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Direct Effects of Temperature on Growth of Different Tropical Phytoplankton Species

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Abstract

Temperature increase may influence competition among phytoplankton species, potentially intensifying cyanobacteria blooms that can be favored by direct and indirect effects of temperature. In this study, we aimed to clarify how cyanobacteria can be favored by the direct effects of increased temperature compared to diatoms and chlorophytes. Strains of the most representative species of a eutrophic coastal lagoon (*Microcystis aeruginosa*, *Planktothrix agardhii*, *Desmodesmus communis*, and *Cyclotella meneghiniana*) were used to test the hypothesis that cyanobacteria would be favored by the direct effect of temperature increase. First, we evaluated the effect of temperature increase on growth in monocultures (batch and chemostats) at 25 and 30 °C and after in mixed cultures (chemostats). In batch monocultures, the cyanobacteria showed higher growth rates in 30 °C than in 25 °C. However, in continuous culture experiments (chemostats), growth rates of *M. aeruginosa* and *P. agardhii* were not affected by temperature, but the strains showed higher biovolume in steady-state with the temperature increase. In continuous mixed cultures, *M. aeruginosa* was always dominant and *C. meneghiniana* was excluded, regardless of temperature tested. *D. communis* was able to coexist with lower biomass. This study shows that rising temperatures can be detrimental to diatoms, even for a tropical strain. Although some studies indicate that the dominance of cyanobacteria in warmer climates may be due to the indirect effect of warming that will promote physical conditions in the environment more favorable to cyanobacteria, the outcomes of mixed cultures demonstrate that the direct effect of temperature can also favor the dominance of cyanobacteria.

Keywords Cyanobacteria · Interspecific variability · Batch cultures · Mixed cultures · Chemostats

Introduction

Climate changes can enhance eutrophication, one of the major environmental problems in freshwater lakes around the world [1, 2]. The expected changes will result in an increase in nutrient levels and temperature and it may strongly influence the aquatic ecosystems [3, 4]. Temperature increase can promote a profound impact on phytoplankton affecting its physiology and primary production, which will lead to a change in the community structure [2, 5, 6].

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The temperature may influence competition among phytoplankton species [7, 8] once it affects their growth, directly influencing the metabolic processes related to photosynthesis and biosynthesis [9]. Temperature effects can also be indirect, as increasing temperature affects the stratification of the water column, reducing vertical mixing and enhancing nutrient efflux from the sediment [2, 10, 11]. Besides, temperature affects organic compounds produced by competitors (allelopathic substances), interfering in competition [7, 12] since they influence metabolism, photosynthesis regulation, and interfere in the cell-cell communication processes [13–15]. All these factors can contribute to the increased occurrence, frequency, and duration of cyanobacterial blooms in several regions of the world [16–18].

Several studies point out that cyanobacteria will be favored by the direct effect of the temperature increase as they reach the highest growth rates above 25 °C [2, 19, 20]. This literature suggests that cyanobacteria have higher growth rates at higher temperatures than other eukaryotic microalgae [2, 10]. For example, some of the most common cyanobacteria

bloom-forming species—*Microcystis aeruginosa* and *Planktothrix agardhii*—show higher growth rates at temperatures above 25 °C [7, 21–24]. Instead, diatoms are mentioned as dominant in the phytoplankton community at lower water temperatures (18–21 °C) [7, 25, 26], and green algae, such as *Desmodesmus* spp., have a wide range of optimum temperature above 27 °C [21]. However, this idea has recently been questioned by a study that suggests the intensification of cyanobacteria blooms in a warming climate is attributed to the indirect effect of temperature, which would affect the mixing regime of the water, making it warmer and stratified [21]. Therefore, more detailed laboratory studies with cyanobacteria bloom species are necessary to better understanding how temperature increase would affect these organisms, as well as knowing the performance of other phytoplankton species living in the same habitat.

In this study, we aimed to clarify how cyanobacteria can be favored by the direct effects of increased temperature comparing to diatoms and green algae. We tested the hypothesis that cyanobacteria would be favored by the direct effect of temperature increase. To this end, we choose the most representative species (*Microcystis aeruginosa*, *Planktothrix agardhii*, *Desmodesmus communis*, and *Cyclotella meneghiniana*) of a eutrophic coastal lagoon in Brazilian southeast.

Methods

Organisms

Experiments were performed with two cyanobacteria—*Microcystis aeruginosa* (Kützing) Kützing (strain MIC-08) and *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek (strain Plank-09); one diatom—*Cyclotella meneghiniana* Kützing (strain Cyclo-01); and one green algae—*Desmodesmus communis* (E. Hegewald) E. Hegewald (strain DELJ-01), formerly *Scenedesmus quadricauda* (Turpin) Brébisson.

The species were isolated from a shallow tropical eutrophic coastal lagoon (Jacarepaguá Lagoon, Brazil, 22° 55′ S and 43° 17′ W) between 2009 and 2011 and were maintained in the culture collection of the Laboratory of Ecology and Physiology of Phytoplankton (LabAlgas), University of Rio de Janeiro State (UERJ). These species usually represent more than 90% of the phytoplankton biomass in this lagoon, where long-lasting blooms of *M. aeruginosa* are frequently observed [26]. *Microcystis aeruginosa* was grown as single cells, except in some mixed cultures where small colonies occurred. Species were not axenic, but regular microscopic inspection revealed that biomass of heterotrophic bacteria remained well under 1% of total biovolume. Both cyanobacteria species produce a variety of microcystins: *M. aeruginosa* (MIC-08) produces dmMC-LR and MC-LR, and *P. agardhii* (Plank-09), mMC-RR, MC-RR, and MC-YR (determined by LC-MS/MS as described in Lürling and Faassen [27]).

