Harmful Algae 90 (2019) 101705

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Subtropical freshwater phytoplankton show a greater response to increased temperature than to increased pCO_2

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ARTICLE INFO

Keywords:
Cylindrospermopsis raciborskii
Raphidiopsis raciborskii
Australia
Strains
Genome comparison
Climate change

ABSTRACT

Global increases in atmospheric CO_2 and temperatures will impact aquatic systems, with freshwater habitats being affected. Some studies suggest that these conditions will promote cyanobacterial dominance. There is a need for a clearer picture of how algal species and strains within species will respond to higher temperatures and CO_2, especially in combination. This study examined two chlorophytes (*Monoraphidium* and *Staurastrum*), and two strains of the cyanobacterium *Raphidiopsis raciborskii* (straight S07 and coiled C03), to determine how the combination of higher temperature and CO_2 levels will affect their growth and maximum cell concentrations. Continuous cultures were used to compare the steady state cell concentrations at 28 °C and 30 °C, and CO_2 partial pressures (pCO_2), 400 and 750 ppm for all cultures, and in addition 1000 ppm at 28 °C for *R. raciborskii* strains. This study showed that, for all species, water temperature had a greater effect than higher pCO_2 on cell concentrations. There were clear differences in response between the chlorophyte species, with *Monoraphidium* preferring 28 °C and *Staurastrum* preferring 30 °C. There were also differences in response of the *R. raciborskii* strains to increasing temperature and pCO_2, with S07 having a greater increase in cell concentration. Genome analysis of *R. raciborskii* showed that the straight strain has five additional carbon acquisition genes (*β-CA*, *chpY*, *cmpB*, *cmpD* and *NdhD4*), indicative of increased carbon metabolism. These differences in the strains' response to elevated pCO_2 will lead to changes in the species population structure and distribution in the water column. This study shows that it is important to examine the effects of both pCO_2 and temperature, and to consider strain variation, to understand how species composition of natural systems may change under future climate conditions.

1. Introduction

Atmospheric CO_2 concentrations are rising at about 0.7 ppm y^{-1} as a result of human actions and may reach 1000 ppm by the year 2100 (Kump, 2002; Royer, 2006; IPCC, 2014). Increasing CO_2 levels will also affect global temperatures, with projections for increases ranging from 1 to 6 °C depending on which models are used, and the willingness of humans to curb greenhouse gas emissions (IPCC, 2014). Much of our understanding of the impact of changes in temperature and CO_2 levels on aquatic systems has come from studies in marine systems, with a focus on vulnerable habitats such as coral reefs (e.g. Hughes et al., 2017). However, other aquatic systems, such as freshwater habitats, are also being impacted by increases in greenhouse gas emissions, directly and indirectly.

Cyanobacterial harmful algae blooms (cyano-HABs) are a risk to the functioning and resilience of freshwater systems worldwide. They also adversely affect water supplies. Cyano-HABs have been increasing in frequency and duration as a result of eutrophication of freshwaters, and future climate conditions, with increasing temperatures and higher CO_2 partial pressures (pCO_2), are likely to favour cyanobacterial growth (Paerl and Huisman, 2008; O'Neil et al., 2012; Visser et al., 2016). One species of concern is the potentially toxic diazotrophic cyanobacterium *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno (basionym *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenayya & Subba Raju) (Aguilera et al., 2018)), because its global range and occurrence has already expanded in recent years, likely due to climate change (Sinha et al., 2012; Wood et al., 2014).

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https://doi.org/10.1016/j.hal.2019.101705
Received 22 July 2019; Received in revised form 22 October 2019; Accepted 6 November 2019
Available online 22 November 2019
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Some studies suggest that increased temperature and associated stratification will promote cyanobacterial dominance over other algal species and lead to increased cell densities (Visser et al., 2016; Sherman et al., 1998). This is because temperature affects algae directly, via their metabolic activities and growth rates, and indirectly via water stratification promoting cyanobacteria capable of regulating their buoyancy (Wood et al., 2017). In countries with low winter temperatures, algal blooms may also commence earlier and persist for longer in the spring and summer months in a warming world (Wiedner et al., 2007).

Some studies have shown that elevated pCO_2 will advantage cyanobacteria (Visser et al., 2016), however, other studies have proposed that increasing CO_2 levels will have greater benefits for species lacking carbon-concentrating mechanisms (CCMs) than for those species that possess them, e.g. cyanobacteria (Raven et al., 2005; Griffiths and Rickaby, 2017). There is a lack of consistency in findings regarding the effect of elevated pCO_2 on diazotrophic cyanobacterial species (Hutchins et al., 2009; Law et al., 2012; Gradoville et al., 2014).

CCMs allow species to actively take up bicarbonate or CO_2 and accumulate CO_2 at the active site of the CO_2-fixing enzyme Ribulose bisphosphate Carboxylase Oxygenase (RubisCO) thus giving them a competitive advantage when CO_2 levels are low (Price et al., 2008; Meyer and Griffith, 2013). However, there is a trade-off between the activity of CCMs and the specificity of RubisCOs. In general terms, organisms such as cyanobacteria, with low RubisCO specificity, require high CCM activity to be competitive, whereas other taxa such as chlorophytes have higher RubisCO specificity and hence can satisfy inorganic carbon requirements with lower CCM activity (Meyer and Griffith, 2013; Tortell, 2000). Therefore, it is likely different species will respond differently to rising pCO_2. Species with less efficient or no inorganic carbon uptake mechanisms, such as chrysophytes, may be favoured in a higher CO_2 world (Maberly et al., 2009). However, some field studies, such as a recent study in a Chinese lake, showed that higher pCO_2 benefitted cyanobacteria (Shi et al., 2017). Li et al. (2016) also showed that increased pCO_2 combined with higher temperature favoured cyanobacteria. Conversely, other studies suggest that elevated CO_2 concentrations will favour chlorophytes over other taxonomic groups, since this is the taxonomic group with the weakest CO_2 concentrating capacity, and relatively low RubisCO specificity (Badger et al., 1998), of the groups studied (Tortell et al., 2000; Low-Décarie, 2014; Low-Décarie et al., 2015).

Furthermore, intraspecific variation will also affect how species respond to environmental changes. *R. raciborskii*, like all phytoplankton species, has multiple strains or ecotypes (for a review see Burford et al., 2016), that co-occur in a waterbody (Willis et al., 2016), and changes in environmental variables can lead to changes in the overall population characteristics, such as average cylindrospermopsin cell quota (Burford et al., 2014). An understanding of the range of strain variation within mixed populations is needed to help explain within-species population responses to environmental changes (Xiao et al., 2017b). *R. raciborskii* is known to flourish under higher temperatures (Briand et al., 2004; Sinha et al., 2012; Thomas and Litchman, 2016), with strains differing in their optimum temperature for growth (Xiao et al., 2017a). Strain differences in response to pCO_2 have also been found, with increased pCO_2 resulting in slightly higher (~9 %) (Pierangelini et al., 2014), or slightly lower (~8 %) growth rates (Lines et al., 2018) depending on strain. This may give *R. raciborskii* a competitive advantage as average global temperatures and pCO_2 continue to increase.

Some studies suggest that increasing atmospheric CO_2 levels will increase primary production in freshwater lakes and reservoirs (Jansson et al., 2012), as algae may be inorganic carbon-limited at present day CO_2 partial pressures (~ 400 ppm). However, Vogt et al. (2017) in their study of 69 boreal lakes with a CO_2 gradient, argue that increased CO_2 is unlikely to change species composition and production. They conclude that environmental context is the key to determining how CO_2 will affect algal species.

Therefore, there is a need for a clearer picture of how algal species respond to the interacting effects of higher temperatures and CO_2 levels. Our study examined three species that are common or dominant in a subtropical reservoir system to determine how the combination of higher temperature and pCO_2 levels affected cell concentration. The species, two chlorophytes and two strains of the cyanobacterium *R. raciborskii,* were isolated from a single water sample and cultured under continuous culture conditions to compare the effects of temperature and pCO_2 on steady state cell concentrations, as a measure of how population density of the species/strains would respond to a changing environment.

