RESEARCH ARTICLE

What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*?

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Abstract

The cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii* are bloom-forming species common in eutrophic freshwaters. These filamentous species share certain physiological traits which imply that they might flourish under similar environmental conditions. We compared the distribution of the two species in a large database (940 samples) covering different climatic regions and the Northern and Southern hemispheres, and carried out laboratory experiments to compare their morphological and physiological responses. The environmental ranges of the two species overlapped with respect to temperature, light and total phosphorus (TP); however, they responded differently to environmental gradients; *C. raciborskii* biovolume changed gradually while *P. agardhii* shifted sharply from being highly dominated to a rare component of the phytoplankton. As expected, *P. agardhii* dominates the phytoplankton with high TP and low light availability conditions. Contrary to predictions, *C. raciborskii* succeeded in all climates and at temperatures as low as 11 °C. *Cylindrospermopsis raciborskii* had higher phenotypic plasticity than *P. agardhii* in terms of pigments, individual size and growth rates. We conclude that the phenotypic plasticity of *C. raciborskii* could explain its ongoing expansion to temperate latitudes and suggest its future predominance under predicted climate-change scenarios.

Introduction

The excessive growth of planktonic cyanobacteria is among the main threats endangering the use of water resources in shallow lakes. Temperature increases in the range of 0.2 °C per decade, and their effects on water mixing regimes, are expected to increase the occurrence, frequency and duration of cyanobacterial blooms in several regions of the planet (Doney, 2006; Falkowski & Oliver, 2007; Markensten *et al.*, 2010). These future changes in climate are also predicted to cause shifts in the species composition of cyanobacterial blooms in favour of invasive species (Mehnert *et al.*, 2010). The

modern global distributions and environmental preferences of cyanobacterial species result from differences in evolutionary adaptations and phenotypic traits (Whitton & Potts, 2000). Understanding the characteristics that allow cyanobacterial taxa to succeed in disparate environments is crucial for predicting future bloom-forming behaviour in warming climates.

Filamentous cyanobacteria such as *Planktothrix* and *Cylindrospermopsis*, as well as colonial genera at *Microcystis*, are the most successful bloom-forming organisms in shallow lakes (Padisák & Reynolds, 1998; Nixdorf *et al.*, 2003; Paerl *et al.*, 2011; Tomioka *et al.*, 2011). In particular, *Planktothrix agardhii* (Order Oscillatoriales) and

Cylindrospermopsis raciborskii (Order Nostocales) can be used as model species because of the extensive information available about their distributions. *Planktothrix agardhii* is a resilient, shade-tolerant species that can produce microcystins and is one of the most common bloom-forming species in temperate lakes (Scheffer *et al.*, 1997). Blooms of *C. raciborskii* are becoming more frequent in tropical (Figueredo & Giani, 2009; Gemelgo *et al.*, 2009), subtropical (Vidal & Kruk, 2008; Everson *et al.*, 2011) and temperate lakes (Hamilton *et al.*, 2005; Stüken *et al.*, 2006) because of the apparently invasive behaviour of the species (Padisák, 1997). The expansion of *C. raciborskii* has generated widespread concern as a result of its potential for producing two toxin types, cylindrospermopsins and saxitoxins (Chorus & Bartram, 1999). To date no consensus exists regarding the main mechanisms that have permitted the expansion of *C. raciborskii* into temperate regions. Proposed hypotheses include climate change–associated water temperature increases (Wiedner *et al.*, 2007), an exceptionally good tolerance of transport (Padisák, 1997), the ecophysiological plasticity of the species (Briand *et al.*, 2004) and the existence of ecotypes with different environmental preferences and tolerances (Chonudomkul *et al.*, 2004; Piccini *et al.*, 2011).

Cylindrospermopsis raciborskii and *P. agardhii* have similar phenotypic traits, including tolerance to continuous mixing of the water column, high phosphorus storage capacity, buoyancy regulation and shade tolerance (Reynolds, 1993; Padisák & Reynolds, 1998; Istvánovics *et al.*, 2000; Padisák, 2003). These similarities are also reflected in their morphology, indicating that they may be functionally equivalent and occupy a similar ecological niche (Kruk *et al.*, 2010). However, some studies show that *C. raciborskii* has higher light requirements for growth (I_k) than *P. agardhii*, suggesting differences in some dimensions of their niches (Briand *et al.*, 2004; Kokociński *et al.*, 2010; Mehnert *et al.*, 2010). Moreover, these two species differ in their capacities to incorporate nitrogen. *Cylindrospermopsis raciborskii* has the capacity to fix atmospheric nitrogen (N_2) through heterocytes, as do other Nostocales, conferring a competitive advantage in nitrogen-depleted environments relative to *P. agardhii*, which cannot fix nitrogen (Whitton & Potts, 2000). The advantages that explain the recent worldwide expansion of *Cylindrospermopsis*, combined with predicted changes because of global warming, may imply an impending shift from *P. agardhii* to *C. raciborskii* at intermediate latitudes.

Although information about *P. agardhii* is extensive, and data about *C. raciborskii* are increasingly available, current knowledge derives either from experiments or field sampling. Very few studies simultaneously compare both species (Dokulil & Teubner, 2000; Wiedner *et al.*,

2007; Kokociński *et al.*, 2010). Functional traits, including morphological and physiological features, govern individual ecological performance and summarize organism responses to the environment (McGill *et al.*, 2006; Violle *et al.*, 2007; Kruk *et al.*, 2010). A comparative approach to studying the morphological and physiological traits and distributions of *P. agardhii* and *C. raciborskii* can provide insight into the behaviour of these key cyanobacterial species. Moreover, this approach can contribute to more general ecological questions such as microorganism invasions (McGill *et al.*, 2006; MacDougall *et al.*, 2009).

Our aim was to evaluate the global distribution and ecological preferences of *C. raciborskii* and *P. agardhii*, and to determine the implications for the geographical expansion of *C. raciborskii*. We assembled a large database spanning wide latitudinal gradients and different climatic regions and carried out laboratory experiments to characterize the morphological and physiological traits of the two species.

