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PRIMARY RESEARCH PAPER

Influence of UV-B radiation on the fitness and toxin expression of the cyanobacterium *Cylindrospermopsis raciborskii*

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Abstract The harmful bloom-forming cyanobacterium *Cylindrospermopsis raciborskii* grows in freshwaters over a wide range of light conditions. This species has increased its global distribution recently. The influence of ultraviolet radiation (UVR) on the fitness and toxin production of *C. raciborskii* has not previously been explored. We performed short-term experiments with three *C. raciborskii* strains (MVCC19, LB2897, and CYP011 K), and we compared their responses with other bloom-forming species (*Microcystis* sp.1 and *Plankthotrix agardhii*) to determine the impact of UV-B radiation on pigments, biomass, and morphological traits. In addition, we analyzed the effect of UV-B on the saxitoxin content and *sxtU* gene expression in the strain MVCC19. *C. raciborskii* strains were stressed differentially by UV-B exposure as evidenced by changes in growth, morphology, and heterocytes number. A significant increase in saxitoxin concentration and *sxtU* gene expression under UV-B suggests that toxin production in *C. raciborskii* can be a response to UV-B stress. In comparison, *Microcystis* sp.1 was more tolerant, while *P. agardhii* was severely impacted by UV-B, indicating also different sensitivities among cyanobacteria to UVR. Our results underscore the influence of UVR on *C. raciborskii* and the differences between strains which showed phenotypic plasticity, which potentially could affect its distribution in freshwaters.

Keywords UVR · *Microcystis* · *Planktothrix* · Traits · Saxitoxin · Gene expression

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Introduction

Nuisance blooms of planktonic cyanobacteria in freshwaters are a worldwide phenomenon. These organisms are expanding geographically apparently due to climate change, and threaten many of the world's largest and most important water bodies (Paerl et al., 2011; Sukenik et al., 2012). Cyanobacteria species that bloom at the water surface are exposed to high levels of solar radiation, including ultraviolet radiation (UVR, 280–400 nm), particularly at the beginning of the seasonal growth phase in transparent waters. UVR can affect many molecules of central physiological importance within cells (Castenholz & Garcia-Pichel, 2000) and has been shown to impair various physiological and biochemical processes that ultimately affect the growth and morphology of the organisms (Sinha et al., 2001; Gao et al., 2008; Singh et al., 2010). UV-B radiation (280–320 nm) at the earth's surface has been increasing in a number of regions across the globe due to ozone depletion (Dionisio-Sese, 2010), and thus may be an important stress factor for primary production in freshwaters.

Cyanobacteria inhabit numerous types of freshwater environments, including those exposed to high UVR, and therefore, differences among algal groups and species in their tolerance to photosynthetic active radiation (PAR) and UVR can be expected (Conde et al., 2002; Bonilla et al., 2009; Häder et al., 2011). For example, *Microcystis aeruginosa* (Kützing) Kützing (colonial, Order Chroococcales) blooms in the epilimnion of stratified eutrophic lakes, and is highly tolerant to high PAR intensities and UVR (Paerl et al., 1985; Deblois & Juneau, 2010). By contrast, *Planktothrix agardhii* (Gomont) Anagnostidis et Komárek (filamentous, Order Oscillatoriales) may be widely dispersed within the water column of eutrophic turbid lakes, and is considered a shade-tolerant species (Padisák & Reynolds, 1998; Oliver & Ganf, 2000), although it is not easily inhibited by high levels of radiation (Oberhaus et al., 2007). Some cyanobacteria grow under high solar radiation intensities because they possess efficient protective mechanisms to counteract harmful UV-A and UV-B effects (Helbling et al., 2006).

There is a special concern with the toxic cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya et Subba Raju (Nostocales), which has emerged as an important bloom-forming species, due to its recent expansion from the tropics to temperate regions (Padisák, 1997; Wiedner et al., 2007; Sukenik et al., 2012), and recent studies suggest that environmental changes can promote its growth (Wood et al., 2014). This species succeeds in shallow and deep lakes and can dominate the phytoplankton community over a wide range of light conditions (Bonilla et al., 2012), including transparent waters more susceptible to UVR. It can dominate the phytoplankton communities which have low total biomass and at high radiation levels (Bonilla et al., 2012). The tolerance to high photosynthetic and UV radiation may be a critical factor determining the ability of microorganisms to grow in, or colonize different aquatic ecosystems. *C. raciborskii*, as well as other planktonic algae, could be affected by UVR at the beginning of its growth cycle under high light penetration conditions, favored by low phytoplankton biomass (Häder, 2000).

C. raciborskii can produce two potent toxins, cylindrospermopsin (cytotoxin) or saxitoxin and its derivatives (neurotoxins), but may also be non-toxic (Haande et al., 2008; Piccini et al., 2011). A current hypothesis suggests there is link between the geographical distribution of *C. raciborskii* strains and the toxins they produce (Sinha et al., 2012; Piccini et al., 2013; Wood et al., 2014). However, it is still not clear what environmental factors are involved in promoting toxin production, and the biological role of toxins remains controversial (Neilan et al., 2012).

