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NOTE

Weak effect of temperature fluctuations on the invasion of *Raphidiopsis raciborskii* (Cyanobacteria) in experimental plankton microcosms

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Abstract

Biological invasions are a major threat for many aquatic ecosystems. In contrast to higher plants and animals, microbial invasions are less obvious and more difficult to detect. One of the most prominent microbial invaders is the cyanobacterium *Raphidiopsis raciborskii*. To better understand the environmental conditions favoring its invasion success, we studied invasion under three different temperature regimes (one constant and two variable) in experimental plankton communities by invader addition experiments. To account for intraspecific variation, we tested four different strains of *R. raciborskii* and the mixture of them. Invasion success of *R. raciborskii* was higher under constant temperature conditions than under fluctuations suggesting that the resident species responded faster to the environmental changes than the invaders. We observed a clear strain-specific effect, demonstrating that strain identity is an important determinant of invasion success. The interaction of temperature fluctuations and strain identity indicates that, among the tested strains, the response to the temperature regimes varied. The mixture of all four strains did not perform better than the best single strain showing no sign of a positive genetic diversity effect. In our experiment, environmental fluctuations did not widen a window of opportunity for the invasion of *R. raciborskii*.

KEYWORDS

biological invasion, *Cephalodella*, chlorophyta, cyanobacteria, *Cylindrospermopsis*, environmental fluctuation, *Raphidiopsis raciborskii*, temperature

Invasive species cause manifold environmental problems and economic costs in their newly occupied habitats (Sala et al., 2000). Therefore, it is of crucial importance to understand the invasion process of spreading species. In contrast to larger animals or plants, microbial invasions are typically less visible, and their invasion process is much more difficult to observe (Litchman, 2010). In addition, a general problem in invasion biology is that, in most cases, invasions are viewed retrospectively, that is, after alien species have successfully entered a new area. Experimental studies on invasions are comparably rare and in many habitats impossible to conduct (Jeschke & Heger, 2018). Some factors that facilitate invasions are environmental changes in the new habitat that open a window of opportunity. These can be perturbations such as nutrient pulses or temperature fluctuations. Compared to the number of invasive macroalgae, relatively few invasive microalgae have been detected. A very prominent one is the cyanobacterium *Raphidiopsis*

Abbreviations: GLM, general linear model; PAR, photosynthetic active radiation.

raciborskii (Padisák, 1997; Sukenik et al., 2012), formerly *Cylindrospermopsis raciborskii* (Aguilera et al., 2018), which is one of two bacteria that were identified among the 100 most threatening invasive species in northern Eurasia (Petrosyan et al., 2023). In many studies, traits were investigated that proved to play a potential role in its invasion success, such as resource use (particularly phosphorus), temperature preference, grazing resistance, and life cycle (e.g., Burford et al., 2016; Istvánovics et al., 2000; Panosso & Lürling, 2010; Soares et al., 2013; Wiedner et al., 2007). Studies on the invasion process in a community context are rather rare (but see Bolius et al., 2019, 2020).

In the present study, we experimentally investigated the invasion of *Raphidiopsis raciborskii* into resident plankton communities consisting of four common phytoplankton species and one microconsumer, the rotifer *Cephalodella* sp., under different temperature regimes. We tested four genetically different isolates alone and in mixture in order to evaluate the effect of genetic identity on the invasion success at constant and fluctuating temperatures. We hypothesized that fluctuating temperatures impact the invasion of *R. raciborskii* and that it is strain-specific. We used four ecologically and genetically different strains of *R. raciborskii* (Mel 07, Zie 11, 26D9, and 27F11) originating from lakes in NE Germany (Bolius et al., 2017) as single invaders and as a mixture. These four strains were chosen because they cover the full range of invasiveness among 10 strains that had been tested in a previous study in different experimental conditions (Bolius et al., 2020). For the resident community, we used the chlorophytes *Monoraphidium minutum* (Göttinger Culture Collection, SAG 243-1), *Chlorella vulgaris* (SAG 211-11b), and *Chlamydomonas reinhardtii* (SAG 11-32b) and the cryptophyte *Cryptomonas* sp. (SAG 26.80). All species are common members of lake phytoplankton communities. *Monoraphidium minutum* and *C. vulgaris* were chosen as representatives of the species-rich group of small coccal chlorophytes, and *Cryptomonas* sp. was chosen because it is known that species from this genus can respond quickly to changing environmental conditions (Stewart & Wetzel, 1986; Weithoff et al., 2000). *Chlamydomonas reinhardtii* was also the food for the consumer stock cultures (see below); thus, a co-transfer of some food algae along with the transfer of the consumer *Cephalodella* sp. did not contaminate the microcosms. In order to allow for nutrient recycling during the experiment, we added the algivorous, microphagous rotifer *Cephalodella* sp. (Seifert et al., 2015), which can feed on all resident algae but cannot ingest filamentous cyanobacteria. It was isolated from a small pond in New Jersey, USA (Altermatt et al., 2011), and has been cultured in the lab for many years. Stock cultures were diluted weekly and fed with *Chlamydomonas reinhardtii*. All stock cultures of the algal species and *Cephalodella* sp. were kept at 20°C in a modified WC medium (Nichols, 1973) at a pH of 8 and a light:-dark cycle of 16:8 h at 85 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} photosynthetically active radiation (PAR).

