



UNIVERSIDADE FEDERAL DO PARANÁ

ELENA FUKASAWA GALVANESE

**CONCOMITANT ACCLIMATION OF *Cylindrospermopsis raciborskii* TO
TEMPERATURE AND PHOSPHATE DEFICIENCY REVEALS DISTINCT STRAIN
PLASTIC RESPONSES**

CURITIBA

2017

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**CONCOMITANT ACCLIMATION OF *Cylindrospermopsis raciborskii* TO
TEMPERATURE AND PHOSPHATE DEFICIENCY REVEALS DISTINCT
STRAIN PLASTIC RESPONSES**

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Ecologia e Conservação, no Curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, da Universidade Federal do Paraná.

Orientador: Prof. Dr. André Andrian Padial

Co-orientador: Prof. Dr. Luis Aubriot

CURITIBA

2017

Universidade Federal do Paraná
Sistema de Bibliotecas

Galvanese, Elena Fukasawa

Concomitant acclimation of *Cylindrospermopsis raciborskii* to temperature and phosphate deficiency reveals distinct strain plastic responses. / Elena Fukasawa Galvanese. – Curitiba, 2017.

32 f.: il. ; 30cm.

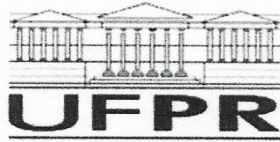
Orientador: André Andrian Padial

Co-orientador: Luis Aubriot

Dissertação (Mestrado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ecologia e Conservação.

1. Cianobacteria. 2. Alga. 3. Ecossistema. I. Título II. Padial, André Andrian. III. Aubriot, Luis. IV. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ecologia e Conservação.

CDD (20. ed.) 574.5



MINISTÉRIO DA EDUCAÇÃO ·
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
Setor CIÊNCIAS BIOLÓGICAS
Programa de Pós-Graduação ECOLOGIA E CONSERVAÇÃO

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de ELENA FUKASAWA GALVANESE intitulada: "Concomitant acclimation of *Cylindrospermopsis raciborskii* to temperature and phosphate deficiency reveals distinct strain plastic responses."

., após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APPROVAÇÃO.

Curitiba, 03 de Março de 2017.

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Dedico este singelo trabalho ao desenvolvimento das forças produtivas materiais e ao rompimento das correntes que nos prendem ainda à pré-história da humanidade.

AGRADECIMENTOS

Agradecer é um gesto simples, que pode tornar-se muito complexo se há uma série muito grande de pessoas a agradecer. Não são apenas contribuições diretas que nos fazem avançar profissionalmente, muitas vezes são pequenas atitudes que nos fazem seguir adiante e poder colher o fruto de nosso trabalho. Assim, agradeço às pessoas que passaram por mim em geral. Entretanto, não posso deixar de mencionar certas pessoas que possuem um papel destacado:

Agradeço aos meus pais e irmão por toda a ajuda e estímulo para eu crescer intelectualmente e pessoalmente, grande parte do que posso oferecer ao mundo é devido essas relações;

Agradeço meus tios, sobretudo o tio Fernando e Grécia, e tia Shizie pelo apoio direto e indireto em todas as fases da vida como estudante;

Agradeço aos míopes Cyro e Glauco, Glauco e Cyro, e Mariana pelos mais de 10 anos de companheirismo e irmandade;

Agradeço ao Mormasso e Galo pelo respiro coletivo numa perspectiva de emancipação desta sociedade e pelo companheirismo;

Agradeço ao prof André Andrian Padiál pela oportunidade de orientação e a sempre afável maneira de orientar-nos, sem enfatizar a hierarquia meramente burocrática;

Agradeço ao prof Luis Aubriot por toda a ajuda e colaboração na execução deste projeto em todas as suas fases, bem como a paciência em explicar muitos conceitos biológicos;

Agradeço Ana, Fernanda, Jaqueline, Juliana, Luis Artur, Suelen e Elielton por toda companhia e ajuda na rotina do LASB;

Agradeço Fatima, Andrea, Signe, Federica, Carmela, Natalie, Bruno, Mariana, Manuel, Andrea Picardo, Tatiana, Nacho, Amelia, Guillermo, Sylvia, Rafael, Federico e Daniel por toda a ajuda, disposição e bons momentos em terras uruguaias;

Agradeço aos colegas Israel, Júlio, Luiz Henrique, Rudá e amigo Thomas pelas discussões, boa companhia e risadas;

Agradeço à Marianel por todo carinho e paciência ao abrir sua casa;

Agradeço à Mariela e Julio toda a ajuda no Uruguai;

Agradeço ao Gabriel o apoio e carinho.

RESUMO

Cylindrospermopsis raciborskii é uma das cianobactérias potencialmente tóxica mais estudada. Efeitos de fatores isolados como aumento da temperatura ou desta combinada com luz têm sido descritos como promotores do crescimento de *C. raciborskii*. Entretanto, ainda não foi descrita a combinação entre deficiência de nutrientes e temperatura. Neste trabalho, as performances de duas cepas de *C. raciborskii* (MVCC19, uruguaia e LP1, brasileira) foram avaliadas sob aclimatação à mudanças de temperaturas (25°C-32°C) e duas transições de suficiência a deficiência de fósforo. Quando sujeita à transição de alta suficiência à deficiência, somente as cepas aclimatadas à alta temperatura cresceram. A cepa MVCC19 mostrou maior taxa de crescimento e reduzida fase curva de crescimento em relação a LP1. Diferenças morfológicas também foram encontradas: MVCC19 cresceu em número de filamentos menores, enquanto LP1 aumentou não só o número de filamentos como também o comprimento e número de células por filamento ao final da fase exponencial. Nós hipotetizamos que atributos ecofisiológicos e morfológicos das cepas podem refletir as condições dos lagos de origem. LP1 foi isolada de um lago oligo-mesotrófico, o que provavelmente explica sua otimização sob deficiência de fósforo; já crescimento explosivo e menores filamentos de MVCC19 podem refletir condições eutróficas e de alta turbidez. O efeito positivo da aclimatação à elevada temperatura pode ser consequência da redução do quota celular mínimo, permitindo aos organismos ajustar e superar a depleção de fosfato nos lagos.

Palavras-chave: cianobactéria, resposta funcional, ecótipos, lago, fisiologia de alga, funcionamento de ecossistema.