First, the direct effect of different temperatures on growth was evaluated in batch and continuous (chemostats) monocultures. After, all tested species were placed in mixed continuous cultures to test the effect of temperature increased on growth and nutrient competition in order to elucidate the dynamics of these species in relation to the environmental conditions of Jacarepaguá Lagoon.

Evaluation of the Effect of Temperature on Growth in Batch Monocultures

Before the beginning of the experiments, cultures were acclimated to each tested temperature for 10–15 days. *M. aeruginosa*, *P. agardhii*, *C. meneghiniana*, and *D. communis* species were set up as triplicate batch monocultures (inoculated at initial biomass of 5×10^4 μm^3 mL^{-1}) in Erlenmeyer flasks containing 100 mL of modified WC medium [28] at 25 °C and 30 °C (Fig. 1). Culture flasks were placed in incubators (SOLAB SL-224) under a light intensity of 60 μmol photons m^{-2} s^{-1} (provided by daylight fluorescent lamps with a photoperiod of 12:12 h light:dark cycle) and shaken twice a day. The lowest temperature (25 °C) tested was chosen based on the actual annual average temperature of surface water in the lagoon from where the species were isolated, and the highest temperature (30 °C) considered an increase of 5 °C to the annual mean predicted by IPCC [29] as a consequence of global warming. Batch monoculture experiment lasted 10 days, and growth was monitored by cell counts of samples taken on alternate days, using a Neubauer chamber. At least 400 individuals were counted (error < 10%; [30]). The biovolume (mm^3 L^{-1}) of each species was estimated from the product of the density population and mean cell volume of each species. The growth rates (r, day^{-1}) were estimated using a solution for the classic logistic growth model [31–34] through non-linear regression over biovolume against time.

$$N_t = \frac{N_0\, K}{N_0 + (k - N_0)e^{-rt}} \quad (1)$$

where N_t = final biovolume; t = time; N_0 = initial biovolume; k = carrying capacity, and r = growth rate.

Evaluation of the Effect of Temperature on Growth in Chemostat Monocultures

A second experiment was run in continuous culture systems (chemostats), performed in Kitasato flasks of 500 mL, with a culture volume of approximately 550 mL and a dilution rate of 0.30 day^{-1}. Light intensity and photoperiod were the same as

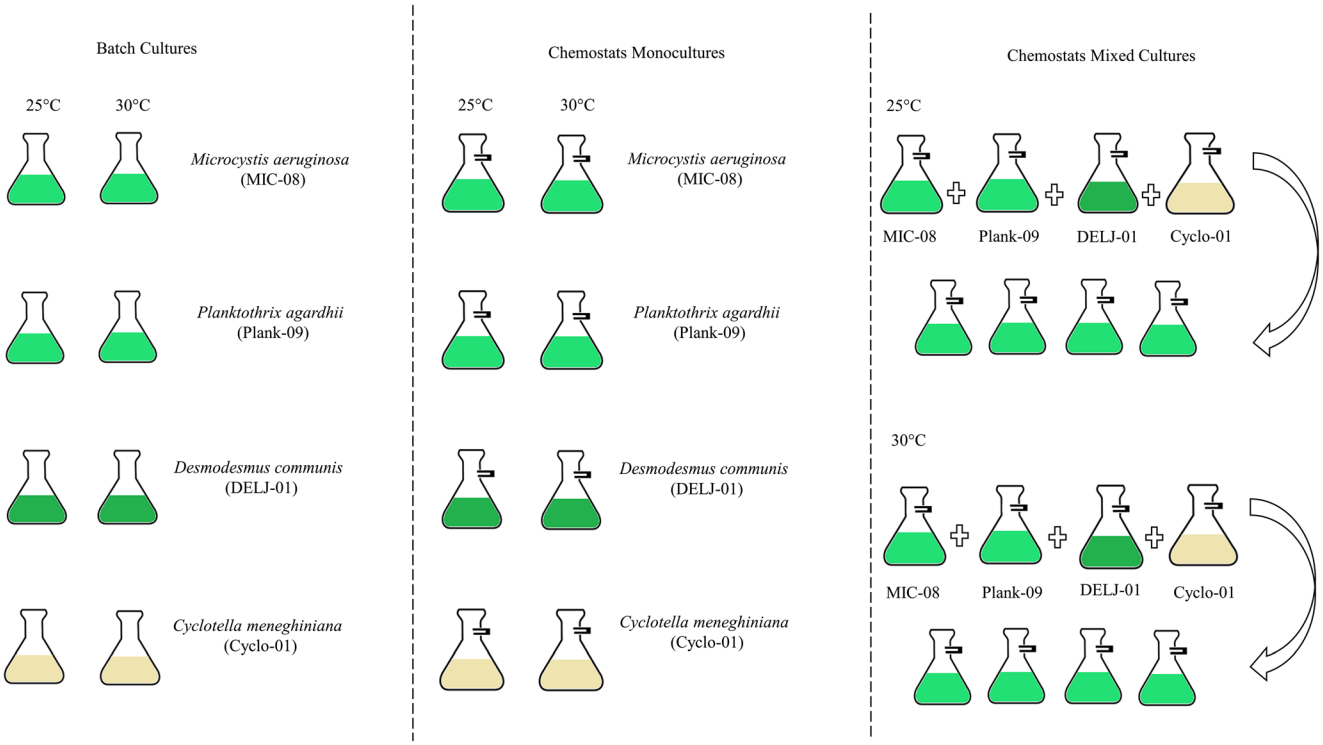

Fig. 1 Experimental design of temperature effect on batch cultures, monocultures, and mixed cultures in chemostats among *Microcystis aeruginosaPlanktothrix agardhiiDesmodesmus communis*, and *Cyclotella meneghiniana*.

batch cultures. Bubbling with sterilized (0.2 μm membrane filters) air ensured both CO_2 supply, trough diffusion from the air, and intense mixing throughout the total volume of the cultures. The CO_2 concentration was not measured, but pH was monitored as a proxy. The pH was monitored using a pH electrode refillable Ag/AgCl (Sensorglass SC-09) calibrated in the range 4.0 to 10.0, with model PH-221 Lutron pH-meter. Since values were in general lower than 8.80, we consider indicative of no carbon (C) limitation [35].

