was measured to be 4-fold higher when dissolved inorganic nitrogen is available (Plominsky et al., 2015).

As outlined in a previous section, *R. raciborskii* is considered a neutrally buoyant species. Kehoe (2009) measured the flotation velocity of 25 straight *R. raciborskii* trichomes, isolated from North Pine Reservoir, Australia, via a particle tracking apparatus (O'Brien et al., 2006). The flotation velocity ranged from 0.1 to 0.66 µm s\(^{-1}\), with the trichome length ranging from 6.5 to 98 µm.

### 1.6.3 Competition between *Microcystis* and *R. raciborskii*

*Microcystis* and *R. raciborskii* are of particular concern because they are dominant species amongst the cyanohABs (Burford et al., 2016; Harke et al., 2016). They frequently dominate successively or simultaneously within the same waterbody (Soares et al., 2009; Yamamoto et al., 2011), and furthermore, *R. raciborskii* may be gradually replacing *Microcystis* as the dominant or co-dominant species in some tropical reservoirs (Marinho and Huszar, 2002). Effective management of both species is vital to protect water quality. An improved ability to predict cyanobacterial population dynamics and species dominance under different environmental conditions will assist in controlling and mitigating these blooms.
When intensified mixing is introduced to eutrophic lakes, phytoplankton, including \textit{Microcystis} spp. and \textit{R. raciborskii}, are mixed to achieve random distributions (Fig. 1-3A). The cells are exposed to similar light conditions and periodically circulated into dark environment. When the mixing intensity is greater than the critical intensity, \textit{Microcystis} might lose its ability to regulate its vertical distribution, while \textit{R. raciborskii} might still be able to sustain its growth as it is adapted to low light. When mixing becomes moderate, large \textit{Microcystis} colonies are able to disentrain from mixing and float up to the water surface (Wallace et al., 2000). Both species could gain a higher primary production after dark-acclimation when mixed back to a well-lit environment (Zevenboom and Mur, 1984; O'Brien et al., 2009).

Under stratified conditions (Fig. 1-3B), mixing is generally restricted to the surface mixed layer with higher temperature and sufficient light. When \textit{Microcystis} spp. and \textit{R. raciborskii} co-occur, the faster flotation velocity may help \textit{Microcystis} to achieve dominance over \textit{R. raciborskii}. This is because the large surface biomass shades light and thereby inhibits the growth of \textit{R. raciborskii}. However, \textit{Microcystis} surface scums might be photoinhibited due to its susceptible to surface light intensities. The higher temperature, higher pH and lack of carbon due to demands of the high biomass might also result in negative environment for sustenance of \textit{Microcystis} colonies (Ibelings and Maberly, 1998). In comparison, \textit{R. raciborskii} may maintain its biomass due to its low light adaption. However, over a long period of stratification, a large surface biomass of \textit{Microcystis} and stronger stratification, can result in \textit{R. raciborskii} finally disappearing from the water column due to light limitation. However, \textit{R. raciborskii} can produce akinetes, resting spores, under adverse conditions, which settle on the floor of the water column, which gives it the ability to regenerate and grow under more suitable conditions.
When moderate mixing is introduced to the lake again, *Microcystis* can benefit by disentInlining from mixing, but *R. raciborskii* is less competitive due to its lower flotation velocity, thus slower to disentain. Hence, determining which species has more favorable growth is subject to the interaction of cells’ net growth rate under varying light, with buoyancy regulation and mixing intensity (Reynolds, 1984).

**Fig. 1-3**: Conceptual model that illustrates the vertical distribution of *Microcystis* spp. and *R. raciborskii*, light gradient and water temperature profile under (A) well-mixed and (B) stratified conditions, respectively. The colors show the temperature gradient, with red indicating warmer while blue cooler. The temperature gradient decreases more sharply in the stratified condition and is more even in deeper layers under the well-mixed condition. The light gradient decreases more rapidly in the stratified condition due to the higher accumulation of *Microcystis* colonies at the water surface compared to the well-mixed condition.

1.6.4 Strain variation

Previous studies have shown a high diversity in cyanobacteria at the intraspecific level. Strains of a single cyanobacterial species vary in their environmental niches, resulting in a population that can adapt and grow in a wider range of environmental conditions than the population of a single strain (Van de Waal et al., 2011; Wang et al., 2015). Strains are typically defined as isolates of individual cells, colonies or trichomes with differing physiology or genetics. They may be named ‘ecotypes’, ‘phenotypes’, ‘genotypes’ and ‘chemotypes’ (Lakeman et al., 2009). Briand et al. (2004) studied 10 *R. raciborskii*
strains collected from seven mid-latitude countries and found that maximum growth rates occurred from 30 to 80 μmol photons m\(^{-2}\) s\(^{-1}\), indicative of high variability of strains of the same species. A recent study of 24 \textit{R. raciborskii} Australian strains (isolates), i.e., 17 straight and 7 coiled isolated from a single surface water sample, showed that each strain exhibited differences in growth rate (0.10 – 0.21 d\(^{-1}\)), toxin cell quota (90.9 – 278.9 fg CYN cell\(^{-1}\)) and cell volume (32.5 – 262.9 μm\(^3\) cell\(^{-1}\)) under the same light, temperature and nutrient conditions (Willis et al., 2016b). Wilson et al. (2006b) measured a large variation in the morphology, toxicity and maximum growth rates of 32 \textit{M. aeruginosa} strains isolated from 12 lakes, in particular, the maximum growth rate differed 5-fold between strains, and the MCs concentrations ranged from 0.07 to 0.98 ng (μg dry mass\(^{-1}\)).

There are few experiments where for each species more than one strain has been used, and these have shown contradictory conclusions. One experiment between two \textit{M. aeruginosa} and two \textit{R. raciborskii} strains under a range of light and phosphorus conditions showed that species competition depended on strains (Marinho et al., 2013). In contrast, another experiment between two \textit{M. aeruginosa} and two \textit{P. agardhii} strains found that \textit{M. aeruginosa} always outcompeted \textit{P. agardhii} when light intensity was below 40 μmol photons m\(^{-2}\) s\(^{-1}\), even though all the strains had similar growth rates in monocultures (Torres et al., 2016). Competition between cyanobacterial species, and particularly \textit{M. aeruginosa} and \textit{R. raciborskii} needs further investigation relating to species and strain variation under variable environmental conditions.

### 1.7 Research aims and thesis structure

The aims of this thesis are to quantify the interspecific and intraspecific variability of growth responses of \textit{M. aeruginosa} and \textit{R. raciborskii} and their strains to physical
conditions, specifically light and temperature. This information is then used to
determine how this variability affects species competition, and to highlight the current challenges in improving prediction of cyanoHABs and species dominance with phytoplankton dynamics models.

This thesis is made up of four chapters, which form a logical sequence in developing the framework for predicting cyanoHABs and species dominance:

**Chapter 2** examines the interspecific and intraspecific variation of growth responses of laboratory cultured *M. aeruginosa* and *R. raciborskii* strains to a range of light and temperature conditions. Four strains of *M. aeruginosa* and eight strains of *R. raciborskii* isolated at the same time from two adjacent reservoirs in Southeast Queensland, Australia, were used. This chapter has been published as ‘Xiao, M., Willis, A., Burford, M.A. 2017. Differences in cyanobacterial strain responses to light and temperature reflect species plasticity. *Harmful Algae* 62, 84–93.’

**Chapter 3** focuses on how the within-species strain variability of *M. aeruginosa* and *R. raciborskii* in responses to light and temperature affects their competition. Growth parameters of the 12 strains were parameterized from the experiment in **Chapter 2** and were used in simulating their pairwise competition. This modelling process modified and improved the previous phytoplankton dynamic growth models by incorporating a Monte Carlo approach to represent parameter variability and uncertainty. **Chapter 3** has been published as ‘Xiao, M., Adams, M.P., Willis, A., Burford, M.A., O’Brien, K.R. 2017. Variation within and between cyanobacterial species and strains affects competition: Implications for phytoplankton modelling. *Harmful Algae* 69, 38–47.’

**Chapter 4** examines whether global distribution and dominance of *Microcystis* spp. and *R. raciborskii* can be predicted from synthesizing the global dataset of culture...
experiments on growth responses of *M. aeruginosa* (as a proxy as *Microcystis*) and *R. raciborskii* to light and temperature. **Chapter 4** uses this information to determine whether the physical factors, i.e., light and temperature, are driving the dominance of *Microcystis* and *R. raciborskii* on a global scale. The predicted species dominance from the phytoplankton dynamics model was compared with the published field observations of cyanoHABs across the globe from 1988 to 2017, to highlight that current culture studies oversimplify the mechanisms driving the worldwide dominance of *Microcystis*.

**Chapter 5** provides a synthesis of work in this thesis, and the current challenges in better predicting the cyanobacterial competition. The chapter suggests the implications with respect to laboratory studies, in terms of studying multiple strains within a species, as well as managing cyanobacterial blooms in terms of more accurate predictions by including this variability. This chapter also suggests there is clearly a lot of research remaining to be done in this relatively new area of endeavor.
2 Differences in cyanobacterial strain responses to light and temperature reflect species plasticity

In Chapter 2, ‘strain’ is used to describe the unicellular cultures from isolation of single cells (isolate), these were then compared in the laboratory studies to determine strain variability to simplify the concept of strain in terms of phytoplankton ecology (Lakeman et al., 2009). All the strains in this study have been identified taxonomically based on morphology, growth rates and cell quotas of different toxins. Additionally, the *R. raciborskii* strains were compared using multiplex randomly amplified polymorphic DNA.
This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors, are:


My contribution to the paper involved: designing and running the laboratory experiments, collecting and analyzing data, writing the manuscript, and addressing the reviewer’s comments from the journal. Anusuya Willis helped in designing the experiments, reviewed the draft and provided useful comments to improve the paper. Michele Burford is the principal advisor of this project, she contributed to developing the experiment, providing ideas in improving the manuscript, and helping with responses to reviewers’ comments.

(Signed) ______________ (Date) 24/10/2018

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(Countersigned) ______________ (Date) 24/9/2018

Supervisor: Prof. Michele Burford
2.1 Introduction

*Microcystis aeruginosa* and *Raphidiopsis raciborskii* are two of the most harmful blooming cyanobacteria in freshwater ecosystems. *Microcystis aeruginosa* has been recorded as an overwhelmingly dominant species in some of the world’s largest lakes, e.g., Lake Taihu (China) (Zhu et al., 2016) and Lake Erie (North America) (Bullerjahn et al., 2016), while *R. raciborskii* is increasingly present in bloom proportions in lakes and reservoirs throughout the world (Antunes et al., 2015). 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, *R. 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).

Previous studies have established that both *M. aeruginosa* and *R. raciborskii* outcompete other cyanobacterial species to be the dominant species in freshwater systems, this is likely a result of their ability to adapt to a wide range of environments (Burford et al., 2016; Harke et al., 2016). *Microcystis aeruginosa* exists mostly as single cells under laboratory culture conditions (Yang et al., 2012; Li et al., 2013), but can form surface ‘scums’ consisting of large colonies (100 – 2000 µm) in the field (Zhu et al., 2014; Rowe et al., 2016). Colony size and morphology determine the vertical flotation velocity of *Microcystis* colonies and whether colonies can disentrain from turbulent mixing to float up towards the water surface and form blooms (Walsby et al., 1995; Wallace et al., 2000). *Raphidiopsis 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 *R. 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., 2006b; Willis et al., 2016b). 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 has 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). It is still unknown whether the variations in strain response may contribute to the highly competitive nature of both species. Therefore, it is 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. *Microcystis aeruginosa* and *R. raciborskii* strains have been found to have different light optima (Briand et al., 2004; Wilson et al., 2006b). *Microcystis 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). *Raphidiopsis raciborskii* develops subsurface blooms at depths of 2 – 3 m in water column (Saker and Griffiths, 2001), or be evenly distributed in the surface mixed layer (O’Brien et al., 2009), and has been shown to have a light optima of ~ 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
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*Litchman, 2016*. *Raphidiopsis raciborskii* has been shown to tolerate temperatures from 19 to 35°C and achieve maximum growth from 29 to 32°C across studies (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 *R. 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). Although 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), 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 and constant light intensities, however, light availability through the water column is actually what drives 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). Resource ratio theory (the $R^*$ rule) predicts that species with the lowest ‘critical’ light requirements will be the superior competitor when light is the only limiting resource (Huisman and Weissing, 1994). The critical light intensity of a species corresponds to the light intensity of a well-mixed water column at which this species can grow. Hence, the superior competitor for light may create a higher biomass that subsequently limits light availability for the inferior competitor (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 *R. raciborskii* co-occur, the accumulation of *M. aeruginosa* on the water surface may reduce the light
from penetrating into the deeper layers where \textit{R. raciborskii} occurs. However, because \textit{R. raciborskii} is adapted to low light the effect of shading by \textit{M. aeruginosa} surface blooms may be minimal. Ibelings and Maberly (1998) has measured that 90% extinction of light through approximately 1 mm water depth. Despite the major impact shading may have on the light availability and thus population dynamics of algae in lakes, there has, to my knowledge, been no quantitative studies on self-shading of individual cyanobacterial species.

In summary, at the species level, both \textit{M. aeruginosa} and \textit{R. 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 strains of \textit{M. aeruginosa} and \textit{R. raciborskii} isolated from two adjacent reservoirs in Southeast Queensland, Australia, to determine the magnitude of strain versus species variability.

\section*{2.2 Material and methods}

\subsection*{2.2.1 \textit{M. aeruginosa} and \textit{R. raciborskii} strains}

Twelve strains were used for this study. Four were \textit{M. aeruginosa} strains: three were single-celled (M2, M3 and M4) and the other one was single-celled with few small colonies (M5). The other eight were \textit{R. raciborskii} strains: five with straight trichomes (C1, C3, C6, WS01 and WS05) and the other three with coiled trichomes (WC03, WC04 and WC07). All the twelve strains were isolated in 2013 by micromanipulation and/or serial dilution, as described in Andersen (2005). All the \textit{M. aeruginosa} strains and \textit{R. raciborskii} strains 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 \textit{R. raciborskii} strains 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 the strains 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 *R. 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: 12 h light/dark cycle using LED lights at a constant temperature of 28°C.

2.2.2 Growth conditions

Two temperatures (T: 20 and 28°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 strains were habituated to each light and temperature treatment for four weeks prior to starting the experiment.

Each strain 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 *R. 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 strains of both species (Table 2-1). Cultures were gently shaken daily.
Table 2-1: Correlations between OD750 measurement and cell count by microscopy for each strain across a range of light and temperature conditions. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

<table>
<thead>
<tr>
<th>Strain</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>C1</th>
<th>C3</th>
<th>C6</th>
<th>WS01</th>
<th>WS05</th>
<th>WC03</th>
<th>WC04</th>
<th>WC07</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^2$</td>
<td>0.76</td>
<td>0.82</td>
<td>0.75</td>
<td>0.78</td>
<td>0.94</td>
<td>0.84</td>
<td>0.83</td>
<td>0.72</td>
<td>0.71</td>
<td>0.89</td>
<td>0.78</td>
<td>0.86</td>
</tr>
</tbody>
</table>

### 2.2.3 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 ($\mu$, d$^{-1}$) was calculated during the exponential phase with the first order rate kinetics (Andersen, 2005):

$$
\mu = \frac{\ln(\text{OD750}_2/\text{OD750}_1)}{t_2-t_1},
$$

Eqn 2-1

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.

### 2.2.4 Cell size and morphology

The diameter of *M. aeruginosa* cells, and cell length, cell width and trichome length of *R. raciborskii* strains were measured at the stationary phase under light microscopy (LEICA ICC50) at ×400 magnification. The cell length and width of *R. 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 *R. raciborskii* strain. The individual cells of
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*M. aeruginosa* and *R. 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.

### 2.2.5 Cell enumeration

Cell enumeration of all cultures was conducted at stationary phase. *Microcystis aeruginosa* cells were counted with a Sedgewick Rafter counter under light microscopy (LEICA ICC50) at ×400 magnification. Small colonies of *M. aeruginosa* strain M5 were dissociated by a boiling pre-treatment method (Joung et al., 2006) prior to enumeration. For *R. raciborskii*, trichome counts were conducted under light microscopy (LEICA ICC50) at ×100 magnification. The cell concentrations (*w*, cells mL\(^{-1}\)) of *R. raciborskii* were calculated from counts of the trichome concentration (trichomes mL\(^{-1}\)), trichome length and the mean cell length with the equation:

\[
\text{Cell concentration} = \frac{\text{trichome concentration} \times \text{Trichome length}}{\text{mean cell length}}.
\]

Eqn 2-2

### 2.2.6 Light attenuation coefficient

Light intensity was measured with a 4-pi sensor (LI-1400, Li-Cor Biosciences) at nine evenly distributed locations inside the flasks and on the culture surface (Fig. 2-1C). \(I_0\) was the initial light intensity for the growth experiment (L10, L30, L50 and L100) illuminated from the bottom of the culture flasks. \(I_{in}\) was measured on Day 0 on the bottom inside the flasks (Fig. 2-1A). \(I_{out}^*\) was measured on the culture surface at the stationary phase (Fig. 2-1B). Light attenuation coefficient (\(k_i\), cells (mL\(^{-1}\))\(^{-1}\) m\(^{-1}\)) of each species was calculated for all the cultures at stationary phase, using the following equation (Huisman et al., 2002b):
where $I_{in}$ ($\mu$mol photons m$^{-2}$ s$^{-1}$) is the incident light intensity for culture to grow, $I_{out}^{*}$ ($\mu$mol photons m$^{-2}$ s$^{-1}$) is the critical light intensity through the culture depth $z$ (m), $w$ (cells mL$^{-1}$) is the cell concentration. Similarly as per Huisman et al. (2002b), in this study, Eqn 2-4 neglects reflection and scattering of photons as well as the spectral distribution of light.

Fig. 2-1: 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.

2.2.7 Statistical analyses

Differences in $\mu$, $k_j$ and cell volume under different light and temperature treatments were tested with two-way ANOVA. Differences in $\mu$ between isolates across the different light and temperature treatments were tested with three-way ANOVA, for each of the three groups: M. aeruginosa, straight R. raciborskii and coiled R. raciborskii. Leneve’s test was used to test the homogeneity of variance, and Shapiro-Wilk test was
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 was 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 $\mu$ between all strains, the deviation of $\mu$ from the species mean for each strain was calculated as following: first, within each species, the mean growth rate of all strains was calculated from all light and temperature treatments; second, the growth rate from all light and temperature treatments of each strain 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 strains under all treatments, while intraspecific variation was calculated from the results for strains within each species under all treatments.

2.3 Results

2.3.1 Growth rate

The twelve strains had a range of growth rates under the same light and temperature conditions, and each strain had different growth rates under each light and temperature condition (Fig. 2-2, Fig. 2-3). The $\mu$ of all strains of *M. aeruginosa* and *R. raciborskii* ranged from 0.16 to 0.55 d$^{-1}$ and 0.15 to 0.70 d$^{-1}$, respectively (Fig. 2-2, Fig. 2-3). In general, *R. raciborskii* strains had greater variation in growth rate than *M. aeruginosa* strains, and straight *R. raciborskii* strains had greater variation than the coiled ones. Of the straight *R. raciborskii* strains, C3 had the largest variation, ranging from $0.22 \pm 0.01$
d\(^{-1}\) to 0.64 ± 0.50 d\(^{-1}\) at L10T28 and L50T28, respectively (Fig. 2-3, Fig. 2-4). The *M. aeruginosa* strain 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, Fig. 2-4). The mean $\mu$ of all *M. aeruginosa* strains, all *R. raciborskii* strains under a specific light and temperature were similar, for example, 0.36, 0.40 d\(^{-1}\), respectively under L100T28 (Fig. 2-5).

![Fig. 2-2](image_url)

*Fig. 2-2*: Growth rates ($\mu$, d\(^{-1}\)) of all *M. aeruginosa* and *R. raciborskii* strains under all light and temperature treatments. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07). Black dots indicate mean growth rates at 20°C (T20), and triangles indicate mean growth rates at 28°C (T28). Error bars indicate standard deviation.

The standard error of the growth rates was always lower when comparing all *M. aeruginosa* to all *R. raciborskii* strains, than when comparing strains within in each species. There was a 75% probability that *M. aeruginosa* had a lower standard error than *R. raciborskii* (Table 2-2).
Table 2-2: Comparison of standard error in growth rate between *M. aeruginosa* and *R. raciborskii*, and between strains within each species in all light and temperature treatments. L10T20 indicated light intensity of 10 µmol photons m$^{-2}$ s$^{-1}$ and temperature of 20°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Between species</th>
<th><em>M. aeruginosa</em></th>
<th><em>R. raciborskii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>L100T28</td>
<td>0.01</td>
<td>0.013</td>
<td>0.014</td>
</tr>
<tr>
<td>L50T28</td>
<td>0.015</td>
<td>0.007</td>
<td>0.023</td>
</tr>
<tr>
<td>L30T28</td>
<td>0.009</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td>L10T28</td>
<td>0.007</td>
<td>0.012</td>
<td>0.01</td>
</tr>
<tr>
<td>L100T20</td>
<td>0.011</td>
<td>0.012</td>
<td>0.016</td>
</tr>
<tr>
<td>L50T20</td>
<td>0.008</td>
<td>0.01</td>
<td>0.011</td>
</tr>
<tr>
<td>L30T20</td>
<td>0.008</td>
<td>0.009</td>
<td>0.011</td>
</tr>
<tr>
<td>L10T20</td>
<td>0.008</td>
<td>0.01</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Fig. 2-3: Deviation from the mean growth rate for all *M. aeruginosa* and *R. raciborskii* strains under all light and temperature treatments. L10T20 indicated light intensity of 10 µmol photons m$^{-2}$ s$^{-1}$ and temperature of 20°C. The dashed lines separate *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

Light and temperature both significantly (*P < 0.05*) affected growth rate of each strain (Fig. 2-3, Table 2-3), but the optimal light and temperature for each strain varied (Fig. 2-3). M2, C6, WS05 and WC03 had the highest growth rate at L100T28; M4, WS01
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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. 2-3). When combining all strains for both species, they had different optimal light and temperature conditions for maximum growth, i.e., *M. aeruginosa* was $0.38 \pm 0.04 \, \text{d}^{-1}$ at L100T20, while straight *R. raciborskii* was $0.45 \pm 0.12 \, \text{d}^{-1}$ at L50T28 and coiled *R. raciborskii* was $0.37 \pm 0.05 \, \text{d}^{-1}$ at L100T28 (Fig. 2-5).

