Raphidiopsis mediterranea (Nostocales) exhibits a flexible growth strategy under light and nutrient fluctuations in contrast to Planktothrix agardhii (Oscillatoriales)

Anabella Aguilera · Luis Aubriot · Ricardo O. Echenique · Jorge L. Donadelli · Graciela L. Salerno

Received: 28 November 2018 / Revised: 6 June 2019 / Accepted: 19 June 2019 / Published online: 26 June 2019
© Springer Nature Switzerland AG 2019

Abstract  Raphidiopsis mediterranea is a freshwater cyanobacterium that forms toxic blooms in eutrophic water bodies. Factors controlling its proliferation have not been explored in detail. We investigated R. mediterranea autecology by (i) analyzing its dynamics in a hypertrophic shallow lake dominated by Planktothrix agardhii (Oscillatoriales) and its relationship with environmental factors; and (ii) studying the effect of light intensity and phosphate availability on R. mediterranea isolates growing in mono or in co-cultures with P. agardhii. The redundancy analysis demonstrated that water temperature, light, and phosphate concentrations were important driving factors for the seasonal succession of the two species. When grown together with P. agardhii, R. mediterranea growth was strongly promoted under the highest light intensity treatment. On the other hand, in monoalgal cultures under phosphorus starvation, both strains exhibited a significant increase in total alkaline phosphatase activity, and changes in the expression of homologs to phoA-like and phoD genes (members of the Pho regulon). However, R. mediterranea showed higher phosphatase activity than P. agardhii, suggesting greater tolerance to phosphate limitation. Taken together, we conclude that physiological features of R. mediterranea play an important role in the coexistence with P. agardhii under environmental changes.

Keywords Phytoplankton dynamics · Alkaline phosphatase · Cyanobacterial blooms · Eutrophic shallow lake · Pho regulon

Handling editor: David Philip Hamilton

Electronic supplementary material  The online version of this article (https://doi.org/10.1007/s10750-019-04002-5) contains supplementary material, which is available to authorized users.

A. Aguilera · G. L. Salerno (✉)
Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET) and Fundación para Investigaciones Biológicas Aplicadas (FIBA), Veytes 3103, 7600 Mar del Plata, Argentina
E-mail: gsalerno@fiba.org.ar

L. Aubriot
Sección Limnología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

R. O. Echenique
División Ficología, Facultad de Ciencias Naturales y Museo (UNLP), CIC-BA, La Plata, Argentina

J. L. Donadelli
Instituto Limnología Dr. Raúl A. Ringelet, UNLP-CONICET, La Plata, Argentina
Introduction

Cyanobacterial blooms in freshwater ecosystems pose a serious ecological and health problem worldwide (Paerl & Otten, 2013). Cyanobacteria tend to dominate in eutrophic lakes due to their eco-physiological traits such as buoyancy, efficient light harvesting, and strategies to face nutrient limitation (Dokulil & Teubner, 2000; Carey et al., 2012). Light and nutrients, in particular phosphorus, are considered primary factors that govern cyanobacterial proliferation (Scheffer et al., 1997; Schindler et al., 2008). Eco-physiological adaptations to face phosphorus limitation include their ability to store it in polyphosphate granules (Gomez-Garcia et al., 2013) and to uptake phosphate from extracellular organic sources after the action of external alkaline phosphatases (Orchard et al., 2009).

*Raphidiopsis mediterranea* Skuja (Nostocales) is a filamentous species that frequently blooms in eutrophic lakes and reservoirs (Rzymski and Poniedziak, 2014; Wilk-Woźniak et al., 2016). Due to its ability to produce potent cyanotoxins such as cylindrospermopsin and anatoxin-a, it is regarded as a potential health risk (Namikoshi et al., 2003; McGregor et al., 2011). In eutrophic shallow lakes, *R. mediterranea* regularly co-occurs with the microcystin producer *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek (Oscillatoriales) (Kurmayer & Gumpenberger, 2006; Yamamoto & Nakahara, 2009; Aubriot et al., 2011), a resilient and shade-tolerant species, whose dominance in eutrophic freshwater systems is commonly attributed to their ability to maintain net growth under self-shading conditions (Scheffer et al., 1997; Bonilla et al., 2012). In temperate regions, *P. agardhii* commonly alternate in dominance with Nostocales under extreme low underwater irradiance and high nutrient concentrations (Havens et al., 2003; Nixdorf et al., 2003; Toporowska et al., 2016). *Planktothrix agardhii* has been studied together with *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno (basionym *Cylindrospermopsis raciborskii* (Aguilera et al., 2018)) either in field or in laboratory experiments (Kokociński et al., 2010; Ammar et al., 2014). However, factors affecting the dynamics of *R. mediterranea* in shallow lakes have been little explored (Aubriot et al., 2011; Aubriot & Bonilla, 2012).

The aim of this study was to investigate the factors controlling the dominance of *R. mediterranea* and contribute to a better characterization of the autecology of the species. We investigated the dynamics of *R. mediterranea* in a turbid and hypertrophic shallow lake dominated by *P. agardhii* located in the Pampean region of central eastern Argentina. Light is particularly relevant in Pampean shallow lakes (Allende et al., 2009; Izaguirre et al., 2015). Field data were complemented with laboratory assays to test whether these two cyanobacterial species have different responses to changes in light and phosphorus availability.