There are many studies focused on the characterization of *C. raciborskii* ecophysiology in order to understand its behavior (for a review see: O'Neil et al., 2012; Sinha et al., 2012). Although there is information about the abiotic requirements and biotic interactions of *C. raciborskii* with other organisms, few studies have been performed to evaluate the response of different bloom-forming cyanobacteria species to UVR, and no specific information is available for the response of *C. raciborskii* to UVR (Bonilla et al., 2012). Physiological tolerance to UVR could give an advantage to *C. raciborskii* over other cyanobacteria or eukaryote phytoplankton and permit successful colonization and growth in new habitats.

We performed this study to address the following question: how tolerant is *C. raciborskii* to UV-B compared with other bloom-forming species? For this purpose, we studied the responses in biomass accumulation and morphological traits of three strains of *C.*

raciborskii, and compared them to *Microcystis* sp. 1 Kützing ex Lemmermann and *P. agardhii* in UV-B stress experiments. We selected three strains of *C. raciborskii* from distant geographical regions (Uruguay, USA, and Australia) and different toxicity profiles to include intraspecific variation. In one of the strains, we also measured the saxitoxin concentration and expression of one of the genes involved in its biosynthesis, *sxtU* (Kellmann et al., 2008) to determine if the concentrations of toxins were affected as well.

Materials and methods

UV-B experiments

Three *C. raciborskii* strains (MVCC19 from Uruguay, LB2897 from USA, and CYP011 K from Australia), *P. agardhii* (MVCC11, from Uruguay), and *Microcystis* sp. 1, from Uruguay, were selected. *Cylindrospermopsis* strains were different ecotypes based on their morphology and toxin production (MVCC19: saxitoxin producer, LB2897: non-toxic and CYP011 K: cylindrospermopsin producer) (Piccini et al., 2011, 2013). Cultures were kept in BG11 modified medium (Ivanikova et al., 2007). 75 ml of cells in the exponential growth phase was transferred to 100-ml quartz flasks. Two treatments were established: PAR (control) and UV-B, with three replicates each, with the exception of the CYP011 K strain that had two replicates (since one replicate failed during the experiment).

After treatments and replicates were settled, the acclimation of all the flasks started. Organism acclimation to the experimental factors is critical to obtain reliable physiological results. In this sense, for acclimation to UV-B, the flasks containing cultures were pre-incubated under UV-B exposure of 30 min per day for 3 days and 1 h per day for another 3 days. In parallel, another set of the same stock cultures was acclimated under PAR (for control treatment). Flasks were kept in a temperature-controlled incubator at 20°C with 80 µmol photon m^{-2} s^{-1} PAR, on a 16 h L:8 h D photoperiod. The UV irradiance was chosen according to Danilov & Ekelund (2000) and provided by Ushio G8T5E UV-B lamp. The radiation on the cultures was UV-B (0.8 W m^{-2}) and UV-A (0.3 W m^{-2}). An International Light radiometer (IL-1400A) with broadband SUL033/W (UV-A) and SUL240/W (UV-B) sensors was used to measure the UVR. Optical density of the cultures was measured daily as absorbance at 750 nm in a spectrophotometer (Thermo Evolution 60).

After the acclimation, and when the cells were in exponential growth phase, the experiments were started (day 0) with an exposure time to the UV-B of 2 h for the first 2 days and 4 h for the last 2 days. The exposure to UV was performed within a 16 h PAR photoperiod during 4 consecutive days ($t = 4$).

All flasks (PAR control + UV-B treatment) were gently shaken every day and randomly moved in the experimental chamber to minimize differences in the light doses between replicates. Treatments were run in the same temperature-controlled incubator used for the acclimation.

Measurements and determinations

To follow population responses daily and to compare changes in relative pigment concentration, phycocyanin (PC) and chlorophyll-*a* (Chl-*a*) were measured daily in vivo with a fluorometer (Turner, Aquafluor), and optical density was measured as absorbance at 750 nm in the spectrophotometer. PC and Chl-*a* data were expressed as fluorescence relative units (FRU).

Biovolume and morphological examination were performed at the beginning of the experiment (day 0), day 2, and 4 based on the analysis of well-mixed 10-ml samples fixed with Lugoĺs solution. Cells, colonies, and filaments (with and without heterocytes) were counted at 400X under an Olympus CKX41 inverted microscope following Utermöhl (1958), until 100 organisms were counted in random fields. Biovolume was calculated according to Hillebrand et al. (1999). Dimensions were gathered for at least 30 specimens of each species in order to determine the individual volume of each species and calculate the fresh weight (biovolume) as a proxy of biomass (µg ml^{-1}). Maximum linear dimension (MLD) of cells and filaments and diameter of the colonies were measured at 1000× under an Olympus BX40 microscope as indicators of size changes.