Materials and methods

Field database

We constructed a database of 940 samples taken from 28 mesotrophic to hypereutrophic lakes where *P. agardhii* and/or *C. raciborskii* were present in at least one sample. In 125 samples neither species was present. Species data were obtained from published (Padisák, 1994; Aubriot *et al.*, 2000, 2011; Kruk *et al.*, 2002, 2010; Marinho & Huszar, 2002; Soares *et al.*, 2009) and unpublished material (kindly provided by F. Bressan, A. Ferreira and S. de Melo). Three climate regions were represented in the lake database: tropical (08°02′–22°33′S), subtropical (34°33′–34°55′S) and temperate (35°30′–38°80′S and 46°50′–52°23′N). Studied lakes in the temperate zone were from Hungary (Balaton Lake), The Netherlands (Deest and Ochten floodplain lakes: D1, D2, D3, D4, D5, O2, O3, O4, O5, O6) and the Argentinean Pampas lakes AR19, AR20, AR29, AR30, AR31 and AR32; in the subtropical zone from Uruguay (Laguna Blanca, Canteras, Chica, Javier, Rodó, Sauce and Ton-Ton); and in the tropical zone from Brazil (Funil, Imboassica, Juturnaíba, Tabocas and Tapacurá). All samples were used to determine the distribution of each species in relation to selected environmental variables, excluding observations with zero biovolume. The environmental variables were lake area (area, ha), maximum depth (Z_{max}, m), mixing depth (Z_{mix}, m), euphotic/mixing depth ratio (Z_{eu}/Z_{mix}), water temperature (T, °C), pH, conductivity (K, µS cm^{-1}), alkalinity (Alk, mg CaCO$_3$ L^{-1}) and total phosphorus (TP, µg L^{-1}). The Z_{eu}/Z_{mix} ratio was used as a proxy of the light available in the environment for phytoplankton

growth (Jensen et al., 1994). The largest number of observations (~ 61%) was from shallow lakes (Z_{max} < 4 m), although several cases corresponded to deep lakes (93 data points, Z_{max} > 20 m, maximum: Funil Reservoir, Z_{max}: 50 m). A wide range of lake areas were included (0.5–7200 ha, plus Balaton Lake which is 59300 ha) but only 19 lakes were smaller than 100 ha. Our data set had an extensive range of TP (12–1653 $\mu g \ L^{-1}$) where few observations ($n = 5$) indicated mesotrophic status (< 30 $\mu g \ L^{-1}$ TP) and 100 were from hypereutrophic conditions (> 200 $\mu g \ L^{-1}$ TP).

Despite the diversity of lakes and locations, phytoplankton sampling always followed routine protocols, and thus, the samples were representative of lake conditions. Samples were obtained at different depths within the mixed, illuminated zone, or in permanently mixed shallow lakes, the whole water column was integrated using sample bottles or tubes. Phytoplankton samples were fixed with Lugol's solution and settled in counting chambers (Utermöhl, 1958); at least 100 individuals of the most frequent species or 400 individuals in total were counted in random fields in an inverted microscope as described in (Kruk et al., 2010). Individual volume (V, μm^3) was calculated for each taxon according to simple volumetric formula, considering the organism as the unit, and biovolume was expressed as $mm^3 \ L^{-1}$. The surface are volume ratio (S/V, μm^{-1}) and filament maximum linear dimension (MLD, μm) were estimated as detailed in Kruk et al. (2010). The community was analysed in terms of species richness (expressed as the number of taxa per sample, S), the absolute biovolume of P. agardhii and C. raciborskii, the relative contribution of each to total biovolume, and their frequency of occurrence (number of observations). Total biovolume was considered low when < 1 $mm^3 \ L^{-1}$, and a species was considered dominant when it represented at least 30% of the total biovolume in a particular sample. The frequency of occurrence, the median and the range of the two species in terms of biovolume were analysed with all samples in the data set (including zero data).

Experimental data

The physiological and morphological responses of the two species were compared using Uruguayan isolates: P. agardhii (MVCC11) and C. raciborskii (MVCC14). Isolate MVCC11 was collected from Lago Rodó (34°55′S, 56°10′W), a eutrophic to hypereutrophic shallow lake used for recreation (Area: 1.5 ha, Z_{max}: 2.5 m, TP: 70–565 $\mu g \ L^{-1}$) (Scasso et al., 2001). Isolate MVCC14 was collected in Laguna Blanca (34°53′ S, 54°20′ W), a eutrophic shallow lagoon (Area: 40.5 ha, Z_{max}: 2.6 m, TP: 86 $\mu g \ L^{-1}$) used as a drinking water supply (Vidal &

Kruk, 2008). Static cultures of the isolates were kept in BG11 medium at 26 °C (±1 °C), as described in the study carried out by Piccini et al. (2011), which is the normal summer water temperature in Uruguayan lakes where the species were isolated (Vidal & Kruk, 2008; Aubriot et al., 2011).

Two set of experiments were performed: light intensity gradient and temperature experiments, and the growth rates (physiological trait) of the two species were determined and compared. In addition, for the light intensity gradient experiments, we evaluated the physiological response of pigment structure change and the morphological trait changes of V, S/V and MLD.

To determine the effect of light intensity on growth rates of P. agardhii, four growth curves under six light intensity levels (from 5 to 180 μmol photons $m^{-2} \ s^{-1}$) were repeated in 5-day experiments at 26 °C (±1 °C). Data for growth curves of C. raciborskii were obtained from Piccini et al. (2011), who performed the experiment under the same conditions. Before beginning the experiments, cultures were acclimated to each light level for 10–15 days and replicated when the biomass was duplicated (three replicates, except for 5 μmol photons $m^{-2} \ s^{-1}$: one). The experiments were run in 100-ml bottles, filled with 80 mL BG11 medium and inoculated with cyanobacterial culture in exponential growth phase. Optical density (OD, absorbance at 750 nm) was used as an indicator of biomass and the initial inoculum for all experiments was 0.1 absorbance units. Absorbance at 440 nm was used to determine the light extinction coefficient (Kirk, 1996) in order to calculate the light intensity inside the bottles. Absorbance measurements were taken in a spectrophotometer (Thermo Evolution 60). The growth rate (μ, d^{-1}) of each isolate was calculated in 24-h intervals during the exponential phase as:

$$\mu = \frac{(\ln OD_f - \ln OD_i)}{(t_f - t_i)}$$

where OD_i and OD_f are the estimated biomasses at initial (t_i) and final (t_f) times, respectively. Maximum specific growth rate, μ_{max}, the initial slope, α, and the irradiance at the onset of light saturation, I_k ($I_k = \mu_{max}/\alpha$), were derived from the fitted model of Jassby & Platt, (1976) for photosynthesis.

