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PRIMARY RESEARCH PAPER

Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria

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Abstract Rising temperatures are expected to favour the growth of bloom-forming cyanobacteria in temperate lakes, but may also change the composition of cyanobacterial communities. To predict future community and bloom dynamics, it is therefore important to understand how bloom-forming species respond to temperature. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju is an invasive, toxin-producing, nitrogen-fixer that may benefit from warming. To understand how changing temperatures will influence its ability to compete against native North American bloom-formers, we characterized the thermal reaction norms and temperature traits of three *C. raciborskii* strains, four strains of *Microcystis aeruginosa* (Kützing) Kützing and one strain of *Anabaena flos-aquae* (Lyng.) Brèb. *C. raciborskii* strains had higher optimum temperatures and survived higher temperatures than toxic *M. aeruginosa* strains, but had no apparent advantage over the non-toxic *M. aeruginosa* strain or *A. flos-aquae*. *M. aeruginosa* strains and *A. flos-aquae* tolerated lower temperatures than *C. raciborskii*, suggesting that fitness differences at low temperature may be important in limiting the latter's spread. Furthermore, we found that nutrient availability strongly influenced thermal reaction norm shape: nitrogen deprivation lowered growth rates and decreased both low- and high-temperature tolerance, but did not affect the optimum temperature in *C. raciborskii*.

Keywords *Cylindrospermopsis* · Temperature · Functional traits · Cyanobacteria · *Microcystis* · Phytoplankton

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Introduction

Global environmental change has led to rising temperatures, which are a major source of stress in natural environments, having already affected most ecosystems on Earth (IPCC Fourth Assessment Report, 2007). Other stressors such as changing nutrient

deposition rates and the spread of invasive species interact with increasing temperatures, making predicting ecosystem responses difficult (Vitousek et al., 2002; Walther et al., 2009). In aquatic ecosystems, one of the major predicted consequences of warmer temperatures is an increase in frequency and severity of HABs (harmful algal blooms), which in lakes are caused mostly by toxic cyanobacteria (Paerl & Huisman, 2009). These blooms can release toxins in high enough concentrations to pose a threat to human health, and may be harmful to algae, zooplankton, and fish, thereby having a negative impact on water quality and ecosystem functioning (Chorus & Bartram, 1999). Rising temperatures may stimulate growth of toxic HAB species both directly and indirectly. Higher temperatures stimulate cyanobacterial growth directly because they are believed to have higher optimum temperatures for growth than other groups of algae (Tilman & Kiesling, 1984; Robarts & Zohary, 1987; but see Lürling et al., 2013). The indirect benefit occurs as a result of increased thermal stratification; cyanobacteria can regulate their buoyancy and take advantage of the high stability of the water column (Jöhnk et al., 2008; Paerl & Huisman 2009).

Lake warming may stimulate growth not only of native species but also invasive cyanobacteria. These have the potential to alter community structure and dynamics in lakes as well as biogeochemical cycling (Litchman, 2010). One such species is *Cylindrospermopsis raciborskii*, a nitrogen-fixing toxic cyanobacterium spreading in temperate regions across the world that is capable of altering local ecosystem processes when dominant (Padisák, 1997; Isvánovics et al., 2000). Recent phylogenetic evidence has suggested that the species originated in the American tropics (Moreira et al., 2015) and its distribution was once thought to be restricted to the tropics and subtropics, where it co-occurs with other bloom-formers such as *Microcystis aeruginosa* and *Anabaena* sp. (Marinho & Huszar, 2002; Molica et al., 2005; Soares et al., 2009; Moisander et al., 2012). However, it has increasingly been found in temperate regions, most recently in Europe and North America (Hong et al., 2006; Conroy et al., 2007; Kling, 2009). It possesses a number of traits that likely make it an excellent competitor in lakes, including nitrogen fixation, low-light tolerance, buoyancy regulation, and strong competitive ability for phosphorus (Padisák, 1997; Isvánovics et al., 2000), the last of which is thought to be atypical for nitrogen-fixers (Smith, 1983). It is also highly successful under fluctuating nitrogen and phosphorus regimes, a factor that likely contributes to its success in dynamic environments (Posselt et al., 2009; Moisander et al., 2012). Some strains of *C. raciborskii* produce a variety of toxins, of which a few have been shown to be allelopathic (Figueredo et al., 2007; Rzymski et al., 2014). Others have been implicated in human poisoning and cattle mortality events (Saker & Griffiths, 2000). The reasons behind its recent invasions into temperate water bodies are as yet unclear, though lake warming has been implicated (Briand et al., 2004; Wiedner et al., 2007; Bonilla et al., 2012; Sinha et al., 2012). However, it is not clear whether rising temperatures will give it an advantage in competition with native species, including other HAB-forming cyanobacteria already adapted to local conditions. The effects of temperature on the growth of *C. raciborskii* and its native competitors are therefore factors that could determine its invasiveness in temperate regions. However, the mechanism by which warming might favour it is unclear: warming may reduce mortality by increasing temperature above the minimum a species can tolerate (Wiedner et al., 2007), and it can also change the difference in (positive) growth rates between species.

