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The effects of phosphorus and temperature on the competitive success of an invasive cyanobacterium

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Abstract Rising lake temperatures and changing nutrient inputs are believed to favour the spread of a toxic invasive cyanobacterium, *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju, in temperate lakes. However, most evidence for these hypotheses is observational or based on physiological measurements in monocultures. We lack clear experimental evidence relating temperature and nutrients to the competitive success of *C. raciborskii*. To address this, we performed a 2 × 2 factorial laboratory experiment to study the dynamics of mixed phytoplankton communities subjected to different levels of temperature and phosphorus over 51 days. We allowed *C. raciborskii* to compete with ten different species from major taxonomic groups (diatoms, green algae, cryptophytes, and cyanobacteria) typical of temperate lakes, under low and high summer temperatures (25 and 30 °C) at two levels of phosphorus supply (1 and 25 μmol L^{-1}). *Cylindrospermopsis raciborskii* dominated the communities and strongly decreased diversity under low-phosphorus conditions, consistent with the hypothesis that it is a good phosphorus competitor. In contrast, it remained extremely rare in high-phosphorus conditions, where fast-growing green algae dominated. Surprisingly, temperature played a negligible role in influencing community composition, suggesting that changes in summer temperature may not be important in determining *C. raciborskii*'s spread.

Keywords *Cylindrospermopsis* · Temperature · Phosphorus · Competition · Cyanobacteria · *Microcystis* · Phytoplankton

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Introduction

Climate change and eutrophication are driving major changes in aquatic ecosystems. They have altered phytoplankton community composition and

decreased their diversity, most obviously by driving increases in harmful algal blooms (HABs) (Heisler et al. 2008; Paerl and Huisman 2009; Kosten et al. 2012). These changes in composition and diversity may impair the ecosystem functioning of the phytoplankton community and also influence higher trophic levels by altering food availability and toxin concentrations (da Costa et al. 2013). They have also been implicated in the spread of invasive species, including microbes, by favouring the growth of species originating in very different environments (Litchman 2010). One species of particular concern is the potentially toxic freshwater cyanobacterium *Cylindrospermopsis raciborskii*, a nitrogen-fixing HAB former spreading through temperate lakes around the world (Padisák 1997; Isvánovics et al. 2000; Burford et al. 2016). *Cylindrospermopsis raciborskii* is responsible for multiple cases of human poisoning and animal mortality (Hawkins et al. 1985), and understanding the drivers behind its competitive success and recent invasion are important goals for protecting human health.

Cylindrospermopsis raciborskii has a number of traits that have contributed to its success, including the ability to fix nitrogen facultatively, tolerate low light, produce akinetes, compete strongly for phosphorus, tolerate high temperatures, allelopathy, and buoyancy (Padisák 1997; Isvánovics et al. 2000; Shafik et al. 2001; Briand et al. 2004; Figueredo et al. 2007; Wu et al. 2009; Moisander et al. 2012; Rzymski et al. 2014; Soares et al. 2013; Burford et al. 2016; Thomas and Litchman 2016). However, none of these traits clearly explains why its invasion has been so recent. A number of hypotheses have been put forth to explain this, including (1) lake warming as a result of climate change opening up new habitable environments (Briand et al. 2004; Sinha et al. 2012), (2) recent selection producing *C. raciborskii* strains that can tolerate the cold temperatures and lower light levels characteristic of temperate lakes (Briand et al. 2004), and (3) anthropogenic dispersal extending the species' range into temperate regions. Despite temperature being a major factor in two of these three hypotheses, competitive success is most probably influenced by its ability to grow at low levels of limiting resources such as phosphorus or light (Tilman 1977, 1982). Crucially, few studies have examined how changes in specific environmental variables influence *C. raciborskii* in competition with other species, and fewer still have

examined how multiple environmental variables interact to influence its competitive ability (Sinha et al. 2012). Since such interactions strongly influence growth rate, nutrient uptake rates, and other physiological traits in phytoplankton, including *C. raciborskii* (Reay et al. 1999; Wu et al. 2012a; Kehoe et al. 2015; Thomas and Litchman 2016), it is highly likely that competitive ability and invasibility are environment dependent (Marinho et al. 2013). Therefore, understanding the conditions under which *C. raciborskii* will succeed requires us to examine how the invader performs while competing against a diverse community. Microcosm experiments with mixed communities are a valuable tool with which to address questions such as these, and experiments with phytoplankton have been used extensively to develop and test ecological theory (Tilman 1977; Tilman and Kiesling 1984; Grover 1988, 1991). Patterns in microcosm experiments have been shown to predict those in natural systems well (Tilman 1977; Drake et al. 1996; Smith et al. 2005). However, few such experiments have been performed with invasive microbes (but see Sperfeld et al. 2010; Chislock et al. 2014). Examining interactions between temperature and nutrients in such experiments is particularly important, because both variables are undergoing strong changes in the environment, and because traits determining competition for nutrients are highly temperature-dependent (Tilman et al. 1981).