Samples were taken at the end of two growth experiments (i.e. 20 and 100 μmol photons $m^{-2} \ s^{-1}$) in order to quantify several characteristics of the two taxa. To compare morphological changes, V, S/V and MLD were calculated for each isolate and replicate, based on microscopic measurements of 60 organisms made under an Olympus BX40 optical microscope at 1000× magnification. To compare changes in relative pigment concentration,

in vivo relative concentrations of phycocyanin and Chl *a* fluorescence were measured in a fluorometer (Turner, Aquafluor), with phycocyanin relative concentration standardized against Chl *a in vivo* fluorescence. Finally, the pigment structure of each taxon was characterized using high-performance liquid chromatography (HPLC). Samples from one replicate of each taxon were filtered onto GF/C glass-fibre filters and kept frozen ($-80\ ^\circ$C) until pigment extraction. HPLC methods and protocols followed those described in Bonilla *et al.* (2005). Carotenoids were detected by diode-array spectroscopy (350–750 nm), chromatograms were obtained at 450 nm (for carotenoids), and Chl *a* was detected by a fluorescence detector (excitation $\lambda = 440$ nm; emission $\lambda = 650$ nm). The identification and quantification of the pigments (Chl *a*, aphanizophyll, β,β-carotene, echinenone and zeaxanthin) was based on commercial standards as detailed in Bonilla *et al.* (2005). Unknown carotenoids were quantified by applying the calibration curves used for β,β-carotene. The final concentration of each pigment is expressed in nmol L^{-1}, and changes in carotenoids were analysed using ratios to Chl *a*.

In order to determine the influence of low temperature on growth rate, 4-day experiments were run for both isolates at three temperatures: 15, 20 and 25 °C ($\pm$1 °C) at both 60 and 135 μmol photons m^{-2} s^{-1}, with four replicates for each condition. The experimental setup and growth conditions were the same as described earlier, with cultures allowed to acclimate to each temperature for 15 days (three times, except at 15 °C: once). Biomass and growth rate were calculated as previously described for light intensity experiments. The parameter Q_{10} (15–25 °C) for each light intensity (60 and 135 μmol photons m^{-2} s^{-1}) was calculated using the maximum average growth rate ($n = 4$) obtained at each temperature for each species.

Statistical analysis

The annual coefficient of variation was calculated to determine the variability of the biomass of the two species in nature, based on temporal data series for 17 lakes in the database, for temperate (11 lakes), subtropical (1) and tropical (5) regions. As the objective of this particular analysis was to determine the amplitude of biomass change, all data, including observations with zero values, were used.

To evaluate the success of the two species in relation to key environmental variables, we examined the maximum relative contribution of each species to total biovolume distribution along gradients of temperature, Z_{eu}/Z_{mix} and TP. For these analyses, data were segregated into groups every one degree Celsius, 0.1 Z_{eu}/Z_{mix} unit, and 10 μg L^{-1}

TP. We then performed linear and nonlinear regressions between species biovolume and each environmental variable. The simple functions with best fit were selected following parsimony criteria of maximum explained variance with the minimum number of parameters and best significance (F-test). Linear relationships with breakpoints, such as those suggested for *Planktothrix* biovolume to Z_{eu}/Z_{mix} and to TP plots, can indicate ecological thresholds (Toms & Lesperance, 2003). We therefore applied a simple piecewise linear regression to these data and breakpoints were determined after 200 iterations. Data were tested for normality and homogeneity of variance prior to analyses and log$_{10}$ transformed when necessary (*P. agardhii* biovolume distribution on Zeu/Zmix gradient).

The three climatic regions were compared by examining data from winter and summer months for each lake and year in the dataset ($n = 445$) in terms of temperature, Z_{eu}/Z_{mix}, TP, and the biovolume of the two species. Differences between environmental and biotic variables were analysed with nonparametric Kruskal–Wallis (K–W) tests, all pairwise multiple comparison tests (Dunn's Method) and Mann–Whitney tests (when *P. agardhii* was present in only two regions).

Differences between physiological and morphological experimental responses of the two species to light and temperature gradients were compared using *t*-test analysis and, when normality failed, with the nonparametric Mann–Whitney (M–W) test. All analyses were performed with the programs STATISTICA 6.0 and SIGMA PLOT 11.0.

Results

Species distributions and their relation to environmental factors

Cylindrospermopsis raciborskii was observed in a higher number of samples than *P. agardhii* (306 and 199 samples, respectively), with the two species co-occurring on 34 occasions (all in Lake Balaton) in a wide range of environmental conditions. We analysed lakes where one of the two species was present on at least one sample. *Cylindrospermopsis raciborskii* was absent in all samples of subtropical (Lago Rodó) and small Dutch temperate lakes, while *P. agardhii* was absent in most of the subtropical Uruguayan lakes (except Lago Rodó) and all tropical Brazilian lakes. Each species reached high biovolume and had a high contribution to total biovolume in several samples (Table 1, Fig. 1a and b). *Cylindrospermopsis raciborskii* was dominant (at least 30% of total biovolume) more frequently than *P. agardhii*, in samples of both high (> 1 mm^3 L^{-1}) and low (< 1 mm^3 L^{-1}) total biovolume. In most cases, *P. agardhii* was a minor

Table 1. Community and environment characteristics (median, minimum–maximum between brackets cursive numbers indicate the number of cases) for *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*, when present

	Lakes with *P. agardhii*	Lakes with *C. raciborskii*	P
Community			
P.agardhii (mm^3L^{-1})	0.79 (0.11–149) *199*	0 (0–10.4) *306*	*
C.raciborskii (mm^3L^{-1})	0 (0–45.0) *199*	2.74 (< 0.01–71.79) *306*	*
P.agardhii (% total BV)	6.56 (0.19–98.8) *199*	0 (0–45.6) *306*	*
C.raciborskii (% total BV)	0 (0–77.80) *199*	32.9 (0.05–97.4) *306*	*
Total BV (mm^3L^{-1})	14.6 (2.22–302) *199*	12.9 (0.07–697) *306*	*
S	27 (3–123) *199*	18 (3–59) *160*	*
Environment			
Temperature (°C)	13.3 (1.70–27.6) *156*	26.7 (11.2–31.6) *154*	*
Area (ha)	1.30 (1.30–6 × 10⁴) *86*	4300 (0.24–6 × 10⁴) *313*	*
pH	8.18 (6.89–9.25) *120*	8.20 (5.49–9.91) *132*	ns
Z_{max} (m)	2.50 (1.40–6.00) *194*	6 (0.3–45) *314*	*
Z_{mix} (m)	2.00 (0.66–5.16) *200*	3.2 (0.3–30) *222*	*
Z_{eu}/Z_{mix}	0.75 (0.17–3.42) *162*	0.68 (0.09–3.78) *61*	ns
K (μScm^{-1})	550 (380–1141) *123*	91.9 (25.6–3457) *129*	*
Alk (mgL^{-1})	160 (65.0–546) *130*	46.5 (0.01–180) *37*	*
TP (μgL^{-1})	130 (50.0–5600) *163*	74.9 (12.4–658) *73*	*

Significant differences (Mann Whitney, *$P < 0.05$) between the measured variables for each species are indicated.
ns, not significant; BV, biovolume; S, species number; Z_{max}, maximum depth; Z_{mix}, mixing zone; Z_{eu}, euphotic zone; K, conductivity; Alk, alkalinity; TP, total phosphorus.

component of the phytoplankton, representing < 10% of total biovolume, but in several cases, it was strongly dominant (> 50% of total biovolume) and its maximum absolute biovolume was one order of magnitude higher than *C. raciborskii* (Table 1). Biovolume variability over time also differed between species. Temporal data series of 17 lakes showed that the annual variation of *P. agardhii* biovolume was significantly higher ($P < 0.05$) than *C. raciborskii*, shifting from low to high values (Fig. 1c and d). Phytoplankton species richness also differed when the dominance of one or the other species occurred; in general, the number of species was higher when *C. raciborskii* was dominant (Fig. 2a and b).