One way to characterize the ability to compete with different species is to examine growth rates under different environmental conditions. Although growth rate does not capture all aspects of resource competition, increases in growth rate decrease R^* in either Monod or Droop equations and therefore strongly contribute to competitive ability (Droop, 1973; Tilman, 1982; Litchman et al., 2015). Growth rates across temperature may be characterized in ectotherms by thermal reaction norms and temperature traits that describe the reaction norms (Kingsolver, 2009; Thomas et al., 2012). The traits we consider here are the optimum temperature for growth (the temperature at which population growth rate is maximized), maximum persistence temperature (T_{max}, the temperature above which population growth rate becomes negative), minimum persistence temperature (T_{min}, the temperature below which population growth rate becomes negative), temperature niche width (the range of temperatures over which population growth rate is positive), and maximum growth rate.

The temperature traits of *C. raciborskii* and its competitors are likely to be important determinants of the species' invasion success, for the following reasons: (1) rising temperatures are thought to have contributed to its spread (Briand et al., 2004; Wiedner et al., 2007; Bonilla et al., 2012), (2) cyanobacterial blooms have been shown to increase the temperature of the water bodies in which they occur by as much as 1.5°C (Kahru et al., 1993), and (3) differences in species' growth rates at different temperatures have been experimentally shown to predict the outcomes of cyanobacterial competition (Fujimoto et al., 1997; Chu et al., 2007). *C. raciborskii*'s thermal reaction norms have been examined in strains from Australia, Europe, Asia, Africa, and South America (Saker & Griffiths, 2000; Briand et al., 2004; Chonudomkul et al., 2004; Mehnert et al., 2010). However, despite its recent invasion into N. America and its potential to disrupt local lake ecosystems, little is known about the physiology of N. American strains of *C. raciborskii*, especially in comparison with its local competitors. Measurements of these temperature traits may help us predict the future pattern of invasion and possible ecosystem changes in temperate North American lakes.

To address this, we examined the effect of temperature on the growth rates of three recently isolated N. American strains of *C. raciborskii*. We compared the performance of *C. raciborskii* across temperatures with that of four strains of *M. aeruginosa*, a non-fixer that co-occurs with *C. raciborskii*, and which presumably competes with it for resources such as light and phosphorus (Conroy et al., 2007; Kormas et al., 2011). *C. raciborskii* appears to be displacing *M. aeruginosa* in some tropical and subtropical lakes (Chapman & Schelske, 1997; Saker & Griffiths, 2001), indicating that this competition may be ecologically important in temperate lakes. We observed almost monospecific blooms of *C. raciborskii* in a lake in Michigan (Litchman et al., unpublished data), a region that commonly experience blooms of *M. aeruginosa*.

Performance of *C. raciborskii* in lakes will also be affected by nitrogen concentration, as nitrogen-fixers are favoured under N-limited conditions (Smith, 1983). However, nitrogen fixation requires an investment of cell resources and, therefore, there is likely to be a fitness cost of N-fixation. As enzyme reaction rates increase exponentially with temperature, the resources devoted to N-fixation may vary with temperature as well, leading to changes in the shape of the thermal reaction norm and differences in performance under nitrogen limitation. To better understand how N-availability and temperature will interactively affect *C. raciborskii*, we characterized its thermal reaction norm under both N-replete and N-free conditions. For comparison, we also estimated growth rates under N-free and N-replete conditions in a common HAB-forming N-fixer, *Anabaena flos-aquae*. Interactions between important environmental variables including temperature, nutrients, and light, are likely to prove important to predicting *C. raciborskii* invasion and the dynamics of phytoplankton communities (Sinha et al., 2012).

Materials and methods

Strains used

We tested three strains of *Cylindrospermopsis raciborskii* (Indiana Lake Lemon, Florida D, and Florida E, hereafter referred to as IN, FL-D, and FL-E, respectively), four strains of *Microcystis aeruginosa* (Gull B-00, Gull K-00, Bear AC-02, Bear AG-02) and a single strain of *Anabaena flos-aquae* UTEX 1444 for growth responses to temperature. The three *C. raciborskii* strains are not known to produce any toxins. *M. aeruginosa* Bear AC-02 lacks the *mcyA* gene necessary for toxin production, while the remaining three *M. aeruginosa* strains (Gull B-00, Gull K-00, Bear AG-02) and *A. flos-aquae* UTEX 1444 possess this gene and produce microcystins detectable in lab assays. The *C. raciborskii* and *M. aeruginosa* strains are recent isolates, with *Microcystis aeruginosa* Gull B-00 and K-00 being isolated in 2000, *M. aeruginosa* Bear AC-02 and AG-02 in 2002, *Cylindrospermopsis raciborskii* strain D in 1999 and *C. raciborskii* strain IN in 2006. *C. raciborskii* strain E was isolated in the decade prior to our experiments, though the precise time of isolation is unknown. The relatively recent isolation increases the likelihood that their growth responses are reflective of performance in natural environments. The *A. flos-aquae* strain has been maintained in laboratory culture since 1967, making it highly likely that adaptation to laboratory conditions has altered its physiology; we therefore do not compare *A. flos-aquae* with the other two species except in the context of nitrogen fixation.

Florida *C. raciborskii* strains were obtained from Dr. Julianne Dyble-Bressie, NOAA (isolated from Lake Dora, Florida) and the Indiana strain was obtained from Dr. Carole Lembi, Purdue University (isolated from Lake Lemon, IN). *M. aeruginosa* strains were obtained from Dr. Alan Wilson, Auburn University and isolated from Gull Lake and Bear Lake in Michigan. *A. flos-aquae* UTEX 1444 was obtained from the UTEX Culture Collection of Algae.