We performed a 2×2 factorial laboratory experiment (three replicates per treatment combination) to examine how changes in peak summer temperature (25 and 30 °C) and phosphate concentrations (1 and 25 μmol L^{-1}) influence the competitive success of *C. raciborskii* and the diversity of the phytoplankton community. We assume that species compete by depleting limiting resources, consistent with an extensive body of theory and experimentation (Tilman 1982). In our case, the limiting resource is expected to be phosphorus in our low-phosphorus treatments, but other resources (such as nitrogen or light) may limit growth in the high-phosphorus treatments. The lower temperature corresponds to peak summer temperatures in moderately-sized temperate lakes. The higher temperature may be reached in these lakes later this century as a result of warming and is already reached in many shallow lakes (de Stasio et al. 1996). *Cylindrospermopsis raciborskii* competed with an assembled laboratory phytoplankton community

consisting of ten species, from four major phytoplankton functional groups: diatoms (*Asterionella formosa*, *Fragilaria crotonensis*, *Synedra acus*), chlorophytes (*Chlamydomonas reinhardtii, Scenedesmus quadricauda, Ankistrodesmus falcatus*), cryptophytes (*Rhodomonas* sp., *Cryptomonas ovata*), and cyanobacteria (*Planktothrix* sp., *Microcystis aeruginosa*). These ten species are native to many temperate lakes and are likely to be encountered by *C. raciborskii* following its invasion. We tracked the dynamics of the community over 51 days, sampling the community every 3 days.

Materials and methods

The isolates we used were largely obtained from culture collections with the exception of *C. raciborskii* strain IN (isolated in 2005 from Lake Lemon, IN, USA), which was provided to us by Dr. Carole Lembi, Purdue University, and *Microcystis aeruginosa* strain Gull B-00, provided to us by Dr. Alan Wilson Auburn University. The remaining strains were *Fragilaria crotonensis* (UTEX strain LB FD496), *Synedra acus* (UTEX strain LB FD340), *Asterionella formosa* (provenance unknown), *Chlamydomonas reinhardtii* (CBSC strain 2935), *Scenedesmus quadricauda* (UTEX strain 614), *Ankistrodesmus falcatus* (provenance unknown), *Rhodomonas minuta* (UTCC strain 344), *Cryptomonas ovata* (UTEX strain LB 2783), and *Planktothrix rubescens* (UTCC strain 507). Isolates were not axenic. Despite differences in isolation location, we expect that competition between these strains will be informative about outcomes in natural systems because there is little evidence for local adaptation in competitive traits within species, i.e. traits appear to be conserved at a species level (Thomas et al. 2012; Boyd et al. 2013). However, it is possible that adaptation to enriched laboratory conditions in some of these strains influenced community dynamics; community experiments should ideally be performed with recently isolated strains, but this is often not feasible.

Experimental set-up

We performed a 2 × 2 factorial experiment with three replicates for each treatment combination, for a total of 12 communities. Temperatures were 30 °C (high) and 25 °C (low), and phosphorus concentrations in the supplied medium were 25 μmol L^{-1} (high) and 1 μmol L^{-1} (low). All communities were grown in 50% WC medium (Guillard 1975) with a nitrate concentration of 500 μmol L^{-1} and a silicate concentration of 100 μmol L^{-1}. The experiment was performed using an irradiance level of 100 μE m^{-2} s^{-1} and a 14:10 light/dark cycle. All species were acclimated to their experimental temperature and phosphate conditions for one week before the experiment. Cultures were only diluted during this week if they reached high densities, which would have reflected a decrease in the phosphate concentration. During this acclimation period, some species increased in density and others remained approximately constant, but none experienced clear population declines, indicating that survival in these conditions was possible for all species.

Each community was inoculated with an approximately equal concentration (in terms of biovolume) of all species, 240.9 μm^3 mL^{-1} in 250 mL of 50% WC medium (Guillard 1975), maintained in 500-mL glass conical flasks. This concentration was chosen based on the maximum concentration obtained in the acclimated monocultures of the most dilute species, *Synedra acus*. After communities were mixed initially, they were immediately returned to their treatment conditions. Growth media was replaced daily to achieve a semi-continuous culture with a dilution rate of 0.2 day^{-1}. Flasks were shaken by hand daily to prevent the influence of spatial structure (e.g. light and nutrient gradients) within the flasks. No wall growth was observed over the course of the experiment. Every 3 days, the volume removed during dilution was preserved for microscopic analysis using Lugol's solution.

Quantifying species dynamics through microscopy

Microscopic inspection of preserved samples showed that cell biovolume changed strongly between treatments and through time (data not shown). This change in biovolume was particularly noticeable for *C. raciborskii* in the low-phosphorus treatments. This made it impossible to use a standard (species-specific) formula to calculate the biovolume contribution of each species based on cell counts; such a comparison would have required characterising the mean biovolume of each species in each community at every time point. Therefore, though expressing our results in

terms of biovolume would have been preferable, we chose instead to compare species dynamics based on cell density. As we saw very strong dominance (>99% by cell density) by 1–2 species at the end of the experiment, we believe that our substantive conclusions would not have changed as a result of using biovolume estimates instead of cell density.