![Boxplots of growth rates](image)

**Fig. 2-4:** Example boxplots of growth rates of all strains of *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07), under three different light and temperature treatments. L10T20 indicated light intensity of 10 µmol photons m$^{-2}$ s$^{-1}$ and temperature of 20°C. Error bars indicate the 25$^{th}$ and 75$^{th}$ percentiles of growth rates.
Table 2-3: Three-way ANOVA comparing growth rates for *M. aeruginosa*, straight *R. raciborski* and coiled *R. raciborski* under eight treatments. Levene’s test was conducted after Boxcox-transformation of the data: F value = 0.50, 0.48, 0.65 and P-value = 0.98, 0.99, 0.87 respectively. Shapiro-Wilk test after data transformation: W = 0.99, 0.99, 0.98 and P-value = 0.64, 0.51 and 0.33 respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Factors</th>
<th>Df</th>
<th>Mean Sq</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. aeruginosa</em></td>
<td>Light</td>
<td>3</td>
<td>0.53</td>
<td>227.05***</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.05</td>
<td>19.34**</td>
</tr>
<tr>
<td></td>
<td>Strain</td>
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<td>0.01</td>
<td>5.02**</td>
</tr>
<tr>
<td></td>
<td>Light: temperature</td>
<td>3</td>
<td>0.04</td>
<td>15.13***</td>
</tr>
<tr>
<td></td>
<td>Light: strain</td>
<td>9</td>
<td>0.01</td>
<td>6.03***</td>
</tr>
<tr>
<td></td>
<td>Temperature: strain</td>
<td>3</td>
<td>0.22</td>
<td>9.27***</td>
</tr>
<tr>
<td></td>
<td>Light: temperature: strain</td>
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<td>0.30</td>
<td>13.02***</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>64</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Straight</td>
<td>Light</td>
<td>3</td>
<td>0.85</td>
<td>205.80***</td>
</tr>
<tr>
<td><em>R. raciborski</em></td>
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<td>1</td>
<td>0.65</td>
<td>157.48***</td>
</tr>
<tr>
<td></td>
<td>Strain</td>
<td>4</td>
<td>0.04</td>
<td>10.73***</td>
</tr>
<tr>
<td></td>
<td>Light: temperature</td>
<td>3</td>
<td>0.02</td>
<td>5.98**</td>
</tr>
<tr>
<td></td>
<td>Light: strain</td>
<td>12</td>
<td>0.08</td>
<td>19.87***</td>
</tr>
<tr>
<td></td>
<td>Temperature: strain</td>
<td>4</td>
<td>0.04</td>
<td>9.30***</td>
</tr>
<tr>
<td></td>
<td>Light: temperature: strain</td>
<td>12</td>
<td>0.04</td>
<td>9.64***</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>79</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Coiled</td>
<td>Light</td>
<td>3</td>
<td>0.09</td>
<td>24.12***</td>
</tr>
<tr>
<td><em>R. raciborski</em></td>
<td>Temperature</td>
<td>1</td>
<td>0.06</td>
<td>16.07***</td>
</tr>
<tr>
<td></td>
<td>Strain</td>
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<td>0.67 NS</td>
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</tr>
<tr>
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<td>0.07</td>
<td>17.76***</td>
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<tr>
<td></td>
<td>Temperature: strain</td>
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<td>11.83***</td>
</tr>
<tr>
<td></td>
<td>Light: temperature: strain</td>
<td>6</td>
<td>0.03</td>
<td>7.06***</td>
</tr>
</tbody>
</table>
Fig. 2-5: The mean growth rates of strains of *M. aeruginosa*, straight *R. raciborskii* and coiled *R. raciborskii* under each light and temperature treatment. Black dots indicate mean growth rates at 20°C (T20), and triangles indicate mean growth rates at 28°C (T28). Error bars indicate standard deviation.

### 2.3.2 Culture cell volume at stationary phase

All strains had different cell volumes under different treatments but *R. raciborskii* strains were more variable than *M. aeruginosa* (Fig. 2-6). The straight *R. raciborskii* strain, WS01, varied the most between treatments, and its maximum cell volume, 82.38 µm³ cell⁻¹, was 4.3-fold higher than its smallest. *R. raciborskii* strain C6 varied the least between treatments compared with other *R. raciborskii* strains, and 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 all the *M. aeruginosa* strains, and its maximum cell volume was 1.9-fold higher than its smallest volume.
Microcystis aeruginosa M3 varied the least compared to all of the studied strains, and its maximum cell volume was 1.6-fold higher than its smallest volume.

2.3.3 Cell concentration at stationary phase

The time to reach the stationary phase depended on light intensity, temperature and the strains. 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 *R. raciborskii* strains under all treatments were compared with cell volume. Cultures with the highest cell concentration at stationary phase had the lowest cell volume (Adj. $R^2 = 0.73$, $P < 0.001$, Fig. 2-7A). Compared with *R. raciborskii*, the cell concentrations of *M. aeruginosa* strains were more similar, except for that of M3, which had the lowest values of around $1.10 \times 10^6$ cells mL$^{-1}$ under L100T20. In general, *M. aeruginosa* strains had higher cell concentrations than *R. raciborskii* under most of the treatments, specifically, L30T20,
L50T20, L50T28 and L100T28 (Fig. 2-7B). Some strains of *R. raciborskii* had comparable cell concentrations to *M. aeruginosa* depended on the strains and treatments, e.g. straight strain WS05 under L10T28 (Fig. 2-7B).

Fig. 2-7: Cell concentration ($10^5$ cells mL$^{-1}$) at stationary phase compared with individual cell volume ($\mu$m$^3$ cell$^{-1}$) (A) and under all treatments (B) when combing *M. aeruginosa* and *R. raciborskii* strains. (A): Solid dots are *M. aeruginosa* and hollow dots are *R. raciborskii*. (B): The eight treatments are separated by short ticks outside the x axis. L10T20 indicated light intensity of 10 µmol photons m$^{-2}$ s$^{-1}$ and temperature of 20°C.

### 2.3.4 Light attenuation coefficient

The light attenuation coefficient ($k_i$) decreased with increasing surface-to-volume ratio for both *M. aeruginosa* and *R. raciborskii* cells under all treatments (Fig. 2-8). The slope of the decrease was lower for coiled *R. raciborskii* than the straight ones. *Raphidiopsis raciborskii* strains had a higher $k_i$ which was also more variable, $4.43 \times 10^{-7}$ to $1.31 \times 10^{-5}$ (cells (mL)$^{-1}$), compared to *M. aeruginosa* strains, $5.2 \times 10^{-7}$ to
$2.2 \times 10^6$ (cells (mL$^{-1}$) m$^{-1}$). *R. raciborskii* strains 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.

![Diagram](image)

Fig. 2-8: Comparison of surface-to-volume ratio (µm$^{-1}$) with $k_j$ (10$^{-7}$ cells (mL$^{-1}$) m$^{-1}$) combining all *M. aeruginosa* and *R. raciborskii* strains across all treatments. On the left-hand side, solid line refers to correlation between $k_j$ and surface-to-volume ratio of straight *R. raciborskii* strains, dashed line refers to that of coiled strains. On the right-hand side, the dashed line refers to correlation within *M. aeruginosa* strains.

### 2.3.5 Interaction of temperature and light

Cell volume and $k_j$ of *M. aeruginosa* strains at stationary phase significantly decreased from T20 to T28 at all light intensities (Fig. 2-9). Coiled *R. raciborskii* strains 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 *R. raciborskii* strains 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 *R. raciborskii* strains also had significantly lower $k_j$ at T20 than T28 under L10.
Fig. 2-9: Boxplots of cell volumes ($\mu$m$^3$ cell$^{-1}$) and $k_i$ ($10^{-7}$ cells (mL$^{-1}$)$^{-1}$ m$^{-1}$) when combining all strains of *M. aeruginosa*, straight *R. raciborskii* and coiled *R. raciborskii* under each of the eight treatments. L10T20 indicated light intensity of 10 µmol photons m$^{-2}$ s$^{-1}$ and temperature of 20°C. The error bar indicates the 25th and 75th percentiles of the growth rates for all strains under each treatment. Significant differences by ANOVA and post-hoc grouping by Tukey test is denoted by a, b, c, d and e.

### 2.4 Discussion

This study demonstrated that even though the *M. aeruginosa* and *R. raciborskii* strains were isolated from two adjacent reservoirs, they had greater intraspecific variation than interspecific variation in growth rate, under the different light and temperature conditions tested. The high intraspecific variation suggests that the occurrence of multiple strains in a population is a key driver of species adaptation to various environmental conditions.

Previous studies have identified variation in strain responses to environmental variables. For example, the CO$_2$-concentrating gene sequences of 20 *M. aeruginosa* strains were investigated (Sandrini et al., 2014; Sandrini et al., 2016), and the authors showed strains currently exist have a selective advantage under higher-CO$_2$ conditions, which will
allow a rapid response to a higher-CO$_2$ world. Higher CO$_2$ levels have been shown to shift populations of *M. aeruginosa* strains from toxic to non-toxic ones (Van de Waal et al., 2011; Sandrini et al., 2016). In other examples, decreasing water transparency and phosphorus concentrations have also been shown to promote the shift from toxic to non-toxic strains of *M. aeruginosa* (Kardinaal et al., 2007; Wang et al., 2015). Similarly, multiple co-occurring strains of *R. raciborskii* have been described (Willis et al., 2016b), and the proportion of toxic and non-toxic *R. raciborskii* strains has also been shown to shift with phosphorus availability (Burford et al., 2014). These studies demonstrate how intraspecific strain variations of the two species result in adaptation to changing environmental conditions, with the result that species appear to have high plasticity.

The greater intraspecific variation compared to the interspecific variation also illustrates the importance of considering the co-occurrence of multiple strains when comparing species. In particular, competition models, which are used to predict the competition between different phytoplankton species (Huisman et al., 2006), could be improved by understanding the intraspecific rather than only the interspecific effects.

### 2.4.1 Interaction of light and temperature for cell growth

The mean growth rate of all *M. aeruginosa* strains and all *R. raciborskii* strains 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 differed, i.e., L100T20 for *M. aeruginosa*, interestingly, L50T28 for straight *R. raciborskii* and L100T28 for coiled *R. raciborskii*. Because of the significant difference in optimal growth conditions for the straight and coiled *R. raciborskii* strains they were grouped separately. This study also
found that the optimal light and temperature conditions for maximum growth rate varied for each strain. There was no trend when comparing the optimal light and temperature to growth rate, for example, L100T20 for WS01 to L30T28 for WC07 within *R. raciborskii*. The extent of variation also differed between strains, 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 across studies, may be a result of different strains used (Li et al., 2015; Burford et al., 2016). The difference in the 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 strains in response to light. Both the cell volume and $k_1$ decreased with the increasing temperature under the same light intensity. Under the higher temperature, the $k_1$ 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, there was no trend between strains with increasing light intensity under the same temperature. This illustrates that the strains have greater physiological variability in their response to temperature than light.

### 2.4.2 Higher species plasticity of *R. raciborskii* than *M. aeruginosa*

*Raphidiopsis raciborskii* had greater variation between strains compared to *M. aeruginosa* in all measurements; i.e. $\mu$, $k_1$ and cell volume. A recent review of *R. raciborskii* has identified it as a highly plastic species based on its range of
physiological responses to light, temperature and inorganic carbon, as well as large differences in toxin cell quota (Burford et al., 2016). The plasticity of the species appears to be a result of multiple co-occurring strains. For example, a recent study of 24 \( R. \) raciborskii isolates, i.e., 17 straight and 7 coiled isolated from a single surface water sample, showed that each isolate exhibited differences in growth rate (0.10 – 0.21 d\(^{-1}\)), toxin cell quota (90.9 – 278.9 fg CYN cell\(^{-1}\)) and cell volume (32.5 – 262.9 \( \mu \text{m}^3 \) cell\(^{-1}\)) (Willis et al., 2016b). While another 10 \( R. \) raciborskii strains isolated from a range of temperate and tropical lakes were also found to have wide light tolerance, from 30 to 400 \( \mu \text{mol photons m}^2 \text{s}^{-1} \), suggesting even greater species plasticity (Briand et al., 2004).

Our study found straight \( R. \) raciborskii strains had more variation than the coiled ones in \( \mu \), \( k_i \) and cell volume, suggesting possible divergence into sub-species. The \( \mu \) and cell volume of straight strains varied by 4.6 and 6.6-fold, while the coiled only by 2.4 and 3.1-fold, respectively. The coiled strains were also less affected by self-shading than the straight strains, indicating greater access to light at lower light intensities. Previous studies have found that coiled strains of \( R. \) raciborskii grew slightly faster than straight ones under low light levels of 10 \( \mu \text{mol photons m}^2 \text{s}^{-1} \) (Pierangelini et al., 2014), which was consistent with our results under L10T20 but not with those under L10T28. These differences between the straight and coiled strains may reflect the typical occurrence of coiled strains at lower depths of the water column compared to the straight strains (Bittencourt-Oliveira et al., 2011).

At the stationary phase, \( M. \) aeruginosa cultures had higher cell concentrations than \( R. \) raciborskii under nearly all of the treatments, leading to higher total biovolumes than \( R. \) raciborskii under most conditions even when accounting for the lower cell volume of \( M. \) aeruginosa. The higher biovolumes reached by \( M. \) aeruginosa may help explain
how *M. aeruginosa* dominates in lentic waterbodies from small ponds to very large lakes worldwide (Visser et al., 2005; Zhu et al., 2016). Yamamoto et al. (2011) investigated 84 eutrophic to hypereutrophic ponds in northern Taiwan and found that *M. aeruginosa* could dominate the phytoplankton community with high cell densities, and when cells accounted for over 98% of the total biovolume very large colonies (>800 cells per colony) formed. Conversely, *R. raciborskii* does not dominate the phytoplankton as completely as *M. aeruginosa* in terms of abundance, but was more likely to have high frequency of occurrence (Yamamoto et al., 2011; Burford et al., 2016).

The higher cell concentration of *M. aeruginosa* in this study compared with *R. raciborskii* may be explained by the lower self-shading compared to *R. raciborskii* strains. Consistent with our study, Baker and Lavelle (1984) also found that smaller cells had less scattering of light. As is found with picophytoplankton, smaller cells have pigment molecules that are more effective at absorbing photons (Raven et al., 2005), which means a higher light availability for smaller cells. Comparing the two species, individual cells of *R. raciborskii* were, on average, over 5-fold larger than *M. aeruginosa*, which suggests that *M. aeruginosa* has higher light availability enabling higher cell concentrations.

### 2.4.3 Species competition in waterbodies

The co-occurrence of *M. aeruginosa* and *R. raciborskii* in freshwater systems suggests resource competition between these species may exist. All the *M. aeruginosa* strains and *R. raciborskii* strains C1, C3 and C6 were isolated from the same reservoir and co-occurred naturally. By comparing the growth responses of these strains from this study, we can examine what the results mean in terms of species competition in waterbodies.
For example, a comparison of a scenario where light competition occurs: using an incident light intensity of 1000 µmol photons m\(^{-2}\) s\(^{-1}\) at the water surface, background light attenuation of the water column of 0.2 m\(^{-1}\), and mean \(k_1\) of \(M.\) aeruginosa and \(R.\) raciborskii cells of \(10^{-6}\) and \(5 \times 10^{-6}\) (cells mL\(^{-1}\)) m\(^{-1}\), respectively. Hence, the light intensity that penetrates through the water column could be calculated based on the Beer-Lambert law (Kirk, 1994).

Hypothetically, a \(M.\) aeruginosa surface bloom occurs over a depth of 0.01 m, with a cell concentration of \(10^8\) cell mL\(^{-1}\). Light intensity beneath the top 0.01 m would decrease to 367 µmol photons m\(^{-2}\) s\(^{-1}\), and then to 246 µmol photons m\(^{-2}\) s\(^{-1}\) at the 2-m depth. Under these conditions, \(R.\) raciborskii in the water column up to 2 m depth still has sufficient light to grow and the two species could co-occur. However, if the \(M.\) aeruginosa cell concentration increased to \(10^9\) cells mL\(^{-1}\) at the top 0.01 m, as occurred in lake Taihu (Zhu et al., 2014), the light intensity would decrease dramatically to nearly 0 just beneath the surface ‘scum’, hence, \(R.\) raciborskii would be unable to grow (Fig. 2-10A). This indicates how \(M.\) aeruginosa, with high cell biomass, can possibly dominate freshwater ecosystems, even though the environment of the surface scum might inhibit the growth of \(Microcystis\) due to the higher temperature, higher light intensity, higher pH and lack of carbon due to the demand of the high biomass (Ibelings and Maberly, 1998).

This first scenario does not consider temperature. It is hypothesized that where the temperature is optimal for \(R.\) 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 light attenuation coefficient of 0.2 m\(^{-1}\), but an initial \(R.\) 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 may be insufficient for *M. aeruginosa* to grow and therefore it would not reach the cell concentration at the water surface that blocks the light and reduces the growth of *R. raciborskii* (Fig. 2-10B). This situation illustrates how *R. raciborskii* could also dominate a freshwater system.

![Diagram](image)

Fig. 2-10: Light intensity (µmol photons m\(^{-2}\) s\(^{-1}\)) calculated through the water column based on Beer-Lambert law from two hypothesized blooms. The initial light intensity on the water surface was 1000 µmol photons m\(^{-2}\) s\(^{-1}\), the light attenuation coefficient of *M. aeruginosa* and *R. raciborskii* were chosen as 10\(^6\) and 5×10\(^6\) (cells mL\(^{-1}\)) m\(^{-1}\), respectively. The background light attenuation coefficient was 0.2 m\(^{-1}\). Fig. 2-10 (A) was calculated based on *M. aeruginosa* blooms at a depth of 0.01 m, and below *M. aeruginosa* surface scum the light attenuated only due to the background light attenuation. Fig. 2-10 (B) was calculated based on evenly distributed cell concentration of *R. raciborskii* at the first 5 m depth through the water column.

Competition studies between *M. aeruginosa* and *R. 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., 2016), suggesting an additional factor favouring *M. aeruginosa* dominance. Therefore, competition between cyanobacterial species needs further investigation relating to species and strain variation under variable environments.
2.5 Conclusions

In conclusion, this study found that the intraspecific variation in $\mu$, $k_I$ and cell volume was greater than the interspecific variation between *M. aeruginosa* and *R. 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 strains having a lower rate of decrease than the straight strains, showing that smaller cells have less self-shading. In general, *M. aeruginosa* reached higher cell concentrations with nearly all of the treatments compared with *R. raciborskii*. However, *R. raciborskii* strains showed greater variation indicating a wider tolerance to environmental variables. Therefore, this study suggests that high frequency of occurrence of *R. 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.
3 Variation within and between cyanobacterial species and strains affects competition: Implications for phytoplankton modelling

Chapter 2 showed that the intraspecific variation of *M. aeruginosa* and *R. raciborskii* was greater than the interspecific variation in their growth responses to a range of light and temperature conditions. However, how the within-species strain variation affects species competition is unclear. Hence, Chapter 3 focuses on how the interspecific and intraspecific variation of growth responses of *M. aeruginosa* and *R. raciborskii* to light and temperature affects species competition. Growth parameters of the twelve strains, parameterized from the experiment in Chapter 2, were used, and their pairwise competition was simulated. This modelling process modified and improved the previous phytoplankton dynamic growth models by including a Monte Carlo approach to represent parameter variability and uncertainty.
This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors, are:


My contribution to the paper involved: analyzing data from laboratory experiments from Chapter 2, running the model simulations, and drafting the whole manuscript. Matthew Adams helped in data analysis, model design and coding, and reviewed the manuscript. Anusuya Willis and Michele Burford helped in data acquisition, reviewed the manuscript and helped give responses to reviewers’ comments. Katherine O’Brien is the principal advisor of this manuscript and contributing to setting the model, analyzing the modelled results, reviewing the manuscript and replying to reviewers’ comments.

(Signed) ___________________________ (Date) 24/10/2018

Name of Student and corresponding author for all listed papers: Man Xiao

(Countersigned) ___________________________ (Date) 24/10/2018

Supervisor: Prof. Michele Burford
3.1 Introduction

Harmful cyanobacterial blooms cause severe water quality issues around the world (Paerl and Otten, 2013). Many cyanobacterial species can co-occur in the same waterbody with overlapping environmental niches (Reynolds, 2006), therefore cyanobacterial population dynamics are affected by the interaction between species which compete for resources (Litchman, 2007). Recent studies have established that cyanobacterial populations are a complex mixture of multiple strains of a single species (Yoshida et al., 2008; Willis et al., 2016b). These multiple strains vary in physiological responses to different environmental conditions (= ecotypes), and vary in toxin cell quota (= chemotypes) (Wilson et al., 2006b; Burford et al., 2016). Yet, how multiple strains within one species may contribute to a species’ competitive ability is poorly understood. Moreover, each individual strain has variable growth responses depending on the environmental conditions, further complicating prediction of which strain will dominate.

Few studies have assessed the effect of variable environmental conditions on species/strain competition (Visser et al., 2016b), and in particular under which circumstances one strain is able to outcompete other strains. Strains of a single cyanobacterial species vary in their environmental niches, which may result in a population that can adapt and grow in a wider range of environmental conditions than the population of a single strain (Van de Waal et al., 2011; Wang et al., 2015).

Cyanobacterial *M. aeruginosa* and *R. raciborskii* dominate and affect water quality in freshwater systems globally (Burford et al., 2016; Harke et al., 2016). These two species co-occur often with successive dominance in natural populations (Soares et al., 2009), however, there is evidence suggesting that *R. raciborskii* is gradually replacing
M. aeruginosa to become the dominant or co-dominant species (Marinho and Huszar, 2002; Moustaka-Gouni et al., 2007). Strains of both species were found to have a wide variation in their growth responses under the same environmental conditions, and different scales of response to changes in environmental conditions (Willis et al., 2016b; Xiao et al., 2017b).

Phytoplankton competition models, based on resource-dependent growth, have been widely used to predict the outcome of competition between two species for one or two resources, such as light, nutrients, and CO2 (Passarge et al., 2006; Van de Waal et al., 2011; Verspagen et al., 2014). Yet, the impact on competition of variation in growth responses within species has not been considered. This is a significant gap, because intraspecific variation in growth in both M. aeruginosa and R. raciborskii can be greater than the interspecific variation (Xiao et al., 2017b).

This study examined competition between pairs of four M. aeruginosa strains and eight R. raciborskii strains, while accounting for variation of growth responses within each strain. A fully-parameterized deterministic model was used to predict competition between strains for light. Unlike previous phytoplankton competition models where fixed values for growth parameters were used, here growth parameters were represented as a statistical distribution to capture their inherent variability, using a Monte Carlo approach (Ogilvie, 1984; Whitehead et al., 2015).

### 3.2 Material and methods

Data from four strains of M. aeruginosa and eight strains of R. raciborskii were used to quantify parameters for growth-irradiance models with and without photoinhibition at 20 and 28°C. These parameters were then applied in a deterministic model of phytoplankton dynamic growth in a well-mixed system, and pairwise competition
between all strains was assessed over 30 days for two incident light levels, two temperature levels, and two different initial conditions. Due to the large variability of growth parameters (as is typical in growth-irradiance models), the dynamic growth model was run 17,000 times for each pairwise competition, with parameter values randomly selected from a statistical distribution using a Monte Carlo approach. All the variables were listed in Table 3-1.