Culture conditions

Non-axenic cultures of every strain were grown in autoclaved 250-ml conical flasks containing approximately 100 ml WC medium (Guillard, 1975). Separate cultures of *A. flos-aquae* and the three *C. raciborskii* strains were maintained in N-replete (1 mmol N L^{-1}, in the form of $NaNO_3$) and N-free WC medium, bringing the total number of cultures to 12. Each culture was maintained in a growth chamber at 20°C under cool white fluorescent lights (EcoLux 20 W). All growth chambers used during the experiment were set to a 14:10 light/dark cycle, with a light intensity of approximately 100 µmol photons m^{-2} s^{-1}. This has been shown to be saturating for most phytoplankton species (Litchman, 2000) and is consistent with past data on these species (e.g. Briand et al., 2004). Cultures were shaken every day by hand and diluted regularly to keep them in exponential growth phase.

Experiment

To measure the thermal reaction norms of all the strains in our study, we measured their population growth rates at six temperatures after acclimation to these conditions. Growth rates were estimated from measurements of chlorophyll-*a* fluorescence (excitation wavelength: 436 nm, emission wavelength: 680 nm) in 24-well microplates over 5 days using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA). Before the experiment, we tested the efficacy of this method by showing that chlorophyll-*a* fluorescence correlated strongly with cell density for all three species above a fluorescence value of 1 (relative fluorescence units, RFU), though the chlorophyll content per cell/colony differed between species.

Cultures were allowed to acclimate for a minimum of three days in growth chambers maintained at a light level of 100 µmol photons m^{-2} s^{-1} and 6 different temperatures (15, 20, 25, 30, 35, and 40°C). Preliminary tests indicated that growth rate remained consistent after this acclimation period at almost all temperatures. This is not the case at extreme temperatures (15 and 40°C, as well as 35°C in the case of *Microcystis*) where growth rate is negative and populations may never truly acclimate. We did not extend the acclimation period further to avoid driving cultures at these extreme temperatures extinct. We began the assay by diluting the cultures to between 1 and 2 RFU in Greiner Bio-One CELLSTAR 24-well microplates (Monroe, NC). Each culture was transferred to two microwells on each of two microplates at every temperature (four replicates for every treatment combination). The microplates were then returned to the growth chambers and chlorophyll-*a* fluorescence was measured every 24 h for 5 days. Before each measurement, microplates were agitated by the microplate reader to ensure that settling did not skew the results. Each well was divided into a 3 × 3 grid and 20 fluorescence measurements were made at each point, with the mean of all 180 measurements being used for further calculations. The microplates were returned to the growth chambers immediately after the measurements.

Calculation of specific growth rate

For each well, the linear regression of the natural log of chlorophyll fluorescence against day number was examined visually, and data points from the end of the growth period were removed if log-fluorescence plateaued before the end of the assay (i.e. culture was no longer experiencing exponential growth). This occasionally occurred when a culture became extremely dense or sparse, at which point it had either exhausted its nutrient supply or was beyond the range in which the instrument registered a linear relationship between chlorophyll fluorescence and biomass. The slope of the resulting regression is the specific growth rate (day^{-1}) of the well culture. The initial cell densities used appear to be too low for accurate measurement of negative population growth rates, as fluorescence levels quickly dropped below the lower detection limit for several cultures at 15, 35, and 40°C. Therefore, we have less confidence in these

measurements than in those involving positive growth. Moreover, due to the rapid decline to below the detection limit, negative growth rate estimates are likely to be underestimates (i.e. the actual rates may be more negative). As this may be a source of bias, when growth rates were negative at both 35 and 40°C, the 40°C measurements were excluded from further calculations of temperature traits and from the figures.

All growth rate measurements from our experiments are included in Supplementary information (Online Resource 1).

Thermal reaction norm characterization and temperature trait estimation

The thermal reaction norm of each strain was characterized as in Thomas et al. (2012) and Boyd et al. (2013), using the equation:

$$f(T) = ae^{bT}\left[1 - \left(\frac{T - z}{w/2}\right)^2\right], \quad (1)$$

where specific growth rate f depends on temperature, T, as well as parameters z, w, a, and b. w is the temperature niche width, while the other three possess no explicit biological meaning. We fit (1) to the growth data for each strain using maximum likelihood to obtain estimates for parameters z, w, a, and b (parameter estimates included in Online Resource 2). We then used the reaction norm equation to numerically estimate four further traits of interest: the optimum temperature for growth, T_{max}, T_{min}, and maximum growth rate. For each culture, Eq. (1) was fit to the data from all temperatures, except where growth was negative at both 35 and 40°C. In these cases, the data for 40°C were omitted during the fitting procedure.

We also estimated confidence intervals on the temperature traits using a parametric bootstrapping approach. For each strain, we fitted (1) to the growth rate measurements and extracted the residuals from this fit. We then performed 1,000 residual bootstraps, a procedure in which the residuals are randomly 'reassigned' to the original growth rate estimates and added to them, thereby generating a slightly different thermal reaction norm. During each iteration, we refitted (1) and re-estimated the reaction norm parameters (z, w, a, b) as well as the derived traits. Examining the distribution of these parameters and traits over the 1,000 bootstraps allows us to quantify the uncertainty in our estimates, which we can then use to generate 95% confidence intervals and examine differences between strains.

Data analysis was performed using R 2.15.2 (R Core Team, 2012).