2. Methods

2.1. Cultures

Staurastrum sp. (Chlorophyta, Desmidiaceae), *Monoraphidium* sp. (Chlorophyta, Spaeropleales) and *Raphidiopsis raciborskii* (Cyanophyceae, Nostocales) strains S07 and C03 were isolated from a surface water sample from Lake Wivenhoe (27°23.6′ S, 152°36.5′ E, Queensland, Australia), as previously described (Willis et al., 2016). Strain S07 has straight trichomes and C03 has coiled morphology; both strains are toxic with 214.09 ± 12.60 and 121.13 ± 5.50 fg combined cylindrospermopsin and deoxy-cylindrospermopsin cell quotas, respectively. Stock cultures were maintained in 250 mL flasks in Jaworski Medium (JM) (Thompson et al., 1988) at 28 °C, under a 12h:12 h light:dark cycle at 15 µmol photon (PAR)·m^{-2} ·s^{-1} using a cool white LED light (Enttec, Australia). Experiments were commenced within a year of isolation of strains and species to minimise physiological changes induced by culture conditions (Lakeman et al., 2009).

2.2. Continuous cultures

The continuous culture apparatus consisted of 1 L elongated glass vessels with rounded ends, diameter 75 mm, height 250 mm, a glass frit located at the bottom and an outlet tube on the upper side. Jaworskii's Medium (JM, Thompson et al., 1988) was continuously added via a peristaltic pump at a rate of 0.1 L d^{-1} throughout the entire experiment. Compressed air (Air Liquide, Australia) had background CO_2 removed by passing through soda lime (Sigma-Aldrich), then CO_2 was added to give final concentrations of 400, 750 and 1000 ppm (5 % CO_2 air, Air Liquide, Australia) controlled via a gas mixer (Blender Model FMA4621A1) and adjusted regularly as needed. The blended mixture was bubbled through the glass frit into culture medium. There was a single continuous culture vessel per treatment, and six replicate samples were taken over two weeks of steady state cell concentrations.

Continuous culture experiments with *R. raciborskii* strains were run using three CO_2 partial pressures (400, 750 and 1000 ppm) at 28 °C, and all other cultures at two CO_2 partial pressures (400 ppm, 750 ppm) representing the current day levels and a projected 2100 future level in IPCC scenario RCP6 and RCP8.5 (IPCC, 2014), and two temperatures (28 °C and 30 °C) a) reflecting current maximum water temperatures in the reservoir from which the cells were isolated, and b) a likely future increase in temperature (Burford and O'Donohue, 2006). Each species and strain were cultured as non-axenic (with minimal bacterial contamination) monocultures under sterile conditions in the continuous culture vessels. The vessels were inoculated with an initial cell concentration of ~3×10^3 cells mL^{-1} in the case of *R. raciborskii*, ~1×10^6 cells mL^{-1} for *Monorapidium* and ~8×10^3 cells mL^{-1} for *Staurastrum*. This was measured spectrophometrically for optical density at 750 nm (OD_{750}) (Novaspec II, Pharmacia Biotech) by calibrating the OD_{750} against the quantified cell densities using a microscope. Cultures were run for 54 days, with steady state cell concentrations attained in the last 14 days and final samples collected within the last 10 days. Room temperature was controlled at two temperatures 28 and 30 °C, under a 10 h:14 h light:dark cycle at 20 µmol photons (PAR) ·m^{-2} ·s^{-1} using cool white LED light (Enttec, Australia) shining into the rear of

the vessels. Continuous cultures were established as chemostats: where inflowing CO_2 concentration was changed between experiments and dilution rates were kept constant; cultures then acclimated and stabilised to the experimental conditions, reaching 'steady-state' where cell concentration remained constant (Andersen, 2005).

2.3. Sampling and sample analysis

Each vessel was monitored for temperature, pH and inflow air CO_2 ppm three times a week throughout the whole experiment. To achieve this, a 100 mL sample was collected from each vessel and pH and temperature were measured immediately with a pH probe (Aqua-pH, TPS, Qld, Australia), CO_2 was monitored using an Infra-Red Gas Analyser (LICOR LI-820 John Morris Scientific Pty Ltd, Australia), slight (~5 - 10 % of the set value) decreases in CO_2 concentrations occurred during a 24 h period and CO_2 inputs were adjusted daily as needed. A 5 mL subsample was used to measure the OD_{750} and maximum quantum yield of Photosystem II using a PHYTOPAM (Walz) after equilibrating samples in the dark for > 20 min. This subsample was then fixed with Lugol's iodine solution (approximately 1 % final concentration) to preserve cells for enumeration. The specific cell division rate (μ_c) was calculated using first order rate kinetics during exponential phase - before steady state and after taking into account the dilution rate, as previously described (Willis et al., 2017). During the steady state the growth rate was equal to the dilution rate.

2.4. Steady state sample analysis

During steady state, a 100 mL sample was collected and pH, temperature, photosynthetic yield using a PHYTOPAM (Walz), and a sample for cell enumeration prepared as outlined above. Cell enumeration was performed as previously described (Willis et al., 2015), and cell volume calculated on a subset of cells based on a cylinder shape for *R. raciborskii,* two cones for *Monoraphidium,* and two truncated cones for *Staurastrum* (Hillebrand et al., 1999).

For alkalinity measurements, a 40 mL subsample was centrifuged for 5 min at 4000 rpm at 4 °C (Eppendorf AG, Hamburg, Germany), the supernatant was collected and filtered through 0.22 µm pore size filters (Minisart, Sartorius, Germany) and stored frozen (-30 °C). The cell pellet was washed twice with 1 mL 15 mmoL L^{-1} sodium chloride solution, transferred to 2 mL Eppendorf tubes and centrifuged for 2 min at ~27,000 *g* (Biofuge-pico, Heraeus, Germany), the supernatant was removed, and the cell pellet stored frozen for C and N analysis. Prior to analysis, the cell pellet was thawed and then dried at 60 °C, and the particulate C and N was analyzed using a mass spectrometer (GV Isoprime, Manchester, UK).

The alkalinity was measured potentiometrically according to the method of Talling (1973) as described and modified by Denny et al. (1983) using a Radiometer Copenhagen RTS80 autotitration system consisting of a PHM63 digital pH meter (with separate glass and calomel reference electrodes), a TTT80 autotitrator and an ABU80 digital autoburette. For analysis, triplicate 2 mL subsamples were titrated with 0.1 N hydrochloric acid that had been standardised to four significant figures against a standardised solution of ignited $NaHCO_3$. Between 3 and 6 pH/voLume data pairs were collected between pH 4.3 and pH 3.7, and the corresponding F-values calculated. Gran plots were constructed from the F-value/volume data pairs and the theoretical alkalinity end point volumes for each titration determined by linear regression (Stumm and Morgan, 1996). Replicate endpoint volumes rarely varied by more than 2 µL. Dissolved inorganic carbon (DIC) speciation and concentrations were then calculated theoretically for each component (free-CO_2, HCO_3^-, and CO_3^{2-}) from measured alkalinity, culture temperature and culture pH (corrected for ionic strength) using the equations given by Mackereth et al. (1989). The salinity of the media was measured using a WP81 salinity probe (TPS, Brendale, Qld, Australia).

2.5. Statistical analysis

To determine statistically significant differences between *R. raciborskii* strains under the three pCO_2 treatments (n = 6 except for cells mL^{-1} and C:N where n = 3), ANOVAs with post-hoc Tukey tests were performed using the software R (R Core team, 2013). To determine statistically significant differences between the measured parameters for each species and strain in different temperature and CO_2 treatments (n = 3), a nested-ANOVA with post-hoc Tukey tests were performed using R (R Core Team, 2013).

2.6. Comparative genomics of Raphidiopsis raciborskii strains

The sequenced genomes of *R. raciborskii* strains C03 and S07 are available on Genbank (accession numbers: C03 NJHU01000000; S07 NJHX01000000; as described in Willis et al., 2019). The carbon acquisition genes were identified in the genomes of both strains by searching the annotated genomes and cross-referencing by blast searches to confirm gene identity using *Microcystis* and/or *Synecococcus* genes (as in Sandrini et al., 2016). Variable genes between C03 and S07 were identified from the list previously identified (Willis et al., 2018), and cross-referenced by BLASTn searches to the genome of *R. raciborskii* strain CS-505 (Fuentes-Valdés et al., 2016).