Species composition and cell densities were determined by microscopic counts. A total of 20 fields of a Palmer cell were counted at $200\times$ or $400\times$ magnification, depending on density, and cell densities estimated (detection limit of approximately 1000 cells mL^{-1}). One sample was lost, a replicate of day 12 of the 25 °C high-phosphorus treatment group. Transient dynamics were completed by approximately 24 days, which we observed during our sample counts. Therefore, we decided to count every third sample thereafter (i.e. every 9 days) to reduce experimental effort without reducing our understanding of the community's trajectory. During the latter half of the experiment, we also performed colorimetric analysis of dissolved nitrate and phosphate concentrations (Lachat QuikChem FIA, Lachat Instruments) in these samples, with a detection limit for phosphate of 1 µg L^{-1} (0.03 µmol L^{-1}) and for nitrate of 10 µg L^{-1} (0.71 µmol L^{-1}).

Statistical analysis

We performed two-way ANOVAs to identify whether temperature and phosphorus influenced the distributions of Shannon diversity (calculated based on species' cell densities) and phosphorus concentration at the end of the experiment. Concentrations of *C. raciborskii* differed by three orders of magnitude between phosphorus treatments. The species also went extinct (i.e. fell below our detection limit) in some of the high-phosphorus replicates, violating the distributional assumptions of ANOVAs, and so we used Welch's unequal variance *t* test to test for differences. Because of deviations of the residual distributions from normality, we avoided the use of a basic ANOVA when examining the effects of temperature and phosphorus on the rate of increase in *C. raciborskii* (i.e. the slope of *C. raciborskii* abundance against time). Instead, we resampled the data to generate 10,000 residual bootstraps of *C. raciborskii* log abundance against time. For each bootstrap, we compared the explanatory power of four models

(temperature alone; phosphorus alone; both main effects but no interaction term; and both main effects and the interaction term). This allowed us to more carefully examine the influence of both variables on *C. raciborskii*'s rate of increase.

We compared the models using small-sample corrected Akaike Information Criterion (AICc), a measure of the relative quality of different models (especially nested ones) when fitted to the same dataset (Burnham and Anderson 2002). Relative to standard AIC, AICc imposes a stronger penalty for increasing the number of model parameters and is preferred for datasets with a small number of points (AIC and AICc values converge at large sample sizes). When comparing two models fit to the same dataset, a lower AIC or AICc value indicates a better model fit. Delta AIC or AICc (i.e. the difference between model AIC or AICc values) is therefore used to characterise relative model quality. By convention, a delta AIC or AICc value of 2 indicates a significant difference in quality (Burnham and Anderson 2002).

We also tested for differences between distributions of dissolved phosphate and nitrate at the end of the experiment using five nested linear models (only intercept; temperature alone; phosphorus alone; both main effects but no interaction term; and both main effects and the interaction term) which we compared using AIC.

All analyses were performed in the R statistical environment (R Core Team 2015).

