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ORIGINAL ARTICLE

The success of the cyanobacterium *Cylindrospermopsis raciborskii* in freshwaters is enhanced by the combined effects of light intensity and temperature

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ABSTRACT

Toxic cyanobacterial blooms in freshwaters are thought to be a consequence of the combined effects of anthropogenic eutrophication and climate change. It is expected that climate change will affect water mixing regimes that alter the water transparency and ultimately the light environment for phytoplankton. Blooms of the potentially toxic cyanobacterium Cylindrospermopsis raciborskii *are expanding from tropical towards temperate regions. Several hypotheses have been proposed to explain this expansion, including an increase in water temperature due to climate change and the high phenotypic plasticity of the species that allows it to exploit different light environments. We performed an analysis based on eight lakes in tropical, subtropical and temperate regions to examine the distribution and abundance of* C. raciborskii *in relation to water temperature and transparency. We then conducted a series of short-term factorial experiments that combined three temperatures and two light intensity levels using* C. raciborskii *cultures alone and in interaction with another cyanobacterium to identify its growth capacity. Our results from the field, in contrast to predictions, showed no differences in dominance (>40% to the total biovolume) of* C. raciborskii *between climate regions.* C. raciborskii *was able to dominate the phytoplankton in a wide range of light environments (euphotic zone= 1.5 to 5 m, euphotic zone/mixing zone ratio <0.5 to >1.5). Moreover,* C. raciborskii *was capable of dominating the phytoplankton at low temperatures (<15°C). Our experimental results showed that* C. raciborskii *growing in interaction was enhanced by the increase of the temperature and light intensity.* C. raciborskii *growth in high light intensities and at a wide range of temperatures, suggests that any advantage that this species may derive from climate change that favors its dominance in the phytoplankton is likely due to changes in the light environment rather than changes in temperature. Predictive models that consider only temperature as a drive factor can therefore fail in predicting the expansion of this potentially toxic cyanobacterium.*

Key words: Harmful algal blooms (HABs); management; climate change; phenotypic plasticity; water transparency.

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INTRODUCTION

Predicted increases in temperature due to climate change will enhance the risk of potentially toxic cyanobacterial blooms in freshwaters (Paerl and Paul, 2012), threatening drinking water, recreation activities and other water uses. Furthermore, changes in mixing regimes and water column transparency that will accompany increased temperatures are also critical factors that will influence future phytoplankton composition and growth (Doney, 2006; Karlsson *et al.,* 2009). A recent major concern is the rise of toxic blooms of filamentous cyanobacteria belonging to the order Nostocales, in particular *Cylindrospermopsis raciborskii* (Sukenik *et al.,* 2012). This species was originally described as a tropical cyanobacterium (reviewed in Padisák, 1997) and has been cited as an invasive species (*sensu* Litchman, 2010) due to its apparent expansion from the tropics to temperate regions, where it now can form dense blooms (Padisák, 1997; Sinha *et al.,* 2012), potentially harmful due to the production of potent toxins (saxitoxin or cylindrospermopsin) (Piccini *et al.,* 2011). Hypotheses that explain the expansion of *C. raciborskii* include an increase in water temperatures due to climate change (Wiedner *et al.,* 2007), phenotypic plasticity that allows the species to exploit a wide range of light conditions due to pigment acclimation (Bonilla *et al.,* 2012; Mehnert *et al.,* 2012) and the capacity to grow under very low phosphate concentrations (Isvánovics *et al.,* 2000; Amaral *et al.,* 2014).

Studies of *C. raciborskii* ecology indicate that the species can dominate the phytoplankton community in a wide range of aquatic environments, deep to shallow, and mesotrophic to eutrophic lakes (Everson *et al.,* 2011; Sinha *et al.,* 2012; Sukenik *et al.,* 2012; Soares *et al.,* 2013). In these environments other cyanobacteria may also commonly alternate in dominance with *C. raciborskii*, including the colonial *Microcystis aeruginosa* (Marinho and Huszar, 2002; Soares *et al.,* 2009) and the filamentous *Planktothrix agardhii* and *Aphanizomenon* sp. (Kokociński *et al.,* 2010; Bonilla *et al.,* 2012). *Planktothrix agardhii* is a filamentous cyanobacterium that can grow forming resilient, dense blooms in shallow eutrophic ecosystems at different latitudes (Scheffer *et al.,* 1997; Bonilla *et al.,* 2012). *P. agardhii* and *C. raciborskii* can also co-occur in the same water bodies (Kokociński *et al.,* 2010; Bonilla *et al.,* 2012) and, therefore, they are excellent model species for use in tests of *C. raciborskii*'s growth capacity. This capacity may be evaluated using experimental studies that assess its response to changing environmental factors in combination with other similar species.

Increased temperatures are often accepted as the main physical factor that regulates the distribution of *Cylindrospermopsis raciborskii* worldwide (Padisák, 1997; Sukenik *et al.,* 2012). However, different temperature values (13°C and 20°C) for akinete germination have been reported in the literature (Padisák, 1997; Rücker *et al.,* 2009), while other studies showed high *C. raciborskii* biomass in freshwaters at lower temperatures (11 to 19°C) (Everson *et al.,* 2011; Bonilla *et al.,* 2012) and experimental evidence has shown that *C. raciborskii* growth rates have high variability at differing temperatures (15 to 40°C) (Briand *et al.,* 2004; Soares *et al.,* 2013). Overall, the available evidence indicates that *C. raciborskii* can succeed across a wide range of water temperatures and does not support the hypothesis that temperature is a limiting factor for its success.

Since the original classification of *C. raciborskii* as a shade-tolerant species (Padisák and Reynolds, 1998) with low saturation light intensities for growth (I_k <26 µmol photon m^{-2} s^{-1}) (Briand *et al.,* 2004), new evidence has shown that this species has a high phenotypic plasticity that may explain its good performance at high light intensities (Saker and Eaglesham, 1999; Piccini *et al.,* 2011; Bonilla *et al.,* 2012; Pierangelini *et al.,* 2014). Thus, light intensity itself may be not sufficient to explain the expansion of *C. raciborskii*, since it was reported to tolerate both low and high light intensities in temperate (Dokulil and Teubner, 2000; Wiedner *et al.,* 2007) and tropical regions (Soares *et al.,* 2009; Gomes *et al.,* 2013).