2.7. Dissolved CO_2 data of Lake Wivenhoe

Weekly data for free CO_2, total $-CO_2$, pH, and alkalinity were obtained from Seqwater for the sampling site 'Dam Wall, Lake Wivenhoe' from January to December 2009. Values are 5 m depth integrated samples taken from within the surface mixed layer, which is about 6 M when the reservoir is stratified.

3. Results

3.1. Raphidiopsis raciborskii strains comparisons under pCO_2 levels of 400, 750, and 1000 ppm

The steady state cell concentrations of the two strains were not significantly different at 400 ppm, but in both cases were significantly ($p < 0.05$) higher at 750 ppm compared to 400 ppm. The increase with the higher pCO_2 was greater for strain S07 compared to C03 (Fig. 1). The cell concentrations of S07 at steady state were significantly ($p < 0.05$) different between each pCO_2 treatment, increasing from $4.1 \times 10^6 \pm 0.61 \times 10^6$ cells mL^{-1} at 400 ppm, to

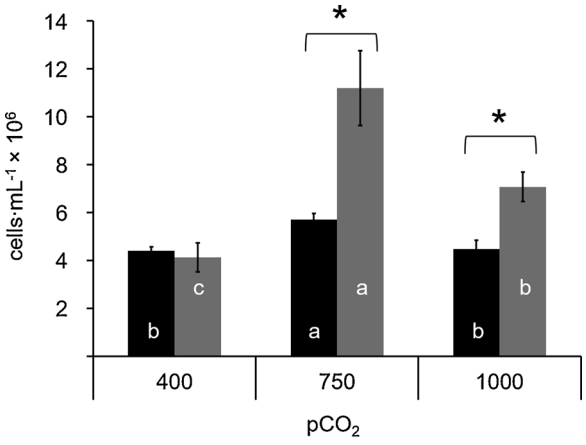

Fig. 1. The cell concentrations (mean ± SD) of *Raphidiopsis raciborskii* strains C03 (black columns) and S07 (grey columns) in continuous cultures during steady state with pCO_2 of 400 ppm, 750 ppm and 1000 ppm. (N = 3; within treatment ANOVA * = $p <$ 0.05; and within strain ANOVA with post-hoc Tukey's test, where different letters equals $p <$ 0.5).

Table 1
Parameters for water chemistry and algal cells within continuous cultures of *R. raciborskii* strains at steady state.

Strain		*R. raciborskii* C03			*R. raciborskii* S07		
		400	750	1000	400	750	1000
Water Chemistry (measured)	pCO_2 (ppm)	27.78 ± 0.08	27.45 ± 0.16	27.19 ± 0.13	27.75 ± 0.13	27.44 ± 0.16	27.35 ± 0.19
	Temp (°C)	7.3 ± 0.12^b	8.0 ± 0.34^a	7.6 ± 0.05^b	6.9 ± 0.05^b	7.9 ± 0.49^a	7.9 ± 0.48^a
	pH	0.74 ± 0.10^a	0.62 ± 0.05^a	0.10 ± 0.01^b	0.68 ± 0.09^a	0.75 ± 0.05^a	0.10 ± 0.01^b
	Alkalinity (meq L^{-1})	0.80 ± 0.10^a	0.61 ± 0.06^b	0.10 ± 0.01^c	0.79 ± 0.11^a	0.74 ± 0.06^a	0.10 ± 0.01^b
Water Chemistry (calculated)	DIC (mmol L^{-1})	0.06 ± 0.00^a	0.01 ± 0.01^b	0.01 ± 0.00^b	0.11 ± 0.01^a	0.01 ± 0.01^b	0.00 ± 0.00^b
	CO_2 (mmol L^{-1})	0.74 ± 0.10^a	0.58 ± 0.06^b	0.10 ± 0.01^c	0.69 ± 0.10^a	0.71 ± 0.06^a	0.10 ± 0.01^b
	HCO_3^-(mmol L^{-1})	2.76 ± 0.70^b	20.79 ± 13.9^a	0.52 ± 0.12^b	1.33 ± 0.25^b	25.46 ± 5.14^a	0.57 ± 0.25^b
	CO_3^{2-}(µmol L^{-1})						
Cell characteristics (measured)	Cells mL^{-1}	4.4 × 10^6 ± 1 × 10^{5b}	5.7 × 10^6 ± 2 × 10^{5a}	4.5 × 10^6 ± 4 × 10^{5b}	4.1 × 10^6 ± 6 × 10^{5c}	11.2 × 10^6 ± 16 × 10^{5a}	7.1 × 10^6 ± 6 × 10^{5b}
	μ_c (d^{-1})	0.13 ± 0.06^a	0.14 ± 0.03^a	0.15 ± 0.10^a	0.12 ± 0.04^a	0.14 ± 0.03^a	0.14 ± 0.04^a
	Cell volume ($µm^3$)	26.4 ± 5.9^b	35.9 ± 7.9^a	24.1 ± 7.7^b	17.8 ± 7.2^a	18.7 ± 4.9^a	16.4 ± 3.2^a
	Biovolume (mm^3 L^{-1})	116.62 ± 4.18^b	205.3 ± 8.57^a	108.1 ± 9.0^c	73.5 ± 10.8^c	209.4 ± 29.2^a	115.6 ± 10.0^b
	C:N (molar)	4.51 ± 0.03^a	4.39 ± 0.07^a	5.11 ± 0.76^a	4.66 ± 0.06^a	4.28 ± 0.04^b	4.84 ± 0.32^a
	Fv/Fm	0.54 ± 0.01^a	0.49 ± 0.02^b	0.55 ± 0.01^a	0.54 ± 0.01^a	0.49 ± 0.02^b	0.55 ± 0.01^a

n = 6 for all values except C:N and cells ml^{-1} where n = 3. Cell division rate was calculated during log phase (first two weeks). ANOVA with Tukey's test, $p < 0.05$. Superscript letters denote significant difference ($p < 0.05$) of within strain and within treatment ANOVA with post-hoc Tukey's test.

11.2 × 10^6 ± 1.6 × 10^6 cells mL^{-1} at 750 ppm CO_2. At 1000 ppm, cell concentrations were 1.7-fold higher than at 400 ppm, i.e. 7.1 × 10^6 ± 0.61 × 10^6 cells mL^{-1}.

The steady state cell concentration of C03 was not significantly different between 400 ppm (4.4 × 10^6 ± 0.16 × 10^6 cells mL^{-1}) and 1000 ppm (4.5 × 10^6 ± 0.4 × 10^6 cells mL^{-1}) but was significantly ($p < 0.05$) higher at 750 ppm (5.7 × 10^6 ± 0.24 × 10^6 cells mL^{-1}).

In the continuous culture of strain C03, the pH (8) was significantly higher ($p < 0.05$) in the 750 ppm treatment compared to both 400 (pH 7.3) and 1000 (pH 7.6) ppm. While in the culture of strain S07, the pH was significantly lower ($p < 0.05$) in the 400 ppm (pH 6.9) treatment compared to the 750 (pH 7.9) and 1000 ppm (pH 7.9) treatments (Table 1). The alkalinity was significantly lower (0.1 ± 0.0; $p < 0.05$) in both strains at 1000 ppm compared to 400 (0.7 ± 0.1) and 750 ppm (0.6 ± 0.1, 0.7 ± 0.1, respectively).

The biovolume of C03 was significantly higher (205.3 ± 8.6 mm^3 L^{-1}; $p < 0.05$) at 750 ppm compared to 400 and 1000 ppm (116.6 ± 4.2 mm^3 L^{-1} and 108.1 ± 9.0 mm^3 L^{-1}, respectively). Strain S07 also had the highest biovolume at 750 ppm (209.2 ± 29.2 mm^3 L^{-1}) and lower biovolumes at 400 and 1000 ppm (73.5 ± 10.8 mm^3 L^{-1} and 115.6 ± 10 mm^3 L^{-1}, respectively). The C:N molar ratio was significantly lower (4.3 ± 0.1; $p < 0.05$) in strain S07 at 750 ppm compared to 400 ppm. However, there was no significant difference ($p > 0.05$) between 1000 ppm and 400 ppm for both strains. The photosynthetic yield, Fv/Fm was significantly lower (0.49 ± 0.0; $p < 0.05$) at 750 ppm for both strains compared to values in cells grown at 400 and 1000 ppm, which were not significantly different from one another.