Prior to the experiment, all four residents and *Raphidiopsis raciborskii* were transferred to a WC medium with reduced nutrient concentrations of 80 μg $\cdot$ L^{-1} phosphorus as K_2HPO_4 and 580 μg $\cdot$ L^{-1} nitrogen as $NaNO_3$ resulting in a molar nitrogen to phosphorus ratio of 16:1 (Redfield ratio). This ratio provides a balanced supply of both nutrients, imposing neither nitrogen nor phosphorus limitation, and reflects moderate eutrophic lake conditions. Thus, neither the residents nor the diazotrophic *R. raciborskii* were particularly favored by this nutrient ratio. Except for the vitamins, the medium is free of organic carbon allowing almost exclusively autotrophic growth of the algae.

The experiment was set up by inoculating 150 mL of medium in 300-mL Erlenmeyer flasks with 0.5 mm^3 $\cdot$ L^{-1} biovolume of each resident species and 15 individuals $\cdot$ mL^{-1} of the consumer. The experimental conditions were the same as for the stock cultures. A light intensity of 85 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} is in the range of the light intensity in the upper 1–3 m of a moderately turbid lake on a sunny day, and it is in the range or below the half saturating light intensity for the residents (Gervais, 1997; Soto-Ramírez et al., 2021; Vejrazka et al., 2013) and for *Raphidiopsis raciborskii* (Mehnert et al., 2010). Thus, all species had good growth conditions during the experiment. We set up four replicates each, amounting for a total of 60 microcosms (four single strains plus one mixture × three temperature treatments × four replicates). Six days later, the invader *R. raciborskii* was added with a biovolume of 0.2 mm^3 $\cdot$ L^{-1} for the single-strain invasions and with 0.05 mm^3 $\cdot$ L^{-1} of each strain for the four-strain invasion experiment. Thus, to all resident communities, the same total amount of invaders was added. Three temperature treatments were run: a constant temperature of 20°C and two treatments with variable temperatures (changed every 2 days) either by first reducing the temperature by 3°C (20° ➡ 17° ➡ 20° ➡ 23° ➡ 20°C, and so on) or by first increasing the temperature by 3°C (20° ➡ 23° ➡ 20° ➡ 17° ➡ 20°C, and so on). Thus, at the time point of the invasion, the temperatures were increasing to 23°C, decreasing to 17°C, or had remained constant at 20°C. Temperature changes in the upper water layer of a shallow lake of 1°C are possible diurnally or via weather changes. Thus, 3°C changes in temperature within 2 days are high but not unrealistic. Every 2 days, 20% of the experimental volume (30 mL) was replaced by fresh medium. The experiment was run in climate-controlled incubation shakers (Infors HT Minitron, Bottmingen Switzerland) with continuous moderate shaking at 36 rounds $\cdot$ min^{-1} to avoid sedimentation. The experiment was terminated after three temperature cycles. The samples were fixed with Lugol's iodine, and the final biovolume of all algae (including the cyanobacterial invader) was determined by the classical Utermöhl technique using inverted light microscopy (Thalheim Spezialoptik, Pulsnitz, Germany). For the resident species, cells were counted, and the number of cells per liter was multiplied by the cell volume. Cell volume was determined by measuring length and width for *Chlamydomonas reinhardtii* and *Cryptomonas* sp. and diameter for *Chlorella vulgaris* and assuming rotation ellipsoids and spheres, respectively, as geometric shapes for volume calculations. For half-moon shaped *Monoraphidium minutum*, a fixed cell volume of 30 μm^3 was used based on previous volume measurements using an electronic particle counter (CASY, Schärfe). For *R. raciborskii*, the cumulative filament length was determined and the width of 40 filaments per treatment was measured and used for calculating the total biovolume assuming a cylindrical shape. In addition, the number of the consumer *Cephalodella* sp. was also quantified by using inverted light microscopy.