In the field, *C. raciborskii* and *P. agardhii* occurrences differed significantly relative to temperature, lake area, maximum depth, mixing depth, conductivity, alkalinity and TP (Table 1). However, no significant differences were found for pH and light availability (Z_{eu}/Z_{mix}) (Table 1). In general, *C. raciborskii* was dominant at higher temperatures than *P. agardhii*. Several occurrences of *P. agardhii* were reported at temperatures below 15 °C, and below 4 °C its contribution varied between 0.2% and 13% of total biovolume (0.1–1.4 $mm^3 L^{-1}$). Almost all data for *C. raciborskii* appeared at temperatures higher than 20 °C. However, it is notable that we observed *C. raciborskii* with high biovolume at 11 °C (2.1 $mm^3 L^{-1}$, 95% of total biovolume) in a subtropical lake (Lago Javier, Uruguay). Both species were dominant in eutrophic to hypereutrophic lakes. *Cylindrospermopsis raciborskii* attained higher biomass under lower TP, and no occurrences of *P. agardhii* were found in samples with < 50 $\mu g L^{-1}$ TP (Table 1).
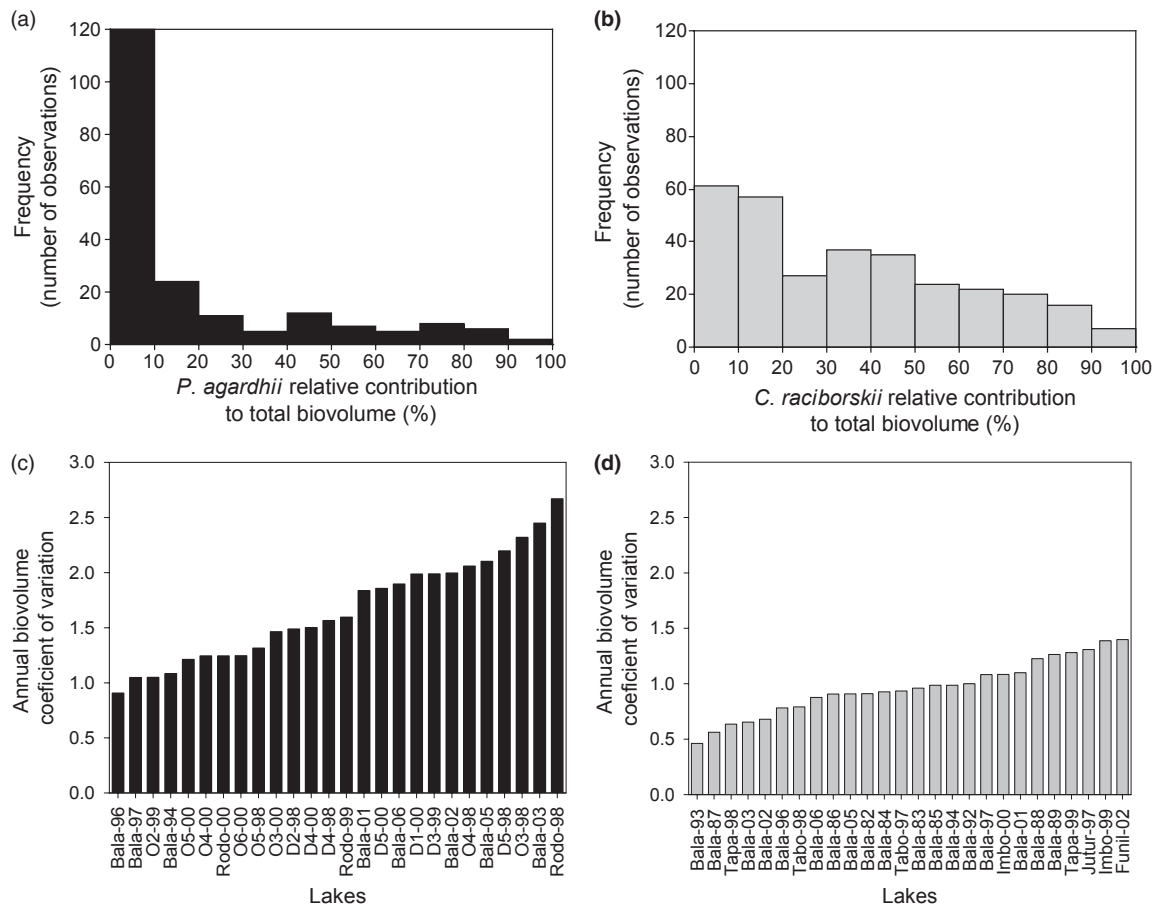
Fig. 1. Frequency of the distribution of the relative biovolume of each species (percentage of total biovolume) for the data set (a: *Planktothrix agardhii*, black bars and b: *Cylindrospermopsis raciborskii*, grey bars). Annual coefficient of variation of biovolume for 17 lakes with temporal data (c: *P. agardhii*, black bars and d: *C. raciborskii*, grey bars). For c and d, the following lakes names are abbreviated: Balaton (Bala), Imboassica (Imbo), Juturnaiba (Jutur), Tabocas (Tabo) and Tapacurá (Tapa). The number after each lake name indicates the last two digits of the year.

Planktothrix agardhii biovolume shifted sharply from high to low values across thresholds in the temperature, Z_{eu}/Z_{mix} and TP gradients (as identified by parameter c in the logistic function and breakpoints in piecewise linear regressions in Fig. 3a, c and e). *Planktothrix agardhii* biovolume decreased abruptly below 11 °C, above 1.62 Z_{eu}/Z_{mix} and above 159 μg L^{-1} TP. *Cylindrospermopsis raciborskii* biovolume was inversely related to TP, and no significant relation was found with temperature or Z_{eu}/Z_{mix} (Fig. 3b, d and f). However, maximum *C. raciborskii* biovolume was observed with Z_{eu}/Z_{mix} values $\leq$ 1. Also, this species had higher biovolumes than *P. agardhii* in fully illuminated water columns (Z_{eu}/Z_{mix}: 3–4).