The four species were grown as monocultures at 25 and 30 °C and samples were taken daily until they reach the steady-state condition and then each 3 days for estimating biovolume, chlorophyll-*a*, and pH. Biovolume ($mm^3\ L^{-1}$) was estimated by optical density (OD) measured at 750 nm calibrated with cell counts in a hemocytometer. Chlorophyll-*a* (Chl-*a*) concentrations ($\mu g\ L^{-1}$) and photosystem II efficiency (ϕ_{PSII}) were measured with the Phyto-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany). Chlorophyll-*a* calibration was undertaken using the studied species extracted in 90% acetone [36] and we used cultures grown at 25 °C and 30 °C under 60 μmol photons $m^{-2}\ s^{-1}$. pH was monitored using a pH electrode Refillable Ag/AgCl (Sensorglass SC-09) calibrated in the range 4.0 to 7.0, with model PH-221 Lutron pH meter. The biovolume ($mm^3\ L^{-1}$) of each species was estimated as described before.

Chemostat monocultures for all species were maintained at steady-state for at least 10–15 days. Biovolume and chlorophyll-*a* were estimated based on the average of measurements during the steady-state period. Growth rates in chemostat monocultures were estimated in order to know the growth velocity of the species until reaching the steady state. The growth rates (r, day^{-1}) were estimated by the logistic model as mentioned before.

The light perceived by the cells (*Ip*) inside the culture flasks was derived from the light extinction coefficient calculated from measurements of absorbance at 440 nm with live samples (spectrophotometer Biospectro, SP-22/model) [37, 38]. *Ip* was calculated from the intensity of the incident light (I_0) and the intensity in the center of the culture flasks (I_c) according to the formulas:

$$Ip = \sqrt{I_0 I_c} \tag{2}$$

$$I_c = I_0 * e^{-K_d * r} \tag{3}$$

$$Kd = \frac{\mathrm{ABS}_{440\ \mathrm{nm}}}{L} \tag{4}$$

where r is the radius of the container and L is the optical path of the cuvette. *Kd* is a constant extinction of PAR (photosynthetically active radiation), and serves to estimate, using the Beer-Lambert law with Abs 440 nm, the light that reaches the center of culture [38].

In this study, we considered limiting only when the value is less than the minimum light requirement (*Ik*) for growth of each species or group.

Evaluation of the Effect of Temperature on Growth in Chemostat Mixed Cultures

The influence of temperature on mixed cultures among *M. aeruginosa* (MIC-08), *P. agardhii* (Plank-09), *D. communis* (DELJ-01), and *C. meneghiniana* (Cyclo-01) were studied in chemostats at 25 °C and 30 °C. The mixed cultures (four species) were set up with the same conditions of monocultures (e.g., dilution rate, light intensity) and run in four replicates of each temperature (Fig. 1). Each species was inoculated with equal biomass of 10^7 μm^3 mL^{-1}. Every 3 days, samples were taken for cell counts, chlorophyll-*a* concentration, photosystem II efficiency, pH, and nutrient measurements. Biovolume was estimated by cell counts in a hemocytometer as already described before. When colony formation was observed (*M. aeruginosa*), mucilage was dissolved using 0.03 M KOH warmed (≈ 50 °C) solution. The biovolume (μm^3 mL^{-1}) of each species was estimated based from the product of the population density and mean cell volume of each species. Cell volumes were calculated according to Hillebrand et al. [39]. Dissolved nutrients, nitrate (N-NO_3) and soluble reactive phosphorus (SRP), were analyzed on filtered samples (GF-3, Macherey-Nagel) in a flow injection analysis system according to manufacturer instructions (FIA lab 2500, FIA lab Instruments Inc., Seattle, WA). Nitrogen (N) and phosphorus (P) limitation to phytoplankton growth was accessed through the nitrate and SRP concentrations, which were compared to those that have roughly been considered to phytoplankton growth based on the half-saturation constants to most of the microalgal species (the nutrients were considered limiting when P < 10 μg P L^{-1} [40] and N < 100 μg N L^{-1} [41]).

Statistical Analysis

A two-way analysis of variance (ANOVA) with temperature and species as factors was performed in order to test whether temperatures affect the growth rate in batch monocultures and biovolume in chemostat mixed cultures. Pairwise multiple comparison procedures (Holm–Sidak method) were applied to distinguish means that were significantly different ($p < 0.05$). A one-way ANOVA with temperature as fixed factor was performed to test whether temperature affect the biovolume, *Ip*, and chlorophyll-*a* content of each species in chemostat monoculture. Pairwise multiple comparison procedures (Holm–Sidak and Dunn's method) were applied to distinguish means that were significantly different ($p < 0.05$). All statistical tests were performed using the tool pack SigmaPlot12.5® (Systat Software, Inc).

Results

Effect of Temperature on Growth in Batch Monocultures

Temperature affected the growth rate of the two cyanobacteria species and the diatom *C. meneghiniana*, but not the green algae *D. communis* (Fig. 2, Table 1). *M. aeruginosa* (0.63 ± 0.02 day^{-1}) and *P. agardhii* (0.64 ± 0.04 day^{-1}) showed the highest growth rates at 30 °C ($p < 0.05$) while *C. meneghiniana* at 25 °C (0.64 ± 0.10 day^{-1}) ($p < 0.05$). *C. meneghiniana* growth rate was higher ($p < 0.05$) than *M. aeruginosa* (0.47 ± 0.00 day^{-1}; $p = 0.005$) and *D. communis* (0.49 ± 0.02 day^{-1}; $p = 0.015$) at 25 °C but reduced its growth rate significantly ($p < 0.05$) at 30 °C (0.47 ± 0.04 day^{-1}; $p = 0.001$). Although *D. communis* also showed somewhat higher growth rate in 30 °C than 25 °C (Fig. 2), this difference was not significant ($p = 0.124$). Besides, no significant difference between cyanobacteria and green algae growth rates was observed when they were incubated at 25 or 30 °C (Fig. 2).