For comparing the single-invader treatments with the four-strain mixture, we calculated the invasion yield (Bolius et al., 2020). The invasion yield is the ratio of the invader biovolume of the four-strain mixture to the mean of the invader biovolume from the four single-strain invasions. In comparison to biodiversity ecosystem function experiments, a higher biovolume of the four-strain mixture than the biovolume of the most successful single invader is called a transgressive invasion yield, and when it is between the highest and the mean of the single invaders, it is called non-transgressive invasion yield. This measure allows to evaluate whether a community of multiple genotypes entering a new habitat is more successful than single genotypes. We applied a generalized linear model (GLM) for testing for differences in mean relative (arcsin square-root transformed) and absolute invader biovolume with strain and treatment as factors and consumer abundance and resident biovolume as co-variates. All statistical calculations were done with SPSS Version 29.

Overall, *Raphidiopsis raciborskii* was observed in all microcosms at the end of the experiment; however, in low absolute biovolume, *Monoraphidium minutum* was by far the dominant species, amounting for, on average, 94.9% (min. 92.1%, max 96.6%) of the total biovolume. The other species had a mean biovolume over all treatments of 2.1% (*Cryptomonas* sp.), 1.1% (*Chlorella vulgaris* and *Chlamydomonas reinhardtii*), and 0.8% (*R. raciborskii*; for raw data, see File S1). Experimental studies have shown that chlorophytes are strong competitors with cyanobacteria either because of high individual growth rates (Lürling et al., 2013) or the community context (Bolius et al., 2019). Despite the low relative share of *R. raciborskii*, the absolute biovolume suggested a successful invasion. Starting with a biovolume of 0.2 $mm^3 \cdot L^{-1}$, after 10 dilutions by 20%, the remaining biovolume with neither growth nor mortality would be 0.017 $mm^3 \cdot L^{-1}$, but *R. raciborskii* had an average biovolume of 0.023 $mm^3 \cdot L^{-1}$, demonstrating a slight net positive growth. For an invasive species, maintaining even a small net positive growth is key for it to persist in a new environment and have the chance to proliferate later when conditions better match the invader's optima. Such a lag phase, lasting for several generations, has often been observed for invasive species (Lockwood et al., 2012). In addition, we observed a treatment and a strain-specific effect (Table 1), an interaction between the two factors, and an effect of total resident biovolume and consumer abundance on the relative amount of *R. raciborskii* (Table 1).

The treatment effect demonstrated that temperature fluctuations have an impact on the invasibility of *Raphidiopsis raciborskii*. Interestingly, at constant temperatures, invasion success was highest (Figure 1). This result suggests that the resident species responded faster to the temperature changes than the invading cyanobacterium, thereby narrowing the window of opportunity for *R. raciborskii*. The tested temperatures were in a range typically below the optimum temperature for many nostocalean cyanobacteria (Mehnert et al., 2010), mimicking early summer temperatures in invaded temperate regions. In many lakes, summer conditions that influence recruitment from akinetes (Wiedner et al., 2007), water column stability (Weithoff et al., 2001), and resource use of the actual phytoplankton community (Sommer et al., 1996) are decisive for the potential of later cyanobacterial blooms. In many temperate lakes, stable summer conditions favor cyanobacterial growth, whereas intermitted mixing often supports growth of chlorophytes or cryptophytes (Steinberg, 1983; Weithoff et al., 2001). Thus, environmental fluctuations did not widen a window of opportunity for *R. raciborskii* invasion in our experiment.