Gene expression and saxitoxin concentration

All American toxic strains of *C. raciborskii* are saxitoxin producers (Piccini et al., 2011; Sinha et al., 2012), and thus we selected the strain MVCC19 to

explore saxitoxin concentration and gene expression. Total RNA was isolated from MCCV19 on the last day of the UV-B experiments by centrifugation (15 min, 4000*g*, 4°C), and the obtained pellet was applied to an RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. The extracted RNA was retrotranscribed to cDNA using the QuantiTect Reverse Transcription kit (Qiagen). The concentration and purity of cDNA were determined spectrophotometrically at 260 and 280 nm (Nanodrop), and tenfold dilution series were then used as a template for quantitative real-time PCR (qPCR). In order to quantify the absolute copy number of *sxtU* gene, qPCR was performed according to Martínez de la Escalera et al. (2014).

Saxitoxin concentration was determined by ELISA (Abraxis, LLC, Warminster, PA, USA) with a detection limit of 0.015 μg l^{-1}. Samples were fixed with a fixator, Concentrated Sample Diluent Abraxis (9:1 volume sample:volume) and frozen until ELISA analyses performed according to manufacturer's instructions. The saxitoxin concentration was expressed as ng ml^{-1} and then standardized against the cell number and expressed as fg $cell^{-1}$. Saxitoxin was measured in MVCC19 prior to the experiment to confirm the strain was expressing the toxin (avg ± SD of 14.1 ± 0.1 ng ml^{-1} saxitoxin, ELISA, with an optical density, OD = 0.34 ± 0.02 absorbance units). Then, the toxin was quantified again during the acclimation period at the beginning of the experiment (day 0) (avg ± SD of 16.31 ± 0.5 ng ml^{-1} saxitoxin, ELISA, with OD = 0.23 ± 0.015 absorbance units). This analysis was performed to confirm that the culture was producing saxitoxin at the beginning of the experiment.

Statistical analyses

To establish differences between treatments (control PAR and UV-B) and over time, three different analyses were carried out. The repeated measures ANOVA was run for in vivo Chl-*a* and OD between days 0 and 4, and the Factorial ANOVA was used to test for differences in biomass, in vivo PC, and frequency of heterocytes between treatments on day 2 versus 4. Finally, a *t* test was used to assess the significance of differences in toxin concentration and morphological measurements between treatments on day 4. Data were tested for heterogeneous variances and non-normal distributions, and transformations were performed when necessary. A confidence level of 95% was used in all analyses. All analyses were performed with the statistical package Infostat (Di Rienzo et al., 2011).

Results

The fitness of *C. raciborskii* strains was negatively affected by UV-B radiation, although differences were found between strains (Figs. 1, 2a–f). A significant interaction between light treatment and time was observed in all *C. raciborskii* strains (Fig. 1a–f): UV-B exposure affected optical density (OD) and in vivo Chl-*a* accumulation during the experiment, showing an increase of these variables in the PAR treatment. CYP011 K, LB2897, and MVCC19 showed a significant decrease in the biomass, phycocyanin contents, and trichome length after UV-B exposure (Figs. 2a–f and 3). The biomass accumulation of these strains was lowest after the first 2 days and showed a recovery on day 4 only in CYP011 K strain. The decrease in the biomass of UV-B relative to PAR treatments after 4 days of exposure was 92% (MVCC19), 95% (LB2897), and 60% (CYP011 K). These biomass changes were statistically significant ($P < 0.05$) indicating an interaction with time (Fig. 2a–f).

P. agardhii also showed significant differences with time and/or light treatment in Chl-*a* and OD values (Fig. 1g–h), and this species' biomass was the most sensitive, being significantly affected by UV-B and exposure time ($P < 0.05$) (Fig. 2h). This species showed a 77% decrease in biomass under UV-B on day 2 and was severely impacted (98% decrease relative to PAR control) on day 4. *Microcystis* sp. 1 was less affected, showing a small difference for in vivo Chl-*a* and OD (although statistically significant) between PAR and UV-B treatments with time (Fig. 1i–j). *Microcystis* sp. 1 also showed a significant decrease in biomass, $P < 0.05$, with a clear difference between UV-B and PAR biomasses, but no time effect (Fig. 2j). Its biomass decreased 45% by day 2, and 49% by day 4.

Significant changes in PC content under UV-B stress were observed for *C. raciborskii* strains, *P. agardhii*, and *Microcystis* sp. 1, although with differences between strains (Fig. 2b, d, f, h and j). MVCC19, LB2897, and *P. agardhii* showed an