The roles of water transparency and light intensity in generating favorable conditions for the colonization of *C. raciborskii* are therefore not clear. The general problem of *C. raciborskii* expansion has not been thoroughly addressed despite the vast literature about this species (for reviews, see Padisák, 1997, Komárek and Komárková, 2003, and Sinha *et al.,* 2012). Most of the available ecological and physiological studies analyze the response of *C. raciborskii* to single variables (*i.e*., temperature *or* light), while few attempts have been made to explore the combined effects of these two factors in the species' performance (Bittencourt-Oliveira *et al.,* 2011; Bonilla *et al.,* 2012; Sinha *et al.,* 2012; Kehoe *et al.,* 2015), which could improve the interpretation of its distribution.

Based on experiments, in a previous study we found that the combination of warm temperature (25°C) with high light intensity (135 µmol photon m^{-2} s^{-1}) resulted in a significant increase in *C. raciborskii* growth rates (Bonilla *et al.,* 2012). These results provide an excellent starting point to explore the growth capacity of *C. raciborskii* in mixed cultures and to analyze its distribution in ecosystems with different water transparency and temperature.

In this study we work with a simple but realistic scenario. We designed short-term interaction experiments in which *C. raciborskii* was added as an inoculum to another cyanobacteria culture (*Planktothrix agardhii*). This species was selected for the interaction experiments because it co-occurs in nature with *C. raciborskii* (Kokociński *et al.,* 2010) and the two taxa have overlapping environmental preferences (*i.e*., turbidity and mixing tolerance, growth in a wide range of temperatures) (Briand *et al.,* 2002; Kokociński *et al.,* 2010; Bonilla *et al.,* 2012). The interaction experiments allow us to test the combined influence of temperature and light intensity on the growth capacity of *C. raciborskii*. We also integrate field and experimental data to study the distribution and growth of *C. raciborskii* and its relationship to water column transparency (*i.e*., the euphotic zone), light intensity and temperature changes. We analyzed a set of temperate, subtropical and tropical lakes where *C. raciborskii* occurred to determine the implications of these abiotic factors for the dominance of the species.

METHODS

Growth experiments under a combination of temperature and light levels

Short-term experiments were performed to compare the growth and morphological changes of *C. raciborskii* under different conditions of light and temperature in monocultures as well as mixed cultures with *P. agardhii*. Two Uruguayan isolates from eutrophic lakes were used: *P. agardhii* (MVCC11) (from Lago Rodó) and *C. raciborskii* (MVCC14) (from Laguna Blanca) and maintained in BG-11 full medium as described in Piccini *et al.* (2011).

To determine the effect of light intensity and temperature on *C. raciborskii* growth rates, triplicate growth curves were obtained in 96 hour experiments under two different light levels (60 and 135 µmol photons m^{-2} s^{-1}) and three temperature levels (low, medium and high: 15, 20 and 25°C, respectively). The light intensities were defined based on previous works (Bonilla *et al*., 2012 and reviewed in Burford *et al*., 2016) and summarized as low (60 µmol photons m^{-2} s^{-1}, below saturation intensity for growth) and high (135 µmol photons m^{-2} s^{-1}, saturation) for comparison. The two factors were combined in a factorial design: low (L_L) and high (H_L) light at low, medium and high temperature (L_T, M_T and H_T, respectively) to obtain six experimental conditions (L_LL_T, L_LM_T and L_LH_T, and H_LL_T, H_LM_T and H_LH_T). Before beginning the experiments, cultures were acclimated to each light intensity and temperature level for 10 to 15 days as described in Bonilla *et al.* (2012). Since we wanted to evaluate the growth of *C. raciborskii* while in interaction with another cyanobacterium, mixed cultures were designed. Cultures in exponential growth phase were selected for the experiment. Interaction experiments were performed by mixing the two species so that the *C. raciborskii* inocula represented 20% to 25% of the initial total biovolume, calibrated both by optical density (absorbance at 750 nm) and by counting under the microscope with a Sedgwick-Rafter chamber (1 mL volume). Before mixing, the density of both cultures was evaluated separately. Then, in 100 ml borosilicate culture bottles with fresh BG-11 medium, inocula of each culture, growing in exponential phase, were added to reach the proportion mentioned above and final culture volume was adjusted to 80 mL. The procedure was repeated three times to obtain three replicates. Bottles were kept with a bubbling system of sterile air and a light:darkness photoperiod of 16:8 h. Growth was evaluated by counting the filaments in 1 mL Sedgwick-Rafter chambers under an Olympus BX40 optical microscope at 200x magnification for each condition and time (initial, 48 and 96 hours). Sixty filaments of each replicate and each species (*C. raciborskii* and *P. agardhii*) were then measured (length and width) under a microscope at 1000x magnification to calculate the maximum linear dimension (MLD), volume, and surface/volume ratio. The individual volume was used to calculate the biovolume of the population (mm^3 L^{-1}). To follow the mono- and mixed cultures absorbance at 750 nm was measured every day and was used as a proxy of bulk biomass. Light intensity within the bottles was derived from the light extinction coefficient calculated from the absorbance at 440 nm according to Kenesi *et al.* (2009) and Kirk (1994). The growth rate (µ, d^{-1}) of each isolate was obtained from the slope of the linear function for each replicate between transformed biovolume (ln) and time. Data for growth curves of the two species in monoculture were obtained from Bonilla *et al.* (2012), who performed experiments in parallel under the same conditions. The growth capacity (*gc*) of *C. raciborskii* was measured as the rate of its population change in mixed cultures, and was calculated for each experiment and each replicate based on Sperfeld *et al.* (2010) using the formula:

$$gc = \log_2\left(\frac{BCrf}{BCri}\right)$$

where BCr*f* and BCr*i* are the species biovolume at 96 (final) and 0 (initial) hours, respectively. When *gc*=0, the species biovolume is unchanged, *gc* >0 indicates an increase in biovolume while *gc* < 0 indicates a negative growing effect in the mixed cultures.