The strain-specific effect showed that different strains vary in their invasion success. These results are partly in line with a previous study in which the strain 26D9 had the lowest success among 10 tested strains and had also the lowest biovolume of all single-strain treatments in this study (Bolius et al., 2020). The other three strains varied in their biovolume among the treatments, which is underlined by the statistical significance of the strain × treatment effect (Figure 1, Table 1). This result suggested differences in temperature performance curves among strains. The mixture of all four strains achieved an intermediate biovolume as compared to single-strain treatments, that is, the mixture had never a higher biovolume than the most successful single strain. In all three treatments, the mixture biovolume was close to the mean biovolume of the four single strains. Thus, the four-strain mixture showed no clear sign of an increased invasion yield (Figure 2). This result showed that the invader identity was a better determinant for invasion success than diversity (Bolius et al., 2020; Weithoff et al., 2017). Within one sample from one lake, many different strains

TABLE 1 Analysis from the GLM with absolute abundance of *Raphidiopsis raciborskii* (*Raphidiopsis*) and arcsin square-root transformed relative abundance of *R. raciborskii* (RelTrans) as dependent variables.

Tests of between-subjects effects						
Source	**Dependent variable**	**Type III sum of squares**	***df***	**Mean square**	***F***	***p***
Corrected model	*Raphidiopsis*	0.006	16.43	3.758-E4	26.228	<0.001
	RelTrans	10.337	16.43	0.646	25.852	<0.001
Intercept	*Raphidiopsis*	1.742-E-4	1.59	1.742-E-4	12.157	0.001
	RelTrans	3.415	1.59	3.415	136.668	<0.001
Residents	*Raphidiopsis*	1.831E−5	1.59	1.831E−5	1.278	0.265
	RelTrans	2.707	1.59	2.707	108.311	<0.001
Cephalodella	*Raphidiopsis*	1.961E−6	1.59	1.961E−6	0.137	0.713
	RelTrans	0.204	1.59	0.204	8.170	0.007
Strain	*Raphidiopsis*	0.002	4.43	4.452-E4	31.074	<0.001
	RelTrans	1.786	4.43	0.446	17.862	<0.001
Temp	*Raphidiopsis*	0.003	2.43	0.001	87.873	<0.001
	RelTrans	1.090	2.43	0.545	21.801	<0.001
Strain * Temp	*Raphidiopsis*	0.001	8.43	3.152-E4	9.435	<0.001
	RelTrans	1.283	8.43	0.160	6.418	<0.001
Error	*Raphidiopsis*	0.001	43	1.433E−5		
	RelTrans	1.075	43	0.025		
Total	*Raphidiopsis*	0.041	60			
	RelTrans	49.336	60			
Corrected total	*Raphidiopsis*	0.007	59			
	RelTrans	11.412	59			

Note: Temperature treatments (Temp) and strain identity (strain) are fixed factors and total resident biovolume (Residents) and *Cephalodella* abundance (*Cephalodella*) are co-variates.

of *Raphidiopsis raciborskii* can be observed: Willis et al. (2016, 2018) described strains that differed in morphology, physiology, and genomics, underlining a high local genetic variability among *R. raciborskii*, a diversity which was also observed in Bolius et al. (2017).

The co-variates total resident biovolume and consumer abundance varied among all replicates by a factor of 2.9 and 2.5 (see File S1), respectively, and they had a significant effect only on relative biovolume of *Raphidiopsis raciborskii*, not on the absolute biovolume (Table 1). This result suggests that both co-variates only fine-tuned the outcome of the experiment whereas the fixed factors of temperature treatment and strain identity were the relevant driving forces for the absolute invasion success.