3.2. Comparative genomics of Raphidiopsis raciborskii strains

The genomes of both strains contain a near complete set of genes involved in carbon acquisition and transport (Supplementary Table 1), including two CCM genes (*ccmL* and *ccmK*), the low -affinity sodium-dependent bicarbonate uptake genes (*bicA*), the putative bicarbonate transporter (*ictB*), the sodium/proton antiporter gene (*nha*), and the carbonic anhydrase (γ-CA) and the CO_2 hydration protein (*chpY)* genes. The low−CO_2 high affinity HCO_3^- - transporter (*cmpABCD*) is present with two copies of *cmpB*, *cmpC* and *cmpD* genes in the variable genome of both strains, strain S07 has an additional carbonic anhydrase (*β*-CA), *cmpB*, *cmpD*, *chpY*, and *ndhD4*. The high-affinity sodium-dependent bicarbonate uptake gene (*sbtA*) was absent from both genomes.

3.3. Comparison of species and strains under pCO_2 levels of 400 and 750 ppm, and temperatures of 28 and 30 °C

Each species and strain varied in its response to the two temperature and pCO_2 levels in the continuous cultures, as measured at steady state. For the cyanobacterium *R. raciborskii* C03, the highest cell concentrations were at the highest temperature (30 °C, $p < 0.001$) with the positive effect of higher pCO_2 levels (750 ppm) being less pronounced than for temperature, but still statistically significant (Fig. 2). The individual cell biovolumes were statistically larger in the higher pCO_2 level (750 ppm) but at the lowest temperature tested (28 °C) (Table 2). However, the maximum quantum yield of PSII, F_v/F_m, was lower in these conditions, while values were similar in all other treatments. C:N ratios did not vary between treatments.

The other *R. raciborskii* strain, S07, had higher cell concentrations at the higher temperature (30 °C), but only at pCO_2 of 400 ppm, and there was a significant interaction between temperature and pCO_2 (Fig. 2). The higher pCO_2 treatment had significantly higher steady state cell concentrations than the lower pCO_2 treatment, but only at 28 °C. Other parameters were similar between treatments, except that the C:N ratio at 750 ppm pCO_2 was lower than that at 400 ppm at a temperature of 28 °C (Table 2).

In the case of the chlorophyte alga, *Monoraphidium* sp., temperature

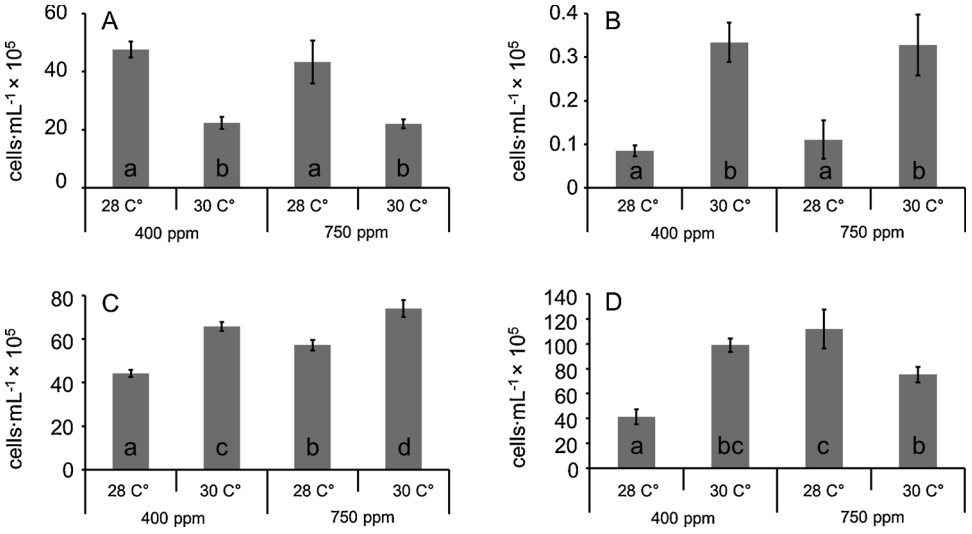

Fig. 2. Cell concentrations (cells mL^{-1}) at steady state in continuous cultures under combinations of two temperatures (28 and 30 °C) and two pCO_2 (400, 750 ppm) treatments: a) *Monoraphidium* sp.; b) *Staurastrum* sp.; c) *R. raciborskii* strain C03; and d) *R. raciborskii* strain S07. Nested ANOVA with post-hoc Tukey's test indicates significant differences between treatments within species, where different letters indicate $p < 0.05$.

affected cell concentrations, but pCO_2 did not (Fig. 2). This species reached higher steady state cell concentrations at 28 °C compared to 30 °C ($p < 0.001$). The C:N ratios were lower in the 750 ppm pCO_2 and 28 °C treatment, compared with other treatments, and F_v/F_m was lower in this treatment compared to that at 400 ppm and 28 °C (Table 2). All other parameters were the same between treatments.

For *Staurastrum* sp., cell concentrations were statistically higher at 30 °C, compared to 28 °C ($p < 0.001$), with pCO_2 level having no effect. For other parameters, there was no statistical difference between treatments (Table 2). The exceptions were lower C:N ratios in the 750 ppm and 28 °C treatment compared with the other treatments. Biovolume was also statistically higher in the 750 ppm and 30 °C treatment compared with the other treatments.

3.4. Dissolved CO_2 data of Lake Wivenhoe

Weekly measurements of the 'Dam Wall' site shows that between 6 January and 31 December 2009, the alkalinity of Lake Wivenhoe varied from 0.98 to 1.58 meq L^{-1} and pH ranged from 7.3–9.6 (Supplementary Fig. 1a-b). Free CO_2 ranged from as low as 0.43 µmol L^{-1} to a high of 157 µmol L^{-1} over the same period. Free CO_2 exceeded air equilibrium for more than 50 % of the time and exceeded the photosynthetic saturation point of ~20 µmol L^{-1} during the winter months. Diel pH variation (data not shown) was typically about 0.3 pH units with pH being lower in the mornings following free CO_2 production from nocturnal respiration, and higher in the late afternoon following net CO_2 consumption during the day. Supplementary Fig. 1b showed that total CO_2 tracked alkalinity, at these pH's most CO_2 exists as bicarbonate and typically accounted for at least 80 %, and regularly between 90 % and 97 % of the total CO_2.

4. Discussion

This study showed that higher water temperature generally had a greater effect on supporting higher cell concentrations at steady state than did increased pCO_2 for the two chlorophyte and one cyanobacterial species. There were clear differences in response between the two chlorophyte species, with *Monoraphidium* having a greater biomass at 28 °C, and *Staurastrum* at 30 °C. There were also differences in response between the two strains of *R. raciborskii,* with the magnitude of the response differing between the strains. The highest cell concentrations occurred at 750 ppm, rather than 1000 ppm, with strain C03 greater at 30 °C compared to S07 at 28 °C. At 28 °C the steady state cell concentration of the straight strain, S07, was double at pCO2 of 750 ppm compared to 400 ppm, while the cell concentration of the coiled strain, C03, only increased by 10 %. However, because the cell volume of C03 was 1.5 times greater than S07 at 400 ppm and increased by 35 % at 750 ppm the resultant biovolume (µm^3 L^{-1}) of the cultures was the same for both strains.

Our results demonstrate within-waterbody strain variability in response to elevated pCO_2, suggesting the possibility that the relative proportion of *R. raciborskii* straight strains in the population will increase compared to coiled strains. This is despite all strains and species being isolated from a single sample from a subtropical reservoir where water temperatures in surface waters in this reservoir in summer are typically 26 °C–28 °C (Burford and O'Donohue, 2006; Burford et al., 2012). The free$-CO_2$ in this reservoir ranges from 0.43–157 µmol L^{-1}, these fluctuations exceed air equilibrium more than 50 % of the time, with increasing pCO_2 the average free$-CO_2$ will increase supporting the phytoplankton that can directly utilise it. Currently, *R. raciborskii* and *Monoraphidium* sp. have been identified as numerically dominant within this reservoir (Burford and O'Donohue, 2006). *Staurastrum* sp. was less common, but our results indicate that this species may increase in abundance as water temperatures increase from the current norm.