Table 3-1: Summary of variables used in this text.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Descriptions</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_{i,i}$</td>
<td>Half-saturation irradiance constant of strain i</td>
<td>$\mu$mol photons m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$I_0$</td>
<td>Mean incident light intensity</td>
<td>$\mu$mol photons m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\bar{I}$</td>
<td>Light intensity averaged over the mixing depth</td>
<td>$\mu$mol photons m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$k_{bg}$</td>
<td>Background light attenuation coefficient</td>
<td>m$^{-1}$</td>
</tr>
<tr>
<td>$k_{li}$</td>
<td>Specific light attenuation coefficient of cells of strain i</td>
<td>(cells mL$^{-1}$)$^{-1}$ m$^{-1}$</td>
</tr>
<tr>
<td>$k_{tot}$</td>
<td>Total light attenuation coefficient of the water column</td>
<td>m$^{-1}$</td>
</tr>
<tr>
<td>$L$</td>
<td>Mortality rate</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$\beta_i$</td>
<td>Photoinhibition parameter of strain i</td>
<td>($\mu$mol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$</td>
</tr>
<tr>
<td>$\mu_{max,i}$</td>
<td>Maximum growth rate at optimal light of strain i</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$\mu_{meas,i}$</td>
<td>Measured growth rate of strain i</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$\bar{\mu}_{net,i}$</td>
<td>Mean net growth rate of strain i</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$w_i$</td>
<td>Cell concentration of strain i</td>
<td>cells mL$^{-1}$</td>
</tr>
<tr>
<td>$\bar{w}_i$</td>
<td>Cell concentration averaged over the mixing depth of strain i</td>
<td>cells mL$^{-1}$</td>
</tr>
</tbody>
</table>
3.2.1 Datasets for parameter estimation

Twelve strains used for this study were isolated from lakes located in southeast Queensland, Australia. Straight \textit{R. raciborskii} strains WS01 and WS05, and coiled \textit{R. raciborskii} strains WC03, WC04 and WC07, were isolated from Lake Wivenhoe ($27^\circ23'38''S, 152^\circ36'28''E$). Straight \textit{R. raciborskii} strains C1, C3 and C6, and \textit{M. aeruginosa} strains M2, M3, M4 and M5, were isolated from Lake Baroon ($26^\circ42'12''S, 152^\circ52'5''E$). Five \textit{R. raciborskii} strains C1, C3, C6, WS01 and WS05 are straight trichomes and three other strains WC03, WC04 and WC07 are coiled trichomes (Xiao et al., 2017b). Three \textit{M. aeruginosa} strains M2, M3 and M4 are single-celled and one strain M5 is single-celled with few small colonies (Xiao et al., 2017b). The buoyancy regulation of all strains has not been measured, but strain variability in buoyancy, positive buoyancy of \textit{M. aeruginosa} (Li et al., 2016a) and neutral buoyancy of \textit{R. raciborskii} (Kehoe, 2009) would be expected. Additionally, all \textit{M. aeruginosa} strains and \textit{R. raciborskii} strain C1 were non-toxic, and the remaining strains were toxic with toxin (CYN + dCYN) cell quotas ranging from $0.4 \pm 0.01$ fg cell$^{-1}$ in C3 to $163.3 \pm 6.1$ (fg cell$^{-1}$) in WC07 ((Willis et al., 2016b); Xiao et. al, unpubl.). All the strains were identified through taxonomy based on morphology, cell quotas of different toxins, as well as multiplex randomly amplified polymorphic DNA (Neilan, 1995).

Data on growth rates of all the twelve strains, under four incident light intensities (10, 30, 50 and 100 \textmu mol photons m$^{-2}$ s$^{-1}$) and two temperatures (20 and 28$^\circ$C), were obtained from monoculture experiments in Xiao et al. (2017b) and additional monoculture experiments using the same methodology as (Xiao et al., 2017b). Additional monoculture experiments were undertaken with strains WC03 and WC04 under three incident light intensities (5, 25 and 40 \textmu mol photons m$^{-2}$ s$^{-1}$) at 20$^\circ$C to expand the available data to better estimate the effect of light intensity on their growth.
responses. This is because, very similar growth rates for two coiled *R. raciborskii* strains WC03 and WC04 were found under lower light intensities at 20°C. For example, the mean growth rates of WC03 were 0.29, 0.26 and 0.27 d⁻¹ under incident light intensities of 10, 30 and 50 µmol photons m⁻² s⁻¹, respectively. The cultures were grown in triplicate until stationary phase. Growth rates in exponential phase, cell concentrations, individual cell biovolumes, and light attenuation properties in stationary phase were measured in the same experimental setup. Thus, the uncertainty that is typically introduced in models by combining studies with different methodologies was avoided.

### 3.2.2 Well-mixed dynamic growth model for phytoplankton competition

Four modelled parameters characterizing phytoplankton growth and mortality were estimated: maximum growth rate $\mu_{\text{max}}$ (d⁻¹) at the optimal light, half-saturation irradiance constant $H_I$ (µmol photons m⁻² s⁻¹), photoinhibition parameter $\beta$ (µmol photons m⁻² s⁻¹⁻¹ d⁻¹) and mortality rate $L$ (d⁻¹), from the growth-irradiance models (with and without photoinhibition). The four parameters were determined from fitting the measured growth rate $\mu_{\text{measure}}$ from the experiments versus the mean light intensity $\bar{I}$ to two growth-irradiance models using non-linear regression, for each of the twelve strains at the two temperatures. The two growth-irradiance models are

\[
\mu_{\text{measure},I} = \mu_{\text{max},I} \frac{1}{1 + \frac{H_I}{I}} - \frac{\beta}{1 + \frac{H_I}{I}} - L, \quad \text{Eqn 3-1}
\]

\[
\mu_{\text{measure},I} = \mu_{\text{max},I} \frac{1}{1 + \frac{H_I}{I}} - L, \quad \text{Eqn 3-2}
\]

where mortality rate $L$ was assumed as 0.1 d⁻¹ as per Huisman et al. (2004). This is because in preliminary estimations for $\mu_{\text{max}}, H_I$ and $\beta$, I attempted to calibrate to the monoculture data for each strain (Xiao et al., 2017b) to estimate the mortality rate.
However, fitting Eqns 3-1 and 3-2 to the data, with the mortality rate kept as a free parameter, led to nonphysical estimates of parameter values because of the small number of independent light values (4) used in the regression.

The growth-irradiance model in Eqn 3-1 assumes that photoinhibition affects phytoplankton growth for the range of incident light levels ($I_0 \leq 100$ µmol photons m$^{-2}$ s$^{-1}$), i.e. $\beta > 0$. Conversely, Eqn 3-2 assumes that photoinhibition can be neglected over the range of incident light levels, i.e. $\beta = 0$. For each strain and temperature, parameters $\mu_{max}$, $H_I$, and $\beta$ were obtained from whichever of Eqn 3-1 or 3-2 had the highest adjusted $R^2$ value for the nonlinear regression against the data. Note that Eqn 3-1 was only used when $\beta$ was positive and significantly different to zero. All the nonlinear regression are shown in Appendix 1A.

$$\bar{I} = \frac{I_0}{k_{tot}z_m}(1 - e^{-k_{tot}z_m}), \quad \text{Eqn 3-3}$$

where $I_0$ is the incident light intensity (µmol photons m$^{-2}$ s$^{-1}$), $k_{tot}$ is the total light attenuation coefficient of the water column (m$^{-1}$), and $z_m$ is the mean mixing depth from the monocultures ($z_m = 0.08$ m). The total light attenuation coefficient $k_{tot}$ is

$$k_{tot} = k_{bg} + \sum_i k_{ji} w_i, \quad \text{Eqn 3-4}$$

where $k_{bg}$ is the background light attenuation coefficient (m$^{-1}$), and $k_{ji}$ is the specific light attenuation coefficient of cells of strain $i$ ((cells mL$^{-1}$)$^{-1}$ m$^{-1}$), $w_i$ is the cell concentration of strain $i$ (cells mL$^{-1}$). The total light attenuation coefficient $k_{tot}$ was firstly calculated from the measured light attenuation properties using Beer-Lambert Law (Kirk, 1985). The $k_{bg}$ and $k_j$ of each strain under all light and temperature conditions were then obtained simultaneously by multiple linear regression using Eqn
3-4. From this regression, the mean and standard deviation of $k_i$ for each strain, at both 20 and 28°C (Table 3-2), were obtained for use in this modelling study.

The dynamic growth model used to predict cyanobacterial competition in this study was adapted from a phytoplankton competition model developed by Huisman et al. (2004). Competition was simulated for only two strains at a time, as the simulated competition outcomes were highly variable with even only two strains competing with each other. Therefore, the dynamic growth model in this study is a system of two ordinary differential equations,

$$\frac{d\bar{w}_i}{dt} = \bar{\mu}_{net,i} \bar{w}_i,$$

Eqn 3-5

where $i = 1, 2$ represents the two competing strains, $\bar{w}_i$ is the cell concentration of strain $i$ (cells mL$^{-1}$), and $\bar{\mu}_{net,i}$ is the net growth rate of strain $i$ (d$^{-1}$). Both $\bar{w}_i$ and $\bar{\mu}_{net,i}$ are averaged over the entire mixing depth $z_m$. The $\bar{\mu}_{net,i}$ was derived from the non-depth-averaged growth-irradiance model (Eqn 3-3) with $\bar{I}$ replaced by $I(z)$, and integrated over a light field of mixing depth $z_m$ as per Huisman and Weissing (1994), to obtain the dependence of cyanobacterial growth rate on light availability as

$$\bar{\mu}_{net,i} = \frac{\mu_{max,i}}{k_{tot}z_m} \ln \left( \frac{H_{i,i} + I_0}{H_{i,i} + I_0 e^{-k_{tot}z_m}} \right) - \beta I - L.$$

Eqn 3-6

Here, $\bar{\mu}_{net,i}$ accounts for growth rates that differ over the light field rather than a growth rate that simply depends on mean light intensity $\bar{I}$ through water column (Eqns 3-3 – 3-4) because the latter is only a valid approximation in the limit of small cell concentrations. In the monoculture experiments, growth rates were measured at the exponential growth phase when cell concentrations were still relatively low (see Xiao et al. (2017b) for details), while in competition this assumption is not always true.
Table 3-2: Mean (± standard deviation) of specific light attenuation coefficient \( k_1 \) \((10^{-7} \text{ (cells mL}^{-1})^1 \text{ m}^{-1})\) and mean cell biovolume \((\mu m^3 \text{ cell}^{-1})\) of \( M. \) aeruginosa and \( R. \) raciborskii strains. The strains are \( M. \) aeruginosa \((M2, M3, M4 and M5)\), and \( R. \) raciborskii \((C1, C3, C6, WS01, WS05, WC03, WC04 and WC07)\). Data were obtained from Xiao et al. (2017b) and the additional monoculture experiments following the same methods in Xiao et al. (2017b). Background light attenuation coefficient \( k_{bg} \) was calculated as 6.42 m\(^{-1}\) from multiple linear regression.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>( k_1 ) ((10^{-7} \text{ (cells mL}^{-1})^1 \text{ m}^{-1}))</th>
<th>Mean cell biovolume ((\mu m^3 \text{ cell}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20°C</td>
<td>28°C</td>
</tr>
<tr>
<td>( M. ) aeruginosa</td>
<td>M2</td>
<td>7.5 ± 0.7</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>8.3 ± 0.7</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>8.9 ± 0.9</td>
<td>5.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>M5</td>
<td>6.7 ± 0.8</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>11.8 ± 7.0</td>
<td>15.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>5.3 ± 3.9</td>
<td>7.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>C6</td>
<td>9.9 ± 3.1</td>
<td>10.4 ± 2.0</td>
</tr>
<tr>
<td>( R. ) raciborskii</td>
<td>WS01</td>
<td>6.7 ± 2.0</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>WS05</td>
<td>4.3 ± 2.3</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>WC03</td>
<td>6.6 ± 3.1</td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>WC04</td>
<td>6.3 ± 1.4</td>
<td>5.8 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>WC07</td>
<td>3.7 ± 2.4</td>
<td>7.4 ± 1.0</td>
</tr>
</tbody>
</table>

Eqns 3-3 – 3-6 constitute the dynamic growth model of competition for light between two strains for this study. The model assumes well-mixed conditions, i.e., cyanobacterial cell concentration is vertically homogeneous throughout the water column. Resources other than light were also assumed to be homogenously distributed throughout the water column and were sufficiently plentiful so that they did not limit the growth of cells. Hence, cell growth in the model depends only on light intensity, which decreases through the mixing depth due to background light attenuation coefficient and self-shading of cells.
The goal of this modelling exercise was to investigate how the growth variability within and between strains affects species competition, rather than simulate cyanobacterial competition in a specific water body. Therefore, a shallow water depth of 0.08 m from the monoculture experiments was chosen. This depth was not representative of a water body typically seen in the field, but enabled us to validate the model against laboratory measurements. The data used to calibrate the model, at the highest incident light intensity tested (100 µmol photons m$^{-2}$ s$^{-1}$), required 72 experiments (12 strains $\times$ 2 temperatures $\times$ 3 replicates; see Xiao et al. (2017b)). In order to simulate field conditions, these experiments needed to be repeated using a higher incident light intensity over larger depths.

### 3.2.3 Competition scenarios

Competition simulations were run at two temperatures (low temperature: 20°C and high temperature: 28°C, as used in the monoculture experiments) and two incident light levels (low light: 30 µmol photons m$^{-2}$ s$^{-1}$ and high light: 100 µmol photons m$^{-2}$ s$^{-1}$), to investigate how light and temperature affect cyanobacterial competition. The low and high temperature scenarios represent typical mean water temperatures in temperate and tropical lakes, respectively (Mowe et al., 2015). The two light intensities were chosen to fall within the range of incident light levels used in the monoculture experiments. 100 µmol photons m$^{-2}$ s$^{-1}$ was defined as ‘high light’ because it resulted in photoinhibition of some strains. Whitelam and Cold (1983) concluded that including photoinhibition in species growth was important for evaluating phytoplankton population dynamics.

Each simulation of pairwise competition was run for 30 days. This time period was chosen because it is sufficiently long for one species to become dominant in a population, or for two species to become successively dominant (Stoyneva, 2003;
Molina et al., 2005). Cyanobacterial cell populations can double in 1.5 to 5 days (Xiao et al., 2017b), so 30 days is also a sufficient timescale for field responses.

For all simulations of pairwise competition, two different initial conditions were trialed to show that the key conclusions of the study were insensitive to these conditions: (1) equal biovolume of $10^5 \, \mu m^3 \, mL^{-1}$ and (2) equal cell concentration of $10^5 \, cells \, mL^{-1}$. The dynamic growth model was run in units of cell concentration (cells mL$^{-1}$), but the predicted competition outcomes were assessed in units of biovolume ($\mu m^3 \, mL^{-1}$) or cell concentration (cells mL$^{-1}$) if the initial condition was equal biovolume or equal cell concentration, respectively. This was to avoid misinterpretation of competition outcomes caused by unit conversion. Conversion between biovolume and cell concentration were as follows:

$$\text{Biovolume} = \text{cell concentration} \times \text{individual cell volume} \quad \text{Eqn 3-7}$$

using the individual cell biovolumes listed in Table 3-2. As biovolume reflects cell size and is closely linked to self-shading effects, in this study, model results obtained from competition with equal initial biovolume are presented in the results; while model results from competition with equal initial cell concentration are presented in Appendix 1B, Table S3-5 – Table S3-8, Fig. S3-2. Moreover, the key model results from both conditions were listed and compared in Table 3-4.

3.2.4 Monte Carlo approach

The dynamic growth model was formulated for a fixed value of each of the four input parameters; however, in practice there is both uncertainty and variability in these parameters. Parameter variability was estimated from fitting laboratory data to the growth-irradiance model. In addition to random experimental error, uncertainty and
variability also arise from model assumptions, unconsidered ecosystem processes, and parameter variability of phytoplankton species through adaptation and acclimation to changing environmental conditions (Gardner and O’Neill, 1983). Growth parameters also vary between individual strains. While there is insufficient information to fully characterize the variability in these growth parameters of all species and strains within one species across the full range of environmental conditions experienced in the field, examining the effect of uncertainty on inter-strain competition provides insight into how uncertainty and variability in growth-irradiance responses affect population dynamics.

Even though the causal mechanisms of variation of the four modelled parameters: $\mu_{max}$, $H_1$, $\beta$ and $k_j$ are not fully established, identifying the variabilities in these parameters on species competition is of great importance. Therefore, in this study, rather than using fixed values for the four input parameters, they were sampled from a statistical distribution which captures their uncertainty and variability using a Monte Carlo approach. The Monte Carlo approach here refers to running the dynamic growth model a large number of times, randomly sampling input parameters from a distribution (Gardner and O’Neill, 1983). The four parameters were assumed to be log-normally distributed instead of normally distributed, so that only positive parameter values were selected in the Monte Carlo process. The mean (m) and standard deviation (s) of the parameters’ log-normal distributions were calculated from the mean ($\mu$) and standard deviation ($\sigma$) of their normal distributions (Table 3-2– Table 3-3) according to (Mood et al., 1974)

$$m = \ln \left( \frac{\mu^2}{\sqrt{\sigma^2 + \mu^2}} \right), s = \sqrt{\ln \left( 1 + \frac{\sigma^2}{\mu^2} \right)}.$$  

Eqn 3-8

In each Monte Carlo simulation of pairwise competition, the dynamic growth model was run 17,000 times, each time with values for $\mu_{max}$, $H_1$, $\beta$ and $k_j$ of competing strains
chosen from their log-normal distribution. The model was chosen to run 17,000 times, because from preliminary simulations this number of model runs was sufficient to assess the effect of parameter variability on strain competition. More specifically, the percentage of model runs where one strain reached a higher biovolume after 30 days than the other strain differed by less than 2%, when a Monte Carlo simulation of 17,000 runs was repeated for a given set of incident light and temperature conditions (data not shown).

### 3.2.5 Assessment of competition outcomes

To assess competition outcomes, several terms which are necessary to interpret results of the Monte Carlo simulations were defined. Most of these terms refer to pairwise competition between two strains, denoted strain A and strain B. Competitiveness (%) of strain A against strain B was defined as the percentage of model runs where strain A reached a higher biovolume (for runs with equal initial biovolume of competing strains) than strain B after 30 days. If strain A’s competitiveness against strain B was greater than 50%, strain A was defined to be ‘more competitive’ than strain B. Furthermore, if strain A’s competitiveness against strain B was greater than 75%, strain A was defined to ‘dominate’ strain B. If strain A’s competitiveness against strain B was between 25 and 75%, strain A was defined to ‘co-exist’ with strain B. After comparing the competitiveness of each competition pair at given light, temperature and initial conditions, all twelve strains were ranked from most competitive to least competitive, and the strain which is ‘more competitive’ than the other eleven strains was defined as the ‘dominant’ strain.
3.3 Results

3.3.1 Growth parameters of *M. aeruginosa* and *R. raciborskii*

At incident light intensities of less than 100 µmol photons m⁻² s⁻¹, three out of the twelve strains were photoinhibited at 20°C, i.e. *R. raciborskii* strains C1, C6 and WC07 (Table 3-3, Appendix 1A, Fig. S3-1). Similarly, four out of the twelve strains were photoinhibited at 28°C, i.e. *R. raciborskii* strains C1 and WC07 and *M. aeruginosa* strains M2 and M5. The relationship between measured growth rate and mean light intensity of two strains – *R. raciborskii* C1 and *M. aeruginosa* M4, both at 20°C – is shown in Fig. 3-1 as an example of the growth-irradiance model with the presence and absence of photoinhibition. The growth rate versus mean light intensity of C1 fitted better to the growth-irradiance model that included photoinhibition (adjusted $R^2 = 0.82$ in Fig. 3-1A, compared to adjusted $R^2 = 0.47$ in Fig. 3-1B), showing that C1 was photoinhibited at incident light intensities of less than 100 µmol photons m⁻² s⁻¹. In contrast, M4 was not photoinhibited (adjusted $R^2 = 0.84$ in Fig. 3-1D), because the predicted photoinhibition parameter $\beta$ was less than zero (Fig. 3-1C).

The four input parameters, i.e., $\mu_{max}$, $H_1$, $\beta$ and $k_1$, varied substantially between strains and with temperature (Table 3-2 – Table 3-3). Across all strains and temperatures, the mean (the best fit of the nonlinear regression when fitting data to the model) of $\mu_{max}$, $k_1$ and $H_1$ varied by factors of 3, 5 and over 10, respectively. The uncertainty in the values of parameters also differed between strains (the mean divided by the standard deviation of parameter, Table 3-3).
Table 3-3: Mean (± standard deviation) of growth parameters in their distribution: maximum growth rate $\mu_{\text{max}}$ (d$^{-1}$) at the optimal light, half-saturation irradiance constant $H_i$ (µmol photons m$^{-2}$ s$^{-1}$), and photoinhibition parameter $\beta$ ((µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$) of all *M. aeruginosa* and *R. raciborskii* strains, obtained from fitting the growth-irradiance models to the data in Xiao et al. (2017b) and the additional monoculture experiments. The strains are *M. aeruginosa* (M2, M3, M4 and M5), and *R. raciborskii* (C1, C3, C6, WS01, WS05, WC03, WC04 and WC07). All the model fits were significant ($P < 0.001$).