Results

Growth in N-replete medium

The three species exhibited strong differences in their thermal reaction norms (Fig. 1). The optimum temperatures of the three *C. raciborskii* strains ranged from approximately 30 to 33°C (Figs. 1, 2; Table 1). *A. flos-aquae* had an optimum above 36°C while *M. aeruginosa* exhibited clear differences between strains. The three toxic *M. aeruginosa* strains had optima of 28–29°C while the single non-toxic strain Bear AC-02 was estimated to growth fastest at 34°C. The species exhibited a similar hierarchy in T_{max}, with *A. flos-aquae* able to tolerate higher temperatures than *C. raciborskii*, and *M. aeruginosa* exhibiting the lowest high-temperature tolerance. However, *M. aeruginosa* and *A. flos-aquae* had lower T_{min}s than the *C. raciborskii* strains (except for *M. aeruginosa* strain Bear AG-02). Maximum growth rate differed strongly between species, ranging from 0.56 to 0.67 day^{-1} in *C. raciborskii*, 0.29 to 0.62 day^{-1} in *M. aeruginosa* and 1.48 day^{-1} in *A. flos-aquae* (Table 1). The growth rates obtained in this study agree well with previously measured rates for the same strains of *M. aeruginosa* (Wilson et al., 2006).

The three *C. raciborskii* strains exhibited relatively little variation in their thermal reaction norms, although the northern strain IN had the lowest optimum temperature of the three and the highest measured growth rates at 20 and 25°C (Figs. 1, 2; Table 1). *M. aeruginosa* showed larger differences in temperature response between strains. Bear AG-02 exhibited the poorest low-temperature tolerance, with an estimated T_{min} above 15°C. The non-toxic Bear AC-02 exhibited an optimum temperature and T_{max} more than 5°C higher than the other strains as well as a maximum growth rate 50% greater. *A. flos-aquae* had the highest estimated optimum temperature and T_{max} of all strains measured (Fig. 2).

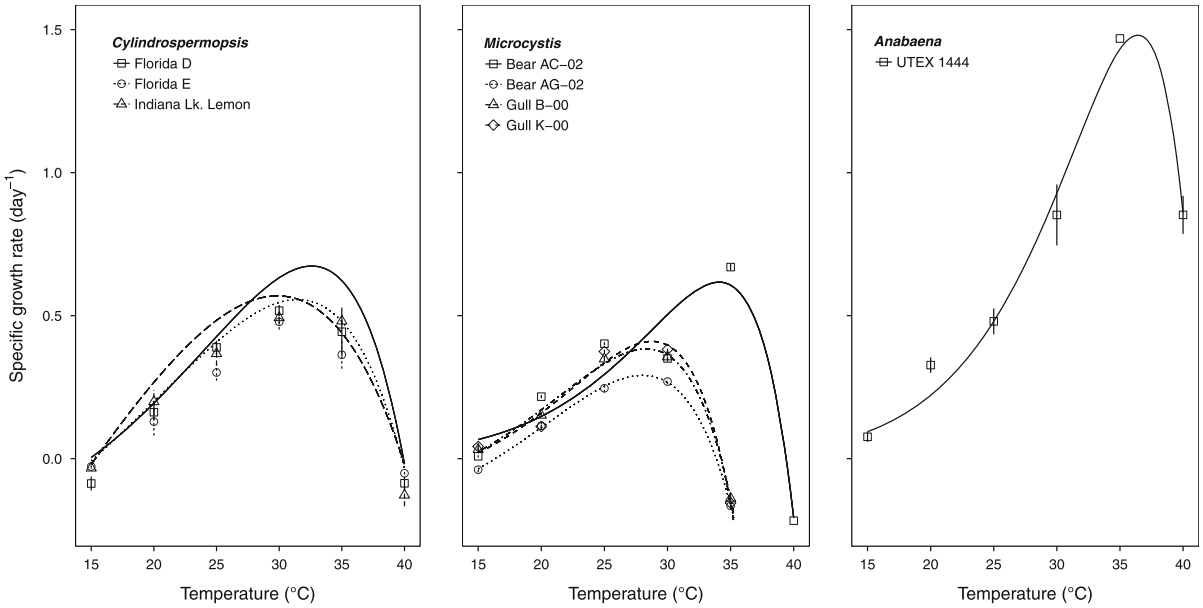

Fig. 1 Specific growth rates (day^{-1}) of *C. raciborskii*, *M. aeruginosa* and *A. flos-aquae* between 15 and 40°C, as well as curve fits to the data based on Eq. (1). *Error bars* indicate standard errors from four replicates

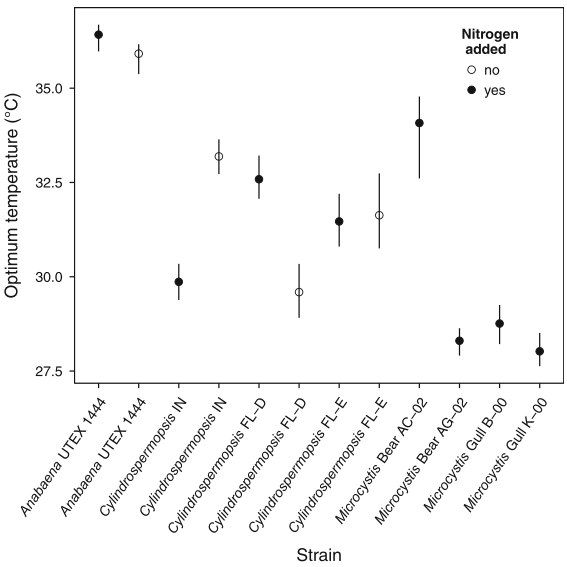

Fig. 2 Optimum temperatures for growth (°C) of all strains. Optima of N-fixers are shown in both N-replete and N-free media. *Error bars* represent 95% confidence intervals estimated by parametric bootstrapping