Field data

We constructed a matrix of 219 data from eight lakes distributed in temperate, subtropical and tropical regions (Tab. 1). Samples were collected following an annual cycle (except for Bnińskie and Bytyńskie lakes, Poland, sampled from July to October) for different years: lakes Funil (2002), Juturnaíba (1996, 1997), Cruzeta (2010, 2011) and Tabocas reservoirs, Brazil (1997, 1998); Javier Lake, Uruguay (2012, 2013); Bnińskie (2004, 2005) and Bytyńskie (2004, 2005) lakes, Poland; and Alte Donau Lake (1993, 1994), Austria. All data were obtained following standard limnological methods with comparable analysis routines. Lake and species data were obtained from published (n=105) (Dokulil and Mayer, 1996; Marinho and Huszar, 2002; Soares *et al.,* 2009; Kokociński *et al.,* 2010; Bonilla *et al.,* 2012; Dokulil, 2015) and unpublished (n=114) data (kindly provided by F. Bressan and L. de Castro Medeiros). The eight lakes in our database varied in size and depth and had mesotrophic to hypereutrophic conditions (Tab. 1). The following environmental variables were used in our analyses: lake area (area, ha), water temperature (T, °C), maximum depth (z_{max}, m), euphotic zone (z_{eu}, m) and the ratio of the euphotic zone to the mixed zone (z_{eu}/z_{mix}). These two last variables (z_{eu} and z_{eu}/z_{mix}) were chosen as proxies to describe the light climate because they are both derived from apparent optical water properties; they are easy to measure, extensively used in the literature and indicate, in relative terms, the light conditions for phytoplankton (Jensen *et al.,* 1994; Reynolds, 2006). A ratio of $z_{eu}/z_{mix} \geq 1$ indicates high light availability for phytoplankton cells, while values <0.2 indicate strong light limitation (Wofsy, 1983; Reynolds, 2006).

Phytoplankton sampling followed routine protocols as detailed in Bonilla *et al.* (2012) and Kokociński *et al.* (2010). Phytoplankton samples were obtained in the euphotic zone and fixed with Lugol's solution. In six lakes (Alte Donau, Javier, Funil, Tabocas, Juturnaiba and Cruzeta) phytoplankton were counted in random fields, using sedimentation chambers and the inverted microscope technique (Utermöhl, 1958), until 100 individuals of the most abundant instead of frequent species was reached, while in Bnińskie and Bytyńskie lakes samples were counted on an upright microscope in 160 fields of a Fuchs-Rosenthal chamber (Kokociński *et al.,* 2010). Individual volume was calculated for each taxon according to the geometric models suggested by Hillebrand *et al.* (1999) and biovolume was expressed as mm^3 L^{-1}. The absolute biovolume of *C. raciborskii* and its relative contribution (%*C. raciborskii*) to total biovolume (BV) were analyzed.

Data analysis

Biological data did not fulfill the assumptions of normality and homogeneity of variance even after simple transformations, and thus a combination of statistical approaches based on non-normal distributions were used to explore relationships between variables. To examine the combined effects of temperature and light we tested the correlation (or absence thereof) between water temperature and z_{eu} and z_{eu}/z_{mix}. To explore trends between variables, the non-parametric Spearman rank order correlation was run between the relative contribution of *C. raciborskii* to total biovolume (hereafter %*C. raciborskii*), the coefficient of variation (CV) of the %*C. raciborskii*, temperature, z_{eu} and z_{eu}/z_{mix}. To test for differences in %*C. raciborskii* between lakes and climates (tropical, temperate and subtropical) a Kruskal-Wallis (K-W) one-way analysis of variance on ranks was performed. Then, when significant differences were found, an all pairwise multiple comparison procedure (Dunn's Method) was applied. Interpolation of raw data using inverse distance weighting was performed to construct a response 3D surface and contour plot of the %*C. raciborskii* (>10% of total biovolume) to water temperature and the z_{eu}/z_{mix} ratio from field information illustrating the relationships between the three numeric variables using the program Grapher 9.0.

Cylindrospermopsis raciborskii responses to temperature, z_{eu}/z_{mix} and latitude were explored by fitting regression models using a Generalized Linear Modeling (GLM) approach appropriate for non-normal error distributions (*i.e*., binomial) (Birks, 2012). Prior to GLM analysis, the dataset was categorized according to *C. raciborskii* contribution to total biovolume into two classes (0, 1). Two datasets were constructed; in the first, class 1 was assigned to samples in which *C. raciborskii* represented ≥10% of total biovolume (non-rare species), and class 0 was assigned to samples <10% of total biovolume. In the second dataset, class 1 included samples ≥40% (dominant species), and class 0 included all data below (<40%). Environmental variables (water temperature, z_{eu}/z_{mix} and latitude in decimal format) were standardized and 'lake' was included as a factor. We created models with binomial error distribution and logit link function that are appropriate for 0-1 data (Birks, 2012) and the model with the best fit was selected based on Akaike's information criterion (AIC; deleting a term whenever ΔAIC >2). The factor 'lake' was not significant in the first models and consequently was removed. Latitude was used as a proxy of climate and differences between lakes (given that all lakes were distinguished by their differing latitudes). To check the goodness of fit of the models we tested for unusual observations with the *leverage* index (Birks, 2012); unusual data were removed and the model was run again. We explored the residual distribution by plotting the deviance residuals against their theoretical quantiles and determining the reference bands to verify the plots were a straight line, following Augustin *et al.* (2012). We selected this method as the best option for a residual analysis of a binomial model (Faraway, 2006; Augustin *et al.,* 2012), that usually does not follow a normal error distribution. The variance explained by the models was summarized with D^2 [(deviance of the null model-residual deviance)/ deviance of the null model*100] (Guisan and Zimmermann, 2000).

A second generalized linear model was employed to detect significant differences in *C. raciborskii* growth rates between the different treatments of mono- and mixed cultures (categorical factors: temperature, light intensity, culture condition). Data distribution was examined before the analysis for normality and equal variance (Shapiro-Wilk and Levene tests, respectively). Data distribution, although with deviation from normality, had equal variance, so the analysis was performed using normal distribution (best fit) and an identity link function. Two-way analysis of variance was performed to test significant differences and interaction between the factors temperature and light intensity on the growth capacity (*gc*) of *C. raciborskii*. All statistical analyses were run with the software Statistica 6.0 and R 3.0.3.6 (function *lmer* enfolded in the package *lme4*).