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>$\mu_{\text{max}}$ (d$^{-1}$) 20°C</th>
<th>$\mu_{\text{max}}$ (d$^{-1}$) 28°C</th>
<th>$H_i$ (µmol photons m$^{-2}$ s$^{-1}$) 20°C</th>
<th>$H_i$ (µmol photons m$^{-2}$ s$^{-1}$) 28°C</th>
<th>$\beta$ ((µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$) 20°C</th>
<th>$\beta$ ((µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$) 28°C</th>
<th>Adjusted R$^2$ 20°C</th>
<th>Adjusted R$^2$ 28°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. aeruginosa</em></td>
<td>M2</td>
<td>0.49 ± 0.02</td>
<td>0.82 ± 0.12</td>
<td>4.15 ± 0.93</td>
<td>10.44 ± 2.72</td>
<td>0</td>
<td>0.0029 ± 0.0015</td>
<td>0.77</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>0.46 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>1.81 ± 0.47</td>
<td>1.74 ± 0.81</td>
<td>0</td>
<td>0</td>
<td>0.64</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>0.57 ± 0.03</td>
<td>0.46 ± 0.02</td>
<td>6.48 ± 1.31</td>
<td>2.02 ± 0.70</td>
<td>0</td>
<td>0</td>
<td>0.84</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>M5</td>
<td>0.43 ± 0.03</td>
<td>0.90 ± 0.17</td>
<td>2.01 ± 0.86</td>
<td>13.35 ± 3.65</td>
<td>0</td>
<td>0.0050 ± 0.0019</td>
<td>0.33</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>0.60 ± 0.05</td>
<td>1.19 ± 0.12</td>
<td>6.15 ± 1.34</td>
<td>13.84 ± 2.17</td>
<td>0.0020 ± 0.0005</td>
<td>0.0068 ± 0.0010</td>
<td>0.82</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>0.51 ± 0.02</td>
<td>0.68 ± 0.11</td>
<td>3.84 ± 1.26</td>
<td>9.19 ± 5.67</td>
<td>0</td>
<td>0</td>
<td>0.56</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>C6</td>
<td>0.62 ± 0.12</td>
<td>0.57 ± 0.02</td>
<td>8.34 ± 3.40</td>
<td>6.53 ± 1.13</td>
<td>0.0023 ± 0.0012</td>
<td>0</td>
<td>0.65</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>WS01</td>
<td>0.50 ± 0.04</td>
<td>0.48 ± 0.02</td>
<td>4.69 ± 2.20</td>
<td>1.04 ± 0.55</td>
<td>0</td>
<td>0</td>
<td>0.40</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>WS05</td>
<td>0.47 ± 0.01</td>
<td>0.60 ± 0.03</td>
<td>5.97 ± 0.74</td>
<td>4.08 ± 1.08</td>
<td>0</td>
<td>0</td>
<td>0.94</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>WC03</td>
<td>0.40 ± 0.02</td>
<td>0.52 ± 0.02</td>
<td>1.45 ± 0.68</td>
<td>3.97 ± 0.83</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>WC04</td>
<td>0.43 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>3.10 ± 1.10</td>
<td>3.39 ± 0.03</td>
<td>0</td>
<td>0</td>
<td>0.45</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>WC07</td>
<td>0.52 ± 0.05</td>
<td>0.61 ± 0.07</td>
<td>2.32 ± 1.12</td>
<td>5.23 ± 1.64</td>
<td>0.0026 ± 0.0006</td>
<td>0.0022 ± 0.0010</td>
<td>0.7</td>
<td>0.71</td>
</tr>
</tbody>
</table>
3.3.2 Competition dynamics

For all pairwise competitions, predicted by the dynamic growth model using the Monte Carlo approach, the biovolumes of both strains increased continuously over the 30 days of simulation (e.g. Fig. 3-2). For example, in the modelled competition between \textit{M. aeruginosa} strain M3 and \textit{R. raciborskii} strain C6 at low light and low temperature conditions, the median biovolume of M3 exceeded that of C6 after 30 days (Fig. 3-2A). While, the 90\% confidence intervals of the biovolume after 30 days overlapped for the two strains, and results were similar when the model was run with equal cell concentration. The 95\textsuperscript{th} percentile of predicted biovolume of M3 after 30 days was nearly an order of magnitude higher than the 5\textsuperscript{th} percentile, for C6 this difference was over three orders of magnitude. In this case, if fixed values were chosen for modelled parameters, either M3 or C6 could have a higher biovolume after 30 days (Fig. 3-2A). Therefore, predicting a clear-cut competition outcome between these two strains is difficult, due to the uncertainty in the parameters that characterize these strains’ growth and light attenuation properties.

3.3.3 Strain competitiveness ranking

The twelve strains were ranked from most competitive to least competitive at given light and temperature conditions (Fig. 3-3). For example, at low light and low temperature conditions, \textit{M. aeruginosa} strain M3 was the most competitive as it was more competitive than all the other eleven strains, whereas \textit{R. raciborskii} strain WS05 was the least competitive as all the other eleven strains were more competitive than WS05 (Fig. 3-3A).

Some strains were predicted to be competitive under all conditions, while others were only predicted to be competitive under some conditions. For example, \textit{R. raciborskii}
strain C3 was predicted to be more competitive than most of the other strains under all incident light and temperature conditions (always ranked in the top four most competitive strains out of twelve) (Fig. 3-3). In contrast, WS05 was predicted to be the second most competitive strain at the high temperature (Fig. 3-3B, D), but was the least competitive under low light and low temperature conditions (Fig. 3-3A).

Fig. 3-1: Examples of the measured growth rate versus mean light intensity (dots) for the *M. aeruginosa* strain M4 and *R. raciborskii* strain C1 at 20°C, fitted to models that either include or exclude photoinhibition (solid lines). Dashed lines represent the 95% confidence interval in the model fits. A: C1 with photoinhibition at 20°C, B: C1 without photoinhibition at 20°C, C: M4 with photoinhibition at 20°C, D: M4 without photoinhibition at 20°C.

In a small number of cases (9% of simulations with equal initial biovolume and 6% with equal initial cell concentration, data not shown), the 90% confidence intervals of the two competing strains’ biovolumes did not overlap (e.g. Fig. 3-2B). In the competition between M3 and WS05 at low light and low temperature conditions, the
biovolume of M3 was always higher than that of WS05 after 30 days. Hence, it is highly likely that M3 outcompetes WS05 at this time, however, the small number of cases indicated that it is uncommon to easily predict the outcome of competition between two strains.

Fig. 3-2: Model prediction of strain biovolume versus time for pairwise competition between strains with equal initial biovolume. A: Competition between M3 and C6 under light intensity of 30 µmol photons m$^{-2}$ s$^{-1}$ and temperature of 20$^\circ$C, B: Competition between M3 and WS05 under light intensity of 30 µmol photons m$^{-2}$ s$^{-1}$ and temperature of 20$^\circ$C. The thick solid and dashed lines represent the median biovolume versus time of the two strains; this is the median of 17,000 model runs used in the Monte Carlo simulation. The red and blue shaded regions represent the 5th to 95th percentiles of the predicted strain biovolume versus time, so 90% of the model runs predicted a strain biovolume within these shaded regions. The purple shaded region represents the overlap between the 5th and 95th percentiles of the biovolumes of the two strains.

The competitiveness between strains also varied with light and temperature conditions. For example, under 30 µmol photons m$^{-2}$ s$^{-1}$ at 20$^\circ$C, the biovolume of M3 was predicted to exceed the biovolume of C6 and WS05 in 59% and 100% of the 17,000 model runs, respectively (Fig. 3-2; Appendix 1B, Table S3-1). Thus, the competitiveness of M3 against C6 and WS05 under these conditions is 59% and 100%, respectively (Fig. 3-3A). At 28$^\circ$C with the same light intensity, however, M3 remained more competitive than C6 (53%), but competitiveness against WS05 reduced substantially to 5% (Fig. 3-3B, Appendix 1B, Table S3-2).
3.3.4 The dominant strain and its co-existing strains

In addition to calculating competitiveness between pairs of strains (Fig. 3-3), the dominant strain at each incident light and temperature condition was determined (Table 3-4). Generally, temperature determined which species dominated, as the dominant strain at 20°C was consistently predicted to be *M. aeruginosa* strains, while at 28°C was consistently predicted to be *R. raciborskii* strains, irrespective of light and initial conditions (Table 3-4). There were typically several *M. aeruginosa* and *R. raciborskii* strains that could co-exist with the dominant strain, and which strains co-existed varied.
substantially with light, temperature and initial conditions (Table 3-4). For example, at
the low light and low temperature condition the dominant strain (*M. aeruginosa* strain
M3) was predicted to co-exist with one *M. aeruginosa* strain and five *R. raciborskii*
strains including C1. Under the same conditions except at a higher temperature of 28°C,
C1 was predicted to dominate, and co-exist with one *M. aeruginosa* strain and two
*R. raciborskii* strains.
Table 3-4: Competitiveness ranking and potential co-existence of cyanobacterial strains predicted after 30 days, using Monte Carlo simulations of our deterministic model of phytoplankton competition. Strains are ordered from the most dominant (left, in italics) to least dominant (right). Strains with a common letter (e.g. ‘a’) are predicted to co-exist after 30 days. The strains are *M. aeruginosa* (M2, M3, M4 and M5), and *R. raciborskii* (C1, C3, C6, WS01, WS05, WC03, WC04 and WC07).

<table>
<thead>
<tr>
<th>Initial condition</th>
<th>Temperature (°C)</th>
<th>Light intensity (µmol m⁻² s⁻¹)</th>
<th>Co-existence of strains (indicated by common letters)</th>
<th>Most competitive strains</th>
<th>Least competitive strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>The same initial biomass (10⁵ µm⁻³ mL⁻¹)</td>
<td>20</td>
<td>30</td>
<td>a</td>
<td>ab</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>a</td>
<td>ab</td>
<td>bc</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>30</td>
<td>a</td>
<td>ab</td>
<td>abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>a</td>
<td>ab</td>
<td>ac</td>
</tr>
<tr>
<td>The same initial cell concentration (10⁵ cells mL⁻¹)</td>
<td>20</td>
<td>30</td>
<td>a</td>
<td>ab</td>
<td>abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>30</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>a</td>
<td>ab</td>
<td>bc</td>
</tr>
</tbody>
</table>
3.4 Discussion

3.4.1 Species and strain variability leads to uncertain outcomes in phytoplankton competition

The competition outcomes predicted by the deterministic dynamic growth model were highly variable for twelve strains of *M. aeruginosa* and *R. raciborskii*, simulated under two constant incident light and two temperature conditions. The dominant strain always belonged to the species *M. aeruginosa* at 20°C and *R. raciborskii* at 28°C, however, there was no absolute ‘winner’ under any of the light and temperature conditions simulated: at least one strain was predicted to co-exist with the dominant strain in each case. This result reflects the co-existence of multiple phytoplankton species in natural lakes (Reynolds, 2006).

Although the twelve strains were originally isolated from two adjacent lakes, and were parameterized under four or seven light levels (depending on the strain) and two temperatures, all input parameters, i.e., maximum growth rate at the optimal light $\mu_{\text{max}}$, half-saturation irradiance $H_1$, photoinhibition parameter $\beta$ and specific light attenuation $k_1$, varied substantially within and between strains. The uncertainty in these parameters was sufficiently large to yield a wide range of biovolumes after 30 days of simulated competition, and therefore there was great variability predicted in strain competitiveness, dominance and co-existence. In comparison, previous studies mostly found competitive exclusion when simulating species competition (Huisman et al., 2004; Passarge et al., 2006). These previous models used fixed growth parameters for each species, even under different environmental conditions, which led to predictions of clear ‘winners’ and ‘losers’ of competition. While, due to parameter uncertainty and variability, results in this study suggest that the outcome of interspecific competition is
less clear than previously thought. Even though *M. aeruginosa* was found to dominate generally at lower temperature than *R. raciborskii* in this study, due to strain variability, *M. aeruginosa* may also dominate in higher temperatures as found in (Xiao et al., 2017b).

By demonstrating how cyanobacterial competition is affected by the variability within and between strains in response to environmental conditions, this study may explain why there are previous contradictory conclusions from competition experiments. For example, one experiment between two *M. aeruginosa* and two *R. raciborskii* strains under a range of light and phosphorus conditions showed that species competition depended on strains (Marinho et al., 2013), which was consistent with the finding in this study. In contrast, another experiment between two *M. aeruginosa* and two *P. agardhii* strains under a range of light intensities found that *M. aeruginosa* always outcompeted *P. agardhii* (Torres et al., 2016). This may have occurred because the strains used in the competition experiment may not have represented the wide range of growth responses of these species.

Since the predicted outcome of competition relied on which two strains were present, it leads to the following questions: Should more strains be parameterized to accurately predict species competition? Would more complex responses of phytoplankton to the local environmental conditions alter the species/strain dominance and co-existence? The challenge is that there is little knowledge about how many strains within one species co-exist in the same lake or across different lakes, nor to confidently quantify the uncertainty and variability of growth responses of these strains under different environmental conditions. For example, O'Brien et al. (2009) found that antecedent light intensity affected primary production and photoinhibition of a *R. raciborskii*-dominated phytoplankton population. This species can adapt to light history with higher primary
production after dark-acclimation compared to a history of higher irradiance.

Accounting for light history effects in determining phytoplankton growth parameters is not simple, due to the uncertainties in short or long timescales of irradiance exposure. Nutrient concentrations can also affect the species growth and thus competition (Geider et al., 1998; Litchman et al., 2003).

Therefore, it is not possible to parameterize the growth responses of all strains under all conditions in a comprehensive dataset. The ‘unknowns’ and uncertainty in ‘true’ values of growth parameters for strains leads to uncertainty in predicted competition. Instead of resolving the ‘unknowns’, here how a Monte Carlo approach can be used to understand the effects of variability in parameters on the predicted species/strain competition has been demonstrated. Although other tools for uncertainty propagation are available, e.g. Mirams et al. (2016), Monte Carlo is a relatively simple approach to propagate the parameter variability in deterministic models, and is relatively free of restrictive assumptions (Gardner and O’Neill, 1983). Where there is low confidence in parameter values, or where variability is high, it makes sense to include variability in the model explicitly, e.g. through a probabilistic approach. In this study, the model parameters have been assumed to have zero covariance when predicting phytoplankton competition for simplicity, but that this is a statistical improvement that can be applied in future work.

The deterministic phytoplankton dynamic growth model in this study was adapted from models developed by Huisman et al. (2004). Their one-dimensional model was simplified by assuming a well-mixed system and focusing on competition for light under constant temperature and incident light intensity. An important aspect of competition for light is the interplay between self-shading and cell growth. In systems that are not well-mixed, however, species-specific buoyancy regulation will likely have
a large impact on the outcomes of competition between phytoplankton species (Huisman et al., 2002a). As a positively buoyant species, *M. aeruginosa* could regulate the vertical distribution and thus access sufficient light (Walsby et al., 1995). In comparison, *R. raciborskii* has been recorded as a neutrally buoyant species (Kehoe, 2009). Thus, while predictions of specific competition outcomes in non-well-mixed systems will differ from the results presented here, the key result of high variability in competition outcomes may generalize to systems that are not well-mixed. For simplicity, the mortality rate in this study was fixed for all strains as 0.1 d$^{-1}$ as per Huisman et al. (2004). In practice, mortality rate of cyanobacteria differed between species and is highly affected by temperature (Whitton, 2012). If the uncertainty in mortality rate was also included in the model, the predicted variability in net growth rate within and between species would be even higher. Therefore, the key conclusion of the study - that variation within and between cyanobacterial strains substantially reduces confidence in model predictions of phytoplankton competition outcomes – is unaffected by the choice of a constant mortality rate.

### 3.4.2 Future work on phytoplankton modelling

Even though multiple strains were involved in this study, the model simulated the competition between two species. In a typical lake, many species are present, but what happens when more species are involved? In models that include multiple phytoplankton species, the species are either grouped, for example by cell size Nogueira et al. (2006), or by functional traits (Mutshinda et al., 2016). Different species’ growth characteristics, however, may not be distinguishable by only one or two functional traits. For example, the N-fixer *Dolichospermum* is positively buoyant (Recknagel et al., 2016), while another N-fixer *R. raciborskii* is neutrally buoyant (Kehoe, 2009), diatoms are non-N-fixers and non-buoyant (Gemmell et al., 2016), and *M. aeruginosa* is a non-
N-fixer and is positively buoyant (Ganf and Oliver, 1982). Using the overlap in functional traits to justify groupings of phytoplankton species in model parameterization may reduce models’ predictive ability, because species with similar functional traits may not behave in exactly the same way (Hellweger, 2017). However, finding the key functional traits to indicate the niche differences within and between species, might be a useful way to improve the model prediction.

To date, studies which involve individual species based on taxonomy have only considered a maximum of two species competing for one or two resources (Ji et al., 2017). Huisman and Weissing (2001) found it impossible to predict the species dominance when simulating competition between more than two species for three or more resources, as transient chaos emerged in the model predictions. Additionally, potential allelopathic interactions between strains and species have been found (Rzymski et al., 2014), but they have not been considered in current phytoplankton models.

While it is possible to add more species/strains and more environmental responses into current phytoplankton models, there are issues in confidently parameterizing these models. The more complex the model becomes, the greater the risk of chaotic interactions. Thus, how to simulate the biodiversity of phytoplankton communities? A number of researchers have suggested that new phytoplankton models are needed, by designing a generic cell that captures the key species traits (such as photosynthesis, respiration, etc.) and trade-offs (operating subject to constraints) to simulate the community dynamics (Allen and Polimene, 2011; Smith et al., 2014). These new models can address the growth variability within and between species, but they require knowledge of intra- and inter-cellular processes that are not yet fully available from current datasets. Some studies have investigated the inner-cellular nutrient availability
on cell growth. For instance, Baird et al. (2013) simulated varying C: Chl ratios on
growth of a diatom and predicted higher ratios for fast-growing large cells compared to
small cells, and subsequently (Robson and Dourdet, 2013) added the effect of nitrogen
fixation on cellular energy use, and hence light response and growth rates. These two
studies demonstrated how mechanistic understanding can be used to predict
phytoplankton growth, but accurate parameterization remains an issue for all
phytoplankton models, and intra- and inter-cellular processes are poorly understood.
Hellweger (2017) suggested that making ecosystem models more complex by including
more information (e.g., variables, processes, parameters, etc.) in the existing models
will increase models’ predictive ability, whereas the meta-analysis of Arhonditsis and
Brett (2004) found that increasing model complexity did not improve model
performance. Conversely, Robson (2014) argued that merging statistical approaches,
even as simple as a Monte Carlo approach, with mechanistic models would significantly
advance understanding of environmental modelling by exploring a wide scenario space
of possibilities.

3.5 Conclusions

In summary, this study simulated pairwise competition between twelve/multiple strains
of two cyanobacterial species *M. aeruginosa* and *R. raciborskii*, using a well-mixed
phytoplankton dynamic growth model merged with a (statistical) Monte Carlo
approach. The Monte Carlo approach accounts for the uncertainty and variability of
growth responses within and between strains. The model results from this study showed
that cyanobacterial competition was highly variable, depending on the strains present,
and the light and temperature conditions. There was no universal ‘winner’: while
*M. aeruginosa* strains dominated at 20°C and *R. raciborskii* strains at 28°C, other strains
were always predicated to co-exist with the dominant strain. These results demonstrate
that within-species strain variability may have a strong influence on phytoplankton population dynamics. Accounting for this variability poses a major challenge to current approaches to phytoplankton modelling.
4 Can the global distribution of cyanoHABs be explained by culture studies of growth responses to light and temperature?

*Microcystis* spp. and *R. raciborskii* have been studied extensively using laboratory cultures to determine their physiological responses to different environmental conditions. I asked the question - Are the outcomes from these numerous laboratory experiments sufficient to confidently predict competition between *Microcystis* and *R. raciborskii* in situ? Hence, Chapter 4 examines whether global distribution and dominance of *Microcystis* spp. and *R. raciborskii* can be modelled from synthesizing laboratory-based culture studies of growth responses to light and temperature, and thus whether these physical factors are driving their dominance on a global scale.
This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors, are:


My contribution to the paper involved: conducting the systematic review of laboratory studies on growth responses and field investigations of cyanobacterial blooms, as well as analyzing data, running model simulations, and drafting the manuscript. David Hamilton helped to lift the whole manuscript, this included checking the models, analyzing model results, and reviewing the manuscript. Katherine O’Brien initiated the idea of reviewing laboratory-based growth studies globally, contributed to checking the models, discussing the model results and reviewing the manuscript. Matthew Adams contributed to building and checking the models, discussing the model results, and reviewing the manuscript. Michele Burford contributed to discussing the model results, improving the ideas in cyanobacterial research and reviewing the manuscript.

(Signed) ___________________________ (Date) 24/10/2018

Name of Student and corresponding author for all listed papers: Man Xiao

(Countersigned) ___________________________ (Date) 24/11/2018

Supervisor: Prof. Michele Burford
4.1 Introduction

Cyanobacterial harmful algal blooms (cyanoHABs) are ubiquitous across lentic freshwater systems worldwide (Paerl and Otten, 2013). These dense blooms, some of which are associated with cyanotoxins, can be costly due to increased water treatment, loss of tourism and recreation revenue, and lower property values (Dodds et al., 2009; Hamilton et al., 2013). The frequency and intensity of blooms appear to be increasing worldwide in response to rising ambient temperatures and CO$_2$ levels, in addition to eutrophication (Paerl and Huisman, 2008; Kosten et al., 2012; O’Neil et al., 2012; Visser et al., 2016b). Understanding and predicting algal population dynamics and their key drivers are essential to manage and control cyanoHABs.

The cyanobacteria Microcystis spp. and Raphidiopsis raciborskii (basionym Cylindrospermopsis raciborskii) are of particular concern because they dominate cyanoHAB events in freshwater ecosystems globally, and have toxin-producing strains (Briand et al., 2009; Antunes et al., 2015; Harke et al., 2016). Microcystis spp. exist as single cells or (more rarely) as paired cells in laboratory cultures but form buoyant colonies that accumulate at the water surface under natural conditions (Xiao et al., 2017c); R. raciborskii is filamentous, with straight and coiled trichomes. Microcystis spp. and R. raciborskii frequently dominate successively or simultaneously within the same waterbody (Soares et al., 2009; Yamamoto et al., 2011), and R. raciborskii is considered to have gradually replaced Microcystis as the dominant or co-dominant species in some tropical reservoirs (Marinho and Huszar, 2002).

Cyanobacterial growth and species dominance are affected by the physical conditions of the waterbody, such as light and temperature, and the nutrient status (Mantzouki et al., 2016). Compared to the effect of latitude on light and temperature (Lewis, 2011),
nutrients are more likely to vary at small scales based on geology, land use and anthropogenic emissions (Paerl et al., 2016). Since light supply also affects the nutrient dependency of algal growth (Litchman et al., 2004), cyanobacterial blooms on a global scale are hypothesized to be controlled primarily by light and temperature.

Laboratory cultures have been used extensively to identify the growth responses of *Microcystis* and *R. raciborskii* strains to a wide range of light and temperature conditions. For example, the optimum temperature for growth of *M. aeruginosa* is between 24 and 32°C (Li et al., 2015; Thomas and Litchman, 2016), and *R. raciborskii* has a range of 29 to 32°C (Briand et al., 2004; Thomas and Litchman, 2016).

*Raphidiopsis raciborskii* has an optimal irradiance for growth of 50 – 120 µmol photons m\(^{-2}\) s\(^{-1}\) across studies (Briand et al., 2004; Dyble et al., 2006), while *M. aeruginosa* is reported to be able to grow at light intensities of up to 400 µmol photon m\(^{-2}\) s\(^{-1}\) (Wiedner et al., 2003). However, it is not known if laboratory experiments conducted on strains provide useful data for predicting growth patterns of *Microcystis* and *R. raciborskii* *in situ* on a global scale.

Hence, in this study, the aim was to predict the global distribution and the relative dominance of *Microcystis* and *R. raciborskii* based on latitude-driven light and temperature conditions. The prediction was done by synthesizing data from the available laboratory-based culture studies of growth responses to these key physical factors.