Effects of N-deprivation at different temperatures

N-deprivation increased the T_{min} (i.e. reduced the low-temperature tolerance) of all three *C. raciborskii* strains (Fig. 1; Table 1); we did not have measurements at sufficiently low temperatures to draw conclusions about its effect on the T_{min} of *A. flos-aquae*. It also slightly decreased the T_{max} (i.e. reduced high-temperature tolerance) in *C. raciborskii*, but had no detectable effect on *A. flos-aquae* (Fig. 1; Table 1). It exhibited inconsistent effects on the optimum temperature and on average had no effect on this (Figs. 2, 3). N-deprivation decreased the optimum in strain FL-D, increased it in strain IN, and did not change it in strain FL-E or *A. flos-aquae*.

N-deprivation did decrease the measured growth rates of *C. raciborskii* and *A. flos-aquae* at most temperatures, by as much as 0.4 d^{-1}; it also reduced their estimated maximum growth rates (Figs. 3, 4; Table 1). However, there were considerable differences in its effects across strains and temperatures. *C. raciborskii* FL-E experienced little to no reduction in growth rate at 20 and 25°C, while all other strains experienced decreases ranging from 0.1 to 0.25 day^{-1}. The largest difference between strains occurred at 35°C, with *C. raciborskii* FL-IN experiencing no detectable reduction in growth while the other three strains experienced reductions of 0.25–0.4 day^{-1}. Differences occurred at 15 and 40°C as well, but since we have less confidence in negative growth rate measurements for reasons explain in the "Materials and methods" section, we do not draw conclusions from them.

Table 1 Temperature traits of the cyanobacterial strains with 95% confidence intervals estimated by parametric bootstrapping

Species	Strain	Nitrogen added	Optimum temperature (°C)	T_{min} (°C)	T_{max} (°C)	Niche width (°C)	Maximum growth rate (day^{-1})
C. raciborskii	IN	Yes	29.9 [29.4, 30.4]	15.3 [14.9, 15.6]	39.9 [39.7, 40.1]	24.5 [24.2, 24.9]	0.57 [0.56, 0.58]
C. raciborskii	IN	No	33.2 [32.7, 33.6]	16.4 [14.9, 17.4]	39.1 [39.0, 39.2]	22.8 [21.8, 24.3]	0.52 [0.50, 0.54]
C. raciborskii	FL-D	Yes	32.6 [32.0, 33.2]	14.9 [13.0, 15.9]	39.9 [39.8, 40.1]	25.1 [24.1, 26.8]	0.67 [0.65, 0.70]
C. raciborskii	FL-D	No	29.6 [28.9, 30.3]	17.7 [17.3, 18.1]	38.6 [38.3, 38.9]	20.9 [20.6, 21.2]	0.41 [0.39, 0.43]
C. raciborskii	FL-E	Yes	31.5 [30.6, 32.1]	15.2 [14.0, 15.9]	39.8 [39.6, 40.0]	24.6 [23.9, 25.8]	0.56 [0.53, 0.58]
C. raciborskii	FL-E	No	31.6 [30.6, 32.4]	17.4 [16.0, 18.2]	39.4 [39.0, 39.7]	22.0 [21.2, 23.3]	0.34 [0.31, 0.38]
A. flos-aquae	UTEX 1444	Yes	36.4 [36.0, 36.7]	−13.2 [−15.2, 10.5][a]	41.4 [41.2, 42.0]	54.7 [31.1, 56.6][a]	1.48 [1.34, 1.60]
A. flos-aquae	UTEX 1444	No	35.9 [35.3, 36.2]	−10.9 [−16.1, 12.6][a]	41.7 [41.4, 42.5]	52.6 [29.6, 57.7][a]	1.07 [1.00, 1.13]
M. aeruginosa	Gull B-00	Yes	28.3 [27.9, 28.7]	14.0 [12.8, 14.8]	34.2 [34.1, 34.3]	20.3 [19.4, 21.4]	0.38 [0.37, 0.40]
M. aeruginosa	Gull K-00	Yes	28.8 [28.2, 29.3]	14.0 [11.7, 15.2]	34.3 [34.1, 34.4]	20.3 [19.1, 22.5]	0.41 [0.39, 0.44]
M. aeruginosa	Bear AC-02	Yes	34.1 [32.6, 34.8]	−6.8 [−17.7, 39.6][a]	39.4 [39.1, 70.8]	46.2 [23.3, 57.0][a]	0.62 [0.55, 0.69]
M. aeruginosa	Bear AG-02	Yes	28.0 [27.5, 28.5]	16.4 [15.8, 16.8]	33.9 [33.7, 34.0]	17.5 [17.1, 18.1]	0.29 [0.28, 0.30]

[a] These point estimates of T_{min} and niche width are inaccurate for these strains due to a lack of measurements at sufficiently low temperatures; however, the 95% confidence intervals overlap reasonable trait estimates