RESULTS

Growth models of *C. raciborskii* in mixed cultures

The culture trichome density variation and the growth rates in short-term experiments showed that light intensity and temperature affected the growth of *C. raciborskii* and that there was interaction between the two factors (Figs. 1 and 2; Tab. 1). However, *P. agardhii* did not change significantly under different light intensities, and temperature was the only factor that explained changes in trichome density and growth rate (Figs. 1 and 2; Tab. 1). *C. raciborskii* grew significantly faster at H_LH_T and second fastest at L_LH_T (Fig. 2). *C. raciborskii* growth was enhanced by higher light intensities and temperatures, as well as by the combined effect of high values of both variables (Tab. 1, Fig. 2). *C. raciborskii* growth rates (d^{-1}) did not differ significantly between monocultures and mixed cultures, and thus no interaction (positive or negative) was detected in the presence of *P. agardhii* (Fig. 2, Tab. 1). In contrast, *P. agardhii* growth responded to increases in temperature, with no differences between mono- and mixed cultures (Fig. 2, Tab. 1). In one treatment, H_LH_T, *P. agardhii* growth in mixed cultures was higher than L_LH_T, suggesting that it was favored by the presence of *C. raciborskii* (Fig. 2).

The growth capacity of *C. raciborskii* in mixed cultures was always positive (*gc* >0), indicating that the species was capable of growth over a wide range of temperatures and in both high and low light intensities (Fig. 3). The expansion of *C. raciborskii* inoculum into *P. agardhii* cultures was significantly promoted by increases in temperature (ANOVA, F =56.09, P<0.001) or light intensity (ANOVA, F =31.45, P<0.001), although no interaction effect was found between the two factors (ANOVA, P>0.05) (Fig. 3).

Distribution of *C. raciborskii* along different temperature and transparency conditions

C. raciborskii reached high biovolume in all regions, at low and high temperatures, and in a wide range of water column transparencies as indicated by euphotic zones (Tab. 2). *C. raciborskii* dominance of BV (*i.e.,* >40% of BV) was found, in general, at z_{eu} >1 m and up to 5 m (Fig. 4), and of >80% of BV was reached at temperatures between 8 and 30°C. The lowest maximum biovolume of the species (>18.5% of BV) was registered in two temperate lakes (Bnińskie and Bytyńskie) (Tab. 2). However, in another temperate lake (Alte Donau), *C. raciborskii* dominated the phytoplankton community (72.3% of BV) at low temperature (1.97°C) (Fig. 5). In the subtropical lake (Javier), the phytoplankton was dominated by *C. raciborskii* at low and high temperatures (15°C: 18 mm^3 L^{-1} *C. raciborskii* and 79.5 %*C. raciborskii*; 25°C: 10 mm^3 L^{-1} *C. raciborskii* and 63.3 %*C. raciborskii*) (Fig. 5). *C. raciborskii* represented a high proportion of total phytoplankton biovolume at low z_{eu}/z_{mix} ratio with temperatures below 25°C and at high z_{eu}/z_{mix} ratio at higher temperatures (Fig. 6). No significant differences were found between regions (K-W, P>0.05) in %*C. raciborskii*, but significant differences were found between lakes (K-W, P<0.05) and most of the lakes' pairwise combinations

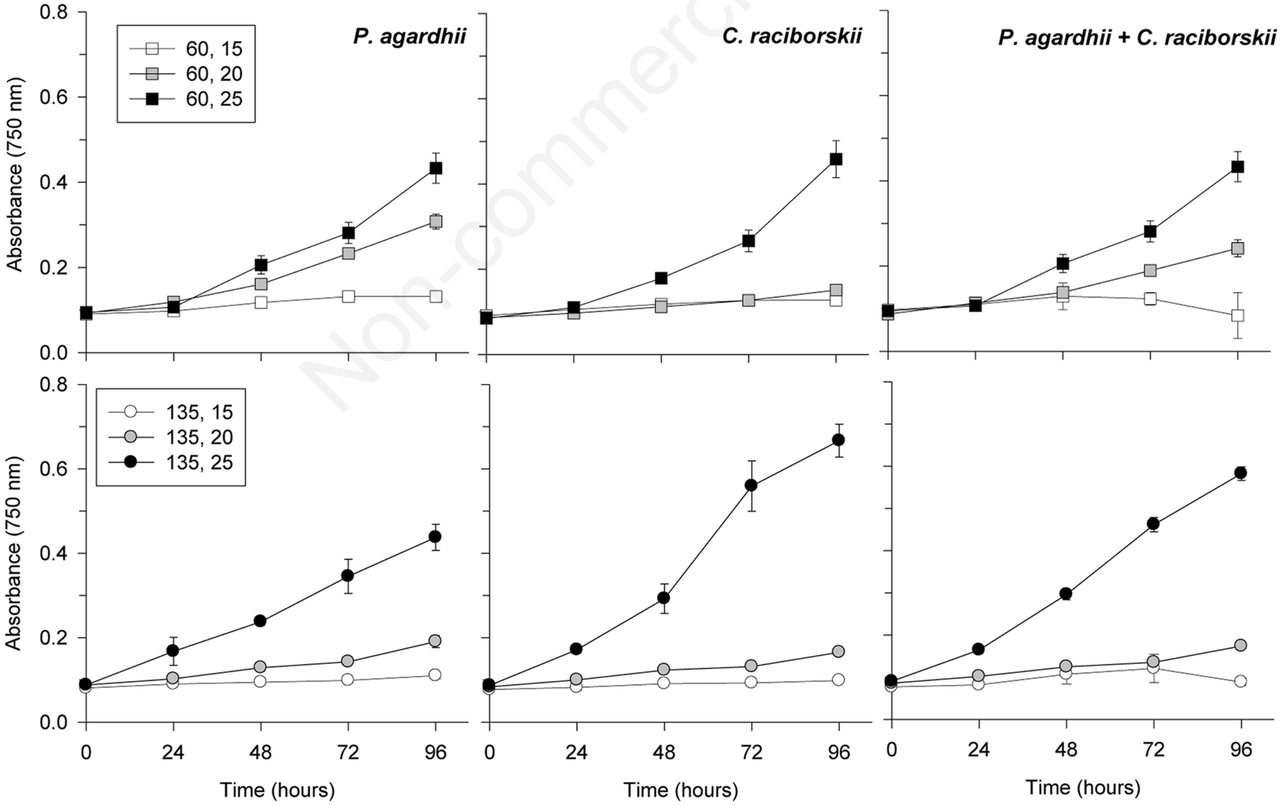

Fig. 1. Growth curves of *Cylindrospermopsis raciborskii* (*C. raciborskii*) and *Planktothrix agardhii* (*P. agardhii*) monocultures and mixed cultures (*C. raciborskii* and *P. agardhii*) at three temperatures (15°C: white, 20°C: grey and 25°C: black) and two light intensities: low (upper panels; 60 µmol photon m^{-2} s^{-1}: squares) and high (lower panels; 135 µmol photon m^{-2} s^{-1}: circles). Data are averages with standard deviations (vertical lines), n=3.