Results

Phytoplankton community composition differed strongly between phosphorus treatments, but not temperature treatments (Fig. 1). *Cylindrospermopsis raciborskii* dominated under low-phosphorus conditions, and two chlorophytes (*Chlamydomonas reinhardtii* and *Scenedesmus quadricauda*) dominated under high-phosphorus conditions. The relative abundance of *C. raciborskii* was therefore highly dependent on phosphorus level (Welch's unequal variances *t* test, $p < 0.001$); under low-phosphorus conditions, it was approximately 99% by cell (or filament) number. Consequently, community diversity declined in all treatments (Fig. 2) and the low-phosphorus treatments had the lowest diversity at the end of the study (Welch's unequal variances *t* test, $p < 0.001$).
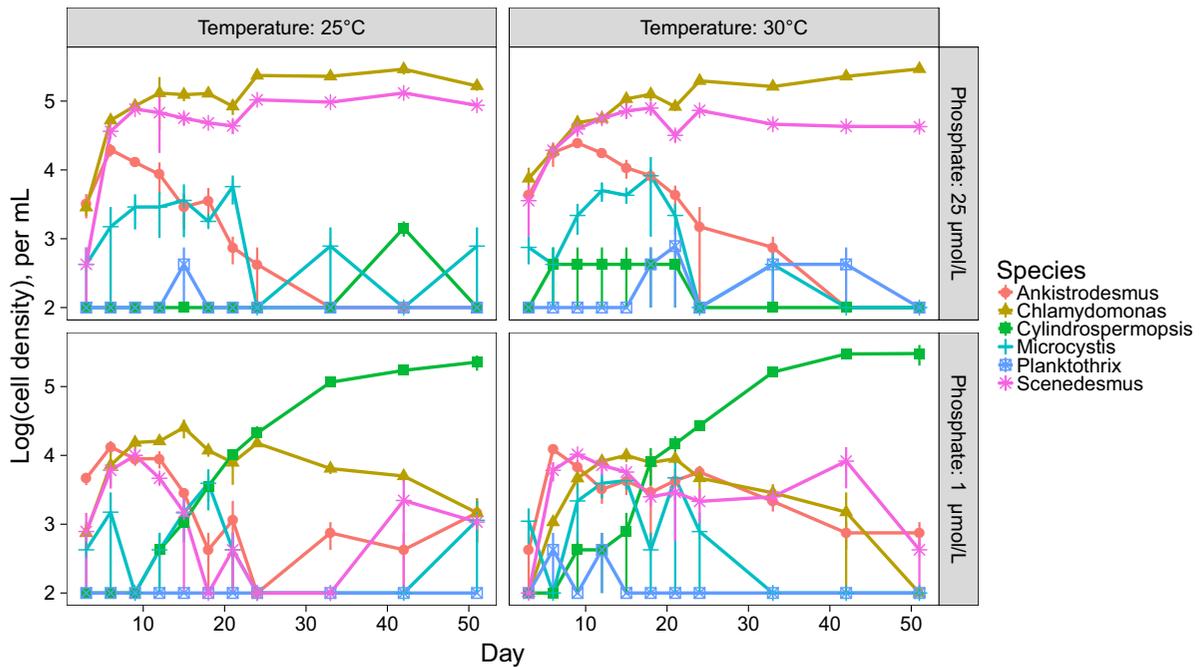
Fig. 1 Community dynamics over the course of the experiment (six most abundant species shown). *C. raciborskii* dominates when phosphorus supply is low, and green algae dominate when phosphorus supply is high. *Error bars* represent standard error of the mean in three replicates. Cell densities below our detection limit (approximately 1000 cells mL^{-1}) were assigned a value of 100 to enable plotting on a log scale. The biovolumes of individual cells/filaments changed over the course of the experiment in low-phosphorus treatments, especially in *C. raciborskii*. This may explain the sharp increase in *C. raciborskii* density in low-phosphorus treatments after day 20
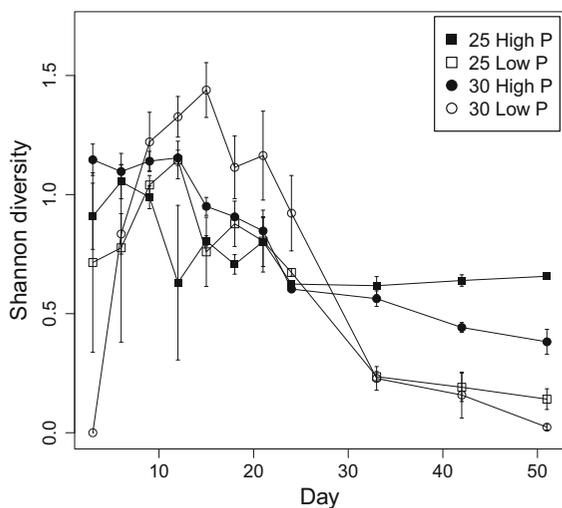

Fig. 2 Dynamics of community diversity (Shannon index) over the course of the experiment. *Error bars* represent the standard error of the mean in three replicates. Note that the estimated diversity values at the earliest sampling dates are biased downwards because most species were below our detection limit at this point, especially in the low-phosphorus treatments

There was no detectable effect of temperature or an interaction on the final abundance of *C. raciborskii*. We used 10,000 bootstrap simulations to test whether temperature and phosphorus influenced the rate of change of *C. raciborskii* within the communities (i.e. the slope of *C. raciborskii* log abundance against time). We compared the explanatory power of four models (temperature alone; phosphorus alone; both main effects but no interaction term; and both main effects and the interaction term) by AICc. In 55% of the bootstraps, the best model contained only phosphorus, while in the remaining 45%, the best model had phosphorus, temperature, and the interaction term. This mixed support indicates that phosphorus was the most important driver of dynamics, but that there may have been a weak role of temperature in influencing *C. raciborskii*'s takeover of the community.

Most species went extinct during the experiment (Table 1). For each species in each treatment, we characterised the 'mean time to extinction' i.e. the duration till the species fell below our microscopy

Table 1 Mean time to extinction for each species in all treatments over the 51-day experiment

Functional group	Species	Average time (in days) to extinction			
		25°/high P	25°/low P	30°/high P	30°/low P
Diatoms	*Synedra acus*	BD	BD	BD	BD
	Fragilaria crotonensis	BD	BD	BD	BD
	Asterionella formosa	15	BD	BD	BD
Chlorophytes	*Chlamydomonas reinhardtii*	–	–	–	–
	Scenedesmus quadricauda	–	–	–	–
	Ankistrodesmus falcatus	33	–	–	–
Cryptophytes	*Cryptomonas ovata*	12	BD	12	12
	Rhodomonas sp.	15	18	24	18
Cyanobacteria	*Microcystis aeruginosa*	–	–	42	33
	Planktothrix sp.	18	BD	–	15
	Cylindrospermopsis raciborskii	–	–	24	–