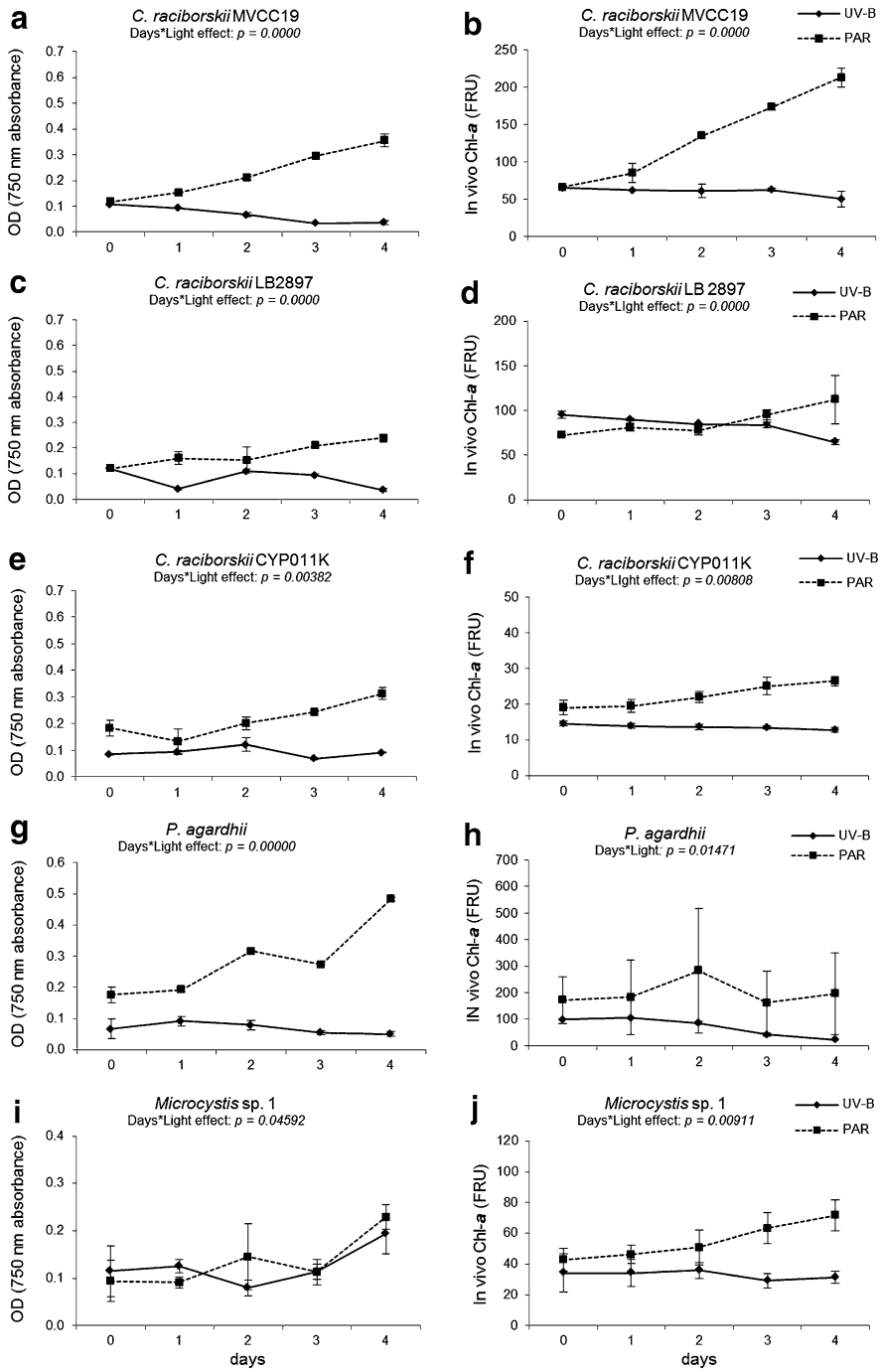

Fig. 1 Daily changes in the in vivo Chl-*a* content (fluorescence relative units, FRU) and the optical density (750 nm absorbance) of *Cylindrospermopsis raciborskii* strains: MVCC19 (**a**, **b**), LB2897 (**c**, **d**), and CYP011 K (**e**, **f**); *Planktothrix agardhii* ($n = 2$) (**g**, **h**) and *Microcystis* sp. 1 (**i**, **j**), exposure to PAR (*squares*, *dotted line*) and UV-B radiation (*circles*). *Vertical lines* show standard deviation ($P < 0.05$). Note different scale on the *y*-axis

interaction effect between light and exposure time ($P < 0.05$), but CYP011 K strain and *Microcystis* sp. 1 only showed a significant light effect ($P < 0.05$).

The maximum linear dimension of the organisms decreased in the UV-B treatment at the end of the experiments (Fig. 3), and significant differences were detected for all strains of *C. raciborskii:* CYP011 K (MLD avg ± SD, for PAR = 142.7 ± 75 µm and for UV-B = 91.4 ± 41 µm), MVCC19 (MLD avg ± SD, for PAR = 249.5 ± 97.8 µm and for UV-B = 80.2 ± 46.5 µm), LB2897 (MLD avg ± SD, for PAR = 127.3 ± 40.7 µm and for