Comparison of the species among climates: temperate, subtropical and tropical

There were significant differences ($P < 0.05$) between geographical regions in terms of temperature, light and

phosphorus. Water temperature was higher in the tropics, more transparent waters were found in tropical systems, and higher trophic states (TP) were found in subtropical lakes (Table 2). *Planktothrix agardhii* occurred only in temperate and subtropical water bodies, where it had a significantly higher average contribution to total biovolume than *C. raciborskii*. *Cylindrospermopsis raciborskii* occurred in the three regions and, notably, had no significant differences in its contribution to total biovolume between tropical and temperate regions (Fig. 4).

Experimental data

Morphology, pigment structure and growth rate of *P. agardhii* and *C. raciborskii* were compared under different light intensities and temperatures (Tables 3 and 4, Fig. 5). Increments of light intensity from 20 to 100 μmol photons m^{-2} s^{-1} induced adaptive morphological responses in *C. raciborskii*. A significant increase in
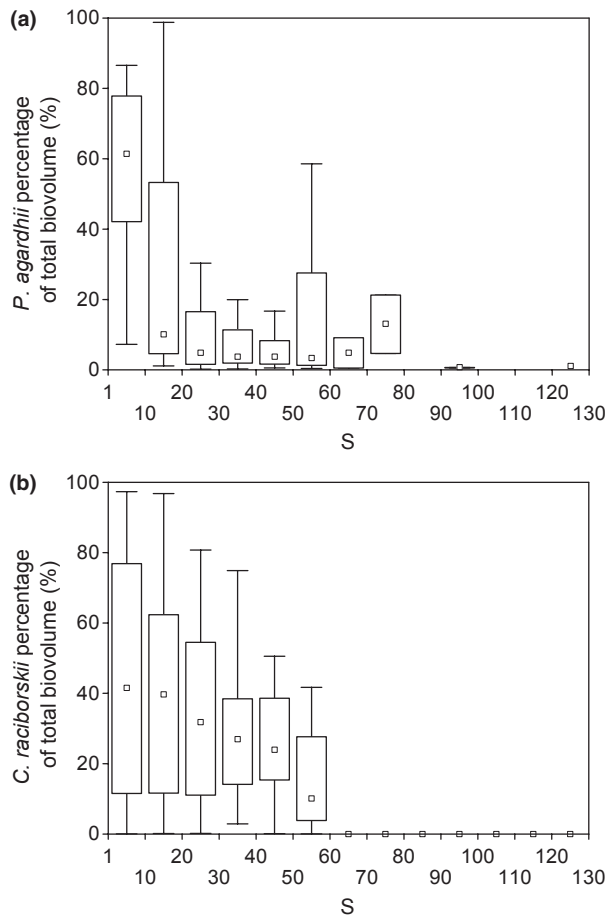
Fig. 2. Contribution of *Planktothrix agardhii* (a) and *Cylindrospermopsis raciborskii* (b) to total biovolume, when each species was > 0, in relation to species number of the community (S). Median (square), percentiles 25% and 75% (box) and range (vertical lines).

C. raciborskii MLD and individual V (M–W, $P < 0.05$) occurred when cultures grew at high light intensity (Table 3).

Lipid pigment composition and responses to light intensity also differed between species. Typical cyanobacterial carotenoids were detected in both species. *Planktothrix agardhii* had higher concentrations of zeaxanthin, β, β-carotene, an undetermined glycosidic carotenoid similar to 4-keto-myxol-2′-methylpentoside (myxol-like) and an unknown carotenoid (car 1), while *C. raciborskii* had high concentrations of aphanizophyll and echinenone (Table 3). In both species, protective and accessory pigment concentrations changed in response to light intensity, with notable differences. The phycocyanin/Chl *a* ratio decreased with higher light intensity in *P. agardhii* and increased in *C. raciborskii*. The magnitude of change in carotenoids/Chl *a* was also higher in *C. raciborskii* than in *P. agardhii* (Table 3). Total carotenoids increased

sixfold in *C. raciborskii*, largely because of aphanizophyll, but also because of echinenone and β,β-carotene. *Planktothrix agardhii* total carotenoids increased 1.5 times with higher light, mainly because of myxol-like and carotenoid 1, whereas β,β-carotene, echinenone and zeaxanthin decreased.

Growth curve experiments performed along a light intensity gradient (from 5 to 180 μmol photons m^{-2} s^{-1}) indicated strong similarities between the two species under light-limited conditions (indicated by α and I_k), although *C. raciborskii* reached significantly higher growth rates ($μ_{max}$) than *P. agardhii* (Table 4, Fig. 5a and b). Temperature growth experiments at two light intensities demonstrated the different behaviour of the two species. *Planktothrix agardhii* growth rates were significantly higher than those of *C. raciborskii* at 15 and 20 °C at low light intensity (60 μmol photons m^{-2} s^{-1}) (Fig. 5c), although no differences were found at 25 °C. However, *C. raciborskii* grew significantly faster than *P. agardhii* (Fig. 5c) at high light intensity (135 μmol photons m^{-2} s^{-1}) at 25 °C. Q_{10} values also showed that *C. raciborskii* growth rate had a higher response to a temperature increase at 135 μmol photons m^{-2} s^{-1} than *P. agardhii* (Table 4).