Communities were sampled every 3 days. Note that some species never increased beyond the detection limit ('BD'), and others never went extinct (indicated with a dash)

BD below detection limit in all samples

detection limit for the final time (in a few cases, it fluctuated around the detection limit for a short period; Fig. 1; Table 1). The diatoms (*Synedra acus*, *Fragilaria crotonensis*, and *Asterionella formosa*) did not reproduce rapidly enough to rise beyond the detection limit despite surviving a week-long acclimation period without declining in density. The temperature conditions may have been at the limits of their tolerance, and the daily dilution therefore drove a rapid decline in their abundance. The cryptophytes (*Cryptomonas ovata* and *Rhodomonas* sp.) persisted for longer (Table 1), but fluctuated at very low densities.

The increase in community cell density through the experiment in all treatments is reflected in the decline in dissolved nutrients (Fig. 3). Phosphate, which was expected to be the limiting nutrient, declined rapidly at the beginning of the experiment, but remained approximately constant from day 18 (we sampled sparsely before this, so this may have occurred at an earlier time point). However, there remained approximately tenfold greater phosphate in the high-phosphorus communities than in the low-phosphorus ones. In contrast, nitrate continued to decline throughout the course of the experiment (Fig. 3). Concentrations of both nutrients at the end were strongly influenced by phosphate supply level ($p < 0.001$), with temperature possibly having a weak effect (AIC of a model including a temperature term is <2 higher than the model with phosphate alone).

Discussion

Our experiments demonstrate that *C. raciborskii* is highly competitive under low-phosphorus conditions and strongly reduces diversity (Figs. 1, 2). Green algae were better competitors under high-phosphorus conditions, suggesting that they were able to take up nutrients quickly and reproduce more rapidly than *C. raciborskii* under similar conditions. Growth may also have been limited by other factors (such as nitrate or light) in the high-phosphorus treatments for which green algae are better competitors.

Previous studies have shown that *C. raciborskii* is able to perform well under phosphorus-depleted conditions (Isvánovics et al. 2000; Wu et al. 2012b) and dominated communities in field mesocosms (Chislock et al. 2014), but this has not been previously demonstrated in a controlled laboratory study. All else being equal, *C. raciborskii* appears capable of competing in low-phosphorus lakes, a habitat infrequently dominated by toxic cyanobacteria, particularly diazotrophs. This may be because phosphorus concentrations in lakes can be far lower than the concentration we supplied, but we note that by the end of each 3-day period, it was depleted to approximately 0.1 µmol L^{-1} in our low-phosphorus treatments. Another possibility is that physical mixing or covariance between environmental factors (such as

Phosphate dynamics
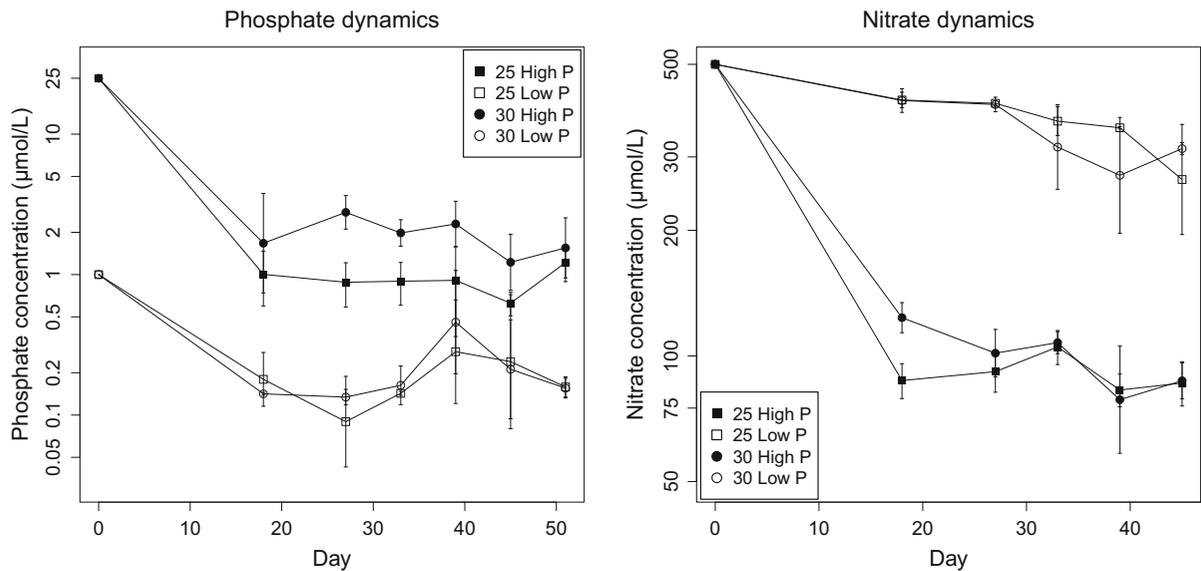
Nitrate dynamics

Fig. 3 Dynamics of dissolved phosphate and nitrate over the course of the experiment. Phosphate concentrations decrease rapidly at the start of the experiment and then stabilise at the point where it limits growth. In the low-phosphorus treatments, nitrate concentrations gradually decrease even after phosphate appears to stop declining. Note that Y-axes are on a log scale