Materials and methods

Field work: sampling and physicochemical parameters

The field study was conducted in Los Patos shallow lake, located in Ensenada city, Province of Buenos Aires, Argentina (34°50′42″S, 57°57′23″W). The region has a temperate humid climate, with 16°C annual average temperature and 1,000 mm annual mean rainfall (Díaz & Mormeneo, 2002; Martínez et al., 2006). Los Patos is an artificial and hypertrophic freshwater body (area 0.25 km², maximum depth 1 m), with dense phytoplankton accumulations dominated by filamentous cyanobacteria. The water body is used for recreational activities and fishing (Bauzá et al., 2014).

Sampling was performed fortnightly from June 2012 to May 2014 in two stations in the littoral zone. Water samples were taken 30 cm below the water surface using a van Dorn bottle and preserved in Lugol’s iodine solution (1%). Surface water temperature, pH, and conductivity were measured in field using portable pH and conductivity meters (Parsec). Water transparency was estimated with a Secchi disk. Meteorological data (air temperature, rainfall, wind direction, and intensity) were provided by the Departamento de Sismología e Información Meteorológica (SIM), Facultad de Ciencias Astronómicas y Geofísicas, Universidad Nacional de la Plata, Argentina.

Chlorophyll, nutrients and phytoplankton determination

Chlorophyll *a* (Chl) was determined from 150-mL samples after filtering through Whatman GF/C glass
fiber filters and extraction with 90% hot ethanol. Chl concentration was determined according to ISO-10260 (ISO, 1992). Dissolved nutrients [dissolved inorganic nitrogen (DIN) = N-NH₄⁺ + N-NO₂⁻ + N-NO₃⁻; Soluble reactive phosphorus (SRP)] were determined after sample filtration through cellulose acetate membranes (0.45 μm, GE Osmonics) following standard analytical methods (APHA, 1985). Total nitrogen (TN) and total phosphorus (TP) were quantified, as per N-NO₃⁻ and SRP, after alkaline persulfate digestion following Tartari & Mosello (1970) and APHA (4500-P E), respectively.

Phytoplankton counting was performed following the Utermöhl (1958) methodology with an inverted microscope (Carl Zeiss AXIOVERT 40C). At least 100 individuals of the most frequent species or 400 individuals in total were counted in random fields (accuracy of 10%) (Lund et al., 1958). Individual volume (μm³) was calculated by measuring at least 30 individuals of each taxa and following volumetric formulae (Hillebrand et al., 1999). Biovolume was calculated by multiplying abundance per individual volume (mm³ l⁻¹). Species were considered dominant when represented at least 30% of the total biovolume in a particular sample.

Co-cultures and strain isolation

The co-culture of P. agardhii and R. mediterranea was obtained from an environmental sample taken in October 2012 by means of serial dilutions in multiwell plates containing sterile MLA medium (Bolch & Blackburn, 1996). The medium was supplemented with cycloheximide to a final concentration of 50 mg l⁻¹ to inhibit eukaryotic algae.

Isolation of R. mediterranea FCC LP and P. agardhii FCC LP strains were carried out from samples collected on October 2014 from single filaments, and using micropipettes under microscope. The identification of the strains was verified after 16S rRNA and cpcBA-IGS sequencing (flanking regions of the cpcB and cpcA genes of the phycocyanin operon) (Aguilera et al., 2018). Isolates are kept in the Fundación para Investigaciones Biológicas Aplicadas (FIBA) Culture Collection (FCC) in Mar del Plata, Argentina.

Co-culture experiments

Co-cultures (R. mediterranea + P. agardhii) were subjected to two phosphate concentration conditions: (i) low phosphate (Pi), called P5 (MLA containing 5 μM P-PO₄) and (ii) high Pi called P200 (MLA, containing 200 μM P-PO₄). To obtain the low Pi concentration we proceeded as follows: cultures in MLA media were diluted 1:1 (volume:volume) with fresh MLA without phosphorus. When cells reached the stationary phase (approximately 10 days later), cultures were diluted 1:1 (volume:volume) with fresh MLA containing 5 μM Pi till reached the next stationary phase. Then, dilution with fresh MLA containing 5 μM Pi was repeated twice a week for 2 months and kept under this Pi-limiting condition until the beginning of the experiments. The coexistence of P. agardhii and R. mediterranea in co-cultures was tested at two Pi concentrations (P5 and P200) and two light intensities (40 and 80 μmol photon m⁻² s⁻¹), LL and HL, hereafter. Experiments were carried out with 80-ml cultures grown for 18 days, and by triplicate. Mean light intensity perceived by organisms (Iₑₑₑ) was calculated from absorbance at 440 nm (Aguilera et al., 2017). During the course of experiments, the co-cultures were refreshed by addition of one volume of MLA containing the respective Pi concentration (5 or 200 μM P-PO₄) every 3 or 4 days. Culture flasks were gently shaken several times a day and grown at 24 ± 2°C under a light/dark (16 h/8 h) cycle. That temperature mimics the conditions found in Los Patos shallow lake during spring or autumn.