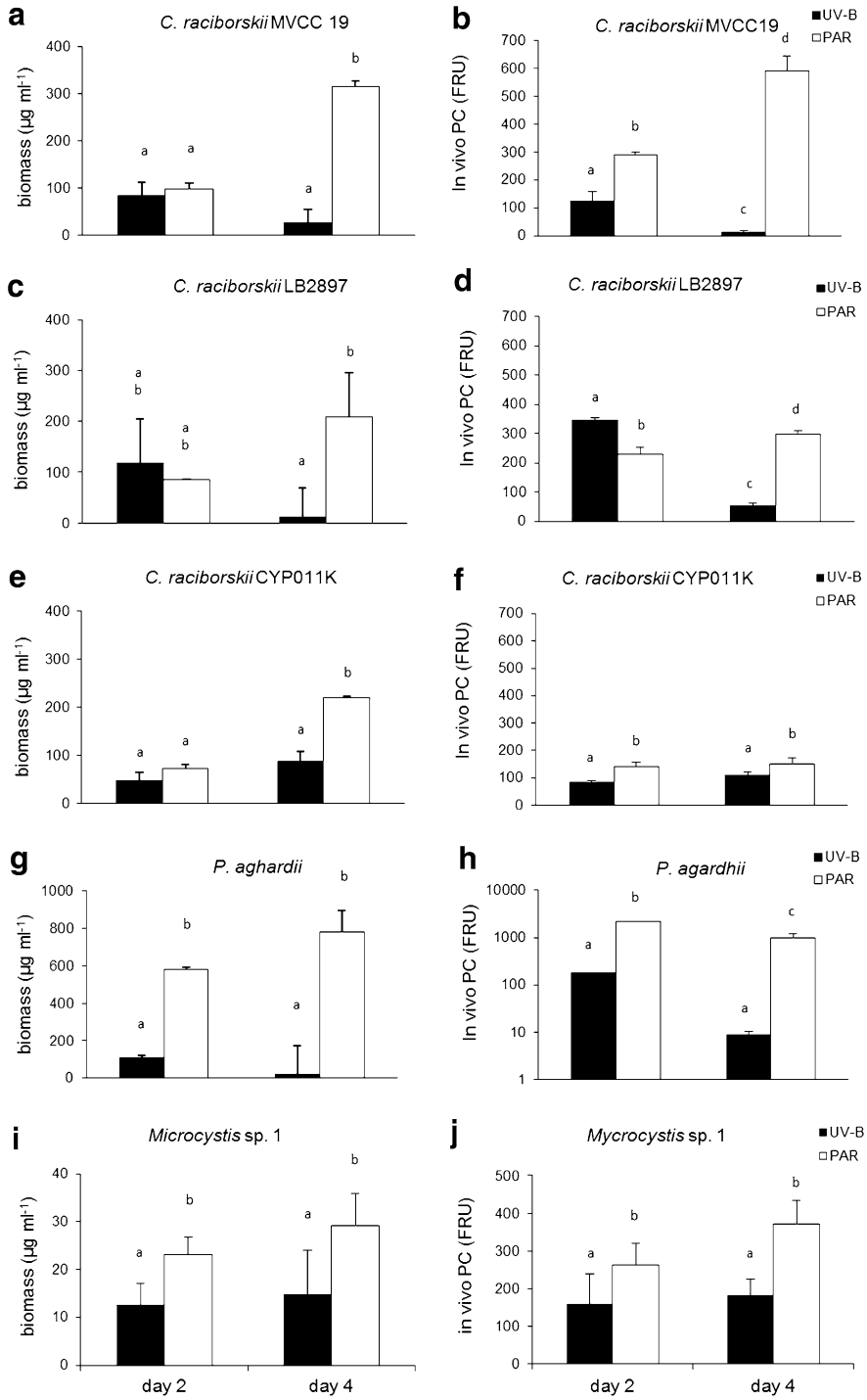

Fig. 2 Changes in the biomass (µg ml^{-1}) and in vivo phycocyanin content (PC) of *Cylindrospermopsis raciborskii* strains: MVCC19 (**a**, **b**), LB2897 (**c**, **d**), and CYP011 K (**e**, **f**); *Planktothrix agardhii* (with logarithmic scale) (**g**, **h**) and *Microcystis* sp. 1 (**i**, **j**), at day 2 and 4 exposure to PAR (*white bars*) and UV-B radiation (*black bars*). *Vertical lines* show standard deviation and *letters denote* significant differences between treatments ($P < 0.05$). Note different scale on the *y*-axis

UV-B = 48.8 ± 18.1 µm), and *P. agardhii* (MLD avg ± SD for PAR = 236.4 ± 80 µm and for UV-B = 23.4 ± 91 µm). *Microcystis* sp. 1 colony diameter was slightly affected by UV-B radiation (MLD avg ± SD for PAR = 169.5 ± 80.6 µm and for UV-B = 116.8 ± 91.1 µm) but differences were not statistically significant ($P > 0.05$). Finally, a decrease in the heterocyte frequency (percentage of filaments with heterocytes in the total population) in the strains of *C. raciborskii* was observed under UV-B treatment with