light and nutrients)—which we largely removed in our experiment—may contribute to their relative lack of success. Unexpectedly, given the attention that temperature has received in explaining its spread (Briand et al. 2004), we found at most a weak effect of temperature on competitive ability (Fig. 1). *Cylindrospermopsis raciborskii*'s dominance in low-phosphorus treatments was reflected in a greater decline in dissolved phosphate in these communities (Fig. 3). Dissolved phosphate in the high-phosphorus treatments declined to levels similar to the starting concentration in the low-phosphorus treatments. However, concentrations in the low-phosphorus treatments were an order of magnitude lower at the end of the experiment. *Cylindrospermopsis raciborskii* can therefore tolerate and grow at lower phosphorus concentrations than the other species in our study, possibly indicating that it has a lower R^* (minimum equilibrium resource concentration; Tilman 1982) for phosphorus at both 25 and 30 °C. If its R^* is indeed lower and there was additional phosphate available in the high-phosphorus treatments, why did *C. raciborskii* not take over in these communities as well and reduce the phosphate concentrations further? We believe that this is due to the fluctuations in phosphate introduced by our semi-continuous culture method.

The evidence for the effects of nutrient pulses on cyanobacteria, and *C. raciborskii* in particular, is unclear and the subject requires additional experimentation to address. Some HAB-forming cyanobacteria are able to physiologically 'anticipate' pulses and optimise their uptake response to them, allowing them to benefit from periodic supply (Aubriot et al. 2011; Aubriot and Bonilla 2012). Field experiments with *C. raciborskii* appear to support a modest increase in its proportion of the community and possibly abundance, with pulsed phosphate additions (although this was not controlled for with non-pulsed phosphate addition) (Posselt et al. 2009). In contrast, experiments with *C. raciborskii* laboratory cultures clearly show that it is negatively affected by large phosphorus pulses (Isvánovics et al. 2000), but possibly benefits from small, frequent ones (Amaral et al. 2014; Willis et al. 2015). The larger phosphate pulses in our high-phosphorus treatment may have effectively increased its R^* for phosphorus in these conditions by preventing *C. raciborskii* from acclimating to the low-phosphorus conditions occurring between dilutions. Additional evidence for this can be found in the slow but steady decline in dissolved nitrogen over the entire course of the experiment (Fig. 3).

The continued decline in nitrate after phosphate concentration had plateaued strongly implies that

phosphorus-use efficiency continued to increase several weeks after the experiment began. We observed a large decline in the filament size (both length and width) of *C. raciborskii* in the low-phosphorus treatments later in the experiment, which further supports this explanation. This suggests that physiological acclimation to low-phosphorus conditions in *C. raciborskii* may give it a strong competitive advantage, although this will require additional work to establish. In contrast, the fast-growing green algae *Scenedesmus quadricauda* and *Chlamydomonas reinhardtii* may be well adapted to resource-rich pulsed conditions and have a weaker acclamatory response.

If our assumptions about the effect of nutrient pulses on *C. raciborskii* are correct, our results imply that if a similar experiment was performed in a chemostat, *C. raciborskii* would dominate by driving the phosphorus concentration well below the level reached in the high-phosphorus treatment. Overall, our results suggest that increases in peak summer temperature are unlikely to have a large effect on *C. raciborskii*'s invasion. This assumes that regular phosphate pulses characterise lake nutrient dynamics adequately. However, in lakes that experience a more steady inflow of nutrients, *C. raciborskii* may prove to be a better competitor.

Peak summer temperature may not be the critical determinant of invasion success, however. Field studies have shown that the period of time during which temperatures are above 15–17 °C may be more important, as this is a threshold below which *C. raciborskii* is not capable of growth (Wiedner et al. 2007). This has also received recent experimental support, with multiple strains of *C. raciborskii* being found to exhibit a T_{min} (minimum persistence temperature, or the temperature below which population growth rate is negative) between 15 and 18 °C (Thomas and Litchman 2016). T_{min} is also nutrient dependent, with nitrogen deprivation increasing this value (i.e. decreasing low-temperature tolerance). Temperature–nutrient interactions may therefore still play an important role in determining *C. raciborskii*'s spread and invasion, but they may be more relevant at temperatures in spring and early summer, when akinete germination occurs.