Effect of Temperature on Growth in Chemostat Monocultures

All species were able to grow in chemostat monocultures and reach steady state at both temperatures (Fig. 3, Table 2). *M. aeruginosa* and *D. communis* showed higher biovolume at steady state when compared to other species. The increase of 5 °C in temperature resulted in higher biovolume of the cyanobacteria species,

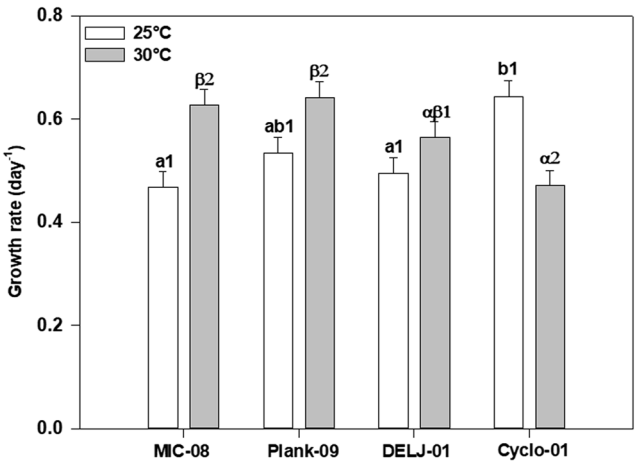

Fig. 2 Growth rates (day-−1) of Microcystis aeruginosa (MIC-08), Planktothrix agardhii (Plank-09), Desmodesmus communis (DELJ-01), and Cyclotella meneghiniana (Cyclo-01) at two different temperatures (25 and 30 °C), grown as batch monocultures. Different letters indicate significant differences (p < 0.05) between species in each temperature tested. Different numbers indicate significant differences (p < 0.05) within each species at different temperatures. Vertical bars are standard deviations (n = 3).

Table 1 Two-way ANOVA table for effects of temperature on growth rates of *Microcystis aeruginosa* (MIC-08), *Planktothrix agardhii* (Plank-09), *Desmodesmus communis* (DELJ-01), and *Cyclotella meneghiniana* (Cyclo-01), grown as batch monocultures

Source	*df*	MS	*F*	*p* value
Temperature	1	0.0101	3.612	0.075
Species	3	0.0036	1.293	0.311
Temperature × species	3	0.0327	11.71	< 0.001
Residual	16	0.0028		
Total	23	0.0071		

M. aeruginosa ($p = 0.001$, $F = 16.529$, $df = 1$) and *P. agardhii* ($p = 0.003$, $F = 12.183$, $df = 1$) (Fig. 3, Table 2), while *D. communis* ($p = 0.205$, $F = 1.664$, $df = 1$) and *C. meneghiniana* ($p = 0.984$, $F = 0.018$, $df = 1$) did not differ. When comparing the biovolume between the species at each temperature tested, 25 °C ($p = 0.001$, $F = 1145.615$, $df = 3$) and 30 °C ($p = 0.001$, $F = 781.302$, $df = 3$), there was only no significant difference between *P. agardhii* and *C. meneghiniana* at 30 °C ($p = 0.581$). Growth rates of the cyanobacteria species were not affected by the temperature, while *D. communis* and *C. meneghiniana* showed lower growth rates at 30 °C (Table 2). *P. agardhii* showed that it can reach steady state at both temperatures tested; however, it took a bit longer at 25 °C. At 30 °C, this strain quickly reached steady state (around the 3rd day), but around the 12th day, it started a slow decline but was not washed out until the end of the experiment.

All four species studied, *M. aeruginosa* ($p = 0.001$, $H = 27.462$, $df = 1$), *P. agardhii* ($p = 0.003$, $F = 12.213$, $df = 1$), *D. communis* ($p = 0.001$, $F = 19.896$, $df = 1$), and *C. meneghiniana* ($p = 0.001$, $F = 50.378$, $df = 1$), significantly increased their chlorophyll contents with increasing temperature. When comparing the chlorophyll contents between the species at each temperature tested, *D. communis* exhibited the highest values of chlorophyll content at both temperatures tested—25 °C ($p = 0.001$, $H = 52.610$, $df = 3$) and 30 °C ($p = 0.001$, $H = 54.662$,