Discussion

Cyanobacteria are believed to have higher optimum temperatures for growth than other groups of phytoplankton (Robarts & Zohary, 1987), though a recent study has suggested that chlorophytes possess similarly high optima (Lürling et al., 2013). The predominance of cyanobacteria when lakes are at their warmest is therefore likely due to a combination of high-temperature optima and traits that are beneficial under stratified conditions, such as buoyancy regulation (Huisman et al., 2004; Paerl & Huisman, 2009). As a number of cyanobacterial species are capable of buoyancy regulation, including the three species considered in this study (Reynolds et al., 1987; Padisák, 1997), differences in temperature response and nutrient competitive abilities may be more important in determining the outcomes of competition between them. Differences in temperature response have been shown to successfully predict the outcomes of competition in cyanobacteria in experiments (Chu et al., 2007) as well as in the field, especially in combination with N:P supply ratio and the species' nutrient traits (Fujimoto et al., 1997).

The optimum temperatures of the three species tested in this study were high and within the range reported for cyanobacteria previously: between 28 and 37°C. The optima of the three *C. raciborskii* strains ranged from 30 to 33°C in N-replete medium, highly similar to estimates from other isolates. Though *C. raciborskii* strains from a number of countries in South America, North America, Europe, Australia, and Asia have now been measured, there is little variation in Topt and no apparent geographical pattern in its distribution (Saker & Griffiths, 2000; Briand et al., 2004; Chonudomkul et al., 2004; Mehnert et al., 2010). This might suggest a lack of local adaptation to temperature differences, but it is important to note that measurements in each of these studies were performed under slightly different conditions, particularly in terms of irradiance. For example, Briand et al. (2004) estimated optima between 29 and 31°C for ten other strains of this species from multiple continents, using an irradiance level of 30 µmol photons m^{-2} s^{-1} and a 16:8 h light/dark cycle. Saker & Griffiths (2000) measured seven Australian strains, also with optima largely around 30°C, but used an irradiance level of 50 µmol photons m^{-2} s^{-1} and a 12:12 h light/dark cycle. Chonudomkul et al. (2004) measured 24 strains from Thailand and Japan and found optima in the range of 30–35°C, using an irradiance level of 40 µmol photons m^{-2} s^{-1} and a 12:12 h light/dark cycle. Given the small amount of apparent variation in Topt, it appears that adaptation to local temperature conditions may be weak at best, though it is difficult to conclude this with confidence due to the differences in experimental methods.

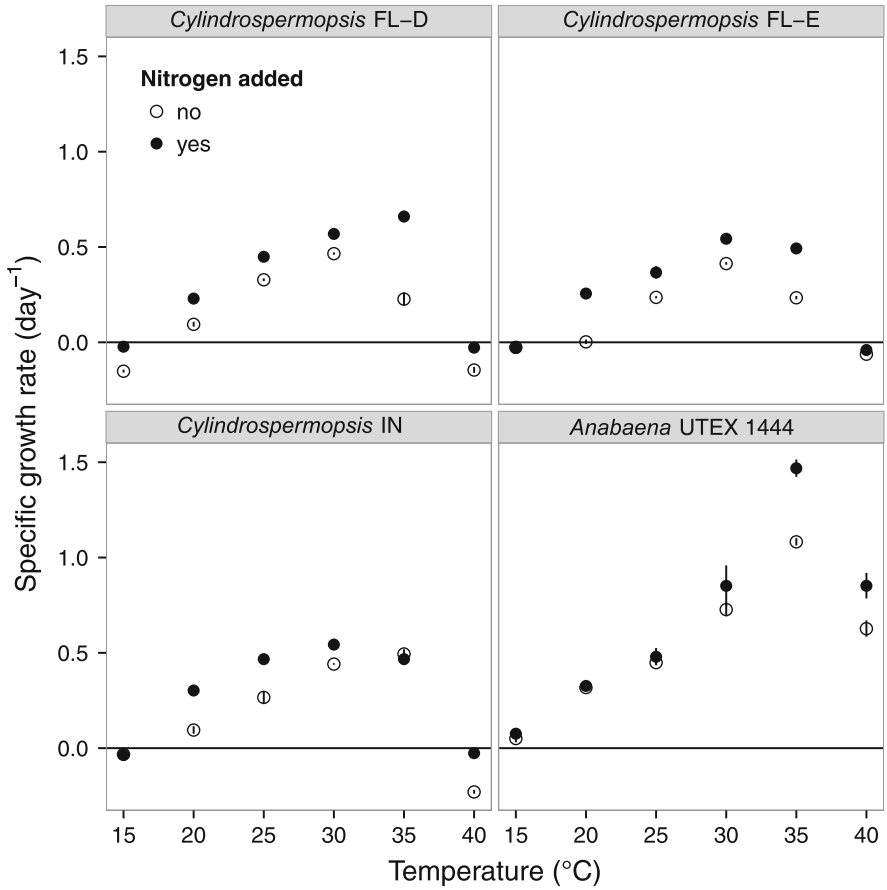

Fig. 3 Specific growth rates of N-fixers under N-replete (*filled points*) and N-free (*hollow points*) conditions at all temperatures. *Error bars* indicate the standard error of the mean