Several mesocosm/microcosm studies in temperate systems, and culture studies of temperate species, suggest that elevated CO_2 concentrations will favour chlorophytes over other taxonomic groups, since this is the taxonomic group with weak carbon-concentrating activity but moderate RubisCO specificity (Tortell, 2000; Badger et al., 1998; Low-Décarie et al., 2014, 2015; Ji et al., 2017). However, the two chlorophytes in our study did not show higher cell concentrations in response to higher pCO_2, suggesting that the increased DIC did not lead to higher net photosynthetic rates and organic carbon production by the cells. *Staurastrum* species can either be exclusive CO_2 users or also take up HCO_3^-, likely affecting their distribution (Spijkerman et al., 2005; Lines and Beardall, 2018), while *Monoraphidium braunii* (Nägeli) Komárková-Legnerová has an inducible HCO_3^- uptake system under low CO_2 (Giráldez et al., 2000). Therefore, the cell concentration response to elevated pCO_2 is likely to be species and strain specific.

Speciation of the DIC components in water is driven by pH and the resulting changes in the proportion of HCO_3^-, the preferred carbon source for the species examined here, can affect phytoplankton growth. The pH of the continuous cultures was influenced by both increases in pCO_2 and algal growth, as they either remove DIC from the water during photosynthesis or release it during respiration. Under the pCO_2 treatments used in this study, the pH at steady state meant that HCO_3^- was the dominant DIC component. Additionally, at 1000 ppm the total DIC was significantly lower than at either 400 or 750 ppm. Interestingly, for both *R. raciborskii* strains, the cell concentration at steady state was lower at 1000 ppm compared to 750 ppm. As the DIC

Table 2
Parameters for water chemistry and algal cells within continuous cultures at steady state with two temperatures (28 and 30 °C) and two partial CO_2 pressures (400, 750 ppm) for the cyanobacterium *Raphidiopsis raciborskii,* strains C03, S07, and the chlorophytes *Monoraphidium* sp, and *Staurastrum* sp. Growth rates were calculated prior to steady state.

Specie		*R. raciborskii C03*				*R. raciborskii S07*			
Water Chemistry (measured)	pCO_2 (ppm)	400	400	750	750	400	400	750	750
	Temp (°C)	27.78 ± 0.08	29.88 ± 0.24	27.45 ± 0.16	29.78 ± 0.08	27.75 ± 0.13	29.91 ± 0.24	27.44 ± 0.16	29.79 ± 0.09
	pH	7.27 ± 0.12^{b}	7.33 ± 0.13^{b}	8.01 ± 0.34^{a}	7.12 ± 0.02^{b}	6.94 ± 0.05^{b}	7.39 ± 0.15^{ab}	7.95 ± 0.49^{a}	7.04 ± 0.10^{b}
	Alkalinity (meq L^{-1})	0.74 ± 0.10^{bc}	0.81 ± 0.15^{ab}	0.62 ± 0.05^{c}	0.93 ± 0.10^{a}	0.69 ± 0.10^{a}	0.97 ± 0.33^{a}	0.75 ± 0.05^{a}	0.87 ± 0.12^{a}
Water Chemistry (calculated)	DIC (mmol L^{-1})	0.80 ± 0.10^{b}	0.85 ± 0.16^{ab}	0.61 ± 0.06^{c}	1.02 ± 0.11^{a}	0.79 ± 0.11^{b}	0.76 ± 0.12^{b}	0.74 ± 0.06^{b}	0.99 ± 0.16^{a}
	CO_2 (mmol L^{-1})	0.06 ± 0.00^{b}	0.05 ± 0.02^{b}	0.01 ± 0.01^{c}	0.09 ± 0.01^{a}	0.11 ± 0.01^{ab}	0.07 ± 0.02^{b}	0.01 ± 0.01^{c}	0.12 ± 0.05^{a}
	HCO_3^- (mmol L^{-1})	0.74 ± 0.10^{bc}	0.80 ± 0.15^{ab}	0.58 ± 0.06^{c}	0.93 ± 0.10^{a}	0.69 ± 0.10^{b}	0.69 ± 0.11^{b}	0.71 ± 0.06^{ab}	0.86 ± 0.12^{a}
	CO_3^{2-}- (µmol L^{-1})	2.76 ± 0.70^{b}	4.21 ± 1.40^{b}	20.79 ± 13.88^{a}	2.87 ± 0.38^{b}	1.33 ± 0.25^{b}	2.19 ± 0.83^{b}	22.33 ± 9.25^{a}	2.02 ± 0.56^{b}
Cell characteristics	Cells. mL^{-1}	$4.4 \times 10^6 \pm 1 \times 10^5$	$6.5 \times 10^6 \pm 2 \times 10^5$	$5.7 \times 10^6 \pm 2 \times 10^5$	$7.4 \times 10^6 \pm 4 \times 10^5$	$4.1 \times 10^6 \pm 6 \times 10^5$	$9.9 \times 10^6 \pm 5 \times 10^5$	$11.2 \times 10^6 \pm 15 \times 10^5$	$7.5 \times 10^3 \pm 6 \times 10^5$
	µ (d^{-1}) *	0.13 ± 0.06^{a}	0.12 ± 0.03^{a}	0.14 ± 0.03^{a}	0.15 ± 0.03^{a}	0.12 ± 0.04^{a}	0.13 ± 0.08^{a}	0.14 ± 0.03^{a}	0.13 ± 0.05^{a}
	Cell Volume ($µm^3$)	26.4 ± 5.9^{b}	28.3 ± 6.4^{b}	35.9 ± 7.9^{a}	30.0 ± 7.9^{ab}	17.8 ± 7.2^{bc}	23.0 ± 7.7^{ab}	18.7 ± 4.9^{ac}	23.9 ± 9.1^{a}
	Biovolume ($µm^3$ L^{-1})	117 ± 4.2^{c}	186 ± 5.8^{b}	205 ± 8.6^{ab}	223 ± 11.7^{a}	73 ± 10.8^{b}	228 ± 12.6^{a}	209 ± 29.2^{a}	180 ± 14.7^{a}
	C:N (molar)	4.51 ± 0.03^{a}	4.52 ± 0.05^{a}	4.39 ± 0.07^{a}	4.39 ± 0.03^{a}	4.66 ± 0.06^{ab}	4.59 ± 0.05^{ac}	4.28 ± 0.04^{c}	4.37 ± 0.05^{bc}
	F_v/F_m	0.54 ± 0.01^{a}	0.52 ± 0.02^{a}	0.48 ± 0.01^{b}	0.53 ± 0.03^{a}	0.54 ± 0.01^{a}	0.56 ± 0.02^{a}	0.49 ± 0.02^{b}	0.53 ± 0.02^{a}
Species		*Monoraphidium sp.*				*Staurastrum sp.*			
Water Chemistry (measured)	pCO_2 (ppm)	400	400	750	750	400	400	750	750
	Temp (°C)	27.32 ± 0.20	29.94 ± 0.31	27.51 ± 0.16	29.87 ± 0.09	27.25 ± 0.28	29.95 ± 0.28	27.53 ± 0.16	29.90 ± 0.10
	pH	7.29 ± 0.06^{b}	6.99 ± 0.11^{b}	8.25 ± 0.49^{a}	6.69 ± 0.07^{b}	6.73 ± 0.09^{b}	6.43 ± 0.05^{ab}	7.03 ± 0.04a	6.27 ± 0.06^{b}
	Alkalinity (meq L^{-1})	0.75 ± 0.12^{bc}	0.51 ± 0.04^{ab}	0.55 ± 0.21^{c}	0.53 ± 0.11^{a}	0.48 ± 0.05^{a}	0.44 ± 0.15^{a}	0.03 ± 0.01^{a}	0.44 ± 0.08^{a}
Water Chemistry (calculated)	DIC (mmol L^{-1})	0.78 ± 0.15^{a}	0.56 ± 0.04^{bc}	0.44 ± 0.09^{c}	0.75 ± 0.16^{ab}	0.59 ± 0.04^{ab}	0.49 ± 0.17^{b}	0.04 ± 0.03^{c}	0.76 ± 0.13^{a}
	[CO_2] (mmol L^{-1})	0.05 ± 0.01^{b}	0.06 ± 0.02^{b}	0.005 ± 0.00^{c}	0.15 ± 0.03^{a}	0.11 ± 0.02b	0.05 ± 0.02^{c}	0.005 ± 0.00^{c}	0.33 ± 0.06^{a}
	HCO_3^- (mmol L^{-1})	0.73 ± 0.14^{a}	0.50 ± 0.04^{bc}	0.41 ± 0.13^{c}	0.60 ± 0.13^{ab}	0.48 ± 0.05^{a}	0.43 ± 0.15^{a}	0.04 ± 0.03^{b}	0.43 ± 0.08^{a}
	CO_3^{2-} (µmol L^{-1})	3.27 ± 0.68^{a}	1.36 ± 0.46^{a}	32.44 ± 49.69 ^{a}	0.71 ± 0.22^{a}	0.64 ± 0.21^{b}	1.06 ± 0.4^{a}	0.09 ± 0.07^{c}	0.18 ± 0.04^{c}
Cell characteristics	Cells mL^{-1}	$4.8 \times 10^6 \pm 2 \times 10^5$	$2.2 \times 10^6 \pm 2 \times 10^5$	$4.3 \times 10^6 \pm 7 \times 10^5$	$2. 2 \times 10^6 \pm 1 \times 10^5$	$8 \times 10^3 \pm 1 \times 10^3$	$33 \times 10^3 \pm 4 \times 10^3$	$11 \times 10^3 \pm 4 \times 10^3$	$32 \times 10^3 \pm 6 \times 10^3$
	µ (d^{-1}) *	0.05 ± 0.17^{a}	0.06 ± 0.13^{a}	0.09 ± 0.13^{a}	0.04 ± 0.13^{a}	0.13 ± 0.15^{a}	0.08 ± 0.15^{a}	0.03 ± 0.18^{a}	0.09 ± 0.12^{a}
	Cell Volume ($µm^3$)	23.37 ± 19.61^{a}	38.58 ± 19.52^{a}	24.48 ± 14.04^{a}	36.67 ± 19.76^{a}	1139.47 ± 322.64^{bc}	931.19 ± 192.68^{c}	1204.87 ± 299.91^{b}	1479.07 ± 281.83^{a}
	Biovolume ($µm^3$ L^{-1})	111 ± 6.5^{a}	86 ± 8^{ab}	106 ± 18.2^{ab}	80 ± 5.7^{b}	10 ± 1.5^{c}	31 ± 4.2^{b}	13 ± 5.4^{c}	48 ± 10.4^{a}
	C:N	6.87 ± 0.07^{bc}	7.29 ± 0.11^{c}	6.56 ± 0.12^{a}	6.89 ± 0.12^{b}	9.28 ± 0.47^{a}	10.64 ± 0.68^{a}	6.89 ± 0.47^{b}	9.77 ± 0.74^{a}
	Fv/Fm	0.58 ± 0.01^{a}	0.57 ± 0.02^{ab}	0.55 ± 0.01^{b}	0.56 ± 0.01^{ab}	0.61 ± 0.02^{a}	0.60 ± 0.03^{a}	0.58 ± 0.02^{a}	0.59 ± 0.02^{a}