ABSTRACT

Cylindrospermopsis raciborskii is one of the most studied potentially harmful cyanobacteria. Effects of single factors like increased temperature or combined with light have been described as promoters of *C. raciborskii* growth. However, until now it was not described for cyanobacteria the combined effects of nutrient deficiency and temperature. Here, the performances of two strains of *C. raciborskii* (MVCC19, Uruguayan and LP1, Brazilian) were evaluated under acclimation to temperatures shifts (25°C – 32°C) and two transitions from phosphorus (P) sufficiency to deficiency. When subjected to the transition from high P sufficiency to deficiency state, only the temperature acclimated strains developed. The MVCC19 strain showed higher growth rate and less prolonged growth phase than LP1. Also morphologically differences were found: MVCC19 strain grew by production of shorter filaments, while LP1 strain increased in length and number of cells per filament at the end of the exponential phase. We hypothesized that ecophysiological and morphological traits of the strains may reflect the originary lake conditions. The LP1 strain was isolated from an oligo to mesotrophic lake, which probably explains its optimization of growth under phosphorus deficiency. The explosive growth and shorter trichomes of MVCC19 may reflect more eutrophic and turbid lake conditions. The positive effect of previous acclimation to elevated temperature may be consequence of the reduction of minimal internal cellular quota, allowing the organisms to adjust and overcome the phosphate depletion in lakes.

Key-words: cyanobacteria, functional response, ecotypes, lake, algae physiology, ecosystem functioning.

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APRESENTAÇÃO

Esta dissertação é apresentada sob forma de um manuscrito que será submetido ao periódico *Harmful Algae*; portanto a formatação segue o que é exigido pela referida revista. O presente trabalho teve como objetivo avaliar o desenvolvimento de duas cepas da cianobactéria *Cylindrospermopsis raciborskii* sob diferentes condições de crescimento, considerando que estudos eco-fisiológicos podem contribuir no entendimento da distribuição e sucesso desta espécie em uma variada gama de condições ambientais. Para tal, foram testadas duas temperaturas, duas transições de suficiência para deficiência de fósforo e dois estados de aclimação.

Foram realizados três experimentos com uma cepa uruguaia (MVCC19) e uma cepa brasileira (LP1), um sob suficiência de fósforo (7 mg total P L⁻¹) e dois com transições de suficiência para deficiência (i.e. diluição da concentração inicial de 7 mg total P L⁻¹ para 0,31 mg total P L⁻¹, e de 1 mg total P L⁻¹ para 0,31 mg total P L⁻¹). Em todos experimentos, eram testadas simultaneamente duas temperaturas e dois estados de aclimação (i.e. aclimação prévia à temperatura ou não). Desta forma, foi possível avaliar o efeito do passado recente sobre o sucesso das cepas em um estado de deficiência nutricional.

Diferenças entre as cepas foram encontradas, sobretudo no experimento de transição de 7 mg total P L⁻¹ para 0,31 mg total P L⁻¹. A cepa uruguaia cresceu de modo mais explosivo que a brasileira, crescendo em número de tricomas ao final da fase exponencial. Por outro lado, a cepa brasileira não atingiu a fase estacionária até o fim do experimento e cresceu não só em número de filamentos como também em comprimento e número de células por tricoma ao final da fase exponencial.

As diferenças entre as cepas provavelmente são relativas aos ambientes de origem de cada uma, e refletem estratégias distintas. Além disso, nossos resultados sugerem mais uma evidência da relação entre a temperatura e o quota mínimo celular, por meio de alterações bioquímicas nas células.

Por fim, os resultados encontrados apoiam a hipótese de distintos ecótipos desta espécie e sugerem uma drástica necessidade de redução da concentração de fósforo para reduzir a ocorrência e intensidade de florações em um cenário de aquecimento global.

Concomitant acclimation of *Cylindrospermopsis raciborskii* to temperature and phosphate deficiency reveals distinct strain plastic responses

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Abstract

Cylindrospermopsis raciborskii is one of the most studied potentially harmful cyanobacteria. Effects of single factors like increased temperature or combined with light have been described as promoters of *C. raciborskii* growth. However, until now it was not described for cyanobacteria the combined effects of nutrient deficiency and temperature. Here, the performances of two strains of *C. raciborskii* (MVCC19, Uruguayan and LP1, Brazilian) were evaluated under acclimation to temperatures shifts (25°C – 32°C) and two transitions from phosphorus (P) sufficiency to deficiency. When subjected to the transition from high P sufficiency to deficiency state, only the temperature acclimated strains developed. The MVCC19 strain showed higher growth rate and less prolonged growth phase than LP1. Also morphologically differences were found: MVCC19 strain grew by production of shorter filaments, while LP1 strain increased in length and number of cells per filament at the end of the exponential phase. We hypothesized that ecophysiological and morphological traits of the strains may reflect the ordinary lake conditions. The LP1 strain was isolated from an oligo to mesotrophic lake, which probably explains its optimization of growth under phosphorus deficiency. The explosive growth and

shorter trichomes of MVCC19 may reflect more eutrophic and turbid lake conditions. The positive effect of previous acclimation to elevated temperature may be consequence of the reduction of minimal internal cellular quota, allowing the organisms to adjust and overcome the phosphate depletion in lakes.

Key-words: cyanobacteria, functional response, ecotypes, lake, algae physiology, ecosystem functioning.

1 Introduction

It is well known that variations in aquatic communities as a response to changes in abiotic phenomena drive the ecosystem functioning (Chase 2010; Hunter-Cevera et al. 2016). The ability to predict the species' composition is particularly searched in managed ecosystems for anthropic uses, i.e. aquatic systems used for water supply. Indeed, a matter of great concern is determining the driving factor for the frequency, intensity and duration of potentially harmful algae blooms in aquatic ecosystems (WHO 1999; UNESCO 2009). In order to adequately control and manage blooms, it is necessary to know the ecological and physiological behavior of harmful algae, as well its dispersion and invasion trends (Carey et al. 2012; Thomas and Litchman 2015).

One of the most studied toxic and widespread distributed cyanobacteria in freshwaters is *Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya et Subba Raju. Recent phylogeographical studies based in genetic markers have indicated that the origin of *C. raciborskii* is the tropical region of America, spreading to Africa while the continents were connected, and then to Australia and Asia, and finally to Europe (Moreira et al. 2015). Several physiological characteristics have been reported as responsible for its worldwide success: wide thermal tolerance (Briand et al. 2004; Bonilla et al. 2016), adjustment to low phosphate concentrations (Aubriot et al. 2011; Amaral et al. 2014), large internal storage of polyphosphate granules and low cell nutrient quotas (Isvánovics et al. 2000), atmospheric N fixing capacity (Kenesi et al. 2009; Yema et al. 2016), wide light tolerance (Bonilla et al. 2016); dormant cells (akinetes) formation (Padisák and Istvánovics 1997) and production of cyanotoxins (Sinha et al. 2014).