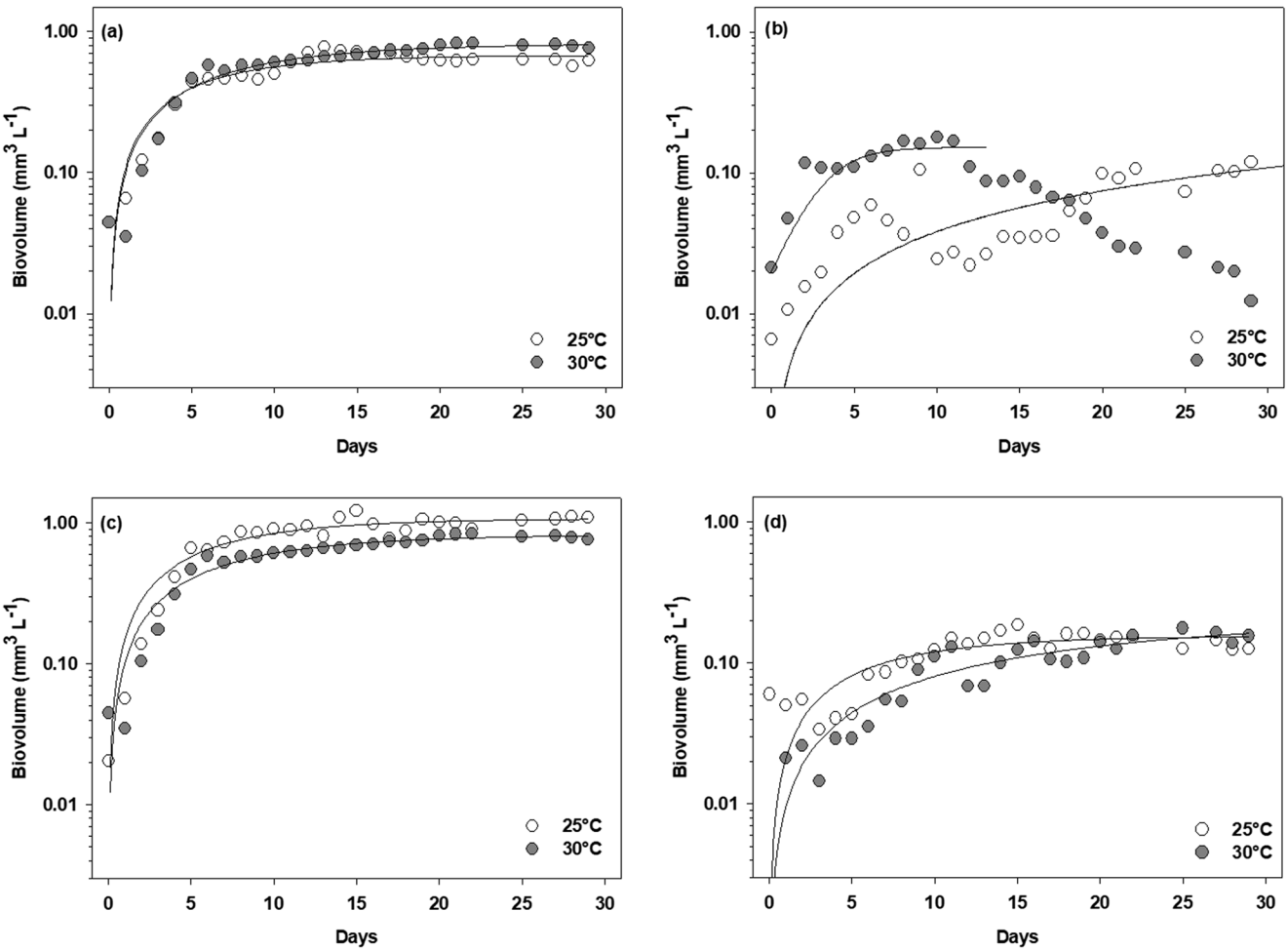

Fig. 3 Growth of (a) *Microcystis aeruginosa* (MIC-08), (b) *Planktothrix agardhii* (Plank-09), (c) *Desmodesmus communis* (DELJ-01), and (d) *Cyclotella meneghiniana* (Cyclo-01) species in chemostats monocultures at 25 °C and 30 °C. The solid line represents the fitted growth by regression, according to the logistic Eq. 1.

Table 2 Steady-state data of each species grown in chemostat monocultures at different temperatures (species abbreviations as in Table 1; *Ip* light perceived inside cultures)

	MIC-08	Plank-09	DELJ-01	Cyclo-01
25 °C				
Biovolume (mm^3 L^{-1})	0.65 ± 0.06	0.10 ± 0.01	0.98 ± 0.11	0.15 ± 0.02
Chlorophyll-*a* content (pg $cell^{-1}$)	0.03 ± 0.01	0.10 ± 0.03	1.07 ± 0.24	0.19 ± 0.02
Growth rate (day^{-1})	0.45	0.39	0.54	0.49
Ip (μmol photons m^{-2} s^{-1})	57.86 ± 0.17	58.72 ± 0.41	58.54 ± 0.25	59.55 ± 0.05
30 °C				
Biovolume (mm^3 L^{-1})	0.74 ± 0.07	0.15 ± 0.02	0.94 ± 0.09	0.15 ± 0.02
Chlorophyll-*a* content (pg $cell^{-1}$)	0.04 ± 0.00	0.12 ± 0.06	1.43 ± 0.23	0.31 ± 0.06
Growth rate (day^{-1})	0.40	0.40	0.39	0.20
Ip (μmol photons m^{-2} s^{-1})	57.59 ± 0.13	58.42 ± 0.2	58.50 ± 0.13	59.51 ± 0.06

$df = 3$), except for *C. meneghiniana* at 30 °C ($p > 0.05$) (Table 2). The growth and increase in biomass of the species in continuous monocultures promoted the reduction of light inside cultures (*Ip*) until the carrying capacity and steady state of each system was achieved. But considering the light perceived by the cells inside the culture flasks (*Ip*), this reduction represented ≤ 4% and was not limiting for growth (Table 2).

Effect of Temperature on Growth in Chemostat Mixed Cultures

At 25 °C, *M. aeruginosa* reached the steady state and dominated the system from the 15th day (Fig. 4). *P. agardhii* and *D. communis* also reached steady state and co-existed with *M. aeruginosa* until the end of the experiment. *C. meneghiniana* could not maintain the growth and gradually decreased the biomass until it was excluded at the 16th day. With the increase of biomass, nitrogen and phosphorus availability was reduced in the culture medium in the first 10 days. But the nutrient concentrations remained above values considered limiting until the end of the experiment. At 30 °C, *M. aeruginosa* grew faster, reached the steady state, and became dominant from the 10th day. *C. meneghiniana* and *P. agardhii* were excluded, and although *D. communis* has not been eliminated from the system, the chlorophyte showed a biomass reduction from the 15th day until the end of the experiment. Due to the increase in total biomass, nitrogen and phosphorus were quickly reduced in the first 6 days of culture, and a potential nitrogen limitation (< 100 μg N L^{-1}) was observed from the 12th day (Fig. 4). Like the observed for monocultures, the reduction of light inside cultures (*Ip*) at both tested temperatures represented < 4%, and it was not limiting for growth. pH varied from 7.25 to 10.21 (8.83 ± 0.39) and 6.93 to 10.84 (9.74 ± 0.58), respectively at 25 and 30 °C.