n = 6 for all values except C:N and cells ml^{-1} where n = 3. *Cell division rate was calculated during exponential phase (first two weeks). ANOVA with Tukey's test, $p < 0.05$. Superscript letters denote significant differences ($p < 0.05$) of within strain and within treatment ANOVA with post-hoc Tukey's test.

speciation was similar in both treatments, this result is likely caused by an imbalance between light availability and CCM activity. Lines et al. (2018) showed that the photosynthetic affinity for CO_2 of *R. raciborskii* is slightly lower at 1000 ppm than 400 ppm, but the change in affinity was less than occurs in other species and suggests constitutive CCM activity. A constitutive CCM may be a disadvantage at the higher pCO_2 and low light intensities, because more bicarbonate would be taken up than could be assimilated, reducing growth. However, low light levels may also restrict the activity of CCMs (Beardall, 1991). This may have occurred in the cultures at 1000 ppm compared to 750 ppm, suggesting that the cells at 1000 ppm were most likely light-limited relative to available carbon (Huisman and Weissing, 1994; Jahn et al., 2018), and accumulated more CO_2 than could be fixed by the available RubisCO. The increase in the particulate C:N, is further indicative of changes in the cellular carbon content, and supports an active CCM with cellular inorganic carbon increased in the cell, but not translating into increased cell concentrations.

Cyanobacteria have been shown to have highly efficient CCMs, counteracting their poor RubisCO specificity, suggesting that they might be more competitive under pCO_2 limitation than other species, and conversely that higher pCO_2 will benefit other species (Raven and Beardall, 2003). However, CCMs can vary, and specifically the range of transporters used for inorganic carbon acquisition can be different across strains and species. Cyanobacteria for instance can express up to five different transporters of inorganic carbon with differing capacity, substrates and affinity, while in eukaryotes, inorganic carbon acquisition can involve active transport across either or both the plasmalemma and the chloroplast envelope (Beardall and Raven, 2017). Sandrini et al., (2014; 2016) and Ji et al. (2017) showed that genotypes of the cyanobacterium *Microcystis aeruginosa* (Kützing) Lemmermann can vary considerably in their inorganic carbon uptake systems with those with a low affinity but high-flux bicarbonate uptake system having a selective advantage when inorganic carbon availability is high.

Genetic differences identified between the *R. raciborskii* strains indicate that possible differences in the carbon acquisition mechanisms exist and are likely the foundation for the different growth responses. The core genomes of strains C03 and S07 both contain a near complete set of genes involved in the CCM and carbon acquisition, the exception is the absence of the *sbtA* gene. Importantly, strain S07, has additional copies of the bicarbonate transporters *cmpB*, *cmpD*, *chpY*, a carbonic anhydrase (*β-CA*), and a NAD(P)H-quinone oxidoreductase subunit D4 (*ndhD4*). The five additional carbon acquisition genes identified in strain S07, and their absence in C03, are typical of the variable genomes of straight and coiled morphotypes previously characterised (Sinha et al., 2014; Willis et al., 2018), suggesting these differences are morphotype specific. The additional carbon acquisition genes in the straight morphotypes may result in a higher rate of bicarbonate (HCO_3^-) uptake and transport within the cell to the carboxysomes and Rubisco enzymes, similar to the response found in *M. aeruginosa* (Sandrini et al., 2014, 2016).

Li et al. (2016) also showed that pCO_2, combined with temperature favoured cyanobacteria over eukaryotic algae. Vogt et al. (2017) in their study of 69 boreal lakes with a CO_2 gradient, agree that environmental context is the key to determining interactions between algal species and CO_2. Indeed, these authors argue that increased CO_2 alone is unlikely to change species composition and production in boreal lakes. Our study showed that increased pCO_2 did increase steady state cell concentrations of two strains of the cyanobacterium, *R. raciborskii,* but the scale of effect differed between the strains and in one case there was an interaction with temperature.

Our study has also shown that examination of the effects of pCO_2 on cell concentrations without also examining the commensurate increased temperatures that will also result from climate change gives a false impression of how species composition of natural systems may change. Burford et al. (2019) argue that studies of cyanobacteria and climate change should examine multiple stressors, rather than focussing on one parameter. They also identify that a range of scientific methods and approaches should be used to understand climate change effects. Shi et al. (2017) used microcosm experiments in a Chinese lake to show that higher pCO_2 levels will preferentially benefit cyanobacteria, which is consistent with our findings. Our study shows it depends on the strain of cyanobacteria being examined. It is likely that the effect shown by Shi et al. (2017) would be more pronounced if pCO_2 had been increased in combination with higher temperatures. Light intensity can also be important in influencing the response of cells to elevated CO_2. Operation of CCMs is energetically expensive so at low light levels where energy is potentially limiting, down-regulation of CCMs could 'free up' energy that can be used for other processes, supporting faster growth (Li and Campbell, 2012; Gao et al., 2012).