Increasing temperature has been pointed out as an important factor favoring the increase in frequency and intensity of blooms in temperate regions (Briand et al. 2004; Antunes et al. 2015). Nutrient load also have been highlighted as a key factor in promoting *C. raciborskii* development (Posselt et al. 2009). Even so, some studies have reported high biomass of *C. raciborskii* even in areas with low phosphorus (Bonilla et al. 2012; Amaral et al. 2014) and nitrogen concentrations (Briand et al. 2002). In general, studies considering simultaneously the effect of more than one factor on *C. raciborskii* growth investigated the roles of combined effects of temperature and light (e.g. Kehoe et al. 2015; Bonilla et al. 2016), although *C.*

raciborskii is known to have a great tolerance to low light availability (Wojciechowski et al. 2016). It is unclear how the interaction of temperature and nutrient load, two key determining factors, affect *C. raciborskii* growth. Considering the fluctuations of nutrients in water (i.e. frequency and concentration), the adaptation of the phosphorus uptake system to a given environment can represent an advantage and consequently a determinant trait (Litchman and Klausmeier, 2008). Therefore, not only the ability of phosphorus storage *per se* is important, but also the response of the uptake system of cells to a new external concentration affects growth optimization (Falkner et al. 2006). In this sense, there are strong evidences that different interacting factors controlling *C. raciborskii* growth acts in a synergistic way (Tonetta et al. 2015; Aguilera et al. 2017). One can thus anticipate that *C. raciborskii* adjust its growth depending on the interacting changes in nutrient and temperature, and also on how sudden changes are recorded.

Another important issue regarding the development of *C. raciborskii* is the differences between strains. Several studies have reported that strains have distinct biological features (e.g. Chonudomkul et al. 2004; Stucken et al. 2009; Piccini et al. 2011) and others highlight the high plasticity of *C. raciborskii* (Briand et al. 2004; Bonilla et al. 2012). There is still no consensus if it is the species plasticity *per se* or the differences among strains that explain the species success in diverse ecosystems, claiming for more physiological investigations of different strains simultaneously (Burford et al. 2016).

This study evaluates the acclimation capability of two strains of *C. raciborskii* to changes in abiotic conditions that may help to explain its success in a wide range of climates and trophic levels. The ecophysiological plasticity and strain performance were evaluated in relation to temperature changes solely and concomitantly with transitions from phosphate sufficiency to phosphate deficiency. This experimental design approaches alternative realistic scenarios to understand the role of multiple interacting factors on the species bloom behavior, persistence and invasion capability.

2 Materials and methods

2.1 *Cylindrospermopsis raciborskii* cultures

We used *C. raciborskii* strains previously isolated from an Uruguayan (MVCC19 – ‘Lago Javier’, 34°51’S, 56°02’W) and a Brazilian (LP1 – ‘Lagoa do Peri’, 27°44’S and 48°31’W) lake, maintained in assay tubes with BG11 medium N-free at 25°C (an usual temperature in which several strains have reasonable growth) and constant light flux of 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ before experiments. Both strains were cultured under the same light, temperature and growth medium for more than one year. The cultures were not axenic, however they were checked routinely to guarantee that other microbial organisms were maintained in low abundance.

2.2 Experimental design

Aiming to compare strains development and growth rate at different temperature and nutrient shifts, the experimental design consisted in two experiments manipulating changes in temperature and phosphate concentration. In all experiments, 80 mL of culture were aseptically placed in 100 mL flasks in thermostatic baths. Temperature shifts occurred when individuals that started the experimental growth were suddenly moved to a different temperature (hereafter ‘not acclimated’). Simultaneously, individuals previously stocked at the same temperature for at least 5 days also started the experimental growth (hereafter ‘acclimated’). Temperature shifts were simulated changing from 25 °C to 32 °C; and from 32 °C to 25 °C. These warming and cooling ranges were chosen considering that 25 °C is around the annual average temperature of lakes, and the maximum growth rate for *C. raciborskii* is reported to be around 32 °C (Briand et al. 2004; Lüring et al. 2013).

Shifts in phosphate concentration were done, always sudden, from a sufficiency to a deficiency state (see levels manipulated below and Amaral et al. 2014). Three replicates were manipulated in each temperature and phosphate concentration treatment.

Experiment 1: The goal was to test for differences in development of *C. raciborskii* strains according to temperatures shifts only (i.e. warming and cooling with or without acclimation). Strains grew in the same modified BG11 (N-free) with 7 mg total P L⁻¹ (a scenario with phosphate sufficiency).

Experiment 2: The goal was to test for differences in development of *C. raciborskii* strains according to temperature shifts (as above) concomitantly with a transition from a phosphate sufficiency to a deficiency state. Two phosphate transitions were tested:

- a) Drastic transition: from 7 mg total P L⁻¹ phosphate sufficiency to 0.31 mg total P L⁻¹ (deficiency *sensu* Amaral et al. 2014) through the dilution of 21.8 mL of the P replete culture with 478.2 mL of N and P-free BG11 medium.
- b) Moderate transition: from 1 mg total P L⁻¹ phosphate sufficiency to 0.31 mg total P L⁻¹ deficiency through the dilution of 155 mL of the P replete culture with 345 mL of N and P-free BG11 medium.

In order to favor the persistence of the cultures, at day 5 in experiment 2a and at day 6 in experiment 2b, a volume around 20 mL was removed from the cultures and added fresh BG11 medium containing the corresponding 0.31 mg K₂HPO₄-P L⁻¹, until completing the 80 mL in every flask.

Every day all flasks were manually shaken and 3 mL was sampled for chlorophyll *a* fluorescence (AquaFluor® Handheld Fluorometer -Turner Designs) and optical density measurements (absorbance in 750 nm, spectrophotometer Thermo Evolution 60). The optical density data were used for calculation of growth rate according to Andersen (2005). After that, around 1.5 mL was conditioned in eppendorfs with acid Lugol's solution for further counts, biovolume estimates and morphological analysis. This was done to investigate differences in morphological traits among strains. However, it was done only for experiment 2a given that this was the one with the highest combined changes in temperature and phosphorus concentration.

The quantitative analysis of trichomes was performed for the initial and final days of growth rate of experiment 2a. A minimum of 100 trichomes was counted in each triplicate in an inverted microscope (Olympus CKX41) with a Sedgewick-rafter chamber. Then, the density was estimated as number of trichomes/mL, and the biovolume was calculated by multiplying the density of trichomes and median volume per trichome according Hillebrand et al. (1999): $Volume(\mu\text{m}^3) = \frac{\pi}{4} \times width(\mu\text{m})^2 \times length(\mu\text{m})$. Also, ten trichomes from each triplicate were measured considering several traits, and summed values per replicate were calculated. The measure was

carried out with an optical microscope (Olympus BX40) at 1000x magnification, and was made for the following traits: length (μm), width (μm), number of heterocytes and number of cells. The length per cell and the volume per trichome were also calculated for the 10 trichomes measured.

2.3 Statistical analysis

Mann-Whitney tests were used (given that assumptions of parametric tests were never met) to compare growth rates between the strains in all experiments. A factorial analysis was not used because the aim was to contrast strains under the same conditions and environmental shifts. Therefore, grow rates were compared:

1) Between strains in the same acclimation condition in order to evaluate if strains differ considering their response to temperature or temperature+nutrient shifts;

2) In the same strain between acclimation conditions in order to evaluate if acclimation affects response to temperature or temperature+nutrient shifts.