Differences between experimental conditions and natural ecosystems are inevitable. The existence of trait and toxin diversity within species, as well as variation in the dynamics of light, temperature, nutrients, and predators, are among the factors likely to play important roles in determining invasion outcomes in specific lakes. Nonetheless, controlled microcosm experiments are valuable because they offer us the ability to disentangle mechanisms, which should lead to better predictive ability on average. Our experiment strongly indicates that phosphate concentrations are more important for *C. raciborskii's* success than variation in summer temperatures, information that is more difficult to come by through monitoring of natural ecosystems. A combination of theory, experiments, and observation is likely to provide us with the most fruitful path towards understanding.

The invasion of *C. raciborskii* has important implications for public health, and understanding the environmental and physiological reasons behind its competitive success and spread is an important challenge for freshwater ecologists. Replicated community experiments such as this allow us to rigorously test hypotheses attempting to explain its spread, as well as forecast the future of the invasion and the consequences for aquatic ecosystems. Our work points towards phosphorus as being a far more important determinant of its success than summer temperature variation. However, we believe that the interaction of nutrients with spring and fall temperatures has the potential to limit *C. raciborskii*'s growth and deserves further investigation.

References

Amaral V, Bonilla S, Aubriot L (2014) Growth optimization of the invasive cyanobacterium *Cylindrospermopsis raciborskii* in response to phosphate fluctuations. Eur J Phycol 49:134–141. doi:10.1080/09670262.2014.897760

Aubriot L, Bonilla S (2012) Rapid regulation of phosphate uptake in freshwater cyanobacterial blooms. Aquat Microb Ecol 67:251–263. doi:10.3354/ame01596

Aubriot L, Bonilla S, Falkner G (2011) Adaptive phosphate uptake behaviour of phytoplankton to environmental phosphate fluctuations. FEMS Microbiol Ecol 77:1–16. doi:10.1111/j.1574-6941.2011.01078.x

Boyd PW, Rynearson TA, Armstrong EA et al (2013) Marine phytoplankton temperature versus growth responses from

polar to tropical waters—outcome of a scientific community-wide study. PLoS ONE 8:e63091

Briand J-F, Leboulanger C, Humbert J-F et al (2004) *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: selection, wide physiological tolerance, or global warming? J Phycol 40:231–238. doi:10.1111/j.1529-8817.2004.03118.x

Burford MA, Beardall J, Willis A et al (2016) Understanding the winning strategies used by the bloom-forming cyanobacterium *Cylindrospermopsis raciborskii*. Harmful Algae 54:44–53

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York

Chislock MF, Sharp KL, Wilson AE (2014) *Cylindrospermopsis raciborskii* dominates under very low and high nitrogen-to-phosphorus ratios. Water Res 49:207–214. doi:10.1016/j.watres.2013.11.022

da Costa SM, Ferrão-Filho ADS, Azevedo SM (2013) Effects of saxitoxin- and non-saxitoxin-producing strains of the cyanobacterium *Cylindrospermopsis raciborskii* on the fitness of temperate and tropical cladocerans. Harmful Algae 28:55–63

de Stasio BT, Hill DK, Kleinhans JM, Nibbelink NP (1996) Potential effects of global climate change on small north-temperate lakes: physics, fish, and plankton. Limnol Oceanogr 41:1136–1149

Drake JA, Huxel GR, Hewitt CL (1996) Microcosms as models for generating and testing community theory. Ecology 77:670–677

Figueredo CC, Giani A, Bird DF (2007) Does allelopathy contribute to *Cylindrospermopsis raciborskii* (Cyanobacteria) bloom occurrence and geographic expansion? J Phycol 43:256–265. doi:10.1111/j.1529-8817.2007.00333.x

Grover JP (1988) Dynamics of competition in a variable environment: experiments with two diatom species. Ecology 69:408–417

Grover JP (1991) Dynamics of competition among microalgae in variable environments: experimental tests of alternative models. Oikos 62:231–243. doi:10.2307/3545269

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chantey MH (eds) Culture of Marine Invertebrate Animals. Plenum Press, New York, pp 29–60

Hawkins PR, Runnegar MTC, Jackson ARB, Falconer IR (1985) Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Appl Environ Microbiol 50:1292–1295

Heisler J, Gilbert PM, Burkholder JM et al (2008) Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8:3–13

Isvánovics V, Shafik HM, Présing M, Juhos S (2000) Growth and phosphate uptake kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in throughflow cultures. Freshw Biol 43:257–275. doi:10.1046/j.1365-2427.2000.00549.x

Kehoe M, Brien KRO, Grinham A, Burford MA (2015) Primary production of lake phytoplankton, dominated by the cyanobacterium *Cylindrospermopsis raciborskii*, in

response to irradiance and temperature. Inland Waters 5:93–100. doi:10.5268/IW-5.2.778

Kosten S, Huszar VLM, Bécares E et al (2012) Warmer climates boost cyanobacterial dominance in shallow lakes. Glob Chang Biol 18:118–126. doi:10.1111/j.1365-2486.2011.02488.x