Phytoplankton populations are composed of a mix of species, including members of the Bacillariophyta, Chlorophyta, and Cyanophyta, which usually have seasonal variation in their relative abundance. Studies have shown a range of responses of different phytoplankton species to elevated pCO_2 (Fu et al., 2007; Li et al., 2016; Pierangelini et al., 2017), therefore changes in species dynamics is expected under future climate conditions (Li et al., 2016; Ji et al., 2017). An increase in cyano-HABs, either as an increase in occurrence or increase in duration and magnitude, typically result in a decrease in the species diversity (Monchamp et al., 2018). Generally, there is significant evidence of an increase in cyano-HABs. However, the the current study shows that strain variability will also lead to changes in the composition of the bloom, with an increase in straight strain morphologies within the population likely to lead to a denser bloom closer to the water surface. This may have follow-on effects for the whole ecosystem functioning (Sukenik et al., 2015), with a denser bloom leading to more self-shading (Xiao et al., 2017a) and a decrease in light penetrating the water column.

Our culture study only focussed on temperature and pCO_2, however, nutrient availability and stratification also affects species population density and composition. Several studies have examined nutrient availability (e.g. Burford et al., 2014; Low-Décarie et al., 2015). However, this is most effectively done in micro- and mesocosm studies, and therefore was not the focus of the present study.

The differences measured between species and strain responses to changes in temperature and pCO_2 highlight the importance of examining multiple cultures under both interacting conditions. The three species in our study came from the same reservoir and were collected at the same time. Despite this, there were clear differences in response, with increased temperature having a greater effect than pCO_2. The difference in response is particularly pertinent for the *R. raciborskii* strains: because strain differences in the population can lead to variation in toxin yield (Burford et al., 2014). Willis et al. (2016) have previously shown that *R. raciborskii* isolates from one reservoir can vary substantially in their growth rates, toxin cell quotas and morphological attributes when cultured under the same environmental conditions. Studies that attempt to generalize about differences in group or class response to climate change may also need to consider the variability of response within species.

Acknowledgements

We wish to thank Stephen Faggotter for maintenance of the CO_2 system, Radislav Bak and the stable isotope laboratory for analysis of carbon and nitrogen, and Man Xiao for the nested-ANOVA analysis. This project was funded by an Australian Research Council Linkage grant with support from Seqwater (LP120100284). [CG]

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.hal.2019.101705.

References

Aguilera, A., Gomez, E.B., Kastovsky, J., Echenique, R.O., Salerno, G.L., 2018. The polyphasic analysis of two native *Raphidiopsis* isolates supports the unification of the genera *Raphidiopsis* and *Cylindrospermopsis* (Nostocales, Cyanobacteria). Phycologia 57, 130–146.

Andersen, R. (Ed.), 2005. Algal Culturing Techniques. Academic Press, London.

Badger, M.R., Andrews, T.J., Whitney, S.M., Ludwig, M., Yellowlees, D.C., Leggat, W., Price, G.D., 1998. The diversity and coevolution of Rubiscos, plastids, pyrenoids and chloroplast-based CO2-concentrating mechanisms in algae. Can. J. Bot. 76, 1052–1071.

Beardall, J., 1991. Effects of photon flux density on the "CO2 concentrating mechanism" of the cyanobacterium Anabaena variabilis. J. Plankt. Res. 13, 133–141.

Beardall, J., Raven, J.A., 2017. Cyanobacteria vs green algae: which group has the edge? J. Exp. Bot. 68, 3697–3699.

Briand, J.F., Leboulanger, C., Humbert, J.F., Bernard, C., Dufour, P., 2004. *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: selection, wide physiological tolerance, or global warming? J. Phycol. 40, 231–238.

Burford, M.A., Davis, T.W., Orr, P.T., Sinha, R., Willis, A., Neilan, B.A., 2014. Nutrient-related changes in the toxicity of field blooms of the cyanobacterium Cylindrospermopsis raciborskii. FEMS Microb. Ecol 89, 135–148.

Burford, M.A., O'Donohue, M.J., 2006. A comparison of phytoplankton community assemblages in artificially and naturally mixed subtropical water reservoirs. Freshw. Rev. 51, 973–982.

Burford, M.A., Green, S.A., Cook, A.J., Johnson, S.A., Kerr, J.G., O'Brien, K.R., 2012. Sources and fate of nutrients in a subtropical reservoir. Aquat. Sci. 74, 179–190.

Burford, M.A., Carey, C.C., Hamilton, D.P., Huisman, J., Paerl, H.W., Wood, S.A., Wulff, A., 2019. Perspective: advancing the research agenda for improving understanding of cyanobacteria in a future of global change. Harmful Algae. https://doi.org/10.1016/j.hal.2019.04.004.

Denny, P., Orr, P.T., Erskine, D.J.C., 1983. Potentiometric measurements of carbon dioxide flux of submerged aquatic macrophytes in pH-statted natural waters. Freshw. Rev. 13, 507–519.

Fuentes-Valdés, J.J., Plominsky, A.M., Allen, E.E., Tamames, J., Vásquez, M., 2016. Complete genome sequence of a cylindrospermopsin-producing cyanobacterium, *Cylindrospermopsis raciborskii* CS505, containing a circular chromosome and a single extrachromosomal element. Gen Announce 4, e00823–16.

Gao, K.S., Xu, J.T., Gao, G., Li, Y.H., Hutchins, D.A., Huang, B., Wang, L., Zheng, Y., Jin, P., Cai, X., Hader, D.-P., Li, W., Xu, K., Liu, N., Riebesell, U., 2012. Rising CO2 and increased light exposure synergistically reduce marine primary productivity. Nat. Clim. Change 2, 519–523.

Giráldez, N., Aparicio, P.J., Quiñones, M.A., 2000. Limiting CO2 levels induce a blue light-dependent HCO3− uptake system in Monoraphidium braunii. J. Exp. Bot. 51, 807–815.

Gradoville, M.R., White, A.E., Bottjer, D., Church, M.J., Letelier, R., 2014. Diversity trumps acidification: lack of evidence for carbon dioxide enhancement of Trichodesmium community nitrogen or carbon fixation at Station ALOHA. Limnol. Oceanogr. 59, 645–659.

Griffiths, H.M.M.T., Rickaby, R.E.M., 2017. Overcoming adversity through diversity: aquatic carbon concentrating mechanisms. J. Exp. Bot. 68, 3689–3695.

Hillebrand, H., Durselen, C.-D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35, 403e424.

Hughes, T.P., Barnes, M.L., Bellwood, D.R., Cinner, J.E., Cumming, G.S., Jackson, J.B.C., Kleypas, J., van de Leemput, I.A., Lough, J.M., Morrison, T.H., Palumbi, S.R., van Nes, E.H., Scheffer, M., 2017. Coral reefs in the anthropocene. Nature 546, 82–90.

Huisman, J., Weissing, F.J., 1994. Light-limited growth and competition for light in well-mixed aquatic environments: an elementary model. Ecology. 75, 507–520.

Hutchins, D.A., Mulholland, M.R., Fu, F., 2009. Nutrient cycles and marine microbes in a CO_2-enriched ocean. Oceanography. 22 128−145.

IPCC, 2014. Climate change 2014: synthesis report. In: Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II [Core Writing Team. IPCC, Geneva Switzerland, 151 and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Jansson, M., Karlsson, J., Jonsson, A., 2012. Carbon dioxide supersaturation promotes primary production in lakes. Ecol. Lett. 15, 527–532.

Jahn, M., Vialas, V., Karlsen, J., Maddalo, G., Edfors, F., Forsström, B., Uhlen, M., Käll, L., Hudson, E.P., 2018. Growth of cyanobacteria is constrained by the abundance of light and carbon assimilation proteins. Cell Rep. 25, 478–486.

Ji, X., Verspagen, J.M.H., Stomp, M., Huisman, J., 2017. Competition between cyanobacteria and green algae at low versus elevated CO_2: who will win, and why? J. Exp. Biol. 68, 3815–3828.

Kump, L.R., 2002. Reducing uncertainty about carbon dioxide as a climate driver. Nature 419, 188–190.

Lakeman, M.B., von Dassow, P., Cattolico, R.A., 2009. The strain concept in phytoplankton ecology. Harmful Algae 8, 746–758.

Law, C.S., Breitbarth, E., Hoffmann, L.J., McGraw, C.M., Langlois, R.J., LaRoche, J., Marriner, A., Safi, K.A., 2012. No stimulation of nitrogen fixation by non-filamentous diazotrophs under elevated CO2 in the South Pacific. Glob. Change Biol. Bioenergy 18, 3004–3014.