Discussion

In this study, we tested the hypothesis that cyanobacteria would be favored by the direct effect of temperature increase, which was in part confirmed. In batch monocultures, the tropical cyanobacteria strains of *M. aeruginosa* and *P. agardhii* showed higher growth rates in 30 °C than in 25 °C, which is in line with their frequent reporting at elevated water temperature [26, 42, 43]. However, in continuous culture experiments (chemostats), growth rates of *M. aeruginosa* and *P. agardhii* were not affected by temperature but the strains showed higher biovolume in steady state with the temperature increase. In continuous mixed cultures, *M. aeruginosa* was always dominant and *C. meneghiniana* was excluded, regardless of temperature tested (25 and 30 °C). These outcomes were expected considering the results of monoculture experiments (batch and chemostats) and support our hypothesis. Nevertheless, *P. agardhii* was excluded at 30 °C, which was an unexpected result.

Cyanobacteria can be favored at high water temperatures since they reach higher growth rates than some other eukaryotic algae [11, 44] or due to longer periods of stratification, since they have the ability to migrate vertically and prevent sedimentation in warmer waters due to global warming [21]. The strains of the cyanobacteria *M. aeruginosa* and *P. agardhii* tested in this study showed higher growth rates when submitted to the elevated temperature in batch cultures. These results agree with former studies that observed *M. aeruginosa* having optimum growth rates around 30 °C [22, 45] and *P. agardhii* around 27 °C [7, 21]. However, the effect of temperature on growth of another *M. aeruginosa* strain (MIJAC-01) isolated from the same lagoon did not show differences in growth rates from 18 to 30 °C [7]. Although *M. aeruginosa* strains exhibit high intraspecific variability in relation to some environmental factors, like different light requirements [33] or different optimal temperatures for growth [7, 46], most of them increase their

growth rates at elevated temperature [21, 47, 48]. In a recent study, a comparison of four *M. aeruginosa* strains and eight *Cylindrospermopsis raciborskii* strains isolated from the same waterbody found greater intraspecific variation than interspecific in growth rate under different light conditions and temperature tested [46]. *M. aeruginosa* is found in Jacarepaguá Lagoon along the whole year, where the water temperature variation extends from 18 to 32 °C [26], since the high intraspecific variation suggests the occurrence of multiple isolates (e.g., strains) in a population and is a key factor of species adapted to various environmental conditions [46].

Unlike cyanobacteria, diatom dominance is reported for systems with low water temperatures, as has been widely shown in field studies [2, 26, 49]. In our experiments, we observed that *C. meneghiniana* had a higher growth in the lower temperature (25 °C) and reduced with the increase in temperature (30 °C). As in the in situ observations, experimental studies in batch culture showed that the highest growth rates of diatoms are associated with low temperatures ranging from 18 to 25 °C, and when submitted to high temperatures, like 30 °C, decrease in growth rates were observed [7, 50].

On the other hand, when we analyzed the green algae performance in batch monocultures in relation to the temperature increase, *D. communis* did not show significant differences on growth rates. Furthermore, growth rates of *D. communis* were not significantly different from both cyanobacteria species at the two temperatures tested. These results are in accordance with another study, which also reported no difference in growth rates at 25–30 °C and argued that cyanobacteria do not grow better than green algae at higher temperatures (> 25 °C) [21].

Chemostat experiments are interesting because nutrients are continuously replenished, simulating a model of natural systems. However, in natural systems, nutrient supply can be sporadically provided in pulses [51]. Therefore, algae growth would not be potentially limited by nutrients, but its growth could be limited by light if they reached steady state with high biovolume. In both chemostat experiments (monocultures and