### 4.2 Material and methods

Four steps were performed (Fig. 4-1) to extrapolate laboratory measurements of growth responses of *Microcystis* and *R. raciborskii* to predict relative dominance of the two species globally using a phytoplankton dynamics model. Firstly, I collated a dataset of
culture studies of growth responses of *M. aeruginosa* (used here as a proxy for *Microcystis* spp. due to the global inconsistencies in species classification), and *R. raciborskii* to light and temperature from across the globe (see Fig. 4-1, Stage 1). The growth rates were then standardized as cyanobacteria were grown under different photoperiods, then growth rates averaged over a day were obtained.

Next, non-linear regression was performed to relate the growth rates to light and temperature values, to parameterize the growth model, including uncertainty in the growth rate predictions (Stage 2a, Fig. 4-1). Similar non-linear regressions of the growth rates for the various light and temperature values for individual strains of *M. aeruginosa* and *R. raciborskii*, were also performed to identify the strain-level variation in growth rate (Stage 2b, Fig. 4-1).

Thirdly, the growth rates of *M. aeruginosa* and *R. raciborskii* predicted from the parameterized growth model, were compared across a range of light and temperature levels, and between the two species (Stage 3, Fig. 4-1).

Finally, population dynamics of *M. aeruginosa* and *R. raciborskii* in summer were predicted by comparing their total biovolumes from the parameterized population dynamics model, assuming well-mixed surface layers, based on latitude-driven summer surface light and temperature globally. The predicted species dominance patterns were then compared to the collated field data on cyanobacterial blooms across the globe from 1988 to 2017 (Stage 4, Fig. 4-1).

All the variables and parameters used in the models are described in Table 4-1. All model equations are listed in Table 4-2. In this study, to represent the uncertainty in the parameterized parameters of the growth model, a Monte Carlo approach was applied for predictions of both growth rate (Stage 3) and population dynamics (Stage 4).
Fig. 4-1: Summary of the methods used in this study. T and I are the temperature and light intensity from laboratory cultures or field investigations, $z_m$ is the surface mixed layer depth, and $k_{bg}$ is the background light attenuation coefficient of water column.
Table 4-1: Parameters and variables used in the model. All parameter values are sourced from the literature and described in *Appendices A – C*.

<table>
<thead>
<tr>
<th>Descriptions</th>
<th>Symbol</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum growth rate at the optimal light and/or temperature</td>
<td>$\mu_{\text{max}}$</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Optimal light for optimal growth</td>
<td>$I_{\text{opt}}$</td>
<td>µmol photons m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Half-saturation irradiance constant</td>
<td>$H_i$</td>
<td>µmol photons m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Photoinhibition parameter</td>
<td>$\beta$</td>
<td>(µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$</td>
</tr>
<tr>
<td>Optimal temperature for optimal growth</td>
<td>$T_{\text{opt}}$</td>
<td>ºC</td>
</tr>
<tr>
<td>Temperature at which cell growth stops</td>
<td>$T_{\text{max}}$</td>
<td>ºC</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>$L$</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Light attenuation coefficient of species i</td>
<td>$k_{i}$</td>
<td>(cells mL$^{-1}$)$^{-1}$ m$^{-1}$</td>
</tr>
<tr>
<td>Background light attenuation coefficient</td>
<td>$k_{bg}$</td>
<td>m$^{-1}$</td>
</tr>
<tr>
<td>Surface mixed layer depth</td>
<td>$z_m$</td>
<td>m</td>
</tr>
<tr>
<td>Latitude</td>
<td>$\text{Lat}$</td>
<td>ºN or ºS</td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature from laboratory cultures or field</td>
<td>$T$</td>
<td>ºC</td>
</tr>
<tr>
<td>Incident light intensity from laboratory cultures / field</td>
<td>$I$</td>
<td>µmol photons m$^{-2}$ s$^{-1}$ / mol m$^{-2}$ d$^{-1}$</td>
</tr>
<tr>
<td>Predicted growth rate</td>
<td>$\mu$</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Total light attenuation coefficient of the water column</td>
<td>$k_{\text{tot}}$</td>
<td>m$^{-1}$</td>
</tr>
<tr>
<td>Cell concentration of species i</td>
<td>$w_i$</td>
<td>cells mL$^{-1}$</td>
</tr>
</tbody>
</table>
Table 4-2: Equations used for the prediction of dependence of growth rate on light and temperature and the phytoplankton population dynamics between *M. aeruginosa* and *R. raciborskii*. All the equations are detailed in *Appendices* 2A – C. The function \( f(T) \) which appears in Eqn 4-5, is defined in Eqn 4-3. The functional form used for dependence of light \( I \) on Latitude (Lat) is empirical and shown graphically in *Appendix* 2C, Fig S4-2B.

<table>
<thead>
<tr>
<th>Description</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependence of growth rate on light and temperature (Growth model)</td>
<td>[ \mu = \mu_{\text{max}} f_I(I) f_T(T) - L ] Eqn 4-1</td>
</tr>
<tr>
<td></td>
<td>[ f_I(I) = \left( \frac{\mu_{\text{max}} + \sqrt{H_I \beta}}{H_I + I} \right)^2 \frac{l}{H_l + I} \beta I / \mu_{\text{max}} ] Eqn 4-2</td>
</tr>
<tr>
<td></td>
<td>[ f_T(T) = \left( \frac{T_{\text{max}} - T}{T_{\text{opt}} \text{opt}} \right) T_{\text{opt}} / (T_{\text{max}} - T_{\text{opt}}) ] Eqn 4-3</td>
</tr>
<tr>
<td>Phytoplankton population dynamics model</td>
<td>[ \frac{d\bar{w}_i}{dt} = \bar{\mu}_i \bar{w}_i ] Eqn 4-4</td>
</tr>
<tr>
<td></td>
<td>[ \bar{\mu}<em>i = \left( \frac{\mu</em>{\text{max}} + \sqrt{H_I \beta}}{H_I + I} \right)^2 \frac{f_T(T)}{k_{\text{tot}} E_m} \ln \left( \frac{H_{l_I+I}}{H_{l_I+I}e^{-k_{\text{tot}} E_m}} \right) - \beta I - L ] Eqn 4-5</td>
</tr>
<tr>
<td></td>
<td>[ \bar{I} = \frac{1}{k_{\text{tot}} E_m} \left( 1 - e^{-k_{\text{tot}} E_m} \right) ] Eqn 4-6</td>
</tr>
<tr>
<td></td>
<td>[ k_{\text{tot}} = k_{bg} + \sum_j k_{ji} \bar{w}_i ] Eqn 4-7</td>
</tr>
<tr>
<td></td>
<td>[ T = -0.0034 \times \text{Lat}^2 + 26.80 ] Eqn 4-8</td>
</tr>
</tbody>
</table>

**Monte Carlo approach**

The Monte Carlo approach here refers to running the model equations multiple times using randomly sampled input parameters from probability distributions for each parameter (Gardner and O’Neill, 1983), to assess the effect of parameter variability on simulated variables. In this study, each parameters’ probability distribution was assumed to be log-normal rather than normal, so that positive parameter values (> 0) were always selected in the Monte Carlo process. The mean and standard deviation of the parameters’ log-normal distributions were calculated from best fit and standard deviation of the parameters’ normal distributions, according to Mood et al. (1974). This Monte Carlo approach was previously employed in Xiao et al. (2017b).
To adequately assess the effect of parameter variability on species growth rate and population dynamics, fifteen-thousand model runs were undertaken for both in Stages 3 and 4, such that result from each simulated condition varied by < 2% with additional runs. Specifically, the uncertainty in the predicted growth rate for each species also included a root mean square error (RMSE), by selecting values from a normal distribution.

**Stage 1: Collation and standardization of laboratory-based culture studies on growth**

Published laboratory studies of growth responses of *M. aeruginosa* and *R. raciborskii* to light and temperature were obtained from *ISI Web of Science* using the search terms ‘*Microcystis aeruginosa*’ and ‘growth’, and ‘*Raphidiopsis raciborskii* (*Cylindrospermopsis raciborskii*)’ and ‘growth’. Studies of *M. aeruginosa* conducted using batch monocultures of unicellular cells were manually selected. Although some studies have successfully induced colony formation in cultures, these colonies are generally not morphologically or physiologically similar to those in field (Xiao et al., 2018), therefore, were excluded from these cases from the analysis. Culture studies of both straight and coiled *R. raciborskii* were included. Studies of: nutrient limitation on cell growth; interactions with biological communities including with other cyanobacteria, bacteria, zooplankton and macrophytes; involving allelopathy; and trace metal impacts, were removed. After data filtering, 20 publications for *M. aeruginosa*, and 16 publications for *R. raciborskii* were used. Reported growth rate, light intensity, light/dark cycle (photoperiod), temperature, light and temperature history, and strain origin (where the strain was originally isolated) were extracted from each study. Data were taken directly from tables where available or otherwise extracted from the graphs in the literature using ScanIt (AmsterCHEM, Almería, Spain).
The light/dark cycle used for culture experiments varied substantially between studies: 24:0, 18:6, 16:8, 14:10, and 12:12 h, thus photoperiod varied between 24, 18, 16, 14 and 12 h, respectively. To make the collected laboratory-based studies comparable and place each study on a common time scale, the measured growth rates were standardized to a photoperiod of 12:12 h, which was also the most widely used light/dark cycle across studies. The standardized growth rates were termed $\mu_d$ (Appendix 2A). Hence, $\mu_d$ represents the growth rate if the culture was grown in a 12:12 h light/dark cycle, and the light intensity adopted in each study represents the light intensity during 12 hours of a day.

**Stage 2: Parameterization of growth rates**

**Stage 2a: Growth parameters of M. aeruginosa and R. raciborskii**

Non-linear regression was conducted on the standardized growth rates $\mu_d$ and their associated light and temperature values for both *M. aeruginosa* and *R. raciborskii* using each strain under each growth condition as a replicate, to obtain parameterized Eqns 4-1 – 4-3. This gave the specific growth parameters based on the best fit and their associated statistical error in the regression, to indicate uncertainty in the growth rate predictions (Table 4-3). The overall uncertainty of growth rate in the non-linear regression represented by the root mean square error (RMSE), was obtained for both species.

In Eqn 4-1, the effects of light and temperature on growth were assumed to be multiplicative as per Nicklisch et al. (2007), where $\mu$ (d$^{-1}$) is growth rate, $\mu_{\text{max}}$ (d$^{-1}$) is the maximum growth rate which occurs at the optimum light and temperature. An assumed mortality rate $L = 0.1$ d$^{-1}$ was used throughout this study following Xiao et al. (2017a). $f_l(I)$ and $f_T(T)$ represented the effects of light and temperature on growth rate,
respectively, and ranged between 0 and 1. $f_T(I)$ in Eqn 4-2 was expressed in the form of a photosynthesis-irradiance curve as per Xiao et al. (2017a), where the maximum growth rate $\mu_{\text{max}}$ occurs at the optimal light intensity $I_{\text{opt}}$, and $H_I$ ($\mu\text{mol photon m}^{-2}\text{s}^{-1}$) is the half-saturation irradiance constant, $\beta$ ($\mu\text{mol photon m}^{-2}\text{s}^{-1}\text{d}^{-1}$) is the photoinhibition parameter. $f_T(T)$ in Eqn 4-3 was based on the temperature-dependent growth curve (Yan and Hunt, 1999; Adams et al., 2017), where $T_{\text{max}}$ ($^\circ \text{C}$) is the maximum temperature above which growth rate ceases, and $T_{\text{opt}}$ ($^\circ \text{C}$) is the optimum temperature at which growth rate is not limited by temperature. The derivation of Eqn 4-2 was provided in Appendix 2B.

**Stage 2b: Strain-level variation in growth parameters**

Similar non-linear regressions were performed for individual strains of *M. aeruginosa* and *R. raciborskii*, to examine intraspecific variation in growth rates. Since most studies were undertaken at fixed light or temperature, or with one varying, dependence of growth rate on light and temperature was parameterized separately for each strain from each study. Note that strains that were cultured under at least five light or temperature levels were selected for parametrization, with all the replicates under a given light and temperature included.

For the individual strains, response to temperature was determined by non-linear regressions on the growth rate, i.e., obtaining $\mu_d$ for each strain by setting $f_T(I)$ to the optimal light $I_{\text{opt}}$, and associated temperature using Eqn 4-1. Similarly, response to light was determined for each strain by setting $f_T(T)$ to the optimal temperature $T_{\text{opt}}$, and associated light using Eqn 4-1.
For parameterization of interspecific (Stage 2a) and intraspecific (Stage 2b) growth models, the value of photoinhibition $\beta$ was chosen to be $> 0$ (significantly different to 0) or $\beta = 0$, depending on whichever $\beta > 0$ or $\beta = 0$ had the highest adjusted $R^2$ value when fitting Eqn 4-1 to the available data.

Strain-level variations in growth responses were also examined under a specific light intensity (100 µmol photon m$^{-2}$ s$^{-1}$) and temperature (20°C), respectively. For both species, the growth curve under variable temperatures at 100 µmol photon m$^{-2}$ s$^{-1}$ was first predicted and compared amongst strains. Similarly, the growth curve under variable light at 20°C was predicted and compared amongst strains.

**Stage 3: Dependence of growth rate on light and temperature**

The growth rates for *M. aeruginosa* and *R. raciborskii* were predicted across a range of temperatures and light intensities, using Eqns 4-1 – 4-3. Temperature ranged from 0 to 40°C (resolved at 0.5°C intervals), covering the range of summer surface water temperatures from 291 lakes in 2009 assembled by Sharma et al. (2015). The light was taken as the mid-month photosynthetically active radiation (PAR) of the northern hemisphere land surface across latitudes (0 – 55 mol m$^{-2}$ d$^{-1}$) (Lewis, 2011). This daily light dose was converted to instantaneous light intensity by assuming a 12:12 h light/dark cycle, equivalent to 0 to 1300 µmol photons m$^{-2}$ s$^{-1}$, resolved it at 5 µmol photons m$^{-2}$ s$^{-1}$ intervals for growth rate predictions. The Monte Carlo approach was applied for predictions of growth rates to represent uncertainty in growth parameters, i.e., $\mu_{\text{max}}$, $H_T$, $T_{\text{opt}}$, $T_{\text{max}}$, $I_{\text{opt}}$, and $\beta$ (Table 4-3). Equations 4-1 – 4-3 were run 15,000 times under each of the selected light and temperature conditions.

The predicted growth rates were then compared quantitatively between *M. aeruginosa* and *R. raciborskii*. If growth rate of *M. aeruginosa* was higher than *R. raciborskii* in <
25% or > 75% of the 15,000 simulation runs under a given light and temperature condition, *M. aeruginosa* was defined to grow slower or faster, respectively, than *R. raciborskii*, otherwise (25 – 75%) there was considered to be no discernible difference in the growth rates of the two species. Note that if both *M. aeruginosa* and *R. raciborskii* had negative growth rates in over 20% of the total runs, no cell growth was assumed and relative growth rate was not calculated.

**Stage 4: Comparison of species dominance globally between model predictions and field observations**

*Global population dynamics and species dominance*

Competition between *M. aeruginosa* and *R. raciborskii* was predicted under latitudinal light and temperature variations (excluding Antarctica) in summer, assuming a surface mixed layer (SML) using a population dynamics model (Eqns 4-4 – 4-8), with incorporation of the parameterized growth Eqns 4-1 – 4-3. The population dynamics model was adopted from Huisman et al. (2002b); Xiao et al. (2017a), and simulates species competition under nutrient-replete conditions with constant mortality (including grazing). Hence, cyanobacterial cells and resources other than light were considered to be vertically homogeneous throughout the SML. Attenuated light intensity was used in simulations and was decreased through the SML depth using a light attenuation coefficient \( k_{tot} \) to account for shading by the cells \( (k_{ji}) \) and a background light attenuation \( k_{bg} \). The \( k_{bg} \) value was chosen as 0.3 and 0.7 m\(^{-1}\) as per Lewis (2011), to test two different values on the growth of the species. Though SML depth varies widely across latitude, altitude and lake size (Lewis, 2011; Woolway et al., 2017), SML depths of 0.01, 1, 3, 6 and 12 m were considered to test its effect on population dynamics. A depth of 0.01 m indicates that cells are concentrated in a very thin surface layer, e.g.,
what might be expected for a surface bloom (Reynolds, 1973); and a depth of 1 m indicates a shallow system or a very shallow thermocline depth which may be representative of a diurnal stratification event. The origin of Eqns 4-4 – 4-7 is explained in Appendix C.

The latitudinal temperature variation has been estimated from non-linear regression on the published summer surface water temperatures of 291 lakes and their latitudes in 2009 (Sharma et al., 2015); see Eqn 4-8 and Appendix 2D, as well as Fig. S4-2A (adjusted $R^2 = 0.47, P < 0.001$). The latitudinal light variation was linearly interpolated from the mid-month PAR on the northern hemisphere land surface at eight latitudes during summer (Lewis, 2011), after correcting for albedo (8% of the global irradiance), see Appendix 2D, Fig. S4-2B. The summer period in the northern hemisphere was considered as July-August-September following Sharma et al. (2015). The light intensity was assumed to be symmetrically distributed between northern and southern hemispheres, i.e., adjusted by six months to correspond to summer in both. For example, 40°S in January was assumed to have the same ambient light intensity as 40°N in July. Since there were no records of surface PAR beyond 70°N or 70°S in Lewis (2011), the light intensity at 85°N or 85°S was adjusted by multiplying the values of 70°N or 70°S with factors of 1.12, 0.97 and 0.40 for the three months of July, August and September, based on NASA’s datasets of monthly-averaged insolation incident on a horizontal surface over the last 22 years (https://eosweb.larc.nasa.gov/cgi-bin/sse/grid.cgi?email=skip@larc.nasa.gov). The mean PAR across latitudes during the summer period (mol m$^{-2}$ d$^{-1}$) was then converted to instantaneous light (µmol m$^{-2}$ s$^{-1}$) following Reynolds (1997) and assuming a 12-h photoperiod across latitudes. The light conversion of the current study was designed to elucidate the relative dominance between $M. aeruginosa$ and $R. raciborskii$ under a range of light and temperature regimes, rather than their absolute growth.
The population dynamics model was run with equal initial total biovolumes \((10^5 \mu m^3 mL^{-1})\) for both \(M. aeruginosa\) and \(R. raciborskii\), and total biovolumes \((\mu m^3 mL^{-1})\) were predicted for 30 days. The initial total biovolumes were converted to cell counts for the purpose of aligning with the units of the population dynamics model. Conversions between biovolume and cell counts were undertaken using the individual cell biovolumes of both species, as per the approach of Xiao et al. (2017b).

The Monte Carlo approach was also applied in predicting the biovolumes, to represent uncertainty in growth parameters, i.e., \(\mu_{max}, H_1, T_{opt}, T_{max}, I_{opt}\), and \(\beta\) (Table 4-3). The growth model represented by Eqns 4-4 – 4-8 was run 15,000 times under each of the selected light, temperature and SML depth simulations. If the total biovolumes of \(M. aeruginosa\) on day 30 was higher than \(R. raciborskii\) in < 25% or > 75% of the 15,000 simulation runs at a given light, temperature and SML depth, \(M. aeruginosa\) was considered to be outcompeted by \(R. raciborskii\), or to outcompete \(R. raciborskii\), respectively, otherwise (25 – 75%) they were considered to co-exist. Note that if both \(M. aeruginosa\) and \(R. raciborskii\) had a declining biovolume (< initial biovolume) in over 20% of the total runs, then the relative dominance was not estimated.

**CyanoHABs and species dominance globally**

The species dominance of \(M. aeruginosa\) and \(R. raciborskii\) was then compared between the predictions of the population dynamics model, and the field observations. A systematic review of field investigations of freshwater cyanobacterial blooms from 1988 to 2017, was undertaken based on publications in *ISI Web of Science*. It was acknowledged that \(R. raciborskii\) was notated as an invasive species in many countries and in some cases is likely to have appeared within the past 30 years (Padisák, 1997; Wood et al., 2014).
Waterbodies were identified that were dominated by *Microcystis* spp. and *R. raciborskii*, either individually, successively or simultaneously, and by other cyanobacterial species. In contrast to laboratory studies, where only data from *M. aeruginosa* cultures were used, for field investigations, data from blooms of all *Microcystis* species was used, including *M. aeruginosa*, *M. wesenbergii* and *M. ichthyoblabe*. This was necessary because there is considerable uncertainty in the identification of these morphospecies due to morphological, biochemical and genetic differences, and classification at species level is contentious and continually being refined (Otsuka et al., 2001; Komárek and Komárková, 2002; Xu et al., 2016b).

On a global scale, the comparison of species dominance was conducted based on climatic zones, where the tropical zone ranges from 23.08°N to 23.08°S, subtropical zone ranges from 23.08°N to 40°N and 23.08°S to 40°S, and temperate zone ranges from 40°N to 85°N and 40°S to 85°S.

### 4.3 Results

#### 4.3.1 Growth parameters of *M. aeruginosa* and *R. raciborskii*

When standardized to 12:12 h light/dark cycle, the maximum growth rate, $\mu_d$, across the culture studies was lower for *M. aeruginosa* (0.87 d⁻¹, Fig. 4-2A) than *R. raciborskii* (1.23 d⁻¹, Fig. 4-2B). Variability in growth responses to light and temperature within and between species was high. *R. raciborskii* was predicted to have a higher $\mu_{\text{max}}$ (0.77 ± 0.47 d⁻¹) at its optimal light and temperature than values for *M. aeruginosa* (0.58 ± 0.36 d⁻¹) (Table 4-3). The half-saturation irradiance constant $I_\text{opt}$ and the optimal light $I_\text{opt}$ for maximum growth of *R. raciborskii* (20.5 ± 12.6 and 124.8 ± 12.9 µmol photons m⁻² s⁻¹) were nearly three-fold and two-fold higher, respectively, than values for *M. aeruginosa* (8.4 ± 5.4 and 67.3 ± 8.2 µmol photons m⁻² s⁻¹). *Microcystis aeruginosa*
had a slightly lower mean value of optimal temperature $T_{\text{opt}}$, and it was not possible to resolve intraspecific differences between the maximum temperature $T_{\text{max}}$, $T_{\text{opt}}$ and the photoinhibition parameter $\beta$ was not substantial due to high levels of uncertainty (Fig. 4-2C, D; Table 4-3).

Growth rates were compared between the model predictions and the standardized growth rates from cultures. The parameterized growth Eqns 4-1 – 4-3 underestimated $\mu_d$ when it was above 0.5 d$^{-1}$ for *M. aeruginosa* (Fig. 4-2A) and 0.7 d$^{-1}$ for *R. raciborskii* (Fig. 4-2B). The simulated light curves at 20°C for each species could not capture the wide distribution of the normalized growth rates of all strains at 20°C (Fig. 4-2E – F). Similarly, the simulated temperature curves at 100 µmol photons m$^{-2}$ s$^{-1}$ could not capture the wide distribution of normalized growth rates of all strains at 100 µmol photons m$^{-2}$ s$^{-1}$ (Fig. 4-2G – H).