Discussion

Our extensive data set and laboratory experiments indicated that although *C. raciborskii* and *P. agardhii* overlap in their distribution relative to temperature, light and trophic status, they differed in their biovolume distributions along these gradients. Our results support the hypothesis that *Cylindrospermopsis* is tolerant to a wide range of climates, from tropical to temperate (Briand *et al.*, 2004). Although many studies have suggested that the optimum water temperature of the species is from 25 to 35 °C (Saker & Eaglesham, 1999; Briand *et al.*, 2004; Mehnert *et al.*, 2010), high biomass has been observed in subtropical lakes at 19 °C (Everson *et al.*, 2011), and *C. raciborskii* was equally dominant throughout the year in a tropical lake independent of water temperature variation (17–24 °C) (Figueredo & Giani, 2009). Still other studies found some strains to be capable of sustaining biomass or growing at temperatures as low as 14–17 °C (Chonudomkul *et al.*, 2004; Piccini *et al.*, 2011). Fabre *et al.* (2010) observed *C. raciborskii* occurrence during winter in a subtropical lake (Lago Javier, Uruguay), and in our database the biovolume of *C. raciborskii* in this lake reached 95% of the total (i.e. 2.2 mm^3 L^{-1}) in winter (water temperature: 11.2 °C). To our knowledge, this is the lowest temperature at which *C. raciborskii* has been observed to reach high biovolume and dominate the phytoplankton. The success of *C. raciborskii* in a wide range
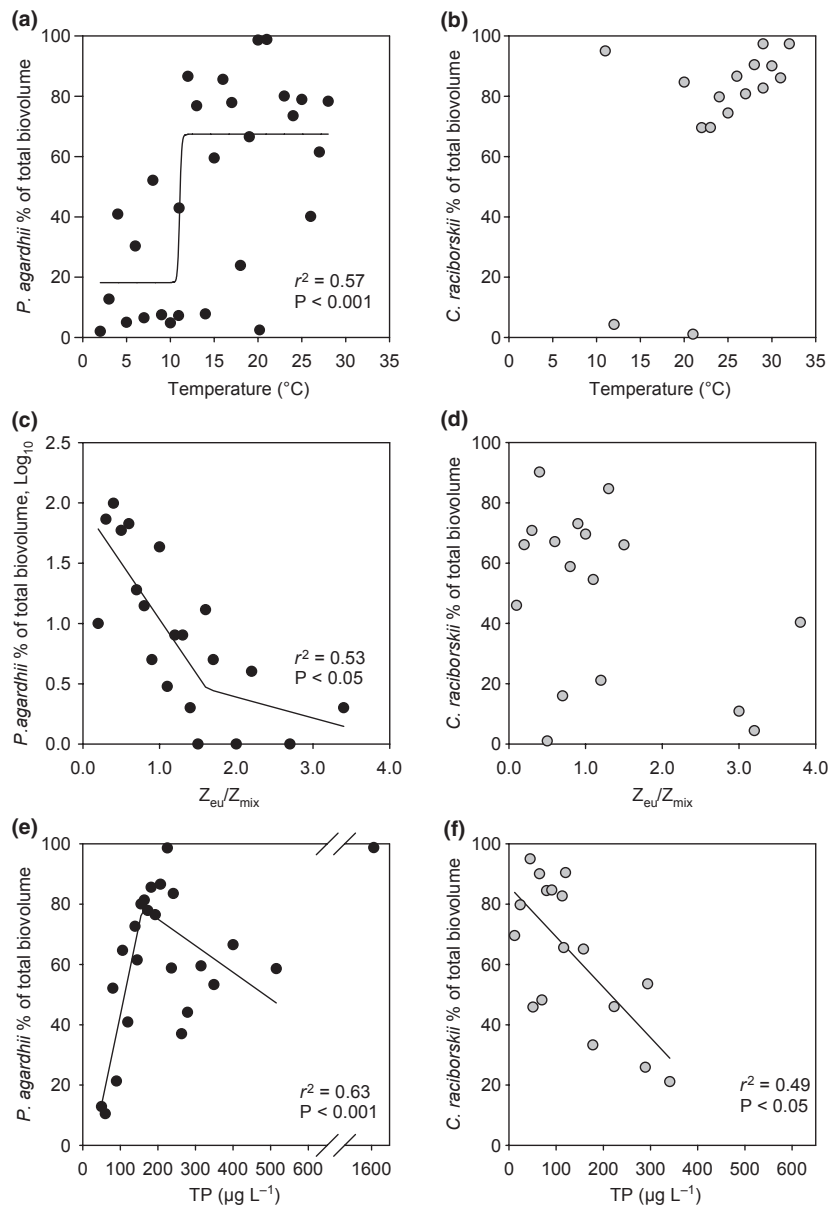
Fig. 3. Maximum values of the contribution to total biovolume of *Planktothrix agardhii* (left, black circles) and *C. raciborskii* (right, grey circles) in relation to water temperature (a and b), Z_{eu}/Z_{mix} (c and d) and TP (e and f). Sigmoidal logistic regression (a, parameters, a: 18.2, b: 49.3, c: 11.1 and d: −99.2), linear piecewise regressions (c, breakpoint: 1.62 and e, breakpoint: 159.2 µg L^{-1}) and linear regression (f) were fitted. No significant model was found for b and d. Coefficient (r^2) and significance (P) is indicated in each plot.

of temperatures observed in our data set and other recent studies (Vidal & Kruk, 2008; Kokociński *et al.*, 2010; Everson *et al.*, 2011) suggests that current concepts of *C. raciborskii* as a tropical species may be due more to a lack of information than to any physiological restriction.

We observed *P. agardhii* only in temperate and subtropical lakes, but in a wide range of temperature conditions. This species can reach high biomass in a range of temperatures from < 2 °C (Toporowska *et al.*, 2010) to

29 °C in tropical ecosystems (Crossetti & Bicudo, 2008; Gemelgo *et al.*, 2009). In our database, *P. agardhii* dominated the phytoplankton of Lago Rodó (subtropical) in all seasons at temperatures ranging from 10 to 31 °C, indicating substantial tolerance to temperature variation.

Our Q$_{10}$ data indicated that *C. raciborskii* grows faster than *P. agardhii* when temperatures shift towards warmer conditions and thus may be favoured by climate warming. Experimental studies showed that *P. agardhii* maximum

Table 2. Water temperature, light availability (Z_{eu}/Z_{mix}) and total phosphorus (TP) of studied lakes grouped by regions (temperate, subtropical and tropical) and based on winter and summer data (median and minimum and maximum between brackets and number of samples in cursive)

	Temperate (35°30′–38°80′S, 46°50′–52°23′N)	Subtropical (34°33′–34°55′S)	Tropical (08°02′–22°33′S)
Temperature (°C)	19.1 (0.50–27.6)a	22.8 (10.0–26.4)b	26.9 (20.0–31.6)c
	207	*40*	*107*
Z_{eu}/Z_{mix}	0.58 (0.17–4.15)a	0.84 (0.17–2.7)a	0.64 (0.09–3.0)b
	257	*75*	*56*
TP (µg L^{-1})	105 (50–1652)a	158 (46–422)b	91.7 (12.4–794)a
	210	*56*	*4*

Significant differences (Mann–Whitney, $P < 0.05$) between regions are indicated with different letters in the table.
ns, not significant; Z_{eu}, euphotic zone; Z_{mix}, mixing zone.