Trichome abundance and biovolume for each species were determined every 2 days using a 1-ml Sedgewick–Rafter chamber (McAlice, 1971). Changes in trichome features in response to light and Pi treatments were analyzed since this information can be useful for understanding the variability of natural populations and the flexible responses to specific environmental variables (Shafik et al., 2003; Miner et al., 2005).

Experiments with mono cultures

To examine the effect of Pi deprivation in R. mediterranea FCC LP and P. agardhii FCC LP
growing separately, exponentially growing cells (optical density, OD750 nm ~ 0.4) were filtered through 0.45-µm cellulose membrane filters (Millipore). Cells were resuspended in MLA or MLA–P (P-PO4 free) media, respectively. Media MLA–P was supplemented with 200 µM KCl to maintain osmotic balance and avoid potassium deficiency. The experiment was carried out at 24 ± 2°C under a 16 h/8 h light/dark cycle (40 µmol m−2 s−1/dark) in an orbital shaker (120 rpm) and in triplicate. Regular microscopic inspection revealed that the biomass of the contaminating bacteria never exceeded 1% of the cyanobacterial biomass in the course of experiments.

Alkaline phosphatase activity was measured daily by using p-nitrophenyl phosphate (pNPP, Amresco) as substrate, following Shen & Song (2007). Enzyme activity was expressed in terms of µmol of pNP released µg−1 chlorophyll a h−1. The flasks were sampled at 24 and 48 h after the start of the experiments for qPCR analysis.

Total RNA was extracted from the cell pellet using the TRIzol Reagent (Invitrogen) according to the manufacturer’s instructions. After digestion with RQ1 RNase-free DNase (Promega, USA), RNA (1 µg) was retrotranscribed using random hexamers (Promega, USA). Then, cDNAs were used in qPCR amplifications to quantify the relative abundance of alkaline phosphatase homologous genes (phoA-like and phoD, members of the Pho regulon) and of homologs to genes involved in polyphosphate synthesis and degradation (ppk and ppa, respectively). Target genes were identified in the genome of Raphidiopsis brookii Hill (strain D9), Planktothrix agardhii NIVA-CYA 126/8 and P. agardhii NIVA-CYA 15 using the integrated microbial genomes portal at the Joint Genome Institute (http://img.jgi.doe.gov/) based on homology. PCR primers were designed using Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast) (Table S1). The qRT-PCR reactions were performed in a Step One real time PCR system (Applied Biosystems) using a Micro Amp Fast Optical 48-well reaction plate with 15 µL reaction volume containing 1 x Power Sybr Green PCR Master Mix (Thermo Fisher), 0.2 µM of each primer, and 1.5 µg of cDNA. Cycling program was: one cycle of 95°C for 10 min, 40 cycles of 95°C for 15 s followed by 40 cycles of 54°C for 1 min. A melting curve analysis was conducted to verify the formation of a single unique product and the absence of potential primer dimerization. To standardize the amount of total RNA in each reaction, 16S rRNA was co-amplified as it considered one of the most suitable internal reference genes for cyanobacteria (Pinto et al., 2012). Samples were run in biological duplicates and technical triplicates. For each primer pair, a standard curve was established by ten-fold dilutions of a PCR template. Linear regression analysis between PCR product concentration and the cycle number (Ct-value) was used to determine primer efficiency and for relative quantification of each gene transcript.

Data analysis

One-way ANOVA was carried out to test whether the environmental and biological parameters were statistically different among sampling stations during the whole sample period (P < 0.05 was considered significant). A preliminary detrended correspondence analysis (DCA) was conducted to know the length of the gradient of species distribution. Because the DCA did not exceed four units of standard deviation, we performed a redundancy analysis (RDA) suitable for species that respond to a linear distribution model (ter Braak & Šmilauer, 2002). The RDA with all the samples (n = 37) was conducted to determine how much variance of the biovolume of dominant species (frequency at least 30% in one sample) is explained by the environmental variables. Species biovolumes were transformed [ln(x + 1)] and environmental data were standardized (ter Braak, 1986). Variables which had a variance inflation factor < 10 were retained in the analysis. The overall significance of the ordination and the significance of the first two axes were tested by a Monte Carlo permutation test (P < 0.05) using unrestricted permutations. The significance of the environmental variables was assessed by forward selection (ter Braak & Šmilauer, 2002). The relationship between the biological variables and the physical–chemical parameters was also analyzed using Spearman’s correlation.

Morphological differences in the strains growing as co-cultures were analyzed with two-way ANOVA, with light intensity and phosphate concentration as factors. The Tukey Test was used for pairwise multiple comparison. Differences in alkaline phosphatase activity and gene expression in the MLA and MLA–P treatments were determined at each time point using a paired t test. Statistics analyses were performed with SigmaPlot version 11 (Systat Software, Inc).
Results

Relationship between R. mediterranea dynamics and environmental factors

During 2012 to 2014, the climate pattern in Los Patos was typical for the region, and characterized by defined seasonality (Fig. 1a). Low transparency and extremely high values of TP, TN, and Chl were registered during the sampling period (Fig. 1b, c; Table 1), with no significant differences between the two stations ($P > 0.05$). TP and SRP showed a strong positive correlation with water temperature ($r = 0.81$ and $r = 0.71$, respectively, $P < 0.01$). Dissolved inorganic nitrogen concentrations were higher from July 2012 to March 2013 (Fig. 1c). Total and dissolved N:P ratios did not vary significantly over the two years ($P > 0.05$). Total N:TP and DIN:SRP ratios were lower than 10 and 15, respectively (Fig. 1d).