The Mann-Whitney test was also used to compare the traits measured for the experiment 2a. Firstly, we checked if traits differed between strains at the beginning of the exponential phase. Then, the change in traits along the exponential growth phase was compared, aiming to evaluate if strains have different behavior when growing. For that, it was used the difference between the final and initial trait values of each replicate. For further exploration of data in experiment 2a, we analyzed the relationship trichomes L^{-1} x chlorophyll a florescence between strains; and compared the relationships between trichome length and number of heterocysts for the two strains using a Nemenyi test. Statistical analyses and one graph were carried out in R environment (R Core Team, 2016). Other graphs were made using SigmaPlot version 11.0 (Systat Software, 2008) and STATISTICA version 7.1 (StatSoft 2005).

3 Results

3.1 Experiment 1: Growth rate responses of strains to temperature shifts

The mean growth curves (as well as the mean growth rates) estimated by the among-replicate averaged optical density data are shown in Figure 1 (as well as results for statistical comparisons). Acclimated strains did not differ at 25 °C, but LP1 had significantly higher growth rate than MVCC19 when strains were not acclimated. Both acclimated and not acclimated MVCC19 strains had higher growth rates at 32°C than in 25°C. On the other hand, LP1 did not attained exponential phase at 32 °C, and no growth rate was calculated (Figure 1).

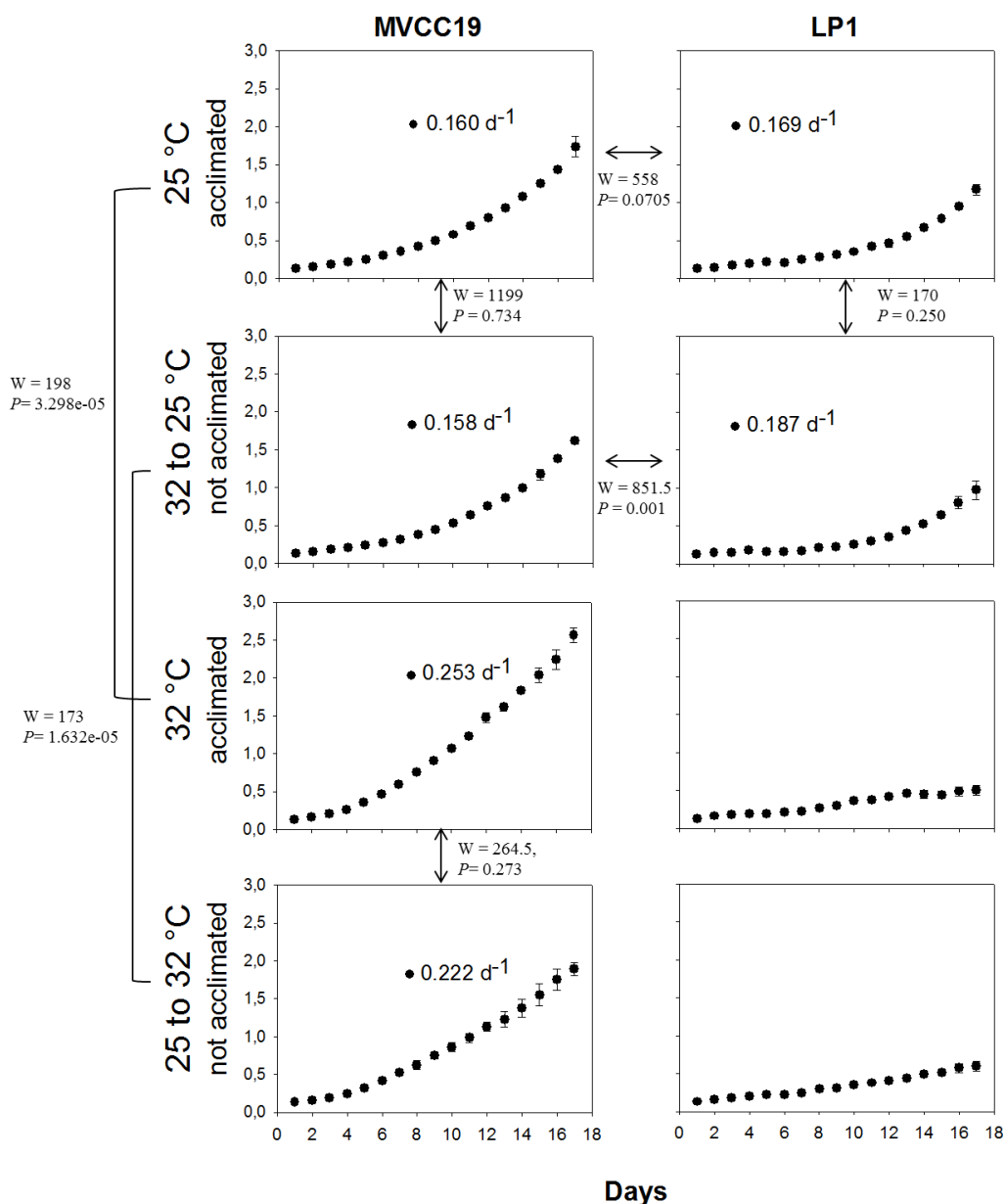


Figure 1. Growth curve of the experiment 1 (phosphorus sufficiency). The curve was obtained with the averaged (\pm standard deviation) values from optical density in 750nm (axis y) for each strain and temperature shift. Results

of Mann-Whitney tests are shown for the intended comparisons (see methods for details). No comparison was made for strains in which no growth rate was calculated.

3.2 Experiment 2: Growth rate responses of strains to temperature and phosphate shifts

Figure 2 shows the growth curves for the data obtained in experiment 2a. In this experiment, both strains did not grow at 25 °C (acclimated or not). At 32 °C, only one not acclimated LP1 replicate grew, which explain the high variation in the curve shown in Figure 2. At this temperature, acclimated strains did grow, and MVCC19 had significantly higher growth rate than LP1 (Figure 2). Strains also differed considering their growth behavior. MVCC19 grew faster while LP1 displayed a slower growth rate but a more prolonged exponential phase (note that LP1 did not achieve the end of stationary phase during the experimental period, Figure 2). This pattern is similar to that of the previous experiment at 25 °C, in which MVCC19 initiates the exponential phase earlier than LP1 and reaches high values of optical density more quickly, even with a lower mean growth rate (see Figure 1).