Table 3. Morphology (average ± standard deviation, $n = 60$) and pigment structure (molar pigment ratios to Chl *a*) of *Planktothrix agardhii* and *Cylindrospermopsis raciborskii* isolates grown under 20 and 100 µmol photons m^{-2} s^{-1}

	P. agardhii MVC11		*C. raciborskii* MVCC14	
Light intensity (µmol photons m^{-2} s^{-1})	20	100	20	100
Size and shape				
Volume ($µm^3$)	3573 ± 1771	6169 ± 3968	414 ± 150	1406 ± 523
MLD (µm)	258 ± 111	318 ± 200	132 ± 48	200 ± 75
S/V ($µm^{-1}$)	0.98 ± 0.09	0.82 ± 0.04	2.02 ± 0.01	1.35 ± 0.03
Carotenoid ratios to Chl *a*				
Myxo-like (497/522)	0.07	0.14	nd	nd
Aphanizophyll	nd	0.18	0.56	3.24
Car 1 (477/505)	0.45	0.67	nd	nd
Zeaxanthin	0.33	0.29	nd	0.05
Lutein	nd	0.24	nd	0.03
Echinenone	0.13	0.08	0.32	0.77
β,β-carotene	0.40	0.25	0.15	0.46
T_{CAR}/Chl *a*	1.53	2.28	1.09	6.74
Phy/Chl *a*	48.9	11.4	77.7	49.4

The maximum absorbance peaks are indicated between brackets for the unknown carotenoids (Myxol-like and Car 1).
S/V, surface/volume ratio; Myxol-like, 4-keto-myxol-2′-methylpentoside-like; T_{CAR}, total carotenoids; Phy, phycocyanin; nd, not detected.

growth occurred between 20 and 25 °C (Post *et al.*, 1985; Sivonen, 1990), and its growth rate increased significantly between 15 and 25 °C (Oberhaus *et al.*, 2007). *Cylindrospermopsis raciborskii* has been shown to benefit more than other cyanobacteria from high temperatures (Mehnert *et al.*, 2010) and also to have higher photosynthetic activity and lower light requirements than other cyanobacterial species (Wu *et al.*, 2009). Dominance of this species, however, cannot be predicted from any single factor. In this sense, our Q_{10} values (at 60 and 135 µmol photons m^{-2} s^{-1}) suggest that *C. raciborskii* could have a competitive advantage over *P. agardhii* at conditions with both high light and high temperature. These differences are attributable to the ability of *C. raciborskii* to increase light-harvesting capacity through changes in shape and pigment composition and their proportions as we demonstrated in our experiments. Different photoprotective responses were also suggested

by pigment changes after light increases. In this sense, the acclimation capacity of *C. raciborskii* is illustrative of its phenotypic plasticity.

Planktothrix agardhii showed a higher competitive capacity than *C. raciborskii* under low light and lower temperatures, which agrees with its broad distribution in turbid temperate lakes (Dokulil & Teubner, 2000; Nixdorf *et al.*, 2003). Based on the biovolume distribution and growth of *P. agardhii* in our study, we suggest that this species has limited plasticity, as its physiological response to temperature increase under high light intensity was less pronounced than that of *C. raciborskii*.

According to our I_k values, both species are shade-tolerant, implying that they can succeed in turbid, eutrophic lakes (Padisák & Reynolds, 1998), as originally proposed for Oscillatoriales (Scheffer *et al.*, 1997). The I_k values that we obtained for both species were lower than those reported in the literature (~ 20 µmol photons m^{-2} s^{-1})

Table 4. Growth parameters under different light intensities (maximum growth rate: μ, slope of the light-limited portion of the curve: α, and subsaturating light: I_k); Q_{10} based on the maximum growth average ($n = 4$) at 15 and 25 °C for both isolates

	P. agardhii MVCC11	*C. raciborskii* MVCC14
Light growth response		
μ max (d^{-1})	0.54 ± 0.03*	0.60 ± 0.02*[†]
α (d^{-1} μmol photons^{-1} m^2 s)	0.08 ± 0.03	0.08 ± 0.03[†]
I_k (μmol photons m^{-2} s^{-1})	7.27 ± 3.15	8.49 ± 3.43[†]
Q_{10} (15–25 °C)		
At 60 μmol photons m^{-2} s^{-1}	2.50	3.80
At 135 μmol photons m^{-2} s^{-1}	3.43	6.46

Mean ± standard deviation for light growth response.
*Significant differences between species (*t*-test, $P < 0.05$).
[†]Data from Piccini *et al.* (2011).

(Talbot *et al.*, 1991; Shafik *et al.*, 2001; Briand *et al.*, 2004) which may support the existence of ecotypes suggested by Piccini *et al.* (2011). The presence of ecotypes with different environmental preferences confers a wide intra-specific variability to *C. raciborskii*; ecotypes are among the hypotheses advanced to explain the species' expansion. The pigments we identified in both species were typical for cyanobacteria (Millie *et al.*, 1990), although they were present in markedly different proportions.

Planktothrix agardhii is favoured in continuously mixed, shallow lakes (Scasso *et al.*, 2001; Kruk *et al.*, 2002; Stüken *et al.*, 2006). Similarly, blooms of *C. raciborskii* are commonly reported in mixed conditions (Bouvy *et al.*, 1999; Huszar *et al.*, 2000; Briand *et al.*, 2002; Figueredo & Giani, 2009) and rarely in stratified deep reservoirs (Padisák *et al.*, 2003). This clearly indicates that both species have a wide tolerance for mixing. While in our study there was no clear relationship between *C. raciborskii* biovolume and light availability (Z_{eu}/Z_{mix}), *P. agardhii* biovolume was higher in turbid conditions below the threshold value of 1.62 Z_{eu}/Z_{mix} ratio, suggesting its dependence on turbidity.

The cyanobacterial contribution to total phytoplankton biomass increases markedly above 30 μg L^{-1} TP in temperate lakes (Watson *et al.*, 1997; Dokulil & Teubner, 2000), and *P. agardhii* biovolume distribution in our data set reflect this general pattern. However, the sudden changes we observed in biovolume distribution in hypereutrophic conditions suggested a threshold near 160 μg L^{-1} TP, above which other factors affected biovolume accumulation. In contrast, there was a negative relationship between trophic status and *C. raciborskii* contribution to total biovolume, with increasing dominance of the phytoplankton below 200 μg L^{-1} TP. Higher ranges of phosphorus cell quota in *C. raciborskii* relative
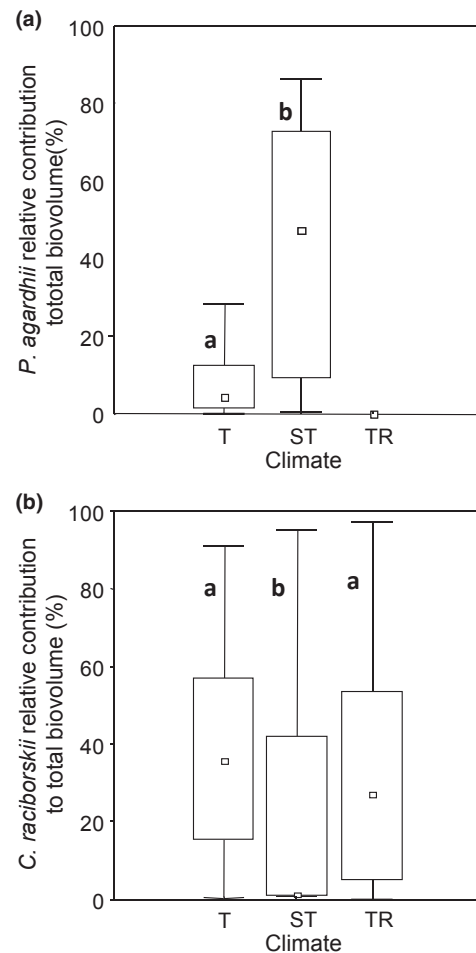