### 4.3.2 Intraspecific variation in growth parameters

The light intensities adopted in culture studies varied widely but were mostly constant and < 150 µmol photons m$^{-2}$ s$^{-1}$ from artificial light sources, and temperature was typically constant and between 20 and 30°C (*Appendix* 2D, Fig. S4-1). Variability in growth response to light and temperature was high between strains (*Appendix* 2D, Tables S4-1, S4-2). For example, across all strains, the mean maximum growth rate $\mu_{\text{max}}$ at optimal temperature, and the mean $\mu_{\text{max}}$ at the optimal light varied by a factor of four (*Appendix* 2D, Table S4-1) and ~3.5 (*Appendix* 2D, Table S4-2), respectively.

Strains of each species isolated from the same waterbody differed in respect to growth parameters. For example, *M. aeruginosa* strains BearAC-02 and BearAG-02, originally isolated from Bear Lake, Michigan, North America (Thomas and Litchman, 2016), had
a 2-fold difference in their mean $\mu_{max}$ at optimal temperature (Appendix 2D, Table S4-1).

Table 4-3: The specific growth parameters with best fit (± standard deviation) for *M. aeruginosa* and *R. raciborskii*: maximum growth rates at: optimal light and temperature $\mu_{max}$ (d$^{-1}$); optimal light for maximum growth $I_{opt}$ (µmol photons m$^{-2}$ s$^{-1}$); half-saturation irradiance $H_I$ (µmol photons m$^{-2}$ s$^{-1}$); photoinhibition parameter $\beta$ ((µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$); temperature for maximum growth $T_{opt}$ (°C); maximum temperature when growth rate ceased $T_{max}$ (°C); and root mean square error of the predicted growth rate (RMSE, d$^{-1}$). This was obtained from fitting Eqn 4-1 to the data collected from 20 and 16 studies, for *M. aeruginosa* and *R. raciborskii*, respectively. Both model fits were significant ($P < 0.001$).

<table>
<thead>
<tr>
<th>Estimated parameters</th>
<th>Species</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>M. aeruginosa</em></td>
<td><em>R. raciborskii</em></td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>0.58 ± 0.36</td>
<td>0.77 ± 0.47</td>
</tr>
<tr>
<td>$I_{opt}$</td>
<td>67.3 ± 8.2</td>
<td>124.8 ± 12.9</td>
</tr>
<tr>
<td>$H_I$</td>
<td>8.4 ± 5.4</td>
<td>20.5 ± 12.6</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.0011 ± 0.0007</td>
<td>0.0010 ± 0.0006</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>29.6 ± 17.9</td>
<td>30.9 ± 18.7</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>40.4 ± 24.5</td>
<td>40.2 ± 24.9</td>
</tr>
<tr>
<td>RMSE of predicted growth rate</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.36</td>
<td>0.38</td>
</tr>
</tbody>
</table>

### 4.3.3 Dependence of growth rate on light and temperature

*Raphidiopsis raciborskii* was predicted to have higher growth rates when the light intensity was above ~150 µmol photons m$^{-2}$ s$^{-1}$, and temperature was above ~25°C (Fig. 4-3). Growth of both species was predicted to be negligible when the temperature was below ~12°C or above ~38°C, or when the light intensity increased to ~600 µmol photons m$^{-2}$ s$^{-1}$ at temperatures below 20°C (Fig. 4-3). For temperatures of up to 25°C or light intensities of up to 150 µmol photons m$^{-2}$ s$^{-1}$, the predicted growth rates were not
substantially different between *M. aeruginosa* and *R. raciborskii*. These findings are consistent across the range of parameters values, since the growth rate Eqns 4-1 – 4-3 were run 15,000 times using the Monte Carlo approach.

### 4.3.4 Species dominance between model prediction and field observations

Using the phytoplankton population dynamics model, *R. raciborskii* was predicted to dominate over *M. aeruginosa* in well-mixed conditions under most light and temperature regimes, surface mixed layer depths (SMLs) and background light attenuation coefficients $k_{bg}$, even accounting for parameter variability (Fig. 4-4, Appendix 2D, Fig. S4-3). In the tropical zones and lower latitudes of the subtropics, *M. aeruginosa* was predicted to be outcompeted by *R. raciborskii* irrespective of SML depth and $k_{bg}$. *Microcystis aeruginosa* was only predicted to dominate over *R. raciborskii* at high latitudes of > 60°N and for SML depth > 6 m. In the low latitudes of temperate zones, co-existence of *M. aeruginosa* and *R. raciborskii* was predicted, and the chance of co-existence was higher with a greater SML depth, $k_{bg}$ and latitude. At the very shallow SML depth (0.01 m), *M. aeruginosa* was predicted to co-exist with *R. raciborskii*, and the chance of co-existence near the equator was much higher compared to other higher SMLs.

The species dominance predicted from the population dynamics model did not match particularly well with the field observations of *Microcystis* and *R. raciborskii* blooms. Reports of cyanobacterial blooms have been published from at least 1130 freshwater ecosystems, including lakes, rivers, reservoirs and ponds across all continents (except Antarctica) from 1988 to 2017 (Fig. 4-5). Over 80% of these systems were found to be dominated by *Microcystis* spp. or *R. raciborskii* on at least one occasion over that time period, with dominance varying throughout tropical, subtropical and temperate zones.
Microcystis dominated by biovolume in more systems than *R. raciborskii* (78% vs. 17%), irrespective of climatic zones. About half of the systems dominated by *Microcystis* are located in the temperate zone (49%), followed by the subtropics (31%) and tropics (20%). In comparison, half of the systems dominated by *R. raciborskii* are located in the tropics (50%), followed by subtropics (31%) and temperate zone (19%). *Microcystis* and *R. raciborskii* were also found to dominate successively or simultaneously (Fig. 4-5), in over 13% of the systems, most of which are located in the tropics (60%), followed by subtropics (23%) and temperate zone (17%).
Fig. 4-2: Comparison of predicted growth rate using the parameterized growth Eqs 4-1 – 4-3, with the published growth rates normalized to 12:12 h light/dark cycle ($\mu_d$): A. *M. aeruginosa* ($R^2 = 0.29, P < 0.001, n = 191$); B. *R. raciborskii* ($R^2 = 0.38, P < 0.001, n = 367$). The diagonal line in A – B represents slope = 1. C: Predicted light curves at the optimal temperature $T_{opt}$ for both species, with associated variability (5th and 95th percentiles of predictions). D: Predicted growth curves at the optimal irradiance $I_{opt}$ for
growth of both species, with associated variability (5th and 95th percentiles of predictions). E – F: Predicted growth curves of both species at 20°C, compared with calibrated growth rates of all strains at 20°C. Colours indicated different temperatures and size corresponds to temperature scaled from 10 to 40°C. G – H: Predicted growth curves of *M. aeruginosa* and *R. raciborskii* at the light intensity of 100 µmol photons m\(^{-2}\) s\(^{-1}\), compared with calibrated growth rates of all strains at this light. Colours indicated different light intensities and size corresponds to light intensity scaled from 10 to 500 µmol photons m\(^{-2}\) s\(^{-1}\).

Fig. 4-3: Percentage of model runs (%) where predicted growth rate of *M. aeruginosa* was higher than *R. raciborskii* under a range of light and temperature conditions, based on Monte Carlo simulation of parameterized growth Eqns 4-1 – 4-3. This Monte Carlo simulation was run 15,000 times for each light and temperature condition. The white area on the heat map indicates conditions corresponding to zero net growth of either *M. aeruginosa* or *R. raciborskii*. The dashed line represents *M. aeruginosa* had higher growth rate than *R. raciborskii* with 25% of 15,000 times under the given light and temperature conditions.
A. Surface mixed layer depth $z_m = 0.01$ m, background light attenuation coefficient $k_{bg} = 0.3$ m$^{-1}$

F. Surface mixed layer depth $z_m = 0.01$ m, background light attenuation coefficient $k_{bg} = 0.7$ m$^{-1}$

B. Surface mixed layer depth $z_m = 1$ m, background light attenuation coefficient $k_{bg} = 0.3$ m$^{-1}$

G. Surface mixed layer depth $z_m = 1$ m, background light attenuation coefficient $k_{bg} = 0.7$ m$^{-1}$
C. Surface mixed layer depth $z_m = 3\, \text{m}$, background light attenuation coefficient $k_{bg} = 0.3\, \text{m}^{-1}$

H. Surface mixed layer depth $z_m = 3\, \text{m}$, background light attenuation coefficient $k_{bg} = 0.7\, \text{m}^{-1}$

D. Surface mixed layer depth $z_m = 6\, \text{m}$, background light attenuation coefficient $k_{bg} = 0.3\, \text{m}^{-1}$

I. Surface mixed layer depth $z_m = 6\, \text{m}$, background light attenuation coefficient $k_{bg} = 0.7\, \text{m}^{-1}$
Fig. 4-4: Model prediction of phytoplankton dominance in summer: percentage of model runs (%) where predicted total biovolumes of *M. aeruginosa* was higher than *R. raciborskii* on day 30, based on Monte Carlo simulations using the population dynamics model (Eqns 4-4 – 4-8) under well-mixed conditions, with latitude-driven surface light and temperature in summer at four surface mixed layer depths \( z_m \): 0.01, 1, 3, 6 and 12 m with two background light attenuation coefficients \( k_{bg} \) (0.3, 0.7 m\(^{-1}\)). This Monte Carlo simulation involved 15,000 model runs for each light, temperature and SML depth. The upper and lower lines in each figure is a threshold beyond which the predictions are less reliable due to the uncertainty in growth parameters for temperatures < 15°C. The middle line in each figure represents the equator.
Fig. 4-5: Global observations of freshwater cyanobacterial blooms, and comparison of dominance between *Microcystis* spp. and *R. raciborskii* from 1988 to 2017, based on published studies.
4.4 Discussion

Based on the synthesis of growth responses of *M. aeruginosa* and *R. raciborskii* cultures to light and temperature, and distributions of these variables across the globe, this study predicted that for a representative surface mixed layer, *M. aeruginosa* would only dominate at high latitudes of temperate regions. *Raphidiopsis raciborskii* was predicted to dominate over *M. aeruginosa* more widely across the globe, with coexistence occurring mostly in lower temperate regions. However, there were differences from field investigations as *Microcystis* spp. dominated in lentic waterbodies more often than *R. raciborskii* (78% vs. 17% dominance of cyanobacterial blooms globally) irrespective of climatic zones. Hence, my study indicates that the light and temperature growth responses of *M. aeruginosa* and *R. raciborskii* from culture studies do not fully explain their relative dominance globally.

The mismatch in species dominance appears to be because laboratory studies fail to capture the physiological mechanisms used by *M. aeruginosa* to regulate its vertical distribution in water column under changing mixing conditions (Visser et al., 2005), or to otherwise adequately mimic conditions *in situ*. It is acknowledged that nutrient availability varies widely across systems and that *M. aeruginosa* and *R. raciborskii* respond differently to this variability (Burford et al., 2017; Xiao et al., 2018). This study chose to test for broad latitudinal differences in species dominance driven by light and temperature. It is acknowledged that nutrients and other biological factors, including those affecting the mortality of cyanobacteria, such as zooplankton grazing and viruses, are also likely to increase the variability of species dominance on a local scale, but were not the focus of this study.
Species dominance between model prediction and the field

Pooling all available laboratory data on growth rates resulted in substantial differences in the maximum growth rate $\mu_{\text{max}}$ and the optimal light intensity $I_{\text{opt}}$ between *M. aeruginosa* and *R. raciborskii*. The estimated higher $\mu_{\text{max}}$ and $I_{\text{opt}}$ of *R. raciborskii* led to the prediction of higher growth rates at temperatures $\geq 25^\circ\text{C}$ and light intensities $\geq 150 \ \mu\text{mol photons m}^{-2} \ \text{s}^{-1}$, compared to *M. aeruginosa*. Given that summer ambient light intensity was $> 150 \ \mu\text{mol photons m}^{-2} \ \text{s}^{-1}$ (equivalent to 6.48 mol m$^{-2}$ d$^{-1}$ assuming a 12:12 h light/dark cycle with constant light intensity) across latitudes (Lewis, 2011), and summer surface water temperatures higher than 25$^\circ\text{C}$ occurred in over 32% of the 291 lakes in 2009 examined in a global study (Sharma et al., 2015), *R. raciborskii* could be predicted to have higher growth rates than *M. aeruginosa* in a large proportion of lentic waters.

Mixing regimes were incorporated into the *R. raciborskii* and *M. aeruginosa* population dynamics model via sensitivity analysis using a range of surface mixed layer (SML) depths from 0.01 to 12 m. Unsurprisingly, given that light and temperature were the only driving factors under the well-mixed conditions imposed in the model, the higher growth rate of *R. raciborskii* gave it a competitive advantage over *Microcystis*, allowing it to accumulate higher biomass and to dominate more often across latitudes. This greater dominance is consistent with studies which have shown that artificial mixing in some reservoirs can boost *R. raciborskii* blooms (Antenucci et al., 2005; O'Brien et al., 2009), but reduce *Microcystis* blooms (Visser et al., 1996b; Heo and Kim, 2004; Antenucci et al., 2005).

The predicted dominance of *M. aeruginosa* over *R. raciborskii* at a given latitude and temperature varied with the background light attenuation coefficient $k_{\text{bg}}$ and SML
depth. This could mostly be explained by the growth response to light of these two species from my synthesis of laboratory-based studies. Compared to *R. raciborskii*, *M. aeruginosa* had a lower optimal light intensity $I_{\text{opt}}$ for maximum growth, suggesting that it is better adapted to lower light intensities (Schwaderer et al., 2011), and under lower light intensities *M. aeruginosa* would more effectively compete with *R. raciborskii*. Therefore, for a higher $k_{\text{bg}}$ and an increasing SML depth, with decreasing mean light exposure of cells, there is a higher probability of *M. aeruginosa* co-existence or dominance over *R. raciborskii*. This is apparent at higher latitudes of temperate zones, where temperatures are lower, which favours *M. aeruginosa* more than *R. raciborskii*, due mostly to the slightly lower optimal temperature value for *M. aeruginosa*.

The prediction of species dominance (Figs 3, 4) in the current study from synthesizing all available culture studies, differs somewhat from culture studies of *R. raciborskii* which have shown that it is adapted to lower light intensities (Pierangelini et al., 2015) and observations of more frequent dominance of *Microcystis* than *R. raciborskii* *in situ* across the globe (see Results). Laboratory-based culture studies on light and temperature growth responses cannot be used to adequately predict competition and dominance of cyanoHAB species.

### 4.4.2 Limitations of laboratory cultures specific to *Microcystis*

A key factor that hinders a more robust prediction of species dominance from culture studies is that *Microcystis* has a variety of mechanisms that allow it to control its vertical position in the water column, and this affects its light and temperature environment. Such factors include colony formation (Xiao et al., 2018), buoyancy regulation (Brookes and Ganf, 2001), and carbon ballast accumulation (Ibelings et al.,
These processes become increasingly important under vertical stratification typical of summer conditions in many lakes. However, in laboratory cultures where homogenous conditions are usually present, *Microcystis* exists mostly as single cells or (more rarely) as paired cells (Yang et al., 2008; Li et al., 2013). Even though formation of *Microcystis* colonies has been successfully induced by adjusting biotic or abiotic factors in some studies, the colonies are not similar to those observed in the field (Xiao et al., 2018).

The global dominance of *Microcystis* has been, in part, attributed to its ability to form colonies (Yamamoto et al., 2011). *Microcystis* colonies have been found to be less susceptible to high irradiance compared to single cells, due to a higher quota of photosynthetic pigments (Wu and Song, 2008; Wu et al., 2011; Zhang et al., 2011) and the ability to protect cells within the colony by self-shading (Xiao et al., 2018). Hence, *Microcystis* cultures of single cells may underestimate the growth of *Microcystis* under light-saturated conditions.

Buoyancy regulation of *Microcystis* colonies under a range of light, temperature and nutrient conditions is likely to confer a competitive advantage to this species (Brookes and Ganf, 2001). Large colony size of *Microcystis* translates into higher floatation velocities; the fastest recorded for freshwater cyanobacteria, with mean velocities almost four magnitudes higher than those of *R. raciborskii* (Xiao et al., 2018). Li et al. (2016a) measured ascent velocities up to 10 m h\(^{-1}\) for an individual *Microcystis* colony with a diameter of 1200 µm. This rapid floatation velocity enables *Microcystis* to optimize light capture (Ganf and Oliver, 1982), and to disentain from the predominant water motions under low to moderate levels of turbulence (Humphries and Lyne, 1988). In nutrient-enriched systems, the large biomass in surface blooms of *Microcystis* could protect sub-surface colonies from high irradiance by shading them, whilst potentially
limiting the growth of \textit{R. raciborskii}, since this species is more evenly distributed through the water column. This is consistent with the dominance of \textit{Microcystis} over \textit{R. raciborskii} in most eutrophic lakes worldwide.

In the modelled scenario of very thin SML depth (0.01 m), similar to a case where colonies accumulate at the water surface (Humphries and Lyne, 1988), my model predicted that \textit{R. raciborskii} would be less likely to outcompete \textit{Microcystis}, compared to deeper SML depths near the equator. The neutral buoyancy of \textit{R. raciborskii} means it is much less likely to be found at the water surface (Kehoe, 2009). This further indicates that mixing regimes, in combination with physiological attributes such as colony formation and buoyancy regulation, may play a key role in the dominance of \textit{Microcystis}.

\textbf{4.4.3 Relevance of laboratory cultures to field conditions}

The existence of strains (also known as ecotypes) leads to a wide intraspecific variation in growth responses of \textit{M. aeruginosa} and \textit{R. raciborskii}. However, some of the variation between studies using different strains may also be attributed to lack of standardization of laboratory techniques, such as photoperiod, which introduces uncertainty in characterizing the growth parameters. Photoacclimation of cells, such that photosynthesis rates are affected, has been found to be important. For example, higher primary production was measured after dark than in photoacclimated \textit{R. raciborskii}-dominated phytoplankton populations (O'Brien et al., 2009). The photoperiod has also been found to interact with temperature and light to affect growth rates of species to varying degrees (Nicklisch et al., 2007). We attempted to standardize conditions for all culture studies, by normalizing growth rates to 12:12 h light/dark cycle but note the wide variations in experimental light regimes.
Light is attenuated exponentially through water depth (Kirk, 1994), and phytoplankton will have varying light exposure at a given point in time under turbulent mixing (Huisman et al., 2004). However, culture studies are typically conducted using constant light. Litchman (2000) found that phytoplankton growth may be depressed or accelerated under fluctuating light conditions and, as a result of differences amongst species, fluctuations may structure phytoplankton communities and affect their diversity (Litchman and Klausmeier, 2001).

Of note in the synthesis of culture studies of *M. aeruginosa* and *R. raciborskii* was that few experiments were conducted at light intensities above 150 µmol photons m\(^{-2}\) s\(^{-1}\) (equivalent to 6.48 mol m\(^{-2}\) d\(^{-1}\)). This light intensity is less than the attenuated light intensity through a 12-m SML depth with a background light attenuation coefficient of 0.3 m\(^{-1}\), based on average daily PAR in summer (Lewis, 2011). These low light intensities in culture studies are because the artificial lights typically used, e.g., fluorescence tubes or LED lights, have a relatively low maximum light intensity. The low light intensities used may lead to an under-representation of photoinhibition, a critical physiological response which affects phytoplankton population dynamics and growth rate differences amongst species (Whitelam and Cold, 1983). The low light intensities adopted in experiments may help to explain why my model prediction of growth rate was negative when light intensity increased to ~600 µmol photons m\(^{-2}\) s\(^{-1}\), and biovolumes declined when the surface mixed layer was assumed to be very shallow (0.01 m) at latitudes close to 20°N or 20°S. These latitudes corresponded to the highest mean irradiance in summer at the water surface (Lewis, 2011). Moreover, few studies of cyanobacterial growth have been carried out at temperatures < 15°C or > 30°C, yet cyanobacterial blooms are frequently recorded for these temperature bands (Utkilen et al., 1996; Giannuzzi et al., 2011).
Chapter 4 CyanoHABs prediction at global scale

Strains of the same species could undergo major evolutionary changes over multiple generations as a result of adaptation to culture conditions (Lakeman et al., 2009). This change in the physiological characteristics of strains compared with field conditions may help to explain the different growth responses of the same strain across studies, such as *R. raciborskii* strain LETC CIRF-01 (Lürling et al., 2013; Soares et al., 2013), and *M. aeruginosa* strain FACHB469 (Li et al., 2013; Li et al., 2014). Therefore, the widely reported light or temperature optima for growth of different strains of the same species might not reflect their ‘real’ optima but rather a conditioning to the environmental conditions at which they were grown. The strain concept for *Microcystis* is further complicated by the description of ‘morphospecies’ or ‘morphotypes’. There are over 50 *Microcystis* morphospecies recognized according to variation in colony form, mucilage structure, cell diameter, etc. (Komárek and Komárková, 2002). There is currently inconsistency between the classical Linnaean taxonomy and modern molecular taxonomy in classifying *Microcystis* species, hence it is impossible to differentiate *Microcystis* strains in laboratory cultures. *Microcystis* strains used across studies may not necessarily be from one morphospecies although a level of standardization was attempted by selecting laboratory studies based on *M. aeruginosa*.

### 4.5 Conclusions

Improving the prediction of growth, dominance and dynamics of harmful cyanobacteria under a changing climate is a growing priority for water authorities around the world. Using a model that synthesized results from laboratory-based culture studies globally, a mismatch of species dominance between *Microcystis* and *R. raciborskii* was found compared to field observations. This study demonstrated that laboratory-derived light and temperature-dependent growth rates do not yield the expected species dominance of *Microcystis* over *R. raciborskii*, and more realistic parameterization of key
physiological processes is required to improve the predictive capability, as well as standardizing growth parameters across studies. The key processes include: colony formation of *Microcystis* which affects buoyancy regulation, how colonies increase the potential for cells to disentrain from the predominant water column advection and mixing processes; how mixing interacts with light and temperature history; and the interaction of these factors with nutrient availability. This study highlights that experimentalists and modellers need to work together, to develop experimental work that better represents conditions *in situ* and identify key process parameters that are most sensitive for predictions of cyanoHABs. The applicability of laboratory studies for field predictions of cyanoHABs will continue to be limited without mode detailed considerations of these processes.
5 General Discussion

Understanding and predicting algal population dynamics and the drivers are critical to manage and control cyanoHABs. The bloom-forming cyanobacterial species, *Microcystis* spp. and *R. raciborskii* are of particular concern due to their worldwide distribution and toxin production (Briand et al., 2009; Antunes et al., 2015; Harke et al., 2016). My collation of global data has shown that from 1988 to 2017 these species dominated in more than 80% of the freshwater ecosystems where cyanobacterial blooms occurred. Importantly, it is increasingly found that populations of *Microcystis* and *R. raciborskii* have co-existing strains, and these strains vary in their physiology and magnitude of growth responses to environmental conditions. This intraspecific variation makes it challenging to predict which species will dominate at a given location under changing environmental conditions.