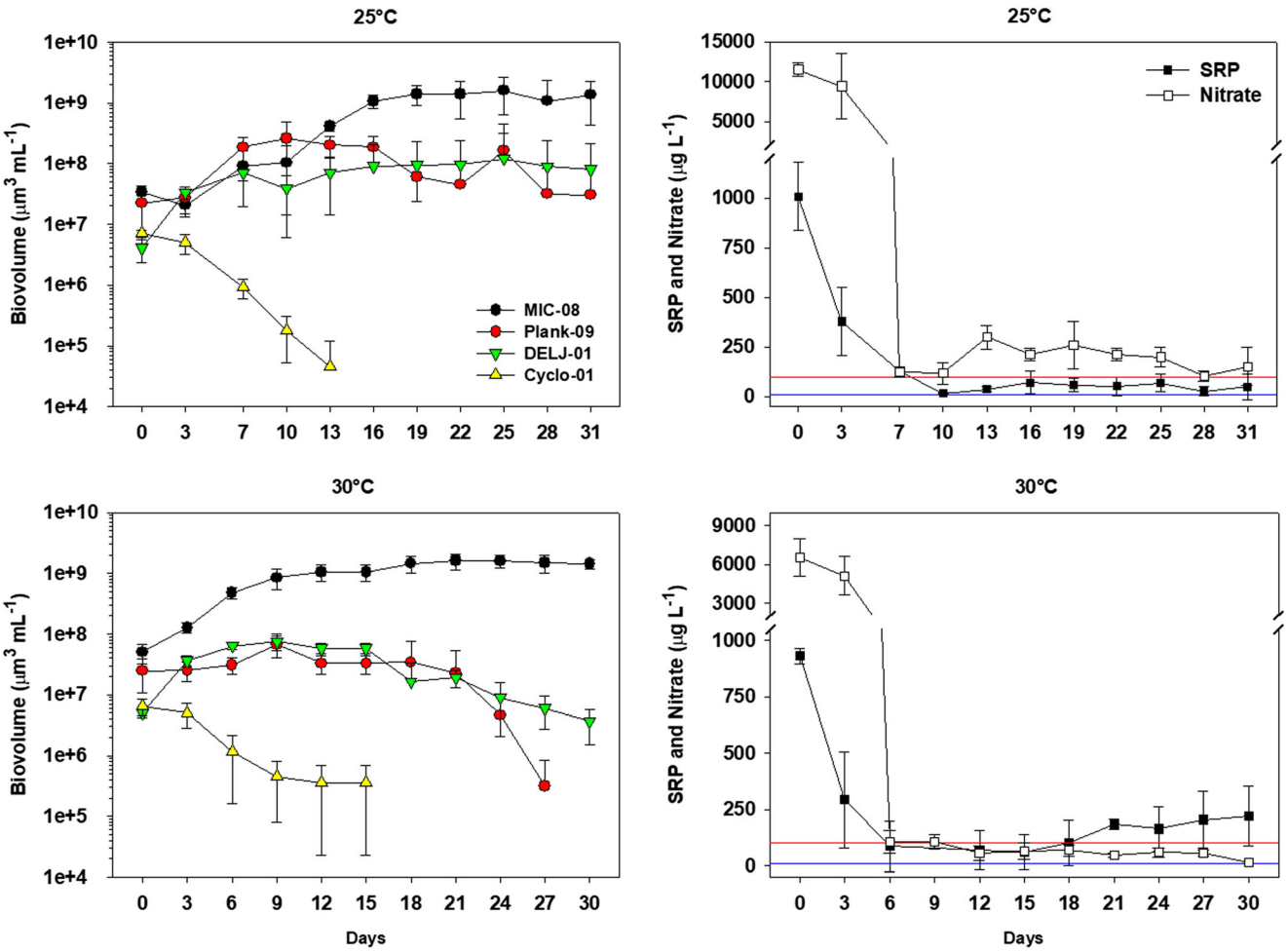

Fig. 4 Growth of *Microcystis aeruginosa* (MIC-08), *Planktothrix agardhii* (Plank-09), *Desmodesmus communis* (DELJ-01), and *Cyclotella meneghiniana* (Cyclo-01) in chemostats mixed culture at 25 °C and 30 °C. Left panels show the biovolume of species. Right panels show soluble reactive phosphorus (SRP) and nitrate concentrations; blue line indicates limiting values of SRP and red line limiting values of nitrate. Vertical bars are standard deviations (n = 4)

mixed cultures) at both tested temperatures, there was no light limitation, since the reduction of perceived light inside the cultures (*Ip*) was ≤ 4% of the incident light. Under light intensity lower than 100 μmol of photons m^{-2} s^{-1}, as we used in this study, more than 80% of the absorbed quantum is used in the photosynthesis process [52]. Then, in our chemostat experiments, no light limitation occurred, and all species were able to invest in cell growth.

In monoculture chemostats, the four studied species were able to grow and reach the steady state at both tested temperatures. So, the direct effect of temperature on growth could be analyzed considering the steady-state traits (Table 2). The negative effect of the increase in temperature on *C. meneghiniana* was again evidenced by the reduction in growth rate at 30 °C, which is consistent with the literature [7, 50]. Nevertheless, although the biovolume in the steady state was not affected, it took longer to be reached at the higher temperature.

When comparing the two cyanobacteria species, no effects on growth rate were observed but both strains showed increase in biovolume pointing to a higher carrying capacity. *M. aeruginosa* reached higher biomass than *P. agardhii*, regardless of the temperature tested. Another experimental study with the same strains (MIC-08, Plank-09) also observed that *M. aeruginosa*, under the same growth conditions of *P. agardhii*, reached higher biovolume at steady state and, consequently, greater carrying capacity [33]. Interestingly, our results also showed that the strain of *P. agardhii* (Plank-09) used in the experiments simply does not support growing in chemostats for a long period at elevated temperature (30 °C). We will need more studies to understand the processes involved.

On the other hand, the increased temperature did not affect *D. communis* biovolume at steady state but reduced its growth rate. It means that *D. communis* took more time to reach the steady state at 30 °C. However, *M. aeruginosa* and *D. communis* reached the steady state with higher values of biovolume than other species. Then, under conditions of no nutrient limitations and the species grow separately, *M. aeruginosa* and *D. communis* will be favored at warmer temperatures.

In chemostat mixed cultures, the cyanobacterium *M. aeruginosa* dominated the system in both temperatures tested. Still, at 25 °C, *D. communis* and *P. agardhii* were capable to reach the steady state and co-existed with *M. aeruginosa*. Considering the results in monoculture chemostats, *D. communis* would be able to dominate the mixed cultures at 25 °C, while it was expected that *C. meneghiniana* would not dominate the mixed cultures at the tested temperatures.

Our study shows that rising temperatures can be detrimental to diatoms, even for a strain isolated from a tropical system. Gomes et al. [7] also pointed the negative effect of temperature on the growth rate of diatoms. Nonetheless, they suggest that the competition results between cyanobacteria and diatoms experimentally tested at different temperatures can be modulated by allelopathic effects. Our experiments were not designed to evaluate possible allelopathic interactions so we cannot ignore this possibility. However, the strong evidence of negative effects on growth rates of the diatom at 30 °C can explain the results of mixed continuous cultures.