Seventy-nine phytoplankton taxa were recorded during the sampling period, and grouped in seven taxonomical classes (Chlorophyceae, 38; Bacillariophyceae, 12; Cyanobacteria, 10). Cyanobacteria dominated during the whole study, and 97% of the total biomass corresponded to filamentous species (period mean 285.0 ± 130.5 mm$^3$ l$^{-1}$) (Fig. 2). Nostocales species [R. mediterranea, Aphanizomenon gracile Lemmermann and Sphaerospermopsis aphani-zomenoides (Forti)] Zapomelova´, Jezberova´, Hrouzek, Hisem, Reháková & Komárková] dominated the phytoplankton together with P. agardhii. The contribution of P. agardhii to the total phytoplankton biovolumen reached up to 99%. Dominant species exhibited seasonal patterns. The most intensive growth of Nostocales was observed before or after the maximum development of P. agardhii (Fig. 3), mainly in summer and early autumn when water mean temperatures were higher (22.0–31.8°C) than those supporting the development of P. agardhii (7.1–19.2°C). Nostocales species, however, did not completely disappear in winter (Figs. 2, 3).

Water transparency values were associated with cyanobacterial blooms. This parameter showed lower values during P. agardhii blooms than during mass development of Nostocales (Fig. 3). R. mediterranea biovolume increased in parallel to a greater light intensity and higher water temperature (summer and early autumn) (Figs. 1a, 3). RDA analysis was used to study the relationship between the abundance of dominant species (Nostocales and P. agardhii) and environmental variables (Fig. 4). Significant variables according to Monte Carlo permutation test ($P < 0.05$) were temperature, Secchi depth, SRP, and N-NO$_3^-$. The analysis showed that the higher water temperatures and SRP were more important for the proliferation of Nostocales than for P. agardhii. Spearman’s correlation analyses confirmed the importance of water temperature, TP, and SRP for the development of Nostocales species (Table S2).

Effect of light and phosphate concentration on P. agardhii and R. mediterranea co-cultures

The coexistence of P. agardhii and R. mediterranea was evaluated in the laboratory after exposing cocultures to two light intensities and two phosphate conditions. Species growth responded differentially to treatments. At the onset of the experiment, P. agardhii biovolume in the co-culture was three-fold higher than that of R. mediterranea (Fig. 5). Planktothrix agardhii grew under high phosphate concentration and light intensity (P200/L$_L$), whereas R. mediterranea notably decreased its biovolume (Fig. 5a). A similar pattern was observed under low phosphate and low light intensity (P5/L$_L$), albeit both species showed lower biovolumes compared to P200/L$_L$ (Fig. 5a, b). High-light treatments significantly promoted the proliferation of R. mediterranea, regardless of the phosphate concentration (Fig. 5c, d). Light intensity changes ($I_{eq}$) inside the cultures are also shown in Fig. 5.

Light intensity and Pi availability treatments led to changes in trichome features. While the average length and volume of P. agardhii trichomes significantly increased under P200/L$_L$ (Tukey’s test, $P < 0.05$), trichome shortness was observed under H$_L$ and trichome fragmentation was noticed under P5/H$_L$ (Table 2). Notably, R. mediterranea trichomes were significantly longer and bigger when cultivated under H$_L$ compared to L$_L$, and were found to be the shorter and smaller under P5/L$_L$ (Tukey’s test, $P < 0.05$) (Table 2).

Responses to Pi deprivation and changes in gene expression in R. mediterranea and P. agardhii

The ability of R. mediterranea FCC LP and P. agardhii FCC LP to cope with Pi deprivation was analyzed by resuspending monoalgal cultures in MLA.
P-free medium. Growth curves of _R. mediterranea_ FCC LP were similar in MLA (200 μM total phosphorous) and MLA_\(\text{P}\) over 12 days (\(P < 0.05\)) (Fig. 6a), whereas cultures of _P. agardhii_ FCC LP grown without Pi displayed slightly slower growth rates compared to MLA (Fig. 6b). In addition, total phosphatase activity was determined along the growth curves. Higher enzyme activities were measured in cultures of both strains growing in MLA_\(\text{P}\) compared to MLA. However, the time course of the enzyme activity differed between species (Fig. 6a, b). Ten-fold higher activity was measured in _R. mediterranea_ FCC LP at day 3 under Pi deprivation compared to MLA, and the activity reached maximal levels.
Table 1  Limnological features of the Los Patos shallow lake, corresponding to the period analyzed (2012 and 2014)