In this study, we focused on temperature variation and strain identity/diversity on invasion success. Other factors, such as the composition of the resident community and the abiotic environment, can also impact an invasion. The resident community can directly reduce or prevent the invasion of *Raphidiopsis raciborskii* when superior competitors are present. Strong competitors are, for example, chlorophytes (Bolius et al., 2019; Lürling et al., 2013) or ecologically similar species, for example, other Nostocales, such as *Anabaena* spp. and *Aphanizomenon* spp. An indirect effect of the resident community was observed in a similar invader-addition experiment, in which the nutrient concentration and the resident diversity were manipulated. When the generalist consumer that also fed upon *R. raciborskii* could proliferate due to high algal productivity, *R. raciborskii* was suppressed, and the fast-growing *Cryptomonas* sp. (the second invader in that experiment) could invade the community. When the inedible chlorophyte *Ankistrodesmus acutus* dominated the community, the consumer was suppressed and *R. raciborskii* successfully invaded (Sperfeld et al., 2010). In the present study, *Monoraphidium minutum* escaped from top-down control and dominated the community. Nutrient concentrations and, in particular, the ratio of nitrogen to phosphorus can also influence the invasion of *R. raciborskii*. A low nitrogen to phosphorus supply ratio (<<16:1, molar) imposes nitrogen limitation on the phytoplankton community and only diazotrophic cyanobacteria can overcome this limitation. High ratios (nitrogen to phosphorus, >>16:1) typically favor non-nitrogen-fixing cyanobacteria and eukaryotic algae. In the present study, we provided nitrogen and phosphorus in a balanced supply at the Redfield ratio of 16:1, which specifically favored neither the residents nor the invader. Thus, we could show that

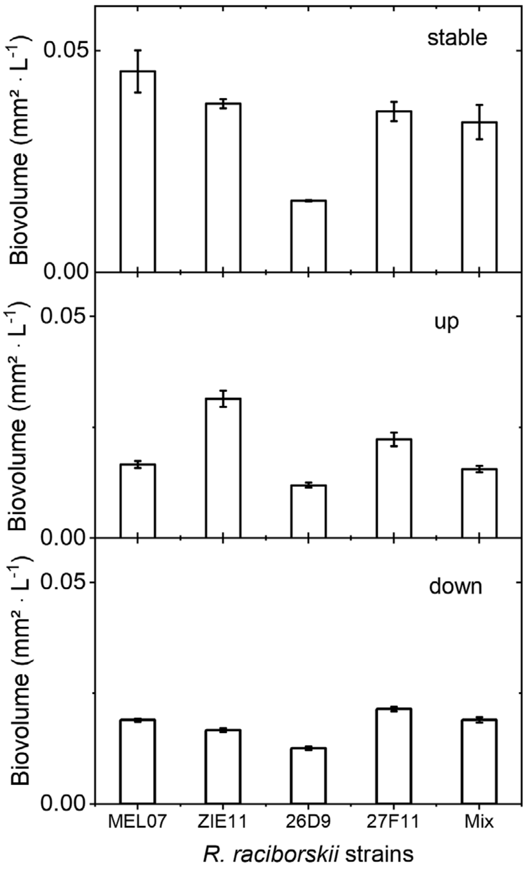

FIGURE 1 Absolute biovolume of the invader *Raphidiopsis raciborskii* into experimental plankton communities. Mel07, ZIE11, 26D9, and 27F11 denote four genetically different strains; mix denotes the mixture of all the four strains. Error bars denote standard error.

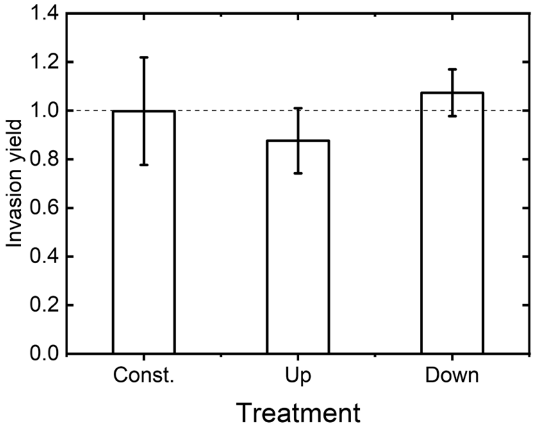

FIGURE 2 Invasion yield of *Raphidiopsis raciborskii* at three temperature regimes, stable at 20°C (top), increasing from 20°C (middle), and decreasing from 20°C (bottom). The horizontal line represents an invasion yield of one, that is, the mixture has reached the same biovolume as the mean biovolume of the four strains in the single strain treatments. Error bars denote 95% confidence interval.

R. raciborskii was able to invade resident plankton communities at our environmental conditions, and the strain identity is the main driving factor.

AUTHOR CONTRIBUTIONS

Guntram Weithoff: Conceptualization (lead); formal analysis (equal); project administration (lead); resources (lead); writing – original draft (lead). **Marley B. Stefan:** Formal analysis (equal); investigation (lead); writing – original draft (supporting).