(Dunn's Method, $P<0.05$, except for Alte Donau *vs* Funil, Javier *vs* Juturnaíba, Funil *vs* Cruzeta and Juturnaiba *vs* Bnińskie, where $P>0.05$).

The coefficient of variation (CV) of %*C. raciborskii* was positively correlated with lake area (Spearman, P=0.727, $P<0.05$) and with its maximum biovolume per lake (Spearman, P=0.738, $P<0.05$). %*C. raciborskii* was also positively correlated with z_{eu} and z_{eu}/z_{mix} ratio (Spearman, P=0.359, $P<0.05$ and P=0.281, $P<0.05$, respectively), while no correlation was found with temperature ($P>0.05$) (Figs. 5 and 6). The presence of *C. raciborskii* when it represented >10% of BV and when it was dominant (>40% of BV) responded negatively to latitude and temperature and positively with light (Tab. 3). When *C. raciborskii* was

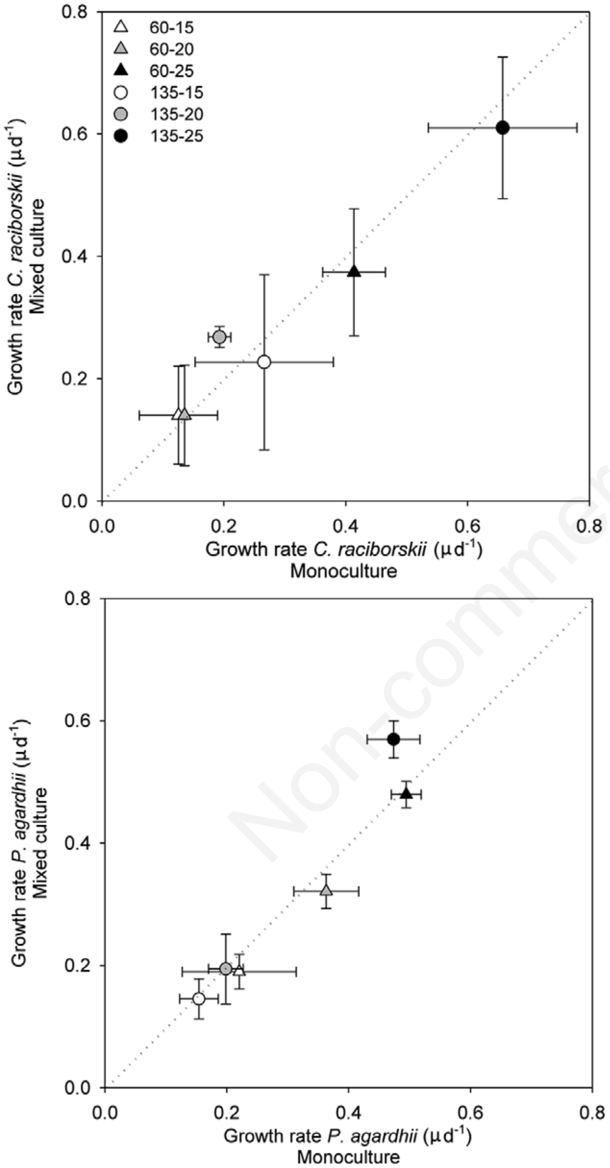

Fig. 2. Growth rates of *C. raciborskii* in monocultures and mixed cultures with *P. agardhii* (upper panel) and *P. agardhii* in monocultures and mixed cultures with *C. raciborskii* (lower panel) under 60 or 135 µmol photons m^{-2} s^{-1} (triangles and circles, respectively) and at 15, 20 and 25°C (white, grey and black, respectively). The solid diagonal line indicates a theoretically perfect correlation 1:1. Data are average with standard deviation, n=3.

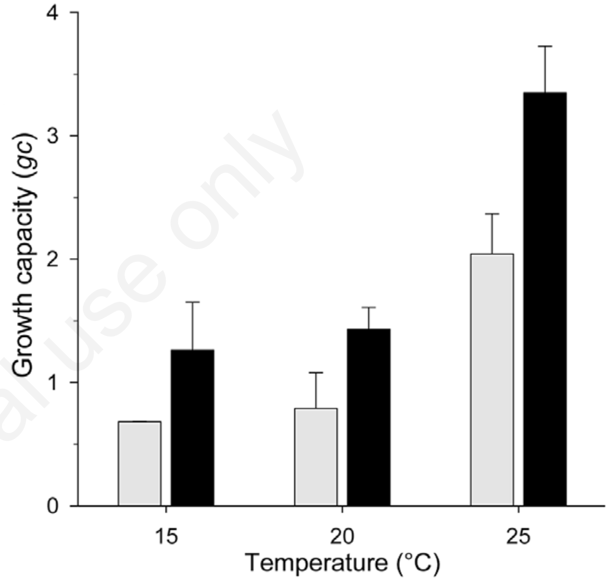

Fig. 3. Growth capacity index for *C. raciborskii* at two light intensities (60 and 135 µmol photons m^{-2} s^{-1}) and three temperatures in mix cultures with *P. agardhii*. Data are averages with standard deviations (vertical lines, grey and black bars, respectively), n=3.