| Variable               | Mean  | SD    | Minimum | Maximum | N  |
|------------------------|-------|-------|---------|---------|----|
| Temperature (°C)       | 19.58 | 6.08  | 7.30    | 33.50   | 74 |
| Conductivity (μScm⁻¹)  | 469.73| 119.10| 322.00  | 949.00  | 74 |
| pH                     | 8.59  | 1.11  | 6.45    | 10.85   | 74 |
| Secchi depth (m)       | 0.11  | 0.03  | 0.07    | 0.17    | 31 |
| Total P (mg l⁻¹)       | 0.71  | 0.19  | 0.45    | 1.29    | 72 |
| Total N (mg l⁻¹)       | 1.66  | 0.38  | 0.72    | 2.79    | 72 |
| P-PO₄ (mg l⁻¹)         | 0.21  | 0.14  | < 0.01  | 0.60    | 74 |
| N-NO₃ (mg l⁻¹)         | 0.18  | 0.14  | 0.05    | 0.64    | 73 |
| N-NO₂ (mg l⁻¹)         | 0.02  | 0.01  | < 0.01  | 0.03    | 74 |
| N-NH₄ (mg l⁻¹)         | 0.15  | 0.17  | < 0.01  | 1.33    | 73 |
| Chlorophyll a (µg l⁻¹)| 398.5 | 149.3 | 115.4   | 902.8   | 73 |

Fig. 2  Relative contribution of main phytoplankton groups and total biovolume in Los Patos lake over the studied period.

Fig. 3  Seasonal variation of biovolume of dominant species and Secchi depth in Los Patos lake over the studied period. Aphanizomenon gracile and Sphaerospermopsis aphanizomenoides were grouped together as “Aphanizomenon-Sphaerospermopsis (Apha-Spha) complex” for the quantitative analyses since the diagnostic features (position and form of the akinetes) needed to distinguish between them were not always present.
(99.7 ± 11.3 μmol μg⁻¹ chlorophyll a h⁻¹) at day 4 (Fig. 6a). On the contrary, alkaline phosphatase activity in P. agardhii FCC LP was five-fold higher at day 10 compared to the control, while the activity reached a maximum at day 11 (Fig. 6b).

The expression of genes involved in phosphorous metabolism was up-regulated after cell transfer to MLA. Transcripts from phoA-like and phoD (members of the Pho regulon, encoding alkaline phosphatases) were approximately 20 and 50% higher after 24 h of Pi starvation, respectively (Fig. 7a, b). In regards to polyphosphate metabolism genes, the highest transcript levels were observed 48 h after transferring cells to MLA, with a 1.5-fold change for pppk transcripts (polyphosphate kinase) in R. mediterranea FCC LP (Fig. 7c, d). Transcripts for the ppa (pyrophosphatase) in R. mediterranea FCC LP and P. agardhii FCC LP increased within 24 h of Pi starvation (Fig. 7e, f).

**Discussion**

This study shows that physiological features of R. mediterranea may play an influential decisive role in coexistence with P. agardhii under environmental changes. This is particularly important because P. agardhii is considered to be successful in creating an environment in which tends to outcompete other cyanobacteria (Kurmay et al., 2016).

The long-standing dominance of cyanobacteria in the turbid and hypereutrophic Los Patos lake was characterized by perennial blooms of P. agardhii (Oscillatoriales) that alternate with blooms of R. mediterranea and other Nostocales species. The increase in P. agardhii biomass from late summer led to a decrease in light availability that, together with the fall in water temperature, could have avoided further development of R. mediterranea in autumn (Figs. 2, 3). Similar cycles of Oscillatoriales and Nostocales were observed in temperate lakes from Europe (Kokociński et al., 2010; Toporowska et al., 2016). Interestingly, the occurrence of R. mediterranea was also registered in autumn and winter albeit in low proportions, indicating that the species was not completely outcompeted by P. agardhii under conditions of low transparency and low temperature.

Analyses integrating physiology and ecology of cyanobacterial species are needed to better predict their dynamics. In this work, complementing field studies and laboratory work highlighted the importance of changes in light, temperature and phosphorus availability in the dynamics of R. mediterranea and P. agardhii. We only used one strain of each species, so the conclusions of the co-culture experiments cannot necessarily be generalized for both species.

When co-cultures were exposed to L₄ (40 μmol photon m⁻² s⁻¹), independently of Pi concentration, P. agardhii growth avoided R. mediterranea proliferation, probably by creating light-limiting conditions (Fig. 5). Similar results were reported in co-cultures of P. agardhii and R. raciborskii, where P. agardhii grew faster and outcompeted the latter under 20 μmol photon m⁻² s⁻¹ (Ammar et al., 2014). Notably, in our experiments, R. mediterranea managed to persist in L₄ treatments (Fig. 5a, b). On the other hand, R. mediterranea grew under both H₄ treatments regardless the initial higher density of P. agardhii (Fig. 6c, d). Taken together, these results reveal that R. mediterranea has certain tolerance to low and medium light intensities. Although little is known about optimal light requirements for R. mediterranea, the phylogenetically related R. raciborskii has been shown to have a high level of flexibility with respect to light and tolerance to a wide range of light.
Fig. 5 Biovolume of *Planktothrix agardhii* and *Raphidiopsis mediterranea* growing under 4 nutritional treatments. 