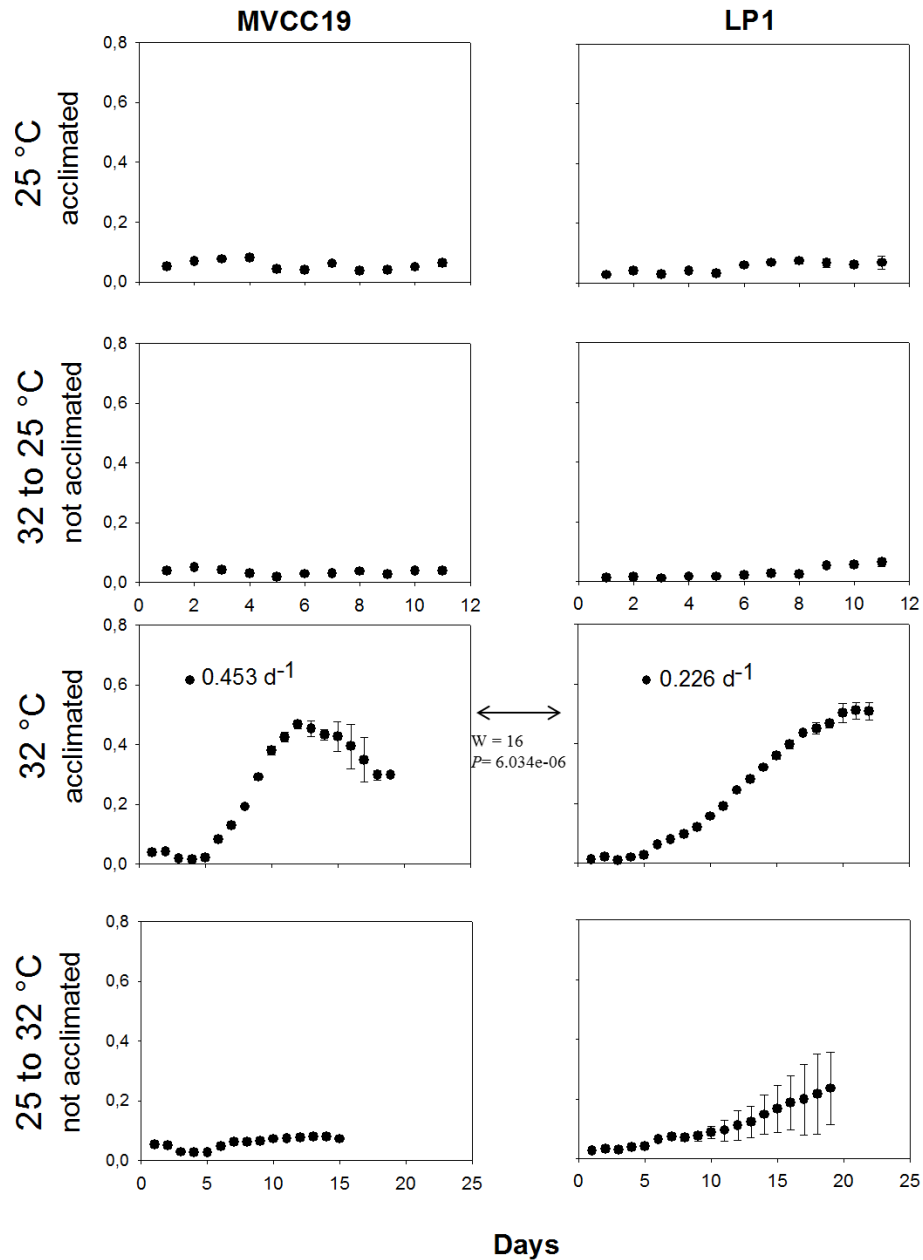


Figure 2. Growth curve of the experiment 2a (depletion from 7 mg total P L⁻¹ phosphate sufficiency to 0.31 mg total P L⁻¹). The curve was obtained with the averaged (\pm standard deviation) values of optical density in 750nm (axis Y) for each strain and temperature+phosphate shift. Results of a Mann-Whitney test is shown for the intended comparisons (see methods for details). No comparison was made for strains in which no growth rate was calculated.

In experiment 2a, several morphological traits and density data were compared at the beginning and ending periods of exponential growth by using acclimated strains at 32 °C (Figure 3, Table 1). Even that LP1 have started exponential growth with lesser trichomes and biovolume, this strain exceeded MVCC19 at the end of exponential phase in those aspects (Table 1).

Table 1. Median values of abundance of trichomes at initial and final days of exponential phase for acclimated strains at 32 °C, according to the second experiment. No statistical analysis is done given that counting data result in only one value per strain.

	STRAIN	
	LP1	MVCC19
Number of trichomes/L initial	1.42 x 10 ⁶	6.24 x 10 ⁶
Number of trichomes/L final	101.93 x 10 ⁶	64.00 x 10 ⁶
Biovolume initial (mm³/L)	0.30	1.72
Biovolume final (mm³/L)	75.42	21.92

Considering the traits measured at the beginning of the exponential growth phase for acclimated strains at 32 °C, only the initial number of heterocytes was significantly higher in MVCC19 strain ($P = 0.043$). LP1 have a higher increase than MVCC19 in length, number of heterocytes, number of cells and volume (Figure 3). Also, it is important to note that only width did not significantly increase in LP1 along the exponential growth phase (see that percentiles crossed the 0 in Figure 3). On the other hand, length, width, number of cells and volume per trichome of LP1 increased along the exponential growth phase, while number of heterocytes decreased for MVCC19 (see percentiles in Figure 3). Therefore, MVCC19 increased its biomass by the number of filaments only (Table 1); meanwhile LP1 also grew in number, length of filaments, number of cells and volume of trichomes as well.

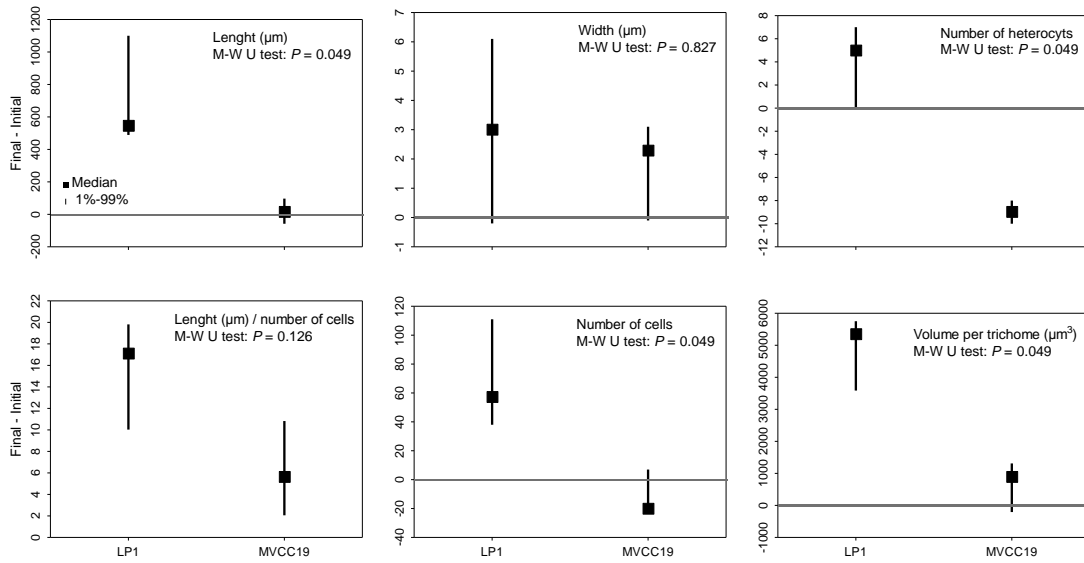


Figure 3. Median (1%-99% percentiles) of the difference between the final and first day of the exponential growth phase for several traits of the two strains – LP1 and MVCC19 acclimated strains at 32 °C (Experiment 2a). Type I error of a Mann-Whitney test comparing the change along the growth phase is also shown.