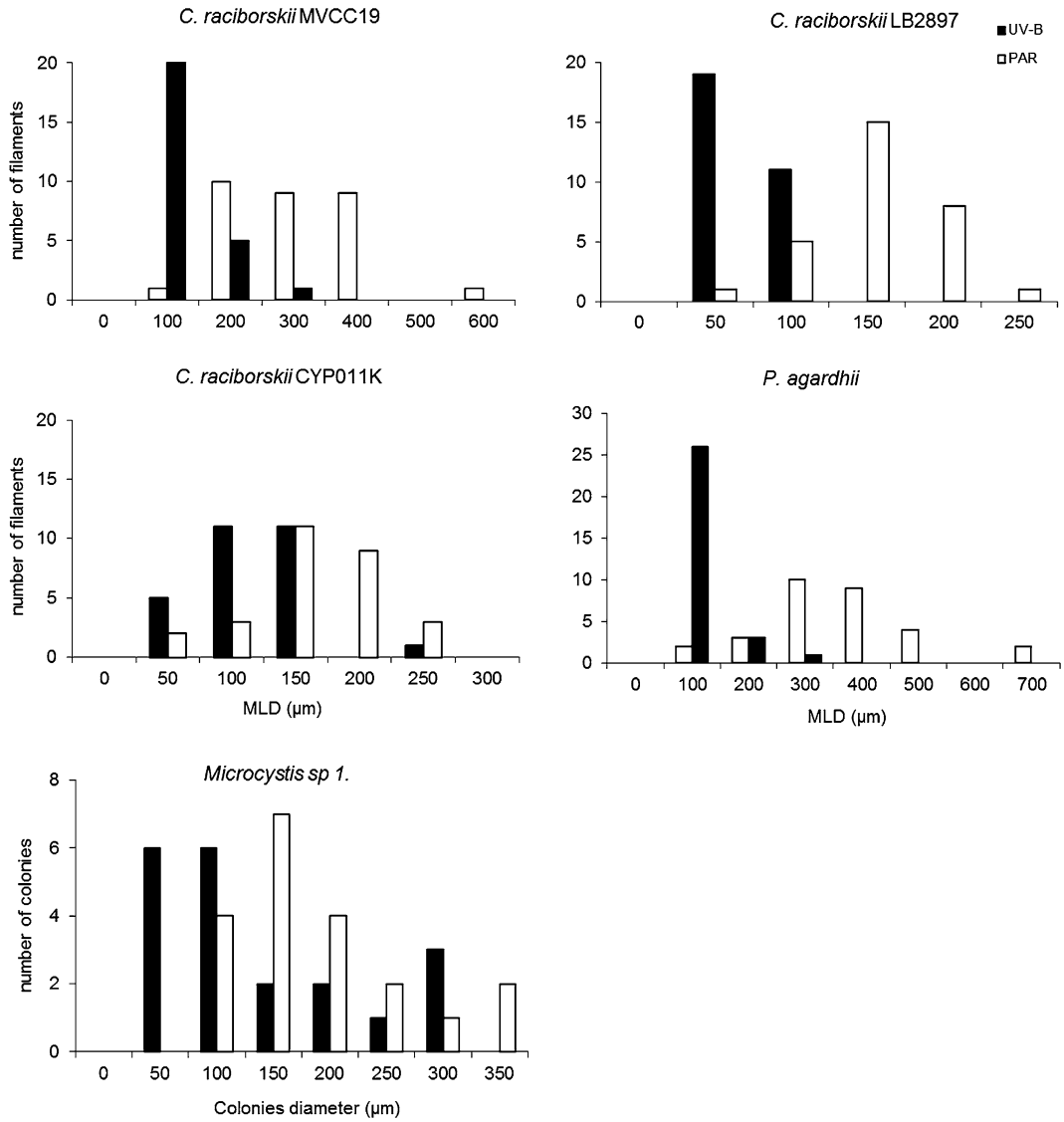

Fig. 3 Frequency distribution of the morphological trait maximum linear dimension (MLD, µm) and diameter of the colonies (µm) with PAR (*white bars*) and UV-B radiation (*black bars*) treatments at the end of the experiments

time, although these changes were significantly different ($P < 0.05$) only for MVCC19 and LB2897 strains (Fig. 4).

Saxitoxin concentrations determined by ELISA in the MVCC19 strain had higher concentrations in the PAR treatment ($P < 0.05$) (Table 1). Although the saxitoxin concentrations standardized against number of cells (fg cell^{-1}) and biomass (ng µg^{-1}) were substantially higher in the UV-B treatment, these differences were not statistically significant ($P > 0.05$) (Table 1). Interestingly, the copy number of *sxtU*, a gene belonging to the sxt cluster that encodes for a putative short-chain alcohol dehydrogenase, increased significantly ($P < 0.05$) per cell unit in the UV-B treatment (Table 1).

Discussion

The data obtained in this study first indicate that *C. raciborskii* physiological status can be depressed under UV-B radiation, which is likely to extend to

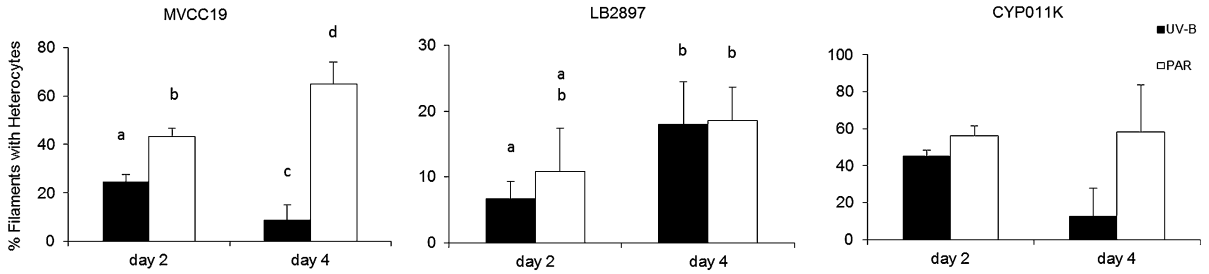

Fig. 4 Changes in the frequency of filaments with heterocytes for each *C. raciborskii* strain. Data represent means ± standard deviations and *letters denote* significant differences between treatments (PAR and UV-B) ($P < 0.05$). Note different scale on the *y*-axis