- **a** 40 μmol photon m$^{-2}$ s$^{-1}$ and 200 μM P-PO$_4$; 
- **b** 40 μmol photon m$^{-2}$ s$^{-1}$ and 5 μM P-PO$_4$; 
- **c** 80 μmol photon m$^{-2}$ s$^{-1}$ and 200 μM P-PO$_4$; 
- **d** 80 μmol photon m$^{-2}$ s$^{-1}$ and 5 μM P-PO$_4$

**Table 2** Morphological features of *Raphidiopsis mediterranea* and *Planktothrix agardhii* growing together under two light intensities (40 and 80 μmol photon m$^{-2}$ s$^{-1}$, low light and high light, respectively) and two phosphate concentrations applied (5 and 200 μM P-PO$_4$, P5 and P200, respectively)

|               | *R. mediterranea* | *P. agardhii* |
|---------------|-------------------|---------------|
|               | Length (μm)       | Volume (μm$^3$) | Length (μm)       | Volume (μm$^3$) |
| **Initial condition (T = 0)** | 79.6 ± 31.0        | 421 ± 202      | 259.3 ± 97.2      | 3,140 ± 1,296  |
| **Low light (T = 15)** |                   |               |                   |               |
| P5            | 78.2 ± 40.1$^a$   | 287 ± 231$^a$  | 225.5 ± 51.7$^a$  | 1,876 ± 555$^a$|
| P200          | 88.9 ± 27.9$^b$   | 559 ± 360$^b$  | 426.9 ± 149.7$^b$ | 4,475 ± 2,070$^b$|
| **High light (T = 15)** |                   |               |                   |               |
| P5            | 151.1 ± 48.2$^c$  | 926 ± 522$^c$  | 169.8 ± 80.3$^a$  | 1,702 ± 1,100$^a$|
| P200          | 137.2 ± 54.1$^d$  | 937 ± 583$^c$  | 112.8 ± 44.2$^c$  | 1,515 ± 699$^c$|

$^a$-$^d$: Designates significantly differences between treatments for the same species (Tuckey’s post hoc test, $P < 0.05$)
Intensities (Pierangelini et al., 2015; Burford et al., 2016). In addition, in field studies, *R. mediterranea* has also been found as the dominant species in turbid, light deficient environments and also in deep water layers (Moustaka-Gouni et al., 2010; Padisák et al., 2009).

*Raphidiopsis mediterranea* and *P. agardhii* growing in co-cultures differed in their morphological responses to different light and Pi environmental conditions. Trichomes lengthened and presented highest volumes under P200/LL, the most favorable growth condition for *P. agardhii*. The shorter trichomes observed under *H* L could be due to high-light intensity stress. Furthermore, physiological stress was enhanced under Pi limitation, which led to trichome fragmentation (Table 2). Accordingly, trichome shortening has been observed in *Planktothrix* cultures when increasing light intensity and decreasing phosphorus concentration (Hasler et al., 2003). On the other hand, *R. mediterranea* showed larger and bigger filaments under *H* L and low Pi availability (P5 treatments), conditions that did not affect trichome development. These results suggest a phenotypic plasticity, which has not previously been reported for this strain (Table 2). The greater variation in the morphology of *R. mediterranea* could imply a higher tolerance to environmental conditions, as suggested for *R. raciborskii* (Xiao et al., 2017).

Additionally, laboratory experiments showed that *R. mediterranea* exhibited flexible features to overcome short periods of phosphate depletion. Even though both strains increased alkaline phosphatase activity, the response of *R. mediterranea* was faster and higher. This could imply an advantage over other organisms under environmental Pi starvation. Accordingly, *R. mediterranea* was shown to coexist with *P. agardhii* due to the rapid regulation of Pi uptake systems under fluctuating Pi availability (Aubriot et al., 2011; Aubriot & Bonilla, 2012).

The expression of genes related to the hydrolysis of phosphomonoesters (phoA-like and phoD) was regulated by external Pi availability in *R. mediterranea* FCC LP and *P. agardhii* FCC LP (Fig. 7), as previously reported in other strains (Orchard et al., 2009; Harke et al., 2012; Bai et al., 2014). On the other hand, the expression of *ppk* and *ppa* genes (polyphosphate synthesis and utilization, respectively) under Pi deprivation suggests that those strains can acclimate to environmental Pi fluctuations by modulating the polyphosphate granule metabolism. To our best knowledge this is the first report on the capacity of *R. mediterranea* to cope with Pi deprivation via phosphatase alkaline activity and rapid expression of *pho* genes. Recent reports showed that *R. mediterranea* is able to coexist with *P. agardhii* over long periods of strong Pi-deficiency, even at Pi concentrations in the nanomolar range (Aubriot & Bonilla, 2018).

Many lake management plans seek to reduce total phosphorus concentrations to mitigate algal blooms (Schindler et al., 2008). Furthermore, the collapse of perennial *P. agardhii* blooms was reported after a decrease in Pi concentrations as a consequence of water management practices performed in a French lake (Catherine et al., 2008). Our results indicate that *P. agardhii* and *R.
**R. mediterranea** respond differently to light and Pi availability. Furthermore, the persistence and dominance of **R. mediterranea** may occur after a change in the environmental conditions, for example, better illuminated water column and low Pi. Our findings indicate that management plans to control cyanobacterial blooms should take into account the dominant species and the changes in their dominances according to their eco-physiological traits.

**Acknowledgements** This work was supported by CONICET, Universidad Nacional de Mar del Plata (EXA793/17) and FIBA, Argentina. We thank María José Cherrez, Mónica Tatiana López, and Hilda Palacio for assistance in field work.