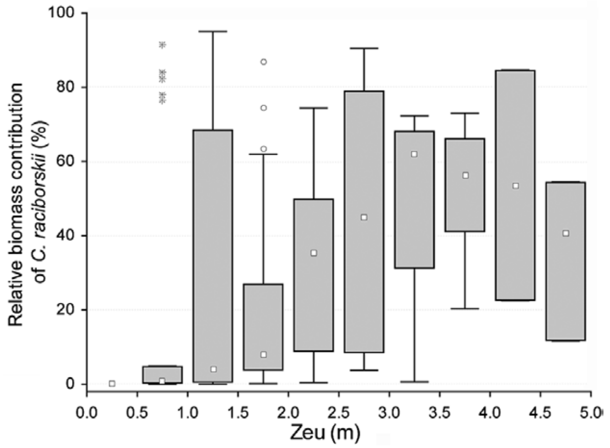

Fig. 4. Contribution of *Cylindrospermopsis raciborskii* to total biovolume in relation to the depth of the euphotic zone for all data and all lakes. Median (white), percentiles 25% and 75% (grey), range (vertical lines), outliers (°) and extremes (*) are shown.

dominant (>40% of BV) there was no significant interaction between factors; when sites where *C. raciborskii* >10% of BV, a combined effect of temperature and latitude was found (Tab. 3).

DISCUSSION

Temperature has been one of the main factors invoked to explain changes in the biogeographic distribution and abundance of *Cylindrospermopsis raciborskii*. In our study we examined temperature, both alone and in combination with light, in an effort to better understand their implications for the expansion of *C. raciborskii*. Our field and experimental approaches provide new data which support the hypothesis that this species is able to grow and dominate the phytoplankton community in a wide range of temperatures, as stated in other studies, and is also significantly promoted by elevated light intensities. This last aspect is critical, since transparent waters, usually at lower trophic levels, may thus also prove to be appropriate habitats for the initial growth and later dominance of this harmful cyanobacterium. Light intensity and water transparency are factors that are often overlooked in water management. Our results suggest that transparent freshwaters from temperate to tropical regions may be suitable environments for the potential increase in population abundance of *C. raciborskii*.

It is usually assumed that cyanobacteria are superior competitors at high temperature relative to other phytoplankton species (Paerl and Huisman, 2008; Paerl and Otten, 2013). However, recent experimental studies found no differences in the growth rates of eight Cyanobacteria (including *C. raciborskii*) and eight Chlorophyta species incubated at higher (35°C) and lower (25°C) temperatures (Lürling *et al.,* 2013). Other studies demonstrated growth at low temperature (15°C) in experiments (Chonudomkul *et al.,* 2004) or maximum growth rates at 24°C, and not at 30°C as generally reported (Saker and Griffiths, 2000).

In our study, *C. raciborskii* dominated the phytoplankton at high and low temperatures (*e.g*., 1.96°C, Alte Donau) and was capable of phytoplankton dominance at different temperatures (Fig. 4), in agreement with other studies (Stüken *et al.,* 2006; Moreira *et al.,* 2015; Dokulil, 2015). Vegetative cells of *C. raciborskii* can survive at low temperatures (<12°C) and low light intensities, as found in Alte Donau Lake (Dokulil, 2015). *C. raciborskii* populations appeared in German lakes when temperature increased above 15 to 17°C (Wiedner *et al.,* 2007), and were negatively correlated with temperature in a survey that analyzed 46 Polish lakes (Kokociński and Soininen, 2012). The experimental and field data, taken together, thus support recent findings that indicate that the species can grow in a wide range of temperatures (Bonilla *et al.,* 2012; Dokulil, 2015). The factors that affect the distribution of *C. raciborskii* are therefore more likely derivative effects of temperature, such as mixing regime changes, instead of temperature itself.

In our dataset, *C. raciborskii* dominated the phytoplankton (>40% of BV) across a wide range of biovolume, with no differences between tropical lakes and those of other latitudes in terms of *C. raciborskii* biomass or its dominance of the phytoplankton. The total

Tab. 1. Results of the three ways ANOVA for differences in growth rate of each species (*C. raciborskii* and *P. agardhii*) under different treatments (light and temperature) and culture conditions (mono- and mixed cultures).

	Df	SS	MS	F-statistic	P
C. raciborskii					
Intercept	1	1.0044	1.0044	3693	0.0003**
Mono-mixed	1	0.0010	0.0010	3.802	0.1905
Light	1	0.0602	0.0602	221.4	0.0045**
Temperature	2	0.2682	0.1341	493.1	0.0020**
Mono-mixed-light	1	0.0001	0.0001	0.363	0.6083
Mono-mixed-temp	2	0.0064	0.0032	11.71	0.0787
Light-temp	2	0.0141	0.0071	25.96	0.0371*
Error	2	0.0005	0.0003		
P. agardhii					
Intercept	1	1.2056	1.2056	2138	0.0005**
Mono-mixed	1	<0.0001	<0.0001	0.007	0.9411
Light	1	0.0092	0.0092	16.38	0.0560
Temperature	2	0.2273	0.1137	201.6	0.0050*
Mono-mixed-light	1	0.0024	0.0024	4.289	0.1742
Mono-mixed-temp	2	0.0025	0.0013	2.264	0.3064
Light-temp	2	0.0163	0.0081	14.45	0.0647
Error	2	0.0011	0.0006		

*Df, degrees of freedom; SS, sum of squares; MS, mean sum of squares; * P<0.05; **P<0.001.*

and relative biovolume of *C. raciborskii* in our subtropical lake (Javier Lake) was similar to those from other subtropical lakes in Brazil (Tonetta *et al.,* 2013), China (Lei *et al.,* 2014) and Australia (Everson *et al.,* 2011). The three temperate lakes in the dataset (Bnińskie and Bytyńskie, Poland; Alte Donau, Austria) were all shallow, polymictic, and eutrophic to hypereutrophic (Kokociński *et al.,* 2010), but they differed markedly in their *C. raciborksii* biovolumes. Similar differences were found in other studies that examined phytoplankton composition and dominance of cyanobacteria species in temperate lakes located within the same region (Briand *et al.,* 2002; Mischke, 2003). The tropical lakes of our dataset (Funil, Juturnaíba, Tabocas and Cruzeta lakes) overlapped in their *C. raciborskii* biovolumes, although they differed in the relative contribution of the species to total phytoplankton biovolume, probably due to local lake characteristics and hydrodynamic constraints (De Senerpont Domis *et al.,* 2013; Soares *et al.,* 2013). These differences underline the importance of local conditions that favor the growth and accumulation of biomass of one particular species.