Table 1 Saxitoxin concentration per volume standardized by cell numbers and by biovolume, and *sxtU* gene copies per cell in *C. raciborskii* MVCC19 strain, under UV-B treatment and control (PAR) at the end of the experiments ($t = 4$ days)

	UV-B treatment	Control PAR
Saxitoxin concentration in the culture (ng ml^{-1})	12.1 ± 2.6	58.7 ± 3.9
Saxitoxin concentration per cell (fg $cells^{-1}$)	13.1 ± 6.5	5.6 ± 2.2
Saxitoxin concentration per biovolume (ng μg^{-1})	0.5 ± 0.3	0.2 ± 0.1
Copy number of *sxtU* gene (*sxtU* copies $cell^{-1}$)	6554.9 ± 2376.4	2.3 ± 2.6

impacts on survival traits and fitness of the species in the environment. Secondly, the data indicate that *C. raciborskii* was more tolerant to UV-B than another filamentous cyanobacterium (*P. agardhii*) that shares a similar ecological niche. It is well documented that UV radiation can limit the growth of cyanobacteria and microalgae in transparent aquatic environments (Karsten, 2008; Retamal et al., 2008). However, there is a wide range of variation in responses to UVR which is partially related to the life strategies of different species (Reynolds, 2006).

Although no previous research on the effect of UVR has been performed for *C. raciborskii*, our results are similar to those reported for other nitrogen-fixing cyanobacteria. For instance, studies performed with *Anabaena* sp. Bory de Saint-Vincent ex Bornet & Flahault (Sinha et al., 1997; Gao et al., 2007), *Nostoc* sp. Vaucher ex Bornet & Flahault, *Scytonema* sp. Agardh ex Bornet & Flahault (Sinha et al., 1997), and *Nodularia spumigena* Mertens ex Bornet & Flahault (Wulff et al., 2007) showed changes in Chl-*a*, PC, growth, and trichome length after UV exposure, suggesting a common response among Nostocales species in relation to UV-B tolerance. Phenotypic plasticity of *C. raciborskii* can play a key role in explaining its survival under environmental stress, including that induced from light intensity and quality. In particular, *C. raciborskii* has a high diversity of carotenoids that protect against light damage, and its concentration of the glycoside aphanizophyll can increase significantly under high light conditions (Bonilla et al., 2012; Mehnert et al., 2012). In our study, differences in the percentage of filaments with heterocytes were also found between *C. raciborskii* strains in the PAR treatment, which supports suggestions of the plasticity of this species (Briand et al., 2004; Piccini et al., 2011; Bonilla et al., 2012). Consequently, the three strains were affected differently in the percentage of filaments with heterocytes under UV-B stress. Gao et al. (2007) found that UVR exposure almost totally inhibited heterocyte formation in *Anabaena* sp. due to inhibition of vegetative cell differentiation into heterocytes. This effect was also found for *Anabaena aequalis* Borge exposed to UV-B radiation for different periods of time, with a delay in the differentiation of vegetative cells into heterocytes (Blakefield & Harris, 1994). Depression of nitrogenase activity has also been shown in *Anabaena* cultures exposed to UV radiation (from 280 to 400 nm at 5 W m^{-2}) (Sinha et al., 1996). Cell differentiation is markedly affected by UV-B, and thus, vegetative cells can be severely limited in their growth under high

water transparency and high UV-B penetration. Differential responses between strains of *C. raciborskii* were found in the MLD of the trichomes, with a significant shortening after UV-B exposure. Fiorda Giardonino et al. (2011) found similar results regarding the morphology of *Anabaena* sp., *Nostoc* sp., and *Arthrospira platensis* Gomont in experiments with natural UVR at water temperature of 18 °C.

Microcystis sp. 1 was the least altered species under UV-B radiation in all measured traits, which was in agreement with previously reported experimental results. For instance, Jiang & Qiu (2005) performed an experiment with *M. aeruginosa* with a similar UV-B irradiance to our experiments (1.05 W m^{-2} of UV-B irradiance 3 h per day) and found a small Chl-*a* inhibition and a greater percentage of PC inhibition, as well as a faster recovery of the cultures after 9 days of exposure. Similar to our results, and with regard to morphology, Fiorda Giardonino et al. (2011) found no differences in the size of *Microcystis aeruginosa* colonies after 4 days of exposure to solar UV radiation at water temperature of 18 and 23°C. In our study, *P. agardhii* was the most sensitive species, very quickly showing significant breakage of the filaments and had almost disappeared after 4 days of exposure to UV-B. This filamentous species is well adapted to highly turbid water where it forms dense buoyant blooms (Dokulil & Teubner, 2000). *P. agardhii* and other Oscillatoriales blooms can last for long periods or become persistent due to low light conditions that promote its dominance and that become a positive feedback mechanism (Scheffer et al., 1997), and thus, a low tolerance to UV-B is in accordance with its shade-tolerant strategy. Another study using experiments with different benthic members of Oscillatoriales (*Phormidium murrayi* (West & West) Anagnostidis & Komárek and *Oscillatoria priestleyi* West & West) also found low tolerance to UV-B irradiance (0.00126 W m^{-2}) after 4 days of exposure, as evidenced by changes in Chl-*a* and PC contents, and growth and motility of the trichomes to avoid light in the experimental cultures (Quesada & Vincent, 1997). Moreover, Wu et al. (2005) observed a shortening of the filaments of two *Arthrospira platensis* strains exposed to 2 h of solar UVR (1.37–1.47 W m^{-2}). These studies and our own results clearly indicate that cyanobacteria exhibit a wide range of responses and sensitivities to UVR, which can have consequences for their biomass and growth under environmental stress.