**References**

Aguilera, A., L. Aubriot, R. O. Echenique, G. L. Salerno, B. M. Brena, M. Pirez & S. Bonilla, 2017. Synergistic effects of nutrients and light favor Nostocales over non heterocystous cyanobacteria. Hydrobiologia 794: 241–255.

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

Allende, L., G. Tell, H. Zagarese, A. Torremorell, G. Pérez, J. Bustingorry, R. Escaray & I. Izaguirre, 2009. Phytoplankton and primary production in clear-vegetated, inorganic-turbid, and algal-turbid shallow lakes from the pampa plain (Argentina). Hydrobiologia 624: 45–60.

Ammar, M., K. Comte, T. D. C. Tran & M. E. Bour, 2014. Initial growth phases of two bloom-forming cyanobacteria (**Cylindrospermopsis raciborskii** and **Planktothrix agardhii**) in monocultures and mixed cultures depending on light and nutrient conditions. Annales de Limnologie – International Journal of Limnology 50: 231–240.

Association, American Public Health, 1985. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.

Aubriot, L. & S. Bonilla, 2012. Rapid regulation of phosphate uptake in freshwater cyanobacterial blooms. Aquatic Microbial Ecology 67: 251–263.

Aubriot, L. & S. Bonilla, 2018. Regulation of phosphate uptake reveals cyanobacterial bloom resilience to shifting N: P ratios. Freshwater Biology 63: 318–329.

Aubriot, L., S. Bonilla & G. Falkner, 2011. Adaptive phosphate uptake behaviour of phytoplankton to environmental phosphate fluctuations. FEMS Microbiology Ecology 77: 1–16.

Bai, F., R. Liu, Y. Yang, X. Ran, J. Shi & Z. Wu, 2014. Dissolved organic phosphorus use by the invasive freshwater diazotroph cyanobacterium, **Cylindrospermopsis raciborskii**. Harmful Algae 39: 112–120.
Bauzá, L., A. Aguilera, R. O. Echenique, D. Andrinolo & L. Giannuzzi, 2014. Application of hydrogen peroxide to the control of eutrophic lake systems in laboratory assays. Toxins 6: 2657–2675.

Bolch, C. J. S. & S. I. Blackburn, 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium Microcystis aeruginosa Kütz. Journal of Applied Phycology 8: 5–13.

Bonilla, S., L. Aubriot, M. C. S. Soares, M. Gonzalez-Piana, A. Fabre, V. L. M. Huszar, M. Lurling, D. Antoniades, J. Padisak & C. Kruk, 2012. What drives the distribution of the bloom-forming cyanobacteria Planktonothrix agardhii and Cylindrospermopsis raciborskii? FEMS Microbiology Ecology 79: 594–607.

Burford, M. A., J. Beardall, A. Willis, P. T. Orr, V. F. Magalhaes, L. M. Rangel, S. M. F. O. E. Azevedo & B. A. Neilan, 2016. Understanding the winning strategies used by the bloom-forming cyanobacterium Cylindrospermopsis raciborskii. Harmful Algae 54: 44–53.

Carey, C. C., B. W. Ibelings, E. P. Hoffmann, D. P. Hamilton & J. D. Brooks, 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Research 46: 1394–1407.

Catherine, A., C. Qublier, C. Yéprémian, P. Got, A. Groelle, B. Vinçon-Leite, C. Bernard & M. Troussellier, 2008. Collapse of a Plankthothrix agardhii perennial bloom and microcystin dynamics in response to reduced phosphate concentrations in a temperate lake. FEMS Microbiology Ecology 65: 61–73.

Díaz, R. A. & I. Mormeneo, 2002. Zonificación del clima de la región pampeana mediante análisis de conglomerados por consenso. Revista Argentina Agrometeorología 2: 125–131.

Dokulil, M. T. & K. Teubner, 2000. Cyanobacterial dominance in lakes. Hydrobiologia 438: 1–12.

Gomez-Garcia, M. R., F. Fazeli, A. Grote, A. R. Grossman & D. Bhaya, 2013. Role of polyphosphate in thermophilic Synechococcus sp. from microbial mats. Journal of Bacteriology 195: 3309–3319.

Harke, M. J., D. L. Berry, J. W. Ammerman & C. J. Gobler, 2012. Molecular response of the bloom-forming cyanobacterium, Microcystis aeruginosa, to phosphorus limitation. Microbial Ecology 63: 188–198.

Hašler, P., A. Pouličková & S. Vařková, 2003. Comparative studies on two strains of the genus Plankthothrix (Cyanophyta, Cyanoprokaryota). Algological Studies 108: 31–43.

Havens, K. E., R. T. James, T. L. East & V. H. Smith, 2003. N: P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. Environmental Pollution 122: 379–390.

Hillebrand, H., C. D. Dürselen, U. Pollinger Kirschkel & T. Zohary, 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403–424.

ISO, 1992. Water quality measurement of biochemical parameters spectrophotometric determination of chlorophyll-a concentration. International Organization for Standardization, Geneva: 1–6.