Li, G., Campbell, D.A., 2012. Rising CO2 interacts with growth light and growth rate to alter photosystem II photoinactivation of the coastal diatom Thalassiosira pseudonana. PLoS One 8, e55562.

Li, W., Xu, X.G., Fujibayashi, M., Niu, Q.G., Tanaka, N., Nishimura, O., 2016. Response of microalgae to elevated CO_2 and temperature: impact of climate change on freshwater ecosystems. Environ. Sci. Poll. Res 23, 19847–19860.

Lines, T., Beardall, J., 2018. Carbon acquisition characteristics of six microalgal species isolated from a subtropical reservoir: potential implications for species succession. J. Phycol. 54, 599–607.

Low-Décarie, E., Bell, G., Fussmann, G.F., 2015. CO_2 alters community composition and response to nutrient enrichment of freshwater phytoplankton. Oecologia. 177, 875–883.

Low-Décarie, E., Fussmann, G.F., Bell, G., 2014. Aquatic primary production in a high-CO_2 world. Trends Ecol. Evol. (Amst.) 29, 223–232.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.

Maberly, S.C., Ball, L.A., Raven, J.A., Sültemeyer, D., 2009. Inorganic carbon acquisition by Chrysophytes. J. Phycol. 45, 1052–1061.

Mackereth, F.J.H., Heron, J., Talling, J.F., 1989. Water Analysis: Some Revised Methods for Limnologists. Second Impression. Freshwater Biological Association, UK 120pp. ISBN 978-0900386-31-2.

Meyer, M., Griffiths, H., 2013. Origins and diversity of eukaryotic CO_2-concentrating mechanisms: lessons for the future. J. Exp. Bot. 64, 769–786.

Paerl, H.W., Huisman, J., 2008. Climate. Blooms like it hot. Science 320, 57–58.

Pierangelini, M., Stojkovic, S., Orr, P.T., Beardall, J., 2014. Elevated CO_2 causes changes in the photosynthetic apparatus of a toxic cyanobacterium, *Cylindrospermopsis raciborskii*. J. Plant Physiol. 171, 1091–1098.

Price, G.D., Badger, M.R., Woodger, F.J., Long, B.M., 2008. Advances in understanding the cyanobacterial CO_2-concentrating mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants. J. Exp. Bot. 59, 1441–1461.

R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/.

Raven, J.A., Ball, L.A., Beardall, J., Giordano, M., Maberly, S.C., 2005. Algae lacking CO_2 concentrating mechanisms. Can. J. Bot. 83, 879–890.

Raven, J.A., Beardall, J., 2003. CO_2 acquisition mechanisms in algae: carbon dioxide diffusion and carbon dioxide concentrating mechanisms. In: Larkum, A.W.D., Douglas, S.E., Raven, J.A. (Eds.), Photosynthesis in the Algae. Advan. Photosyn. (Series Editor, Govindjee)., Kluwer, pp. 225–244.

Royer, D.L., 2006. CO_2-forced climate thresholds during the Phanerozoic. Geochim. Cosmochim. Acta 70, 5665–5675.

Sandrini, G., Ji, X., Verspagen, J.M.H., Tann, R.P., Slot, P.C., Luimstra, V.M., Schuurmans, J.M., Matthijs, H.C.P., Huisman, J., 2016. Rapid adaptation of harmful cyanobacteria to rising CO_2. Proc. Nat. Acad. Sci. 113, 9315–9320.

Sandrini, G., Matthijs, H.C.P., Verspagen, J.M.H., Muyzer, G., Huisman, J., 2014. Genetic diversity of inorganic carbon uptake systems causes variation in CO_2 response of the cyanobacterium *Microcystis*. ISME 8, 589–600.

Sherman, B.S., Webster, I.T., Jones, G.J., Oliver, R.L., 1998. Transitions between *Aulacoseira* and *Anabaena* dominance in a turbid river weir pool. Limnol. Oceanogr. 43, 1902–1915.

Shi, X., Li, S., Wei, L., Qin, B., Brookes, J.D., 2017. CO_2 alters community composition of freshwater phytoplankton: a microcosm experiment. Sci. Tot. Environ. 607-608, 69–77.

Sinha, R., Pearson, L.A., Davis, T.W., Burford, M.A., Orr, P.T., Neilan, B.A., 2012. Increased incidence of *Cylindrospermopsis raciborskii* in temperate zones – is climate change responsible? Water Res. 46, 1408–1419.

Sinha, R., Pearson, L., Davis, T.W., Muenchhoff, J., Pratama, R., Jex, A.R., Burford, M.A., Neilan, B.A., 2014. Comparative genomics of *Cylindrospermopsis raciborskii* strains with differential toxicities. BMC Genomics 15, 83.

Spijkerman, E., Maberly, S.C., Coesel, P.F.M., 2005. Carbon acquisition mechanisms by planktonic desmids and their link to ecological distribution. Can. J. Bot. 83, 850–858. https://doi.org/10.1139/B05-069.

Stumm, W., Morgan, J.J., 1996. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. Wiley-Interscience, NY.

Talling, J.F., 1973. The application of some electrochemical methods to the measurement of photosynthesis and respiration in fresh waters. Freshw. Rev. 3, 335–362.

Thomas, M.K., Litchman, E., 2016. Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria. Hydrobiologia 763, 357–369.

Thompson, A.S., Rhodes, J.C., Pettman, I., 1988. Culture collection of algae and protozoa, catalogue of strains, National Environmental Research Council, Swindon. UK.

Tortell, P.D., 2000. Evolutionary and ecological perspectives on carbon acquisition in phytoplankton. Limnol. Oceanog. 45, 744–750.

Visser, P.M., Verspagen, J.M.H., Sandrini, G., Stal, L.J., Matthijs, H.C.P., Davis, T.W., Paerl, H.W., Huisman, J., 2016. How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae 54, 145–159.

Vogt, R.J., St-Gelais, N.F., Bogard, M.J., Beisner, B.E., del Giorgio, P.A., 2017. Surface water CO2 concentration influences phytoplankton production but not community composition across boreal lakes. Ecol. Lett. 20, 1395–1404.

Wiedner, C., Rücker, J., Brüggemann, R., Nixdorf, B., 2007. Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. Oecologia 152, 473–484.

Willis, A., Adams, M.P., Chuang, A.W., Orr, P.T., O'Brien, K.R., Burford, M.A., 2015. Constitutive toxin production under various nitrogen and phosphorus regimes of three ecotypes of *Cylindrospermopsis raciborskii* ((Wołoszyńska) Seenayya et Subba Raju). Harmful Algae 47, 27–34.

Willis, A., Chuang, A.W., Dyhrman, S., Burford, M.A., 2019. Differential expression of phosphorus acquisition genes in response to phosphorus stress in two *Raphidiopsis raciborskii* strains. Harmful Algae 82 19 -25.

Willis, A., Chuang, A., Woodhouse, J.N., Neilan, B.A., Burford, M.A., 2016. Intraspecific variation in growth, morphology and toxin quotas for the cyanobacterium, Cylindrospermopsis raciborskii. Toxicon 119, 307–310.

Willis, A., Posselt, A., Burford, M.A., 2017. Variations in carbon-to-phosphorus ratios of two Australian strains of Cylindrospermopsis raciborskii. Euro. J. Phycol. 52, 303–310.
Willis, A., Woodhouse, J.N., Ongley, S.E., Jex, A.R., Burford, M.A., Neilan, B.A., 2018. Genome variation in nine co-occurring toxic *Cylindrospermopsis raciborskii* strains. Harmful Algae 73, 157–166.
Wood, S.A., Borges, H., Puddick, J., Biessy, L., Atalah, J., Hawes, I., Dietrich, D.R., Hamilton, D.P., 2017. Contrasting cyanobacterial communities and microcystin concentrations in summers with extreme weather events: insights into potential effects of climate change. Hydrobiologia 785, 71–89.
Xiao, M., Willis, A., Burford, M.A., 2017a. Differences in cyanobacterial strain responses to light and temperature reflect species plasticity. Harmful Algae 62, 84–93.
Xiao, M., Adams, M.P., Willis, A., Burford, M.A., O'Brien, K.R., 2017b. Variation within and between cyanobacterial species and strains affects competition: implication for phytoplankton modelling. Harmful Algae 69, 38–47.