The outcome of chemostat mixed cultures showed the dominance of cyanobacteria in both temperatures tested, what argues in favor of our hypothesis. Conversely, this result cannot be fully explained based on the growth rates of monoculture experiments as observed in another study [33], since the *M. aeruginosa* and *P. agardhii* were not affected by the temperature increase. However, other factors may have contributed to the dominance of *M. aeruginosa*. For example, the dilution rate may be one of the reasons favoring *M. aeruginosa* at both temperatures since it has already been shown that *Microcystis* spp. can dominate the green algae *Scenedesmus quadricauda* at competition under low dilution rates (< 0.65 day^{-1}) [51].

Another factor that could explain the outcome of the experiments and the dominance of *M. aeruginosa* in both temperatures tested may be related to the ability for nutrient acquisition. In the mixed cultures, phosphorus and nitrogen availability was quickly reduced in the first days of the experiment and growth was potentially limited by nitrogen from the 10th day to the end of the experiment at 30 °C. It is known that high temperatures accelerate the cell metabolism, resulting in the greater assimilation of nutrients and consequently rapid cell growth [9]. The affinity of many cyanobacteria for nitrogen or phosphorus is higher than other photosynthetic organisms [53]. For example, *M. aeruginosa* strain has the ability to assimilate nutrients faster than a diatom strain isolated from the same reservoir [23] and cyanobacteria are able to dominate in high and low phosphorus concentrations [54]. Although diatoms as *Cyclotella* spp. should outcompete for nitrogen, because their growth affinity is higher than cyanobacteria taxa [55], a competition study [56] showed that *M. aeruginosa* had lower half-saturation constant (Ks) for nitrogen (16 μg L^{-1}) than *Cyclotella* sp. (234 μg L^{-1}). So, the exclusion of *C. meneghiniana* from both chemostats was not due to lack of nutrient, but because of the lower growth rate and consequently the low production of biomass at high temperatures. *M. aeruginosa* is also known to have lower Ks (N) than *D. communis* (former *Scenedesmus quadricauda*) [51] and *P. agardhii* [57]. Therefore, assuming that *M. aeruginosa* is a stronger competitor to assimilate nutrients than the other species studied, we suggest that the dominance of *M. aeruginosa* was due to the ability to assimilate nutrients and increase its biomass in a period of time faster than the others at the two temperatures tested.

In addition, other environmental factors could explain the success of *M. aeruginosa* in chemostat experiments, such as pH and biotic interaction. The pH of the water may influence the dominance of a specific phytoplankton group, since cyanobacteria species are stronger competitors at low levels of CO_2, while eukaryotic phytoplankton species may be better competitors at higher levels [48, 58–60]. Confirming the previous idea, laboratory experiments showed that *M. aeruginosa* prefers neutral to slightly alkaline waters [48] because this species can absorb and directly use HCO^{-3} [60], while green algae are generally mediated by carbonic anhydrases [50]. On the other hand, a recent study demonstrated that *Microcystis* sp. is a relatively poor competitor at low CO_2 levels and is a strong competitor at high levels [61]; green algae like *Scenedesmus* sp. coexist with *Microcystis* sp. in high levels of CO_2 but may also be an efficient competitor at low CO_2 levels [61]. In addition, these authors observed that *Microcystis* sp. was the only species that increased its competitive ranking at elevated CO_2 while green algae did not change [61]. However, *D. communis* is an efficient competitor in the obtained pH range (6.93 to 10.84), since it coexisted with *M. aeruginosa* in both temperature tests.

Biotic interaction also could be an important factor to explain the dominance of *M. aeruginosa* in mixed chemostat experiments. Although *P. agardhii* simply cannot grow and remain in steady state for a long period of time (Fig. 3) at 30 °C, its exclusion may have been due to the biotic interaction with the other species. *P. agardhii* grew well in monocultures, and its growth rate was positively influenced by temperature. However, when grown as mixed cultures, it was excluded at 30 °C. Previous study with *P. agardhii* and *M. aeruginosa* has shown that temperature can influence the degree of competition between them [7]. Moreover, *D. communis* could also be influenced for *M. aeruginosa*, since the *D. communis* showed lower biomass in both mixed chemostats, and previous studies have also been demonstrated that *M. aeruginosa* affects the growth of green algae and diatom [62, 63].

Several studies have shown that temperature affects the structure of phytoplankton communities [3, 4, 7]. Cyanobacteria would be a strong competitor due to direct effect, promoting higher growth rates at elevated temperatures [11, 45], faster assimilation of nutrients than other phytoplankton groups [9, 23, 54], and also interferes in the production of allelopathic compounds [7, 62, 63]. The indirect effect of temperature as the waters become warmer and stratification stands for longer periods [21]. However, no study showed the direct effect of temperature would affect the behavior of different species isolated from the same habitat. The Jacarepaguá Lagoon, the site from where the strains were isolated, is a high-seasonally dynamic system and changes in phytoplankton composition have been associated with the temperature variation when the dominance of cyanobacteria (*M. aeruginosa* and *P. agardhii*) are closely related to higher temperatures [26, 64] and diatoms as *C. meneghiniana* at a colder temperature [26]. Our results were consistent with the dynamics that occur in this system and showed that the direct effect of increasing temperature intensified the dominance of *M. aeruginosa*, and at 30 °C not only resulted in the exclusion of the diatom but also of *P. agardhii*.

Conclusion

Looking only at the growth rate of the isolated strains (monoculture), warming is expected to increase the cyanobacteria and green algal biomass, whereas the diatom is expected to dominate in colder temperatures (around 25 °C). This is in fact also what is being observed annually in the lagoon where the species have been isolated from [7]. Lürling et al. [21] indicate that there is no significant difference between the growth rates of cyanobacteria and chlorophytes as a function of temperature increase. And that the dominance of cyanobacteria in warmer climates may be due to the indirect effect of warming that will promote physical conditions in the environment more favorable to cyanobacteria. However, contrary to what Lürling et al. [21] concluded, our results demonstrate that the direct effect of temperature can also favor the dominance of cyanobacteria.

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