Our data show that light environment is critical for understanding the ecology and distribution of *C. raciborskii.* The species can exploit high light conditions, as found in field (Fabbro and Duivenvoorden, 1996; Dokulil and Mayer, 1996) and experimental studies (Wu *et al.,* 2009; Mehnert *et al.,* 2010). Our analysis showed maximum *C. raciborskii* dominance of the phytoplankton in both low (z_{eu}/z_{mix} <0.2) and high (z_{eu}/z_{mix} >1) transparency conditions, reinforcing the species' capacity for success in different light environments due to its phenotypic plasticity (Piccini *et al.,* 2011; Mehnert *et al.,* 2012). Furthermore, our experimental data clearly show that light intensity is a critical factor in *C. raciborskii* performance, as growth rates indicated a clear optimization of species fitness at high temperatures and light intensities (Fig. 2). At high light intensity *C. raciborskii* overcame the limitations imposed by low temperatures and was able to expand. However, *C. raciborskii* blooms are also highly tolerant of self-generated low light intensities. This was observed in low transparency conditions (*i.e.*, z_{eu}/z_{mix} <0.2) where the species dominated the phytoplankton community (Bonilla *et al.,* 2012). Acclimation capacity of the species to dif-

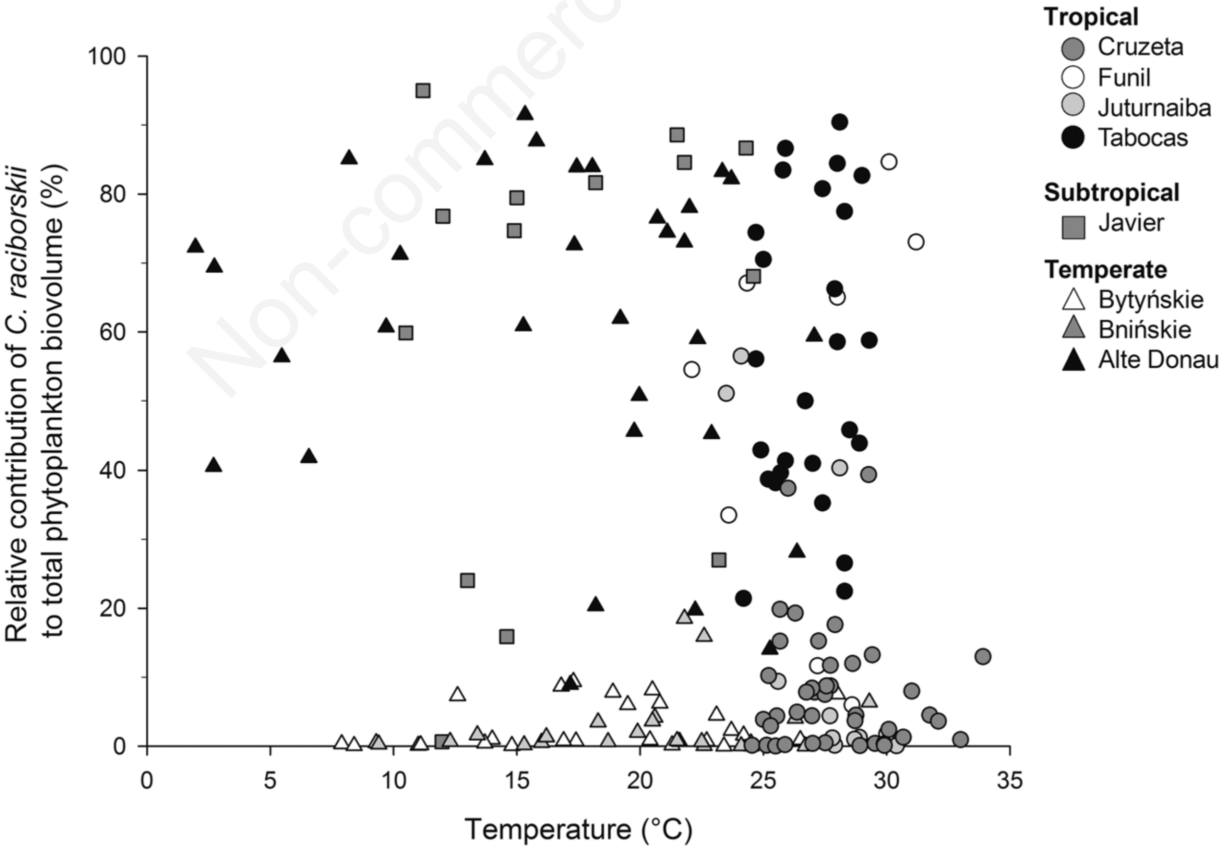

Fig. 5. Contribution of *Cylindrospermopsis raciborskii*, when present, to total biovolume, in relation to the water temperature for all data and the eight lakes.

Tab. 2. Characteristics of the studied lakes and euphotic zone (Z_{eu}), temperature and *Cylindrospermopsis raciborskii* biovolume analyzed in this study. Trophic state based on total phosphorus.

Lake name	N	Latitude	Climate	Area (ha)	Trophic state	z_{max} (m)	z_{eu} (m) min-max	Temperature (°C) min-max	*C. raciborskii* (mm^3 L^{-1}) min-max	Max %*C. r.* biovolume
Bnińskie*	31	52°12'N	Temperate	226	HE	8.5	0.96-2.40	9.3-29.3	0.02-2.1	18.5
Bytyńskie*	31	52°29'N	Temperate	309	HE	7.0	0.60-2.28	7.9-28	0.05-2.9	9.4
Alte Donau°	32	48°23'N	Temperate	158	HE	6.8	0.63-4.75	1.9-27.1	0.54-52.5	91.5
Javier	14	34°51'S	Subtropical	235	M	10	0.61-3.05	11-25.8	0.21-34.9	95
Funil	11	22°30'S	Tropical	4000	E	45	0.6-9.0	20-31.6	0.01-11.7	97.4
Juturnaíba	26	22°33'S	Tropical-humid	4300	E	5.6	0.67-1.89	20.9-30.4	0.01-12.5	56.5
Tabocas	26	08°06'S	Tropical-humid	161	E	18	1.05-4.2	24.2-29.3	0.6-17.7	90.4
Cruzeta	48	06°24'S	Tropical semi-arid	616	E	6.5	1.2-2.64	24.6-30	0.02-39.4	39.4

*E, eutrophic; HE, hypereutrophic; M, mesotrophic; min, minimum; max, maximum; N, number of data; Max %C. r., maximum relative contribution of C. raciborskii to total phytoplankton biovolume; *Kokociński et al., 2010; °Dokulil and Teubner, 2003 and Dokulil, 2015.*

Tab. 3. Results of the final generalized linear model (GLM) analyzing the effects of location (latitude), temperature and light for the presence of *C. raciborskii* when >10% of total biovolume (*C.r.*>10%) (D^2=37.3%, AIC=132.7) and when *C. raciborskii* >40% of total biovolume (*C.r.*>40%) (D^2=22.9%, AIC=169.9). Results are shown for estimated coefficients, their standard errors (SE) and the statistical significance P for the explanatory variables.