Anabaena flos-aquae exhibited the highest optimum temperature of 36°C, towards the higher end of the 27–39°C range of estimates for this species (Uehlinger, 1981; Novak & Brune, 1985). The three toxic *Microcystis aeruginosa* strains exhibited optima around 28°C, while the non-toxic strain Bear AC-02 possessed an optimum of 34°C. These measurements are slightly more extreme than estimates from earlier studies, which are between 30 and 32°C (Nalewajko & Murphy, 2001; Imai et al., 2009), possibly indicating important intraspecific variability. Some of these optima are higher than the temperatures these species are likely to experience in their natural environments, a pattern that has been observed in earlier studies of phytoplankton and other taxa (Barker, 1935; Karentz & Smayda, 1984; Kingsolver, 2009; Thomas et al., 2012). These are likely to be adaptive responses to environmental temperature variation, given the physiological constraints that these phytoplankton experience (i.e. an exponential increase in maximum growth rate with temperature and skewness of thermal tolerance curves). An eco-evolutionary model of phytoplankton growth in the ocean found that the best strategy under typical patterns of temperature variation was to have an optimum several degrees above the mean temperature (Thomas et al., 2012). Variability in the temperature environment may select for higher optima, as growth rates decrease more rapidly above the optimum temperature than below it (Martin & Huey, 2008). Our findings support the high-temperature preference of subtropical and temperate cyanobacteria (Reynolds, 2006), which implies that rising lake temperatures will promote cyanobacterial dominance (Paerl & Huisman, 2009; Kosten et al., 2012).

Maximum growth rates of *C. raciborskii* grown on nitrate have ranged from 0.3 to 1.3 day^{-1} in previous studies (Saker & Griffiths, 2000; Shafik et al., 2001; Briand et al., 2004; Mehnert et al., 2010). This places the N. American strains (0.56–0.67 day^{-1}, Fig. 1; Table 1) at the lower end of the range, below measured Australian and Hungarian strains (Saker &

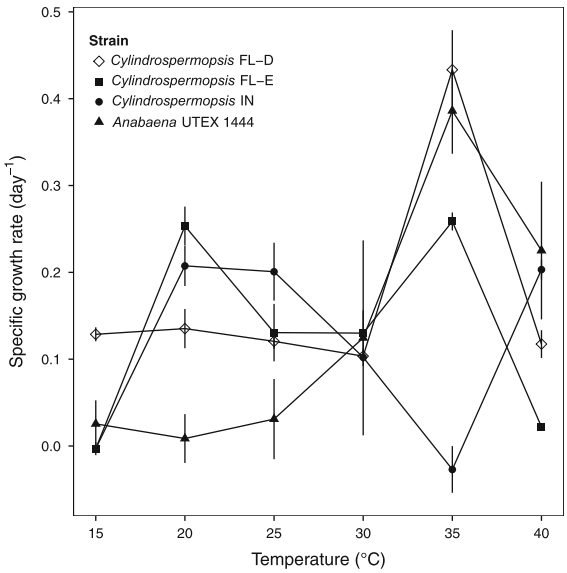

Fig. 4 The difference in specific growth rate (day^{-1}) of N-fixers between N-replete and N-free conditions at all temperatures. *Error bars* indicate standard error of the difference between growth rates

Griffiths, 2000; Shafik et al., 2001) but above a number of European, American and African strains (Briand et al., 2004; Mehnert et al., 2010). However, our earlier caveats about differences in experimental methods in these studies apply here as well. Variation in T_{max} is limited, with all studies finding values between 35°C and just above 40°C (Saker & Griffiths, 2000; Briand et al., 2004; Chonudomkul et al., 2004). However, T_{min} exhibited notable differences, with the three *C. raciborskii* strains in our study dying at 15, unlike in earlier studies. These previous studies found that *C. raciborskii* can tolerate temperatures between 10 and 15°C (Briand et al., 2004; Chonudomkul et al., 2004; Mehnert et al., 2010). The consistency of these earlier findings across geographical regions suggests that their difference to our findings do not reflect strain differences, but an interaction between temperature and irradiance. The irradiance we used was considerably higher than that used in earlier studies; our experiments were conducted at 100 µmol photons m^{-2} s^{-1}, approximately the optimum light intensity at intermediate temperatures, as determined by Briand et al. (2004) and Shafik et al. (2001) at 25 and 27°C. Irradiance level has been shown to alter the response to temperature in many phytoplankton, including *C. raciborskii* (Dauta et al., 1990; Kehoe et al., 2015), which suggests that the inability of the *C. raciborskii* strains in our study to survive at extreme temperatures is likely due to the different light environments used in the two studies. Alternatively, the differences may be indicative of local adaptation to more variable temperature conditions; ecotypic differences have previously been suggested to explain intraspecific variation in light response in this species (Piccini et al., 2011; Pierangelini et al., 2014).

Climate change and *C. raciborskii*

Our data suggest that climate change is likely to favour the invasive *C. raciborskii* over the native temperate cyanobacterium *M. aeruginosa* in temperate North America. *A. flos-aquae* performed better than both these species, but because it has been maintained in laboratory cultures since 1967, this may be due to adaptation to laboratory conditions, which leads to important changes in physiology and genome architecture (Swan et al., 2013), making the comparison unreflective of performance differences in natural environments. However, its temperature response does inform our understanding of the constraints on adaptation to high temperatures under highly favourable growth conditions. Therefore, we restrict discussion of *A. flos-aquae* to the effect of N-deprivation on thermal reaction norm shape, as the cost of nutrient deprivation is more likely to be conserved. However, if the temperature response has not changed significantly since its isolation, our results would lead us to predict that warming will facilitate the invasion of subtropical *A. flos-aquae* strains in temperate lakes.