Fig. 4. Contribution of *Planktothrix agardhii* (a) and *Cylindrospermopsis raciborskii* (b) to total biovolume, in relation to the three geographical regions when each species was > 0. Median (square), percentiles 25% and 75% (box) and range (vertical lines). T, temperate; ST, subtropical and TR, tropical regions. Different letters indicate significant differences (K–W, $P < 0.001$) between regions.

to *P. agardhii* may permit *C. raciborskii* to better exploit low P environments (Ducobu *et al.*, 1998; Istvánovics *et al.*, 2000). Some studies suggest that *P. agardhii* growth is greatly dependent on high-frequency phosphate availability (Catherine *et al.*, 2008; Crossetti & Bicudo, 2008; Kokociński *et al.*, 2010; Aubriot *et al.*, 2011), while *C. raciborskii* is able to dominate with small, low-frequency phosphate inputs (Posselt & Burford, 2009). Phytoplankton from oligotrophic and mesotrophic ecosystems may thus be sensitive to a replacement by *C. raciborskii* as a dominant species under small nutrient enrichments.

According to our data, *C. raciborskii* can dominate the phytoplankton at lower overall biovolume than *P. agardhii*, giving insight into the ability of *C. raciborskii* to colonize and rapidly succeed in new habitats. While variations of *C. raciborskii* biovolume during the year were gradual,
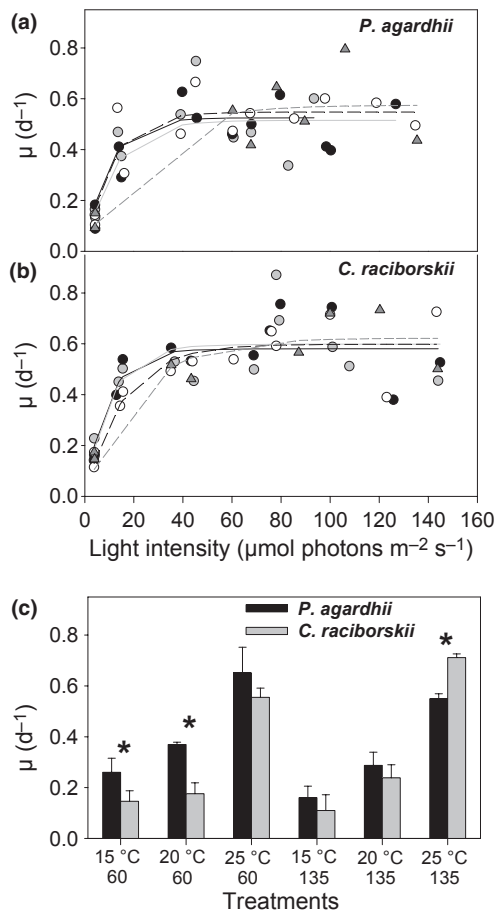
Fig. 5. Experimental growth curves of *Planktothrix agardhii* (a) and *Cylindrospermopsis raciborskii* (b) under a gradient of light intensities. For each species, four curves were performed (white circles, grey circles, black circles and grey triangles) and corresponding function fits are shown (solid line, dotted line, dashed line and short dashed line). (c) Maximum growth rates of *P. agardhii* (black bars) and *C. raciborskii* (grey bars) under three temperatures (15, 20 and 25 °C) and two light intensities (60 and 135 µmol photons m^{-2} s^{-1}) experiments. Bars are averages, and vertical lines are standard deviations. *Significant differences (*t*-test, *P* < 0.05) between species at the corresponding experimental level of light and temperature.

P. agardhii was either dominant or scarce in phytoplankton. Moreover, the dominance of *P. agardhii* in the phytoplankton appears to occur in more eutrophic conditions (i.e. higher phytoplankton biovolume). Scheffer *et al.* (1997) observed similar behaviour in eutrophic temperate shallow lakes and proposed hysteretic mechanisms to explain the distribution and resilience of *P. agardhii*.

Our data indicated that higher diversity (as taxonomic richness) is supported under dominance of *C. raciborskii* than that of *P. agardhii*. This suggests a higher capacity of *C. raciborskii* for co-existence with other species (Kokociński *et al.*, 2010) and may support the hypothesis of its greater plasticity. Sperfeld *et al.* (2010) demon-

strated experimentally that the invasion and success of *C. raciborskii* was not affected by the diversity of the host phytoplankton community. The relatively higher diversity associated with *C. raciborskii* dominance also has implications for food webs, as some studies also found positive correlations between the biomass of *C. raciborskii* and zooplankton (Bouvy *et al.*, 2001; Soares *et al.*, 2009). Conversely, the lower diversity of communities dominated by *P. agardhii* may result from a capacity of this species to generate limiting conditions (i.e. high turbidity) for potential phytoplankton competitors.

The greater plasticity of *C. raciborskii* in response to key environmental factors (temperature and light intensity) may explain its gradual response to changing environments. Conversely, the lower plasticity of *P. agardhii* fits with its narrower distribution in nature. Aquatic environments are highly variable habitats in terms of light and nutrient resources at the time scale of phytoplankton life spans. Reversible plastic phenotypes represent an advantage for organisms in highly variable environments (Piersma & Drent, 2003), allowing the adjustment of their functional responses and increasing their invasive potential (Litchman, 2010). Ecotypes with differing environmental tolerances such as those shown in *C. raciborskii* (Piccini *et al.*, 2011) further strengthen its aptitude for invasive behaviour and success in different climates.

In summary, *C. raciborskii* and *P. agardhii* behaved differently as a result of contrasting strategies for responding to environmental constraints. Further research is required to determine whether this pattern may represent differing strategies in bloom-forming filamentous cyanobacteria of the orders Oscillatoriales and Nostocales. Differences between *P. agardhii* and *C. raciborskii*, as well as between other organisms with comparable strategies, will likely affect the future distribution of these species in projected warming climates where blooms will be enhanced. The high phenotypic plasticity of *C. raciborskii*, and its wide tolerance ranges to key environmental factors, explains its current expansion to temperate latitudes and forecasts its further increase in the future.

Acknowledgements

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