In figure 4 is shown the chlorophyll a fluorescence in relation to the number of trichomes. Even with lower and lesser trichomes, MVCC19 produced more chlorophyll a than LP1 in the exponential phase.

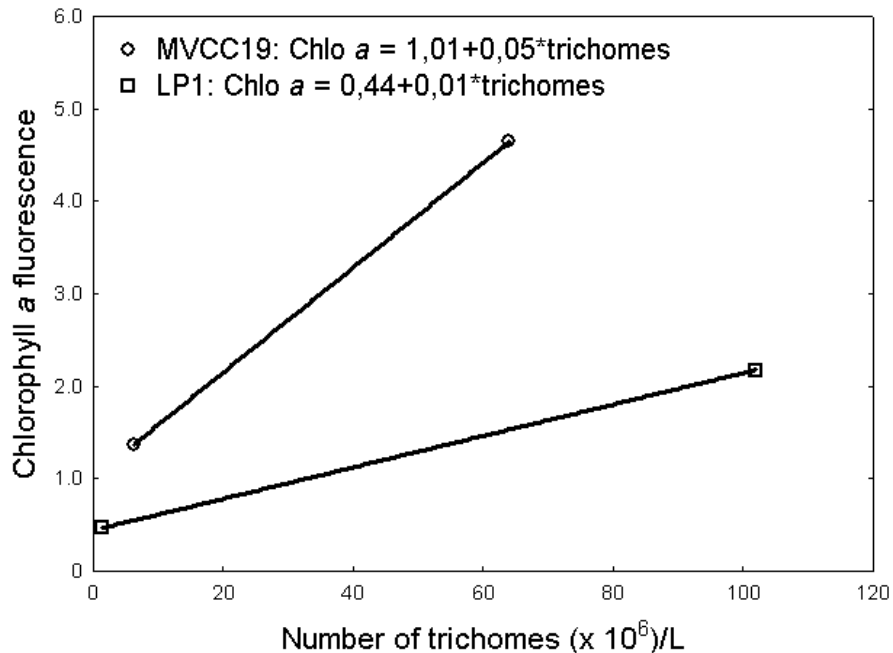


Figure 4. Scatter plot of chlorophyll a fluorescence in relation to number of trichomes in LP1 and MVCC19 obtained in the experiment 2a (the data used correspond to first and last days of exponential phase).

Figure 5 also explore further the data by showing the relationship between length of trichomes and number of heterocysts of all trichomes counted in experiment 2a. For LP1, longer trichomes had higher number of heterocysts (Nemenyi Test, $p < 0.05$), what was not observed for MVCC19.

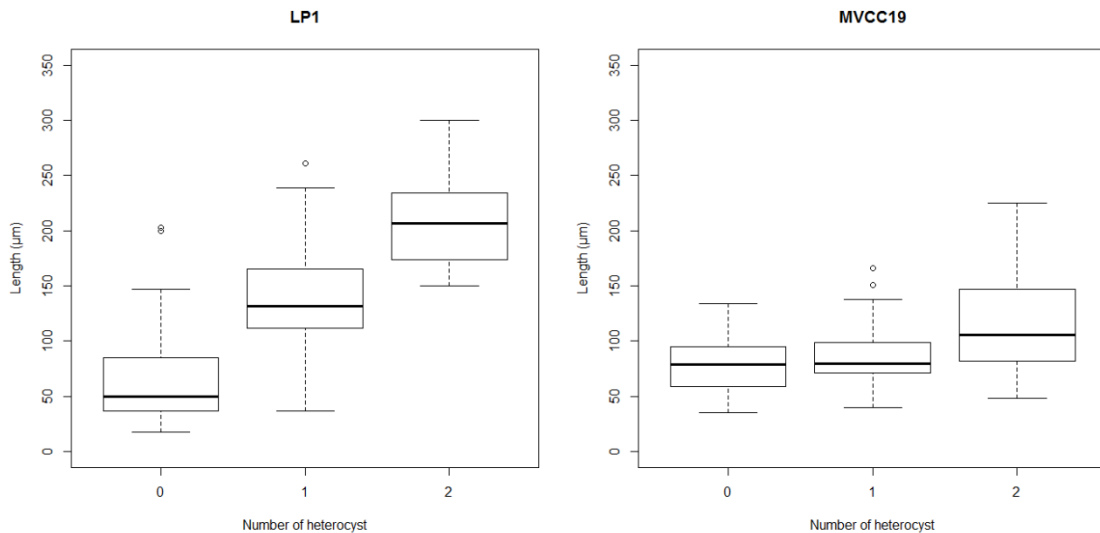


Figure 5. Distribution of trichomes length in relation to the number of heterocysts. All trichomes counted in experiment 2a were used for this graph.

Figure 6 summarize the growth curves obtained in experiment 2b. Distinct from experiment 2a, the temperature was not a determinant factor to strains' growth, since both MVCC19 and LP1 grew at 25 °C and 32 °C. In this experiment, the acclimation effect was not clear: under 25 °C, the acclimated strain of MVCC19 did not grow, while the acclimated LP1 grew; under 32°C only the acclimated MVCC19 grew, meanwhile grow was observed in both acclimated and not acclimated LP1 triplicates. Moreover, the growth of MVCC19 at 32°C had large deviation, suggesting that growth was close to suboptimal conditions.