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REFERENCES

Aguilera, A., Gómez, E. B., Kaštovský, J., Echenique, R. O., & Salerno, G. L. (2018). The polyphasic analysis of two native *Raphidiopsis* isolates supports the unification of the genera *Raphidiopsis* and *Cylindrospermopsis* (Nostocales, Cyanobacteria). *Phycologia*, *57*, 130–146. https://doi.org/10.2216/17-2.1

Altermatt, F., Schreiber, S., & Holyoak, M. (2011). Interactive effects of disturbance and dispersal directionality on species richness and composition in metacommunities. *Ecology*, *92*, 859–870.

Bolius, S., Morling, K., Wiedner, C., & Weithoff, G. (2020). Genetic identity and herbivory drive the invasion of a common aquatic microbial invader. *Frontiers in Microbiology*, *11*, 1598. https://doi.org/10.3389/fmicb.2020.01598

Bolius, S., Wiedner, C., & Weithoff, G. (2017). High local trait variation of a globally invasive cyanobacterium. *Freshwater Biology*, *62*, 1879–1890. https://doi.org/10.1111/fwb.13028

Bolius, S., Wiedner, C., & Weithoff, G. (2019). Low invasion success of an invasive cyanobacterium in a chlorophyte dominated lake. *Scientific Reports*, *9*, 8297. https://doi.org/10.1038/s41598-019-44737-8

Burford, M. A., Beardall, J., Willis, A., Orr, P. T., Magalhaes, V. F., Rangel, L. M., Azevedo, S. M., & Neilan, B. A. (2016). Understanding the winning strategies used by the bloom-forming cyanobacterium *Cylindrospermopsis raciborskii*. *Harmful Algae*, *54*, 44–53.

Gervais, F. (1997). Light-dependent growth, dark survival and glucose uptake by cryptophytes isolated from a freshwater chemocline. *Journal of Phycology*, *33*, 18–25.

Istvánovics, V., Shafik, H. H., Présing, M., & Juhos, S. (2000). Growth and phosphate uptake kinetics of the cyanobacterium *Cylindrospermopsis raciborskii* (Cyanophyceae) in through-flow cultures. *Freshwater Biology*, *43*, 257–275. https://doi.org/10.1046/j.1365-2427.2000.00549.x

Jeschke, J. M., & Heger, T. (2018). *Invasion biology: Hypotheses and evidence*. CABI.

Litchman, E. (2010). Invisible invaders: Non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. *Ecology Letters*, *13*, 1560–1572. https://doi.org/10.1111/j.1461-0248.2010.01544.x

Lockwood, J. L., Hoopes, M. F., & Marchetti, M. P. (2012). *Invasion ecology* (2nd ed.). Wiley-Blackwell.

Lürling, M., Eshetu, F., Faassen, E. J., Kosten, S., & Huszar, V. L. M. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology*, *58*, 552–559. https://doi.org/10.1111/j.1365-2427.2012.02866.x

Mehnert, G., Leunert, F., Cirés, S., Jöhnk, K. D., Rücker, J., Nixdorf, B., & Wiedner, C. (2010). Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. *Journal of Plankton Research*, *32*, 1009–1021. https://doi.org/10.1093/plankt/fbq033

Nichols, H. (1973). Growth media – Freshwater. In J. R. Stein (Ed.), *Handbook of phycological methods: Culture methods and growth measurements* (pp. 7–24). Cambridge University Press.

Padisák, J. (1997). *Cylindrospermopsis raciborskii* (Woloszynska) Seenayy et Subba Raju, an expanding highly adaptive cyanobacterium: World-wide distribution and review of its ecology. *Archiv für Hydrobiologie Supplementband Monographische Beiträge*, *107*, 563–593.

Panosso, R., & Lürling, M. (2010). *Daphnia magna* feeding *Cylindrospermopsis raciborskii*: The role of food composition, filament length and body size. *Journal of Plankton Research*, *32*, 1393–1404. https://doi.org/10.1093/plankt/fbq057

Petrosyan, P., Osipov, F., Feniova, I., Dergunova, N., Warshavsky, A., Khlyap, L., & Dzialowski, A. (2023). The TOP-100 most dangerous invasive alien species in northern Eurasia: Invasion trends and species distribution modelling. *Neobiota*, *82*, 23–56. https://doi.org/10.3897/neobiota.82.96282

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T., Walker, B. H., Walker, M., & Wall, D. H. (2000). Global biodiversity scenarios for the year 2100. *Science*, *287*, 1770–1774.