Comparing the response of *C. raciborskii* with *P. agardhii* and *Microcystis* sp., our results are in agreement with the different ecological strategies of these species (Chorus & Bartram, 1999). Filamentous *C. raciborskii* is described as tolerant to frequent mixing cycles (weekly and/or seasonally), while *P. agardhii* is described as a species that tolerates continuous mixing and low light intensities (Scheffer et al., 1997; Wiedner et al., 2007). Considering our results and those of previous studies (Dokulil & Teubner, 2000; Nixdorf et al., 2003; Bonilla et al., 2012), *C. raciborskii* may have a competitive advantage over *P. agardhii* under conditions of both high light and warm temperatures. These differences are attributable to the different photoprotective responses of *C. raciborskii* and are probably related to its phenotypic plasticity. This plasticity could offer a clear advantage for *C. raciborskii* dispersion (Padisák, 1997). A different ecological strategy was found in *Microcystis* sp. 1, the most tolerant strain, which is in agreement with other studies that demonstrate a high tolerance to high light intensities and UVR (Sommaruga et al., 2009; Tomioka et al., 2011). These colonial taxa accumulate, forming scums on the water surface where they can be exposed to high irradiances. The effect of UV radiation stress is also differentially affected by other kinds of stress factors in the environment (Singh et al., 2012). In this sense, the high phenotypic plasticity of a species, such as *C. raciborskii*, can be an advantage for survival in changing environments (Carey et al., 2012). In particular, differential responses between strains and species may result more from differences in the adaptive capacities than from an acclimation to UV radiation. According to Wu et al. (2005), there are different scales of acclimation and adaptation when cells receive full solar radiation. For instance, on the scale of days, the morphological changes to counteract UV damage (breakage of the filaments, reduced size of colonies) are associated with acclimation; however, on decadal time scales, adaptation involves changes in the spiral structure, from a rather elongated helix to a very compressed one.

The increased saxitoxin concentration under UV-B exposure showed for the first time that this environmental stressor can enhance the toxicity of *C. raciborskii*. The biological role of these toxins for the organisms remains controversial, with hypotheses including defense against predators (Neilan et al.,

2012) and chemical communication (Bar-Yosef et al., 2010). Light intensity and photoperiod also appear as an important factor influencing saxitoxin or cylindrospermopsin concentrations (Dyble et al., 2006; Carneiro et al., 2009). This was also the case in our study, where we found higher saxitoxin concentrations per cell (fg saxitoxin cell^{-1}) and biomass (ng saxitoxin μg^{-1}), together with a significantly increased copy number of the *sxtU* gene after UV-B irradiation. It has been suggested that the transcription of toxin biosynthesis and transport genes is constitutive and related to population growth rather than directly to specific environmental conditions (Stucken et al., 2014). Although little is known about the ecological role of saxitoxins, our results showed that under UV stress the number of transcripts per cell increased significantly, suggesting that saxitoxin production may be linked in some way to cellular stress. Pomati et al. (2003) concluded that saxitoxin-producing *C. raciborskii* could prevent cell lysis caused by sodium pump inhibitors. Moreover, Soto-Liebe et al. (2012) have shown that in *Raphidiopsis brookii* Hill, saxitoxin is exported out of the cell in response to increases in extracellular Na^+ and K^+, suggesting that saxitoxin can have a role in ensuring homeostasis against salinity variations. It is well known that UVR promotes cellular stress and lysis (Karentz et al., 1994), so the potential role of saxitoxin in ensuring cellular homeostasis could also explain the finding that UV stress increased not only the expression but also the production of saxitoxin. Further research regarding the role of this toxin in stress at a cellular and population level should be conducted.

Particular ecophysiological characteristics have been proposed to explain *C. raciborskii* success and expansion in freshwaters, including its tolerance to a wide range of light conditions, better exploitation of energy than other cyanobacterial species, high growth rates at high light intensities, and efficient phosphorus uptake and toxin production, among others (Piccini et al., 2011; Bonilla et al., 2012; Amaral et al., 2014). The response of *C. raciborskii* to UV-B may also have consequences for the initial growth phase in transparent water columns as growth may be inhibited if UV-B penetration is high. Among the ecophysiological characteristics described for *C. raciborskii*, the response to UVR may require more detailed study in order to evaluate its impact on *C. raciborskii* fitness and population dynamics.

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