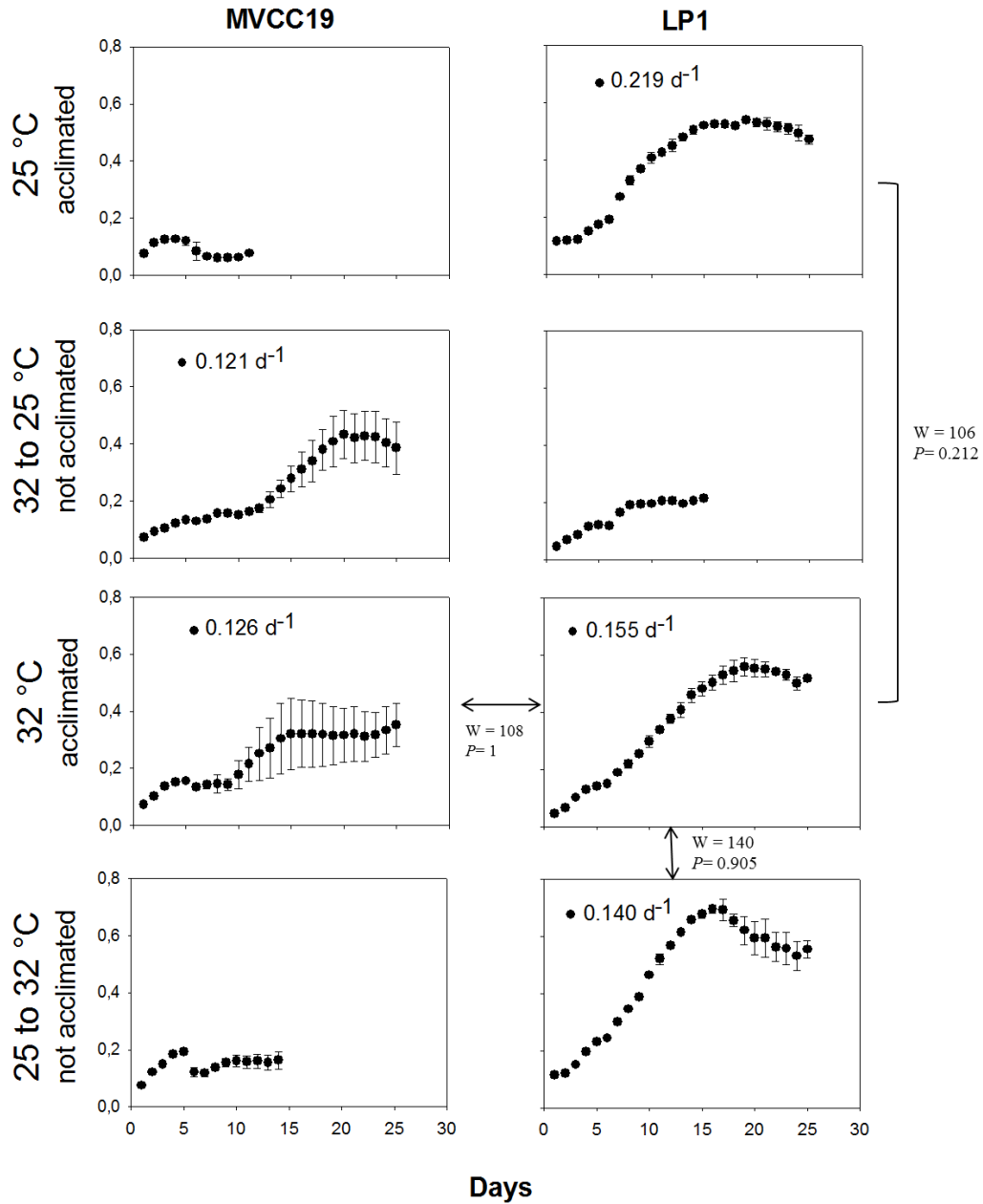


Figure 6. Growth curve of the experiment 2b (depletion from 1 mg total P L⁻¹ phosphate sufficiency to 0.31 mg total P L⁻¹). The curve was obtained with the averaged (\pm standard deviation) values of optical density in 750nm for each strain and temperature+phosphate shift. Results of Mann-Whitney tests are shown for the intended comparisons (see methods for details).

4 Discussion

Two main issues can be drawn from this study: *C. raciborskii* strains behave differently, but both were affected by temperature and phosphate shifts depending on acclimation treatments. Taken together, our results are in line with previous studies suggesting that strains of *C. raciborskii* have markedly distinct biological features (Chonudomkul et al. 2004; Stucken et al. 2009; Piccini et al. 2011). Considering the studied strains (LP1 and MVCC19), the results can be summarized as follows: under transition to phosphate deficiency, MVCC19 was explosive resembling bloom-forming growth behavior; whereas LP1 was slower with prolonged exponential growth phase. Moreover, MVCC19 increased its biomass by increasing the number of filaments, while LP1 increased not just the number of filaments but also the number of cells per trichome, total length and length per cell. As a consequence, LP1 reached a higher biomass at the end of exponential phase. Regardless, both strains under high transition from P sufficiency to P deficiency only could grow at 32°C when acclimated, pointing to a common aspect of uptake system adjustment. Our results suggest that the acclimation capabilities and strains distinct growth behavior, and morphological flexibility may reflect suitable plastic responses to succeed in different lake conditions (see also Burford et al. 2016; Bonilla et al. 2016).

4.1 Understanding LP1 and MVCC19 differences

It is important to note that both strains have been cultivated under the same laboratory conditions for at least one year, so it can be assumed that the differences found may reflect the morphological and physiological adequacy to the original environment. 'Lago Javier' (MVCC19) and 'Lagoa do Peri' (LP1) have only slight differences in average depth and temperature. 'Lagoa do Peri' has an average depth of 4.2 m (Hennemann and Petrucio, 2016) and an average temperature of 22.9 °C (Tonetta et al. 2017). 'Lago Javier' has an average depth of 6.8 m and an average temperature of 19.3 °C (F. Hirsch, pers. com.). Both lakes have light limitation, given that the euphotic zone:mix layer ratio is below c. 1 (Fabre et al. 2010; Tonetta et al. 2013). The main differences between lakes are their trophic state and homogeneity. 'Lagoa do Peri', is a meso to oligotrophic lake, vertical and horizontally homogeneous (Hennemann and Petrucio 2011) with higher transparency than 'Lago Javier'. Blooms

of *C. raciborskii* in 'Lagoa do Peri' have inspired many studies to understand which factors are involved in it (Tonetta et al. 2015; Hennemann and Petrucio 2016). On the other hand, 'Lago Javier' is an eutrophic to hipereutrophic and monomictic lake (Vidal and Kruk 2008; Fabre et al. 2010; Aguilera et al. 2017). The stratification and high organic matter produces an anoxic condition and high nutrients concentration in the hypolimnion (Aguilera et al. 2017), generating nutrient inputs by partial mixing more similar to pulse pattern.

Compared to LP1 in 'Lagoa do Peri', MVCC19 in 'Lago Javier' is subjected to rapid changes in environmental conditions, for example, exposure to low light or dark environment, due to restricted euphotic zone, and nutrient pulses due to partial water mixing of deep layers. In line with our results, nutrient pulses are frequently related to the occurrence of high growth rate organisms with high nutrient uptake rates (Litchman and Klausmeier 2008). Sudden changes in the position at the water column can also explain the higher pigment content of MVCC19 and the lower filaments length than LP1. Given that the pigment production is not limited by nutrient concentration, MVCC19 can increase pigment production and light absorption, another feature that highlight the plasticity of the species considering low light tolerance (Burford et al. 2016; Wojciechowski et al. 2016). Concomitantly, MVCC19 increased individual density by producing relatively (to LP1) short filaments and few heterocysts (see Figures 3 and 5). All such features suggest that compared to LP1, MVCC19 increase its chance of success as a *r* strategist (see Reynolds 2006).