Seifert, L. I., Weithoff, G., Gaedke, U., & Vos, M. (2015). Extreme heat changes post-heat wave community reassembly. *Ecology and Evolution*, *5*, 2140–2148. https://doi.org/10.1002/ece3.1490

Soares, M. C. S., Lürling, M., & Huszar, V. L. M. (2013). Growth and temperature-related phenotypic plasticity in the cyanobacterium *Cylindrospermopsis raciborskii*. *Phycological Research*, *61*, 61–67. https://doi.org/10.1111/pre.12001

Sommer, U., Gliwicz, M., Lampert, W., & Duncan, A. (1996). The PEG-model of seasonal succession of planktonic events in fresh waters. *Archiv für Hydrobiologie*, *106*, 433–471.

Soto-Ramírez, R., Tavernini, L., Zúniga, H., & Poirrier, P. (2021). Study of microalgal behaviour in continuous culture using photosynthetic rate curves: The case of chlorophyll and carotenoid production by *Chlorella vulgaris*. *Aquaculture Research*, *52*, 3639–3648.

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.

Steinberg, C. (1983). Effect of artificial destratification on the phytoplankton populations in a small lake. *Journal of Plankton Research*, *5*, 855–864.

Stewart, A. J., & Wetzel, R. G. (1986). Cryptophytes and other microflagellates as couplers in planktonic community dynamics. *Archiv für Hydrobiologie*, *106*, 1–19.

Sukenik, A., Hadas, O., Kaplan, A., & Queseda, A. (2012). Invasion of Nostocales (Cyanobacteria) to subtropical and temperate freshwater lakes – Physiological, regional and global driving forces. *Frontiers in Microbiology*, *3*, 86. https://doi.org/10.3389/fmicb.2012.00086

Vejrazka, C., Janssen, M., Benvenuti, G., Streefland, M., & Wijffels, R. H. (2013). Photosynthetic efficiency and oxygen evolution of *Chlamydomonas reinhardtii* under continuous and flashing light. *Applied Microbiology and Biotechnology*, *97*, 1523–1532.

Weithoff, G., Lorke, A., & Walz, N. (2000). Effects of water-column mixing on bacteria, phytoplankton and rotifers under different levels of herbivory in a shallow eutrophic lake. *Oecologia*, *125*, 91–100.

Weithoff, G., Taube, A., & Bolius, S. (2017). The invasion success of the cyanobacterium *Cylindrospermopsis raciborskii* in experimental mesocosms: Genetic identity, grazing loss, competition and biotic resistance. *Aquatic Invasions*, *12*, 333–341. https://doi.org/10.3391/ai.2017.12.3.07

Weithoff, G., Walz, N., & Gaedke, U. (2001). The intermediate disturbance hypothesis – Species diversity or functional diversity? *Journal of Plankton Research*, *23*, 1147–1155.

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. https://doi.org/10.1007/s00442-007-0683-5

Willis, A., Chuang, A. W., Woodhouse, J. N., Neilan, B. A., & Burford, M. A. (2016). Intraspecific variation in growth, morphology and toxin quotas for the cyanobacterium, *Cylindrospermopsis raciborskii*. *Toxicon*, *119*, 307–310.

Willis, A., Woodhouse, J. N., Ongley, S. E., Jex, A. R., Burford, M. A., & Neilan, B. A. (2018). Genome variation in nine co-occurring toxic *Cylindrospermopsis raciborskii* strains. *Harmful Algae*, *73*, 157–166.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

File S1 Data from the invasion experiment. Biovolume of the individual resident species (*Monoraphidium minutum, Chlorella vulgaris, Cryptomonas* sp., *Chlamydomonas reinhardtii*), their sum (Sum of residents), and the invader *Raphidiopsis raciborskii*, its relative biovolume, and its transformed percentages (arcsin (sqrt (%))) and consumer abundance (*Cephalodella* sp.). Temp refers to temperature treatment for constant temperature (Temp), first increasing temperatures (up) and first decreasing temperature (down).

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