To address the inherent challenges of incorporating intraspecific variation in population dynamics between species of cyanoHABs, this thesis investigated within-species strain variation in growth responses to a range of light and temperature conditions. *Microcystis aeruginosa* and *R. raciborskii* were used as case studies as they commonly co-occur, dominating freshwaters successively and/or simultaneously (Marinho and Huszar, 2002; Soares et al., 2009; Yamamoto et al., 2011). Specifically, this thesis integrates: laboratory experiments (Chapter 2); a deterministic model (phytoplankton dynamics model) merged with a statistical model (Monte Carlo approach) (Chapter 3); synthesis of published laboratory-based growth studies, field investigations worldwide and model predictions (Chapter 4). This thesis has provided important new insights into key physical factors affecting cyanobacterial growth and how this links with their population dynamics. The thesis has also highlighted inadequacies in current
experimental approaches linking physical conditions to cyanobacterial growth responses in modelling.

5.1 Key findings of this thesis

This thesis had a number of key outcomes as outlined below:

1) There was higher intraspecific variation than interspecific variation in growth responses to light and temperature when comparing *M. aeruginosa* and *R. raciborskii* cultures (Chapter 2). This was despite the fact that the strains co-occurred, being isolated from two samples from two neighbouring lakes. The high intraspecific variation indicates high species plasticity of the population. Overall, *M. aeruginosa* had higher growth rates at the higher light intensity but the lower temperature, while *R. raciborskii* with straight trichomes had higher growth rates at the lower light intensity but the higher temperature. The coiled *R. raciborskii* strains had higher growth rates at the higher light intensity and the higher temperature. *Raphidiopsis raciborskii* had greater variation in growth rates, light attenuation coefficients and individual cell volumes between strains than *M. aeruginosa*, whereas *M. aeruginosa* strains had higher final cell concentrations than *R. raciborskii* under all the tested light and temperature conditions.

2) Including intraspecific variation in the deterministic phytoplankton dynamics model showed that the outcomes of species competition depended on the strains present, and resulted in species co-existence. The intraspecific variation was included by incorporating a Monte Carlo approach with the deterministic model (Chapter 3). This study predicted that when *M. aeruginosa* and *R. raciborskii* competed, there was no absolute ‘winner’ as there would always be strains predicted to co-exist with the
dominant strain. Additionally, one strain did not always win under all conditions, indicating dominance of a particular strain depending on the environmental conditions.

Therefore, this study suggests that when investigating species competition, a single strain cannot adequately represent a species, nor a single parameter set adequately represents a species/strain. This highlights the importance of strain-level variation in the global distribution of cyanoHABs. The dominance of a species may be driven by the successive dominance of strains that respond to and dominate under different environmental conditions.

3) There was a poor match in the dominance of *Microcystis* between the model prediction (based on collated laboratory data across the globe), and the field observations of cyanoHABs (Chapter 4). My collation of field studies showed that over the last 30 years, of over 1130 freshwater systems with cyanoHABs, 78% were dominated by *Microcystis* spp. and 17% were dominated by *R. raciborskii* on at least one occasion. In general, *Microcystis* dominated over *R. raciborskii* irrespective of climatic zones. Approximately 13% of these systems were found to be co-dominated by *Microcystis* and *R. raciborskii*, successively and/or simultaneously.

From the collated culture studies of *M. aeruginosa* (as a proxy of *Microcystis*) and *R. raciborskii*, the dependence of growth rate on light and temperature was predicted, including uncertainty in the growth rate predictions. Overall, *R. raciborskii* had a higher $\mu_{max}$ (1.23 d$^{-1}$) at its optimal light and temperature than that of *M. aeruginosa* (0.87 d$^{-1}$). There was no substantial difference between their maximum temperature ($T_{max}$) at which growth ceased, optimal temperature ($T_{opt}$) for maximum growth, and photoinhibition parameter ($\beta$). The growth rate differences were thus driven by light parameters, i.e., the half-saturation irradiance ($I_{1}$) and optimal light ($I_{opt}$) for maximum
growth. *Raphidiopsis raciborskii* had nearly three-fold and two-fold higher, respectively, $H_f$ and $I_{opt}$ than those of *M. aeruginosa*. Unsurprisingly, *R. raciborskii* was predicted to have higher growth rates at temperatures $\geq 25^\circ C$ or light intensities $\geq 150 \mu mol$ photons m$^{-2}$ s$^{-1}$. For temperatures of up to $25^\circ C$ or light intensity of up to $150 \mu mol$ photons m$^{-2}$ s$^{-1}$, the predicted growth rates were not substantially different between the two species.

Consequently, by incorporating the growth rate predictions into a population dynamics model, with latitude-driven surface light and temperature as forcing inputs, *R. raciborskii* was predicted to dominate over *M. aeruginosa* more often. *Microcystis aeruginosa* would outcompete *R. raciborskii* at high latitudes of temperate zones, but be outcompeted by *R. raciborskii* at tropical and subtropical zones, with co-existence in the lower temperate zones. This model prediction poorly matches the field observations of dominance of *Microcystis* over *R. raciborskii* irrespective of climatic zones.

The poor match highlights the key factor that has hindered a more robust prediction of cyanobacterial dominance patterns observed in the field.

Overall, this thesis sheds new light on the interspecific and intraspecific variations of *M. aeruginosa* and *R. raciborskii* with respect to light and temperature. By merging numerical models with statistical approach, population dynamics were predicted between *M. aeruginosa* and *R. raciborskii* under a range of physical conditions.
Through comparing the model prediction with literature review of field investigations, this thesis highlights the effect of high within-species strain variability in their physiological responses to changing environment on interspecific competition.

### 5.2 Implications of this thesis

The study has implications with respect to laboratory studies, in terms of studying multiple strains within a species, as well as managing cyanobacterial blooms in terms of more accurate predictions by including this variability.

1) This thesis has shed new light on the differences of growth responses between culture studies of two cyanobacterial species – due to strain differences, an area that has been largely ignored in current phytoplankton models when predicting population dynamics (Huisman et al., 2006; Passarge et al., 2006; Elliott, 2010; Baird et al., 2013). This may explain why the competitive exclusion theory (i.e., the most effective competitor dominates the resource and drives all the other species in the system to extinction) does not occur in reality, where a single millilitre of water may contain dozens of phytoplankton species, known as ‘the paradox of the plankton’ (Hutchinson, 1961). This thesis included the strain variability, resulting in no absolute ‘winner’, as there were always some strains co-existing with the dominant strain, as seen in Chapter 3, Xiao et al. (2017a). Therefore, including the within-species strain variation in deterministic models may substantially increase the confidence in predicted outcomes of species dynamics.

However, due to the complex environmental conditions and co-existing strains within a species, it is impossible to parameterize the responses of all species under all conditions in a comprehensive dataset. The ‘unknowns’ and uncertainty in ‘true’ values of growth responses leads to uncertainty in model predictions. Instead of resolving the
‘unknowns’, in this study, the simple statistical approach – the Monte Carlo approach provided a solution to propagate the parameter variability in deterministic models, and to help explore more scenarios in prediction than measured. Thus, for any deterministic models that have great uncertainty or variability in modelled parameters, Monte Carlo approach could be used to propagate the uncertainty and variability explicitly, and is relatively free of restrictive assumptions (Gardner and O’Neill, 1983).

Current phytoplankton models have included multiple species, these species are either grouped, for example by cell size (Nogueira et al., 2006), or by functional traits (Mutshinda et al., 2016). The problem is that growth characteristics of different species may not be distinguishable by only one or two functional traits. For example, the N-fixer, *R. raciborskii* is filamentous while the non-N-fixer, *Microcystis*, forms colonies. Thus, including more than one key traits in model parameterization may improve the predictive ability of phytoplankton models.

2) *Microcystis* remains the most widely spread harmful cyanobacterial species, dominating in over 78% of the systems that have had cyanoHABs over the last 30 years. Even though other cyanobacterial species, e.g., *R. raciborskii*, are increasingly reported, the proportion of systems in which *Microcystis* has become dominant may be higher. Moreover, as the most effective way so far to reduce *Microcystis* blooms, artificial mixing has sometimes failed to control *Microcystis* blooms, but meanwhile boosted the growth of other cyanobacterial species, including *R. raciborskii* (Antunes et al., 2015), thus possibly altering dominance amongst species. Nevertheless, controlling *Microcystis* blooms is still the priority in water management of the dominated systems. This is because, *Microcystis* forms thick surface scums in millimetres Reynolds (1973), which substantially decreases the light availability through to deeper layers, affecting the growth of other living creatures and hence the whole ecosystem. Over time,
Microcystis could predominate the plankton, take over the ecosystem with annual blooms, resulting in loss of biodiversity. With intensified mixing, Microcystis colonies are pushed to deeper layers, and not confined to the water surface. Thereafter, species that have higher growth rates than Microcystis, such as green algae and diatoms, may recover sufficiently to overcome the dominance of Microcystis.

5.3 Future work

While my studies have made significant inroads in understanding species population dynamics between the most problematic cyanobacterial species M. aeruginosa and R. raciborskii and their strains, there is clearly a lot of research remaining to be done in this relatively new area of endeavor.

5.3.1 Revisiting the concept of strain variability

In this thesis, ‘strain’ is used to describe the unicellular cultures based on isolation of single cells (isolates), these were then compared in the laboratory studies to determine strain variability. However, the strain concept in phycology is complex, because it often concludes that the genetic differences are representative of the natural environmental selective pressures, but disregards the selection pressure and continued genetic evolution that occurs under laboratory conditions (Lakeman et al., 2009). In field conditions, strains can differ in environmental niche (ecotype), differ in physiology (phenotype), differ in genetics (genotype), and/or differ in biochemistry (chemotype). Each ‘-type’ can be inclusive of the others and is used relative to the experimental description applied. The strains used in this study were characterized with variable phenotypes (morphology and growth (Chapter 2)), and these phenotypic differences in the R. raciborskii strains had a genetic base, with strains having a ~15% variable genome (Willis et al., 2018). The fact that these R. raciborskii strains co-occur in the
field and likely undergo genetic recombination leading to the appearance of new strains and disappearance of others, indicates that specific strains may only exist transiently in wild populations. This suggests that individually isolated strains may not represent natural condition, but because the strains were isolated at the same time and kept in the same conditions (thus limiting divergence arising from laboratory selective pressures) the variability between the strains may still represent the natural variability (Lakeman et al., 2009).

The strain concept in *Microcystis* species is further complicated by the description of ‘morphospecies’ or ‘morphotypes’. There are over 50 *Microcystis* morphospecies recognized according to variation in colony form, mucilage structure, cell diameter, cell arrangement within a colony, ratio of the pigments phycocyanin and phycoerythrin, and the seasonal life cycle of colonies (Komárek and Komárková, 2002). There is still no agreement between the classical Linnaean taxonomy and modern molecular taxonomy in classifying *Microcystis* species. Due to this taxonomic issue, *Microcystis* strains used across studies may not necessarily be from one morphospecies.

Given that many strains of the same species co-occur in natural populations, with temporal and spatial evolution and adaptation, future research on species dynamics should consider this within-species strain variation and inform the design of laboratory studies using multiple strains. Moreover, there are questions remaining: how variable are the strains in a population or around the globe? Is there any possibility that the traditional way of grouping species, subspecies or strains is problematic? Functional traits have been widely used to indicate the niche differences within and between species (Litchman et al., 2007; Litchman et al., 2010; Mutshinda et al., 2016), therefore, could it be useful to improve the understanding of species diversity by finding key functional traits?
5.3.2 The paradox of plankton

This thesis highlights that multiple strains (genotypes) that co-occur within a population can lead to species co-occurrence, and one single strain does not always win under all environmental conditions. Hence, in the same population, if strains of one species respond differently to different environmental conditions, the population genetic diversity may be maintained as all genotypes experience favourable and unfavourable periods when environmental conditions vary (Gsell et al., 2012). For example, strains of the same species differ in their temperature preferences, so they may successively dominate as temperatures alternate between cooler and warmer conditions.

Besides this genotype variation, there are also other factors that may explain the paradox of the phytoplankton. For example, interactions with other biological factors such as parasitism, which is a type of consumer-resource interaction. The most competitive phytoplankton host is attacked preferentially by the parasites, while the less competitive hosts are driven to grow better. The modern theory of co-existence also shows that the stable co-existence of species is an interaction between two opposing forces: stabilizing mechanisms, which maintains diversity via niche differentiation, and fitness differences between species, which drive the best-adapted species to exclude others within a particular ecological niche (Chesson, 2000).

5.3.3 Laboratory studies

Laboratory growth studies investigating the extreme growth conditions would be helpful in capturing the complete growth curves, to improve cyanobacterial growth prediction. The temperatures below 10°C represent the high latitudes’ condition, high temperature of above 35°C represent the warming climate, and high light levels of > 500 µmol photons m\(^{-2}\) s\(^{-1}\) will fill the knowledge gap on species photoinhibition.
Moreover, cyanobacterial blooms have frequently been recorded within these temperature bands (Utkilen et al., 1996; Giannuzzi et al., 2011; Ndlela et al., 2016).

One of the challenges of culturing *Microcystis* in the laboratory is that it exists as single cells or (more rarely) as paired cells, but under natural conditions forms colonies. Given the key role of colony formation (Xiao et al., 2018), buoyancy regulation (Brookes and Ganf, 2001), and carbon ballast accumulation (Ibelings et al., 1991) in bloom formation and dominance of *Microcystis*, future work in *Microcystis* colonies should involve: developing techniques to induce laboratory colonies with morphologies similar to those seen in the field; quantifying the growth responses of colonies to different environmental conditions, and colony formation with interaction of mixing regimes.

### 5.3.4 Phytoplankton modelling

Current phytoplankton models have predicted population dynamics mostly at the level of single species with respect to their acclimated (short-term) physiological responses to a few driving factors (Riebesell and Gattuso, 2014). This ‘acclimation’ is the flexibility within a species to adapt to conditions in the short term. It is, however, the long-term response of communities, which involves the genetic mutation in cells allowing them to adapt to new and more variable conditions. CyanoHABs may be the result of cyanobacterial species having years of adaptation under the changing environment. Therefore, in the context of predicted phytoplankton dynamics, using days as the timescale does not evaluate a long-term adaptation of species responses in an ecosystem. Instead, to find out the mechanisms driving species’ dominance, long-term research with more realistic time periods (scale of years) needs to be considered in future phytoplankton prediction.
Loss rate measurements are essential in determining the net growth of cells under different environmental conditions. However, in current phytoplankton models, including this study, loss rate was assumed to be a fixed value based on other studies, e.g., 0.1 d$^{-1}$ or 0.01 h$^{-1}$ (Huisman and Sommeijer, 2002; Xiao et al., 2017a). While loss rate is complex, including respiration, zooplankton grazing and mortality due to cyanophages and heterotrophic bacteria, more accurate field measurements are still needed. Colonial *Microcystis* is expected to have a lower loss rate than *R. raciborskii*, as large colonies are better protected from zooplankton grazing than single filaments. Hence, given a specific loss rate for each species, the confidence in predicting phytoplankton dynamics might be improved.

Specifically, in the prediction of *Microcystis* blooms or competition between *Microcystis* and any other phytoplankton species, future models need to incorporate: (1) *Microcystis* colony formation and how colonies respond under various mixing conditions; (2) competition mechanisms between *Microcystis* and other less buoyant or sinking phytoplankton on timescales of years, with an emphasis on the effects of shading by *Microcystis* colonies and explicit loss rates between these species. This information may possibly resolve the mismatch in dominance of *Microcystis* between model prediction based on culture studies and field observations.

From pooling the cyanobacterial culture studies globally, we quantified the growth variability of *M. aeruginosa* and *R. raciborskii* and used this information in phytoplankton models to predict global scale on latitudinal-light and temperature conditions (Chapter 4). However, our modelled results of dominance of *Microcystis* did not match the observed cyanohAB events across the globe. This mismatch leads to the questions about model complexity and predictability, in particular: (1) Would growth responses of phytoplankton to a greater suite of environmental conditions, other
than only light and temperature, alter the species/strain dominance and co-existence? (2)

How do we determine whether model processes and parameters should be simpler or more complex? (3) Can we achieve greater realism in models to match the real world without greater complexity? (4) What data do we need to support more complex models and how to collect these data in a comprehensive way?

There are always arguments about how complex models need to be to ensure predictability. Hellweger (2017) suggested that making ecosystem models more complex by including more information, e.g., variables, processes, parameters, in the existing models will increase model predictability. In contrast, the meta-analysis of (Arhonditsis and Brett, 2004) found that increasing model complexity did not improve model performance.

In addition to physical factors such as light, temperature and turbulent mixing, nutrient availability is also a key factor affecting the magnitude and duration of cyanobacterial blooms. *Microcystis* and *R. raciborskii* have been shown to possess different strategies to utilize nutrients. Compared to unicellular cells, *Microcystis* colonies have a lower requirement for nutrients and are less affected by nutrient-limitation compared to single cells of the same species (Shen and Song, 2007; Wang et al., 2014). Colonial morphology also increased the persistence of *Microcystis* in fluctuating P conditions (Shen and Song, 2007). *Microcystis* colonies also attract microbial consortia, such as bacteria, fungi and other algae, which can enhance nutrient and carbon cycling (and recycling) (Paerl and Millie, 1996). In comparison, *R. raciborskii* has been more competitive than other cyanobacterial species under low and/or variable phosphorus and nitrogen availability (Burford et al., 2017). Studies found that increasing nitrogen concentrations resulted in a higher growth rate of *R. raciborskii*, while increasing phosphorus did not increase the growth rate but sustained the cells for longer (Willis et
al., 2015). Moreover, including nutrient availability to cell growth and population
dynamics may allow us to explain why different species dominate at different systems
within small geographical locations (Chapter 4).

Moreover, there are always misconnections between what we measure in the laboratory,
what we use for prediction in models, and what we seek to explain and predict based on
field investigations. However, there are currently no concrete solutions to resolve the
misalignments between them so we could start by designing experiments or developing
new techniques, in the context of these challenges. As a great example, Puddick et al.
(2016) developed a novel way to measure millimetre-scale surface-scum of Microcystis
in situ, and provided an accurate way to measure the very fine surface cell concentration
of buoyant species for the boundary and initial conditions in phytoplankton modelling.
It seems promising to improve the techniques in in situ studies to improve model
predictability for cyanoHABs.

5.3.5 Model validation

For this thesis, the modelling studies in Chapters 3 and 4 were designed to answer
specific questions based on the physical drivers of light and temperature only. Chapter
3 was to test how strain variability could possibly affect the species competition, and
Chapter 4 predicted how variable the distribution of Microcystis and R. raciborskii
could be under a range of light and temperature conditions across latitudes. These two
physical factors are mostly driven by latitude on a global scale. Future research could
test laboratory competition experiments and field-based studies, e.g., mesocosm or
succession studies in a single lake, thereby enhancing knowledge on strain variability
and improving/refining the phytoplankton models. Experiments could be undertaken to
compare and contrast strain-level succession of Microcystis and R. raciborskii under
different environmental drivers, and compare with laboratory studies involving growth responses of single strains. Manipulative mesocosm experiments could be undertaken where species compete directly with each other, to assess the performance of species succession in a more realistic environment.

5.4 Conclusions

In summary, this study thesis provided new insights into the importance of understanding within-species strain variability to a range of physical conditions from laboratory-based growth studies, and how this variability affects cyanobacterial competition and dominance on a global scale. Yet, currently, laboratory-based culture studies oversimplify the environmental conditions, and the current phytoplankton models oversimplify the variability and uncertainty of modelled parameters that are partly caused by within-species strain variability. All these have hindered a more robust prediction of cyanoHABs. Future research is required to resolve these issues to better understand and predict cyanobacterial population dynamics, and to manage and control cyanoHABs.
Appendix 1: Supplementary materials for Chapter 3

1A. Measured growth rate versus light intensity for all *M. aeruginosa* and *R. raciborskii* strains

A. M2 without photoinhibition at 20°C

B. M2 with photoinhibition at 28°C

C. M3 without photoinhibition at 20°C

D. M3 without photoinhibition at 28°C
E. M4 without photoinhibition at 20°C

F. M4 without photoinhibition at 28°C

G. M5 without photoinhibition at 20°C

H. M5 with photoinhibition at 28°C

I. C1 with photoinhibition at 20°C

J. C1 with photoinhibition at 28°C
K. C3 without photoinhibition at 20°C

L. C3 without photoinhibition at 28°C

M. C6 with photoinhibition at 20°C

N. C6 without photoinhibition at 28°C

O. WS01 without photoinhibition at 20°C

P. WS01 without photoinhibition at 28°C
Q. WS05 without photoinhibition at 20°C

R. WS05 without photoinhibition at 28°C

S. WC03 without photoinhibition at 20°C

T. WC03 without photoinhibition at 28°C

U. WC04 without photoinhibition at 20°C

V. WC04 without photoinhibition at 28°C
Fig. S3-1: Measured growth rate versus mean light intensity (dots) for all *M. aeruginosa* and *R. raciborskii* strains at two temperatures 20 and 28°C, fitted to models that either include or exclude photoinhibition (solid lines). The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07). Dashed lines represent the 95% confidence interval in the model fits.
1B. Predicted competitiveness of strains from the Monte Carlo simulation

Table S3-1: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 30 µmol photons m\(^{-2}\) s\(^{-1}\), temperature of 20\(^{\circ}\)C, and equal initial biovolume. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

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Table S3-2: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 30 μmol photons m$^{-2}$ s$^{-1}$, temperature of 28°C, and equal initial biovolume. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

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Table S3-3: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 100 µmol photons m\(^{-2}\) s\(^{-1}\), temperature of 20°C, and equal initial biovolume. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

<table>
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<th>M5</th>
<th>C1</th>
<th>C3</th>
<th>C6</th>
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<th>WS05</th>
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<td>4</td>
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Table S3-4: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 100 µmol photons m\(^{-2}\) s\(^{-1}\), temperature of 28°C, and equal initial biovolume. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

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<td>C1</td>
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<td>C3</td>
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<tr>
<td>C6</td>
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<tr>
<td>WS01</td>
<td>48</td>
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<td>WS05</td>
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Table S3-5: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 30 µmol photons m\(^{-2}\) s\(^{-1}\), temperature of 20°C, and equal initial cell concentration. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

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<td></td>
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<td>M4</td>
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<td>C1</td>
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</table>
Table S3-6: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 30 µmol photons m\(^{-2}\) s\(^{-1}\), temperature of 28°C, and equal initial cell concentration. The strains are \(M.\ aeruginosa\) (M2, M3, M4 and M5), straight \(R.\ raciborskii\) (C1, C3, C6, WS01 and WS05) and coiled \(R.\ raciborskii\) (WC03, WC04 and WC07).