In contrast to Javier limnological conditions, the constant vertical homogenization of water column in 'Lagoa do Peri' (Hennemann and Petrucio 2011; Tonetta et al. 2013; Hennemann and Petrucio 2016) possibly permitted an optimization of LP1 growth under mesotrophic nutrient state but with a better light environment than in 'Lago Javier'. This apparent strategy of growth may be reflected in the larger trichomes, probably by reallocating the nutrients and improving buoyancy to fix nitrogen and grow. It can be hypothesized that larger filament length and lower photosynthetic pigment content of cells may reflect a more stable environment with a higher light availability, while the opposite is probably more adequate for a more heterogeneous water column, with sudden changes in nutrient inputs and light availability.

On one hand, more heterocysts per trichome are produced in LP1 than in MVCC19; while pigment production is higher in MVCC19 than in LP1. Taken together, such results may reflect a trade-off between energy investment in pigment production or nitrogen fixing (Yema et al. 2016). Contrary to our results, Kenesi et al. (2009) investigated the effects of different nitrogen forms on *C. raciborskii* growth under reduced phosphorus concentration in chemostats and found a higher frequency of longest filaments without heterocysts. The explanation of the authors was the heterocysts position on terminal parts and the relative low uptake by long filaments. The discrepancy between the results may be not just the distinct methods (i.e. batch *versus* chemostat), but also the constant dilution and subsequent different grow rates compared by Kenesi et al. (2009); whose represent different conditions and optimizations. Also, such contrast may highlight that other *C. raciborskii* strains may have different behaviors to environmental changing.

Beyond the high P affinity and storage capacity of *C. raciborskii*, its success in a mesotrophic environment can be a result of P inputs like phosphate release from sediments and by heavy rainfalls. In fact, Henneman and Petrucio (2016) propose the sediments as an important P source to *C. raciborskii* in 'Lagoa do Peri'. The same explanation is given by Pádisak and Istvánovics (1997) for the discrepancy between the nutrients concentration decrease in the water column and the increased biomass of *C. raciborskii*. This temporal and spatial uncoupling between nutrient uptake and growth draws attention to an important aspect: the role of recent history of nutrient exposure of organisms. This highlights the role of previous exposure of organisms to a nutrient condition which affects the adjustment to the next nutrient status (Falkner et al. 1989; Plaetzer et al. 2005; Aubriot et al. 2011). When this phenomenon is considered, a consequent better correlation between nutrients and phytoplankton biomass is obtained when analyzing with delay in time (Wojciechowski and Padiál 2015). In our experiments, we tested the intensity of this effect in the third experiment (i.e. 2b). In contrast to experiment 2a, in 2b the strains could grow at 25°C and the acclimation treatment was not determinant on the success of the strains.

Relatedly, metabolic changes can be affected by temperature changes. For instance, the marine cyanobacterium *Synechococcus* from different regions had distinct genes expressions and proteins abundances under distinct temperatures,

what could explain the higher effect of the low temperature on the tropical clade of this taxon (Varkey et al. 2015). This represents intraspecific adaptations, which will determine a set of metabolic responses available for the organisms under stress. In our case, other environmental changes and consequent morphophysiological adaptations may act as determinants in how organisms overcome the stress. Since the functioning of P uptake system is dependent of ΔpH in thylakoid membrane (Falkner et al. 1989), it cannot be discarded the influence of pigment content in the process of adjustment to a new P external concentration. This could cause dissimilar reaction times of MVCC19 and LP1, explaining that MVCC19 may have an advantage in environments under pulses of P (Amaral et al. 2014). Given that it was not our goal, we suggested that plasticity in photoadaptation (i.e. capacity to change the pigments composition) must be further investigated in *C. raciborskii* (see also Bonilla et al. 2012; Bonilla et al. 2016). Also considering the *Synechococcus* clades, the increase in the expression of proteins involved in membrane transport occurred at low temperature (Varkey et al. 2015). According to the authors, this can indicate the influence of the temperature on nutrient uptake efficiency and/or increased internal demand. Therefore, relationship between molecular reactions and temperature may occur in other taxa, and is a possibility to be studied to explain the general responses of MVCC19 and LP1 to temperature shifts and acclimation treatments.

4.2 Common effects of temperature and acclimation on strains

Although all differences described above for the studied strains, both were capable to growth under severe P deficiency only when acclimated at the highest temperature. The high temperature tested in this study favored the adjustment capability of both strains to P deficiency. Shatwell et al. (2014), investigating the effect of temperature, photoperiod and P limitation on the growth of the diatoms *S. minutulus* and *N. acicularis* found the positive effect of higher temperature when the cultures were P limited. Higher temperature may favor overcoming P-limitation by adjusting the internal minimum cellular quota of organisms towards its reduction as temperature increases. Ahlgren (1987, 1988) described the same relationship; and the author affirms that when the growth conditions are not the optima, the minimal

quota cellular of the limiting (and sometimes the not limiting) nutrient increases. Organisms have a higher cost to adapt to a new stressing condition, reinforcing the role of recent environmental context in the success of the organism.

The tolerance to lower temperatures and photo inhibition is associated with the higher polyunsaturated lipid molecules (Varkonyi et al. 2000). Probably, the reduction in membrane fluidity by lower temperature will consequently alter the nutrient uptake machinery (Varkey et al. 2015), requiring more nutrients just for the maintenance of the cell. This increase in nutrient requirement could explain the reduction in thermal tolerance under N deficiency found by Thomas and Litchman (2015). In the opposite way, the success of *C. raciborskii* in low temperature (Vidal and Kruk 2008) can be considered as a result of trophic state compensating the negative effects of temperature. It may be concluded that the positive effect of temperature on nutrient deficiency may help explain the persistence of bloom-forming cyanobacteria during summer stratified periods in which phosphate have been depleted to undetectable levels at the euphotic zone.

5 Conclusion

Aiming to understand the drivers of *C. raciborskii* growth, two strains were submitted to simultaneous effect of temperature shift and phosphorus deficiency. The strains showed different growth strategies even when cultivated under the same laboratory conditions for more than one year, supporting the hypothesis of the existence of distinct ecotypes. Our results also support the role of interacting factors promoting the success of this species. This study showed that *C. raciborskii* is able to better overcome phosphorus deficiency under higher temperatures, which help explaining its persistency in oligo-mesotrophic lakes. The combined positive effect of temperature on nutrient deficiency tolerance may imply a drastic reduction in nutrient loads to constrain *C. raciborskii* development in warmer aquatic environments.

Acknowledgements

All experiments were executed in Univeridad de La Republica, Sección de Limnología. We acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) by the fellowship granted. We also thank F. Martigani for technical support and help during all experiments and Dr L. F. Fernandes for assistance and microscope use. A. A. P also acknowledges CNPq for continuous financial support.

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