Izaguirre, I., M. L. Sánchez, M. R. Schiaffino, I. O’Farrell, P. Huber, N. Ferrer, J. Zunino, L. Lagomarsino & M. Mancini, 2015. Which environmental factors trigger the dominance of phytoplankton species across a moisture gradient of shallow lakes? Hydrobiologia 752: 47–64.

Kokociński, M., K. Stefaniak, J. Mankiewicz-Boczek, K. Izydorczyk & J. Soininen, 2010. The ecology of the invasive cyanobacterium Cylindrospermopsis raciborskii (Nostocales, Cyanophyta) in two hypereutrophic lakes dominated by Planktothrix agardhii (Oscillatoriales, Cyanophyta). European Journal of Phycology 45: 365–374.

Kurmayer, R. & M. Gumpenberger, 2006. Diversity of microcystin genotypes among populations of the filamentous cyanobacteria Planktothrix rubescens and Planktothrix agardhii. Molecular Ecology 15: 3849–3861.

Kurmayer, R., L. Deng & E. Entfellner, 2016. Role of toxic and bioactive secondary metabolites in colonization and bloom formation by filamentous cyanobacteria Planktothrix. Harmful Algae 54: 69–86.

Lund, J. W. G., C. Kipling & E. D. Le Cren, 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 143–170.

Martínez, O. R., M. A. Hurtado & J. E. Giménez, 2006. Caracterización ambiental de los humedales costeros del Río de la Plata. Provincia de Buenos Aires, Argentina. Revista UnG – Geociencias 5: 55–64.

McAlice, B., 1971. Phytoplankton sampling with the Sedgwick-Rafter cell. Limnology and Oceanography 16: 16–28.

McGregor, G. B., B. C. Sendall, L. T. Hunt & G. K. Eaglesham, 2011. Report of the cyanotoxins cylindrospermopsin and deoxy-cylindrospermopsin from Raphidiopsis mediterranea Skuja (Cyanobacteria/Nostocales). Harmful Algae 10: 402–410.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla & R. A. Relyea, 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution 20: 685–692.

Moustaka-Gouni, M., K. A. Kormas, P. Polykarpou, S. Gkelis, D. C. Bobori & E. Vardaka, 2010. Polyphasic evaluation of Aphanizomenon issatschenkoi and Raphidiopsis mediterranea Skuja in a Mediterranean lake. Journal of Plankton Research 32: 927–936.

Namikoshi, M., T. Murakami, M. F. Watanabe, T. Oda, J. Yamada, T. Tsujimura, H. Nagai & S. Oishi, 2003. Simultaneous production of homoanatoxin-a, anatoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium Raphidiopsis mediterranea Skuja. Toxicon 42: 533–538.

Nixdorf, B., U. Mischke & J. Rücker, 2003. Phytoplankton assemblages and steady state in deep and shallow eutrophic lakes – an approach to differentiate the habitat properties of Oscillatoriales. Hydrobiologia 502: 111–121.

Orchard, E. D., E. A. Webb & S. T. Dyhrman, 2009. Molecular analysis of the phosphorus starvation response in Trichodesmium spp. Environmental Microbiology 11: 2400–2411.

Padisák, J., L. O. Crossetti & L. Naselli-Flores, 2009. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. Hydrobiologia 621: 1–19.

Paerl, H. W. & T. G. Otten, 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microbial Ecology 65: 995–1010.
Pierangelini, M., S. Stojkovic, P. T. Orr & J. Beardall, 2015. Photo-acclimation to low light – changes from growth to antenna size in the cyanobacterium Cylindrospermopsis raciborskii. Harmful Algae 46: 11–17.

Pinto, F., C. C. Pacheco, D. Ferreira, P. Moradas-Ferreira & P. Tamagnini, 2012. Selection of suitable reference genes for RT-qPCR analyses in cyanobacteria. PLOS ONE 7: e34983.

Rzymski, P. & B. Poniedziałek, 2014. In search of environmental role of cylindrospermopsins: a review on global distribution and ecology of its producers. Water Research 66: 320–337.

Scheffer, M., S. Rinaldi, A. Gragnani, L. M. Mur & H. van Nes, 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78: 272–282.

Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng & S. E. M. Kasian, 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences 105: 11254–11258.

Shafik, H. M., L. Vörös, P. Spröber, M. Présing & A. W. Kovács, 2003. Some special morphological features of Cylindrospermopsis raciborskii in batch and continuous cultures. Hydrobiologia 506: 163–167.

Shen, H. & L. Song, 2007. Comparative studies on physiological responses to phosphorus in two phenotypes of bloom-forming Microcystis. Hydrobiologia 592: 475–486.

Tartari, G. À., & R. Mosello, 1970. Metodologie analitiche e controlli di qualità nel laboratorio chimico dell’istituto Italiano di Idrobiologia. Doc. Dell’istituto Ital. Hidrobiol. No. 60 28048 Verbania Pallanza: 57–60.

ter Braak, C. J. F., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67: 1167–1179.

ter Braak, C. J. F. & P. Šmilauer, 2002. CANOCO reference manual and CanoDraw for Windows user’s guide: software for canonical community ordination (version 4.5). Biometris, Wageningen.

Toporowska, M., B. Pawlik-Skowronska & R. Kalinowska, 2016. Mass development