Microcystis aeruginosa strains tolerated low temperatures better than *C. raciborskii*, with estimated T_{min}s around 14°C. This low-temperature advantage of *M. aeruginosa* may be an important factor in limiting the invasion of *C. raciborskii*; if lakes spend a greater proportion of time above the *C. raciborskii* T_{min} of 15–18°C, it may strongly favour their invasion and growth. This 15–18°C threshold corresponds closely with the 15–17°C range identified as crucial to favouring the growth of lake populations of *C. raciborskii* (Wiedner et al., 2007), providing a strong link between physiological tolerance and performance in natural environments. Above 20°C, the three *C. raciborskii* strains had higher growth rates than the toxic *M. aeruginosa* strains. The non-toxic *M. aeruginosa* strain Bear AC-02 experienced comparable growth rates at to *C. raciborskii* at all temperatures,

suggesting that there might be a trade-off between toxin production and growth rate or high-temperature performance in *M. aeruginosa*. As our study was not designed to test this difference and lacked statistical power in this regard, any difference between toxic and non-toxic strains may be purely coincidental. However, the potential implications for such a trade-off are important: it would suggest that higher summer temperatures may favour non-toxic strains over toxic ones. Therefore, we hope that this question will be addressed more rigorously with carefully designed experiments.

The performance of a cyanobacterial species in the 20–30°C range may be a useful indicator of future success and invasibility in temperate regions, because phytoplankton communities are frequently dominated by cyanobacteria at these temperatures, and intermediate-sized lakes are expected to spend a greater proportion of the year in this temperature range in the future (de Stasio et al., 1996; Magnuson et al., 1997). This invasion may alter lake ecosystems and communities through a variety of pathways—changes in nitrogen supply (as a result of N-fixation), changes in phosphorus concentration (as *C. raciborskii* is an excellent phosphorus competitor), changes in the light environment (due to its shade tolerance), altered zooplankton community abundance and composition (as a result of changes in toxin load and type) (Padisák, 1997; Istvánovics et al., 2000). Each of these can alter the selective environment and may lead to both ecological and evolutionary changes in the local community (Litchman et al., 2010). The outcomes of competition between these species in lakes will depend on other factors as well, including nutrient and light response.

Interactions between these factors and the role of natural cycles in environmental variables may prove to be important in drive dynamics in natural systems. For example, our study (and most studies of this kind) used constant temperature and binary light/dark conditions, while taxa in natural environments experience daily cycles in both temperature and light intensity. Especially due to the highly nonlinear effects of temperature and light on growth rate (Litchman, 2000; Kingsolver, 2009; Edwards et al., 2015), the effects of interacting, cycling variables may be highly complex. Early examinations of the effects of fluctuating light have found strong influences on growth rate, but at most a weak interaction with temperature, possibly making the job of prediction easier (Litchman, 2000; Shatwell et al., 2012). Many important questions remain unresolved, however. Most importantly, can measurements made under constant conditions be used to accurately predict growth under fluctuating conditions? Current models appear to have some predictive power, but do an inadequate job of capturing the effect of fluctuations on growth (Litchman, 2000). The development of better models that account for physiological acclimatization should therefore a priority, as they could guide us in developing experiments and assays to collect more useful data, with the goal of improving forecasts of phytoplankton dynamics in natural systems.

Effects of N-deficiency

N-deficiency showed inconsistent effects on the growth of nitrogen-fixers. It reduced low-temperature tolerance (increased T_{min}) and high-temperature tolerance (decreased T_{max}) in all *C. raciborskii* strains. This suggests that eutrophication may favour spread and dominance of *C. raciborskii* by altering their response to environmental temperatures. If true, this leads to the testable prediction that *C. raciborskii* should be found in lakes with higher nutrient concentrations earlier in the season (i.e. at lower temperatures) than those with lower nutrient concentrations. It further points towards a physiological mechanism by which a combination of eutrophication and warming will have a strongly interactive effect on the success of the species, and may have already done so. However, the fact that N-deprivation altered optimum temperatures and growth rates in an unpredictable manner (Fig. 2; Table 1) indicates that predicting the outcome of the interaction will be challenging, and will likely require more extensive experiments with measurements across a range of nutrient concentrations.

Conclusion

Our study indicates that warming of temperate lakes is likely to favour *C. raciborskii* over the native *M. aeruginosa* due to *C. raciborskii*'s inability to survive at low temperatures and higher growth rates at warmer temperatures. *M. aeruginosa* currently has a strong advantage in temperate North American lakes due to its ability to tolerate colder temperatures. By

beginning its growth earlier in the season than *C. raciborskii*, it may have access to nutrients at a time when the latter is unable to grow, thereby negating the strong nutrient competitive abilities of *C. raciborskii*. However, warming above the 15–18°C temperature range will strongly favour *C. raciborskii*; the presence of this temperature threshold suggests that a nonlinear transition between *Microcystis*-dominated and *Cylindrospermopsis*-dominated communities is a possibility. Management of nutrient pollution in lakes may also play an important rule in delaying or preventing *C. raciborskii*'s spread, due to the effect of N-deprivation on T_{min}. Understanding the nature of the interaction between nutrients, light, and temperature, particularly under fluctuating conditions, will likely improve our ability to predict *C. raciborskii* invasion as well as the composition and dynamics of phytoplankton communities.

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