Litchman E (2010) Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. Ecol Lett 13:1560–1572. doi:10.1111/j.1461-0248.2010.01544.x

Marinho MM, Souza MBG, Lürling M (2013) Light and phosphate competition between *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* is strain dependent. Microb Ecol 66:479–488. doi:10.1007/s00248-013-0232-1

Moisander PH, Cheshire LA, Braddy J et al (2012) Facultative diazotrophy increases *Cylindrospermopsis raciborskii* competitiveness under fluctuating nitrogen availability. FEMS Microbiol Ecol 79:800–811. doi:10.1111/j.1574-6941.2011.01264.x

Padisák J (1997) *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. Arch für Hydrobiol Suppl Monogr Beiträge 107:563–593

Paerl HW, Huisman J (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ Microbiol Rep 1:27–37. doi:10.1111/j.1758-2229.2008.00004.x

Posselt AJ, Burford MA, Shaw G (2009) Pulses of phosphate promote dominance of the toxic cyanophyte *Cylindrospermopsis raciborskii* in a subtropical water reservoir. J Phycol 45:540–546. doi:10.1111/j.1529-8817.2009.00675.x

R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reay DS, Nedwell DB, Priddle J, Ellis-Evans JC (1999) Temperature dependence of inorganic nitrogen uptake: reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Appl Environ Microbiol 65:2577–2584

Rzymski P, Poniedziałek B, Kokociński M et al (2014) Interspecific allelopathy in cyanobacteria: cylindrospermopsin and *Cylindrospermopsis raciborskii* effect on the growth and metabolism of Microcystis aeruginosa. Harmful Algae 35:1–8. doi:10.1016/j.hal.2014.03.002

Shafik HM, Herodek S, Présing M, Vörös L (2001) Factors effecting growth and cell composition of cyanoprokaryote *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenayya et Subba Raju. Arch für Hydrobiol Suppl Algol Stud 140:75–93

Sinha R, Pearson LA, Davis TW et al (2012) Increased incidence of *Cylindrospermopsis raciborskii* in temperate zones—is climate change responsible? Water Res 46:1408–1419. doi:10.1016/j.watres.2011.12.019

Smith VH, Foster BL, Grover JP et al (2005) Phytoplankton species richness scales consistently from laboratory microcosms to the world's oceans. PNAS 102:4393–4396

Soares MCS, Lürling M, Huszar VLM (2013) Growth and temperature-related phenotypic plasticity in the cyanobacterium *Cylindrospermopsis raciborskii*. Phycol Res 61:61–67. doi:10.1111/pre.12001

Sperfeld E, Schmidtke A, Gaedke U, Weithoff G (2010) Productivity, herbivory, and species traits rather than diversity

influence invasibility of experimental phytoplankton communities. Oecologia 163:997–1010. doi:10.1007/s00442-010-1594-4

Thomas MK, Litchman E (2016) Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria. Hydrobiologia 763:357–369. doi:10.1007/s10750-015-2390-2

Thomas MK, Kremer CT, Klausmeier CA, Litchman E (2012) A global pattern of thermal adaptation in marine phytoplankton. Science 338:1085–1088

Tilman D (1977) Resource competition between plankton algae: an experimental and theoretical approach. Ecology 58:338. doi:10.2307/1935608

Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton

Tilman D, Kiesling RL (1984) Freshwater algal ecology: taxonomic trade-offs in the temperature dependence of nutrient competitive abilities. In: Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology: proceedings of the third international symposium on microbial ecology. American Society for Microbiology, Washington, DC, USA, pp 314–319

Tilman D, Mattson M, Langer S (1981) Competition and nutrient kinetics along a temperature gradient: an experimental test of a mechanistic approach to niche theory. Limnol Oceanogr 26:1020–1033

Wiedner C, Rücker J, Brüggemann R, Nixdorf B (2007) Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. Oecologia 152:473–484. doi:10.1007/S00442-007-0683-5

Willis A, Adams MP, Chuang AW et al (2015) Constitutive toxin production under various nitrogen and phosphorus regimes of three ecotypes of *Cylindrospermopsis raciborskii* ((Wołoszyńska) Seenayya et Subba Raju). Harmful Algae 47:27–34. doi:10.1016/j.hal.2015.05.011

Wu Z, Shi J, Li R (2009) Comparative studies on photosynthesis and phosphate metabolism of *Cylindrospermopsis raciborskii* with *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. Harmful Algae 8:910–915. doi:10.1016/j.hal.2009.05.002

Wu Z, Zeng B, Li R, Song L (2012a) Combined effects of carbon and phosphorus levels on the invasive cyanobacterium, *Cylindrospermopsis raciborskii*. Phycologia 51:144–150. doi:10.2216/10.87.1

Wu Z, Zeng B, Li R, Song L (2012b) Physiological regulation of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) in response to inorganic phosphorus limitation. Harmful Algae 15:53–58. doi:10.1016/j.hal.2011.11.005