	C.r.>10%			*C.r.*>40%		
	Estimate	SE	P	Estimate	SE	P
Intercept	2.3936	0.6498	0.0002**	-0.0477	0.3794	0.8999
Temperature	-2.8537	0.7787	0.0003**	-1.3731	0.3613	0.0001**
Latitude	-2.4242	0.6034	<0.0001**	-1.8605	0.4867	0.0001**
Light	1.7157	0.3894	<0.0001**	0.5712	0.2155	0.0080*
Interactions						
Temp:Light	0.6379	0.5066	0.2080	0.4803	0.2903	0.0980
Lat:Light	1.008	0.7455	0.1764	0.3351	0.4750	0.4805
Temp:Lat	2.271	0.7034	0.0012*	0.5405	0.3699	0.1439

Lat, latitude; Temp, temperature; Light, Z_{eu}/Z_{mix}. *$*P<0.05$; $**P<0.001$.*

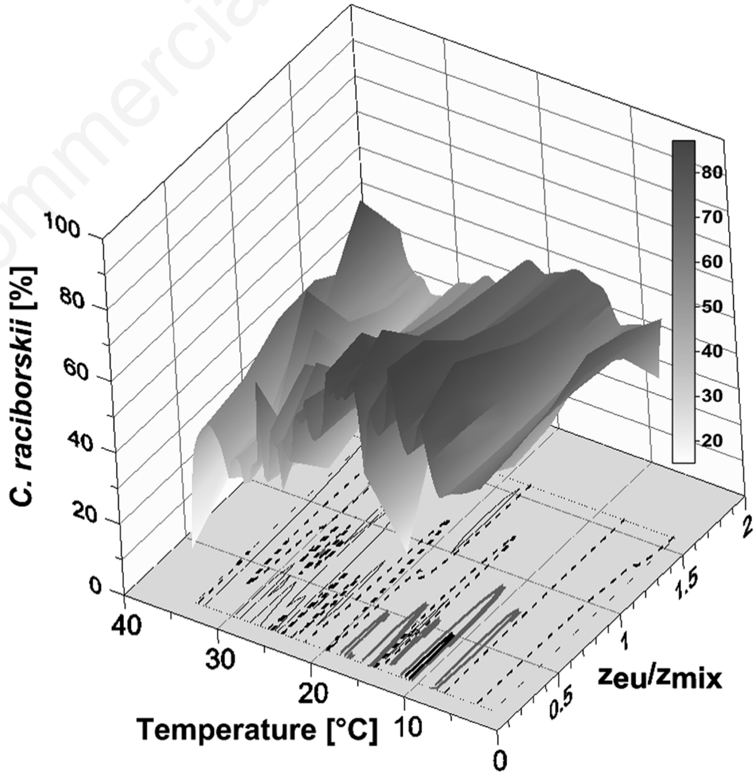

Fig. 6. Surface plot found from interpolation of the raw data (inverse distance weighting) of %*C. raciborskii* (grey gradient of the mesh) water temperature and euphotic zone to mixing zone ratio (zeu/zmix) of the eight studied lakes. Lines in the bottom indicates isobaths for %*C. raciborskii* (solid grayline, 80%; solid black line, 90%).

ferent light conditions may explain high primary production rates in dark-acclimated populations, as shown in experiments with natural phytoplankton (O`Brien *et al.,* 2009). Short term field experiments with *C. raciborskii* blooms showed maximum photosynthesis at high temperature (28°C) with saturation at low light intensity, indicating a strong interaction between light and temperature (Kehoe *et al.,* 2015).

CONCLUSIONS

In our study, there was no clear negative interaction between *C. raciborskii* and *P. agardhii* in the mixed culture short term experiments. Moreover, *P. agardhii* is a shade adapted species and its growth was favored by the presence of *C. raciborskii* at high light intensity, which might be related to the shading effect of *C. raciborskii* biomass in the mixed culture (Padisák and Reynolds, 1998; Bonilla *et al.,* 2012). The tolerance of *C. raciborskii* to changes in light intensity may also help to explain its growth under a combination of environmental stresses. Light intensity and UV radiation can have consequences for *C. raciborskii* toxin expression, as shown by a recent study where greater UVB exposure enhanced *C. raciborskii* saxitoxin gene expression and production (Beamud *et al.,* 2016). Also, in factorial experiments with phosphate concentrations and light intensity, Marinho *et al.* (2013) found that *C. raciborskii* can outcompete or be displaced by *Microcystis aeruginosa*, depending on the combination of the two factors and on the strains. More experimental studies including light intensity and its fluctuations are needed to identify the capacity of *C. raciborskii* to overcome combinations of stress factors.

Future predicted effects of climate change include dramatic shifts in seasonal water stratification-mixing cycles that will directly affect the euphotic depth and light intensity in lakes (Karlsson *et al.,* 2009; Posch *et al.,* 2012). These physical changes will affect phytoplankton composition, resulting in changes in species dominance (Rühland *et al.,* 2008; Posch *et al.,* 2012). In this context, the importance of light as a critical factor for determining the initial growth and expansion of *C. raciborskii* has significant implications in the context of changing climates. The species is increasingly being found in freshwater water bodies around the world, indicating no climate restrictions (Burford *et al*., 2016), except for extreme polar regions. According to our data, although temperature regulates *C. raciborskii* growth in general, there is no clear temperature limitation controlling its expansion, and changing stratification dynamics in temperate lakes, due to climate change, may therefore present opportunities for the future success of this species. Water management programs must therefore take into account that even slight changes in light transparency can enhance the presence of this harmful cyanobacterium.

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