<table>
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<tr>
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<tbody>
<tr>
<td></td>
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<td>M4</td>
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<td>WC04</td>
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<td>WC07</td>
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Table S3-7: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 100 µmol photons m\(^{-2}\) s\(^{-1}\), temperature of 20\(^{\circ}\)C, and equal initial cell concentration. The strains are \textit{M. aeruginosa} (M2, M3, M4 and M5), straight \textit{R. raciborskii} (C1, C3, C6, WS01 and WS05) and coiled \textit{R. raciborskii} (WC03, WC04 and WC07).

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</tr>
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<tbody>
<tr>
<td></td>
<td>M2  M3  M4  M5  C1  C3  C6  WS01  WS05  WC03  WC04  WC07</td>
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<td>M2</td>
<td>– 19 15 75 74 24 70 47 94 89 89 90</td>
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<td>M3</td>
<td>81 – 36 93 87 47 77 66 100 98 98 95</td>
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<td>M4</td>
<td>85 64 – 93 89 63 79 73 100 98 98 95</td>
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<td>M5</td>
<td>25 7 7 – 61 10 65 30 70 72 69 85</td>
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<tr>
<td>C1</td>
<td>26 13 11 39 – 16 58 27 50 52 51 74</td>
</tr>
<tr>
<td>C3</td>
<td>76 53 37 90 84 – 76 65 99 97 96 95</td>
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<td>C6</td>
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<tr>
<td>WS01</td>
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<td>WS05</td>
<td>6 0 0 30 50 1 59 17 – 60 55 82</td>
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<td>WC03</td>
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<td>WC04</td>
<td>11 2 2 31 49 4 57 18 45 54 – 79</td>
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<tr>
<td>WC07</td>
<td>10 5 5 15 26 5 40 11 18 22 21 –</td>
</tr>
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</table>
Table S3-8: The competitiveness (%) of strain A against strain B predicted by the Monte Carlo simulation of our deterministic model of phytoplankton competition, for light intensity of 100 µmol photons m$^{-2}$ s$^{-1}$, temperature of 28°C, and equal initial cell concentration. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborskii* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborskii* (WC03, WC04 and WC07).

<table>
<thead>
<tr>
<th>Strain A</th>
<th>Strain B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M2</td>
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<tr>
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<td>–</td>
</tr>
<tr>
<td>M3</td>
<td>42</td>
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<td>WC04</td>
<td>29</td>
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<tr>
<td>WC07</td>
<td>33</td>
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</tbody>
</table>
Supplementary materials

Fig. S3-2: Heat maps of competitiveness (%) in pairwise competition between all strains with equal initial cell concentration, predicted by Monte Carlo simulations of our deterministic model of phytoplankton competition. A: Strain competitiveness under light intensity of 30 μmol photons m\(^{-2}\) s\(^{-1}\) and temperature of 20°C; B: Strain competitiveness under 30 μmol photons m\(^{-2}\) s\(^{-1}\) and 28°C; C: Strain competitiveness under 100 μmol photons m\(^{-2}\) s\(^{-1}\) and 20°C; D: Strain competitiveness under 100 μmol photons m\(^{-2}\) s\(^{-1}\) and 28°C. The strains are *M. aeruginosa* (M2, M3, M4 and M5), straight *R. raciborski* (C1, C3, C6, WS01 and WS05) and coiled *R. raciborski* (WC03, WC04 and WC07). Strains are ordered based on their competitiveness, which is different for each light and temperature condition tested, on the x- and y-axes. The color bar was scaled to 50 – 100% instead of 0 – 100%, as strains have been ranked the strains from most dominant to least dominant.
Appendix 2: Supplementary materials for Chapter 4

2A. Growth photoperiods standardized to 12 h

To make the collected laboratory-based studies comparable and place each study on a common time scale, we assumed that the photoperiod affects the growth rate linearly (Geider et al., 1997; Reynolds and Irish, 1997). The measured growth rates in each study were adjusted to an equivalent 12:12 h light/dark cycle:

\[ \mu_d = \frac{\mu_m}{T_{ex}} \times 12, \quad \text{Eqn S4-1} \]

where \( \mu_d \) (d\(^{-1}\)) is the 12-h standardized growth rate, \( \mu_m \) (d\(^{-1}\)) is the measured growth rate, \( T_{ex} \) (h) is the photoperiod for individual culture studies, and 12 (h) is the correction factor. Hence, \( \mu_d \) represents the growth rate if the culture was grown in a 12:12 h light/dark cycle, and the light intensity adopted in each study represents the light intensity for 12 hours of the day.
2B. Derivation of light-limited growth \( f_1(I) \)

The derivation of the light-limited growth curve was to identify the values of the optimal light intensity \( I_{\text{opt}} \) for maximum growth. The photosynthesis-irradiance model for this study was modified based on the model in Xiao et al. (2017a) as:

\[
\mu = m \frac{1}{H_I + I} - \beta I, \quad \text{Eqn S4-2}
\]

where the value of \( m \) \((\text{d}^{-1})\) is chosen so that \( \mu \) equals the maximum growth rate \( \mu = \mu_{\text{max}} \) at the optimal light level \( I = I_{\text{opt}} \), corresponding to:

\[
\frac{d\mu}{dt}(I_{\text{opt}}) = 0, \quad \text{Eqn S4-3}
\]

thus,

\[
m\left(\frac{1}{H_I + I_{\text{opt}}} - \frac{I_{\text{opt}}}{(H_I + I_{\text{opt}})^2}\right) - \beta = 0. \quad \text{Eqn S4-4}
\]

Given that \( I_{\text{opt}} > 0 \), then

\[
I_{\text{opt}} = \sqrt{\frac{mH_I}{\beta}} - H_I, \quad \text{Eqn S4-5}
\]

substituting Eqn S4-5 and \( \mu = \mu_{\text{max}} \) into Eqn S4-2 we expect \( m > \mu_{\text{max}} \), then:

\[
m = (\sqrt{\mu_{\text{max}}} + \sqrt{H_I\beta})^2. \quad \text{Eqn S4-6}
\]

Substituting Eqn S4-6 into Eqn S4-5 then gives:

\[
I_{\text{opt}} = \sqrt{\mu_{\text{max}}H_I/\beta}. \quad \text{Eqn S4-7}
\]
The Eqns S4-2 and S4-5 are combined to yield Eqn 4-2 in the model.
2C. Global population dynamics model

The phytoplankton dynamic growth model in this study is a system of two ordinary differential equations as per Xiao et al. (2017a):

\[
\frac{d\bar{w}_i}{dt} = \bar{\mu}_i \bar{w}_i, \tag{Eqn S4-8}
\]

where \( i = 1, 2 \) represents \( M. \ aeruginosa \) and \( R. \ raciborskii \), \( \bar{w}_i \) (cells mL\(^{-1}\)) is the cell concentration and \( \bar{\mu}_i \) (d\(^{-1}\)) is the net growth rate. Both \( \bar{\mu}_i \) and \( \bar{w}_i \) are averaged over the surface mixed layer (SML) depth \( z_m \). \( t \) (d) is the simulation period, and 30 days is used in this study. \( \bar{\mu}_i \) was derived from the integrated irradiance over the SML similarly to a mathematical derivation carried out in Huisman and Weissing (1994), to obtain the dependence of cyanobacterial growth rate on light availability:

\[
\bar{\mu}_i = \frac{m_{r(T)}}{k_{tol}z_m} \ln \left( \frac{H_{11}+l}{H_{11}+l e^{-k_{tot}z_m}} \right) - \beta_i \bar{I} - L, \tag{Eqn S4-9}
\]

where \( m \) and \( f_T(T) \) are given in Appendix B, Eqn S4-6 and Eqn S4-3. The depth-integrated light intensity \( \bar{I} \) is:

\[
\bar{I} = \frac{1}{k_{tot}z_m} \left( 1 - e^{-k_{tot}z_m} \right), \tag{Eqn S4-10}
\]

where \( I \) (\( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \)) is the incident light intensity at the water surface, \( k_{tot} \) is the specific light attenuation coefficient:

\[
k_{tot} = k_{bg} + \sum_i k_{ji} \bar{w}_i. \tag{Eqn S4-11}
\]

The background light attenuation coefficient \( k_{bg} \) was chosen 0.3 and 0.7 m\(^{-1}\) as per Lewis (2011), and the specific light attenuation coefficient of \( M. \ aeruginosa \) and
R. raciborskii cells were chosen as $6.7 \times 10^{-7}$ and $7.0 \times 10^{-7}$ (cell mL$^{-1}$)$^{-1}$ m$^{-1}$ following Xiao et al. (2017b).
Fig. S4-1: The 20 and 16 laboratory-based published culture studies to obtain parameterized growth equations for *M. aeruginosa* (A) and *R. raciborskii* (B), respectively. This nonlinear regression was performed with all replicated growth rates of each strain under each light and temperature condition. Colors indicate different strains.
Fig. S4-2: Latitudinal surface water temperature and incident light variations in summer across latitude. A: surface water temperatures collected from 291 sites in 2009 assembled by Sharma et al. (2015) (dots); solid line is predicted mean surface water temperature across latitudes, and the dashed lines are the uncertainty (5th and 95th percentiles) in the predictions. B: incident light intensity interpolated linearly from the mid-month photosynthetically active radiation (PAR) of the northern hemisphere land surface in summer periods of July, August and September as per Lewis (2011), after correcting for albedo (8% of the global irradiance). The extended values at 85°N was calculated by multiplying the values of 70°N with factors of 1.12, 0.97 and 0.40 for the three months, based on NASA’s datasets of monthly-averaged insolation incident on a horizontal surface over the last 22 years (https://eosweb.larc.nasa.gov/cgi-bin/sse/grid.cgi?email=skip@larc.nasa.gov).
A. Background light attenuation coefficient $k_{bg} = 0.3 \text{ m}^{-1}$

Fig. S4-3: Percentage of model runs (%) that predicted total biovolumes of *M. aeruginosa* was higher than *R. raciborskii* on day 30, based on Monte Carlo simulation of phytoplankton dynamics model (Eqns 4-4 – 4-8) under well-mixed conditions, four surface mixed layer (SML) depths with two background light attenuation coefficients $k_{bg}$ (A: 0.3 m, B: 0.7 m$^{-1}$). The purple, white, red, blue and yellow dots were predictions from the four SML depths of 0.01, 1, 3, 6 and 12 m. The dashed lines are the cut-off ratios 25% and 75% for comparison: below 25% *M. aeruginosa* could be outcompeted by *R. raciborskii*, above 75% *M. aeruginosa* could outcompete *R. raciborskii*, and in between 25 – 75% the two species could co-exist.

B. Background light attenuation coefficient $k_{bg} = 0.7 \text{ m}^{-1}$
Table S4-1: The specific growth parameters with the best fit (± standard deviation) for individual strains of *M. aeruginosa* and *R. raciborskii*: maximum growth rate at its optimal temperature $\mu_{\text{max}}$ (d$^{-1}$), optimum temperature $T_{\text{opt}}$ (°C), maximum temperature $T_{\text{max}}$ (°C), and root mean square error of the predicted growth rate (RMSE, d$^{-1}$), obtained from fitting Eqn 4-1 to the data available, after normalizing to the optimal light of the species. All the model fits were significant ($P < 0.001$). L/D means light/dark cycle.

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<th>Species</th>
<th>Strain</th>
<th>Maximum growth rate at the optimal temperature, $\mu_{\text{max}}$ (d$^{-1}$)</th>
<th>Optimum temperature $T_{\text{opt}}$ (°C)</th>
<th>Maximum temperature $T_{\text{max}}$ (°C)</th>
<th>RMSE of predicted growth rate (d$^{-1}$)</th>
<th>Adjusted $R^2$</th>
<th>Origin</th>
<th>Notes</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><em>M. aeruginosa</em></td>
<td>BearAC-02</td>
<td>0.62 ± 0.38</td>
<td>32.2 ± 19.6</td>
<td>39.7 ± 24.1</td>
<td>0.1</td>
<td>0.82</td>
<td>Bear Lake, Michigan, North America</td>
<td>100 µmol photons m$^{-2}$ s$^{-1}$, 14:10 L/D</td>
<td>Thomas and Litchman (2016)</td>
</tr>
<tr>
<td></td>
<td>BearAG-02</td>
<td>0.36 ± 0.22</td>
<td>28.0 ± 17.0</td>
<td>34.7 ± 21.0</td>
<td>0.01</td>
<td>0.99</td>
<td>Bear Lake, Michigan, North America</td>
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<tr>
<td></td>
<td>GullB-00</td>
<td>0.44 ± 0.27</td>
<td>27.9 ± 16.9</td>
<td>34.9 ± 21.2</td>
<td>0.02</td>
<td>0.99</td>
<td>Gull Lake, Michigan, North America</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GullK-00</td>
<td>0.46 ± 0.28</td>
<td>28.1 ± 17.1</td>
<td>34.8 ± 21.1</td>
<td>0.03</td>
<td>0.97</td>
<td>Gull Lake, Michigan, North America</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>NIVA-CYA140</td>
<td>PCC7941</td>
<td>R. raciborskii</td>
<td>PMC 98.14</td>
<td>PMC 99.12</td>
<td>CYLI-53</td>
<td></td>
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<tr>
<td><strong>Chlorophyll</strong></td>
<td>0.83 ± 0.51</td>
<td>1.77 ± 1.08</td>
<td>0.56 ± 0.38</td>
<td>0.54 ± 0.33</td>
<td>0.53 ± 0.32</td>
<td>0.43 ± 0.26</td>
<td></td>
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<tr>
<td><strong>Cyanobacteria</strong></td>
<td>30.9 ± 18.7</td>
<td>32.1 ± 19.5</td>
<td>28.5 ± 17.3</td>
<td>31.4 ± 19.0</td>
<td>21.7 ± 13.1</td>
<td>28.6 ± 17.5</td>
<td></td>
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<tr>
<td><strong>Total</strong></td>
<td>38.2 ± 23.2</td>
<td>41.9 ± 25.5</td>
<td>42.0 ± 25.5</td>
<td>46.0 ± 27.9</td>
<td>37.7 ± 22.8</td>
<td>40.1 ± 24.3</td>
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</tr>
<tr>
<td><strong>Growth</strong></td>
<td>0.04</td>
<td>0.16</td>
<td>0.02</td>
<td>0.04</td>
<td>0.02</td>
<td>0.05</td>
<td></td>
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</tr>
<tr>
<td><strong>Growth Rate</strong></td>
<td>0.96</td>
<td>0.80</td>
<td>0.97</td>
<td>0.92</td>
<td>0.98</td>
<td>0.90</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Location and Conditions**

- **NIVA-CYA140**: Bendig's Pond, Canada, North America. 80 µmol photons m⁻² s⁻¹, 16:8 L/D.
- **PCC7941**: Europe. 16:8 L/D.
- **R. raciborskii CYP-026J**: Bourke reservoir, Australia. 30 µmol photons m⁻² s⁻¹, 16:8 L/D.
- **ITEP-O18**: Tabocas reservoir, Brazil, South America. 16:8 L/D.
- **PMC 98.14**: ‘Francs-Pêcheurs’ pond, France, Europe.
- **PMC 99.12**: Lake Chanteraînes, France, Europe.
- **CYLI-53**: Lake Melangsee, Germany, Europe.
<table>
<thead>
<tr>
<th>Location</th>
<th>PMC</th>
<th>Form1</th>
<th>Form2</th>
<th>Form2</th>
<th>Form2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epazote lake, Mexico, North America</td>
<td>0.62 ± 0.38</td>
<td>29.6 ± 17.9</td>
<td>39.6 ± 24.0</td>
<td>0.08</td>
<td>0.90</td>
</tr>
<tr>
<td>Lake Guiers, Senegal, Africa</td>
<td>0.44 ± 0.27</td>
<td>28.9 ± 17.5</td>
<td>40.1 ± 24.3</td>
<td>0.05</td>
<td>0.90</td>
</tr>
<tr>
<td>Lake Lemon, Indiana, North America</td>
<td>0.57 ± 0.35</td>
<td>31.6 ± 19.1</td>
<td>40.0 ± 24.2</td>
<td>0.03</td>
<td>0.97</td>
</tr>
<tr>
<td>Lake Dora, Florida, North America</td>
<td>0.58 ± 0.35</td>
<td>31.4 ± 19.1</td>
<td>38.9 ± 23.6</td>
<td>0.04</td>
<td>0.97</td>
</tr>
<tr>
<td>Lake Dora, Florida, North America</td>
<td>0.48 ± 0.29</td>
<td>31.5 ± 19.1</td>
<td>40.4 ± 24.5</td>
<td>0.03</td>
<td>0.97</td>
</tr>
<tr>
<td>Funil Reservoir, Brazil, South America</td>
<td>0.76 ± 0.46</td>
<td>30.5 ± 18.5</td>
<td>40.6 ± 24.6</td>
<td>0.03</td>
<td>0.93</td>
</tr>
<tr>
<td>Solomon Dam, Australia</td>
<td>1.04 ± 0.63</td>
<td>29.5 ± 18.0</td>
<td>36.9 ± 22.4</td>
<td>0.16</td>
<td>0.52</td>
</tr>
<tr>
<td>Solomon Dam, Australia</td>
<td>1.41 ± 0.86</td>
<td>31.0 ± 18.8</td>
<td>37.2 ± 22.6</td>
<td>0.25</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Table S4-2: The specific growth parameters with the best fit (± standard deviation) for individual strains of *M. aeruginosa* and *R. raciborskii*: maximum growth rate at the optimal light $\mu_{\text{max}}$ (d$^{-1}$), optimal light for maximum growth $I_{\text{opt}}$ (µmol photons m$^{-2}$ s$^{-1}$), half-saturation irradiance $H_I$ (µmol photons m$^{-2}$ s$^{-1}$), photoinhibition parameter $\beta$ ((µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$) and root mean square error of the predicted growth rate (RMSE, d$^{-1}$), obtained from fitting Eqn 4-1 to the data available, after normalizing to the optimal temperature of the species. All the model fits were significant ($P < 0.001$). L/D means light/dark cycle.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Maximum growth rate at the optimal light, $\mu_{\text{max}}$ (d$^{-1}$)</th>
<th>Optimum light intensity $I_{\text{opt}}$ (µmol photons m$^{-2}$ s$^{-1}$)</th>
<th>Half-saturation irradiance constant, $H_I$ (µmol photons m$^{-2}$ s$^{-1}$)</th>
<th>Photoinhibition parameter, $\beta$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ d$^{-1}$</th>
<th>RMSE of predicted growth rate (d$^{-1}$)</th>
<th>Adjusted $R^2$</th>
<th>Origin</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. aeruginosa</em></td>
<td>MIC-03</td>
<td>0.41 ± 0.25</td>
<td>137.8 ± 20.1</td>
<td>6.5 ± 4.0</td>
<td>0.00001 ± 0.00009</td>
<td>0.02</td>
<td>0.81</td>
<td>Jacar epaguá Lagoon, Brazil, South America</td>
<td>24°C, 24:0 L/D</td>
<td>Torres et al. (2016)</td>
</tr>
<tr>
<td></td>
<td>PCC7806</td>
<td>0.64 ± 0.38</td>
<td>146.4 ± 36.9</td>
<td>9.2 ± 5.7</td>
<td>0.0003 ± 0.0002</td>
<td>0.06</td>
<td>0.71</td>
<td>The Netherlands, Europe</td>
<td>22°C, 12:12 L/D</td>
<td>(Wiedner et al., 2003)</td>
</tr>
<tr>
<td><em>R. raciborskii</em></td>
<td>CYP-026J</td>
<td>0.74 ± 0.45</td>
<td>120.7 ± 16.2</td>
<td>11.0 ± 6.9</td>
<td>0.0006 ± 0.0004</td>
<td>0.05</td>
<td>0.76</td>
<td>Bourke reservoir, Australia</td>
<td>25°C, 16:8 L/D</td>
<td>Briand et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>ITEP-A3</td>
<td>0.81 ± 0.49</td>
<td>121.5 ± 6.9</td>
<td>17.5 ± 10.8</td>
<td>0.0010 ± 0.0006</td>
<td>0.03</td>
<td>0.95</td>
<td>Riacho do Pau reservoir, Brazil, South America</td>
<td>24°C, 24:0 L/D</td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>r</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
<td>Location</td>
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<tr>
<td>PMC 98.14</td>
<td>0.74 ± 0.45</td>
<td>96.6 ± 7.6</td>
<td>8.0 ± 4.9</td>
<td>0.0006 ± 0.0004</td>
<td>0.03</td>
<td>0.83</td>
<td>Francs-Pêcheurs’ pond, France, Europe</td>
<td></td>
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<tr>
<td>PMC 99.12</td>
<td>0.65 ± 0.40</td>
<td>176.0 ± 69.1</td>
<td>7.4 ± 4.5</td>
<td>0.0002 ± 0.0001</td>
<td>0.02</td>
<td>0.92</td>
<td>Lake Chanteraïmes, France, Europe</td>
<td></td>
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<tr>
<td>ACT-9502</td>
<td>0.71 ± 0.43</td>
<td>123.0 ± 5.8</td>
<td>12.1 ± 7.4</td>
<td>0.0006 ± 0.0003</td>
<td>0.02</td>
<td>0.96</td>
<td>Lake Balaton, Hungary, Europe</td>
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<tr>
<td>PMC 117.02</td>
<td>0.63 ± 0.38</td>
<td>NA</td>
<td>6.76 ± 4.20</td>
<td>NA</td>
<td>0.05</td>
<td>0.73</td>
<td>Lake Guiers, Senegal</td>
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</tr>
<tr>
<td>Form1</td>
<td>1.40 ± 0.85</td>
<td>102.7 ± 8.5</td>
<td>14.6 ± 9.0</td>
<td>0.0019 ± 0.0011</td>
<td>0.15</td>
<td>0.80</td>
<td>Solomon Dam, Australia</td>
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<tr>
<td>Form2</td>
<td>0.96 ± 0.59</td>
<td>118.0 ± 24.4</td>
<td>13.5 ± 8.8</td>
<td>0.0009 ± 0.0006</td>
<td>0.018</td>
<td>0.42</td>
<td>Solomon Dam, Australia</td>
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<tr>
<td>MVCC14</td>
<td>0.63 ± 0.38</td>
<td>71.5 ± 16.2</td>
<td>12.7 ± 8.2</td>
<td>0.0016 ± 0.0012</td>
<td>0.09</td>
<td>0.61</td>
<td>Laguna Blanca, Uruguayan</td>
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</tbody>
</table>

NA: no photoinhibition was found with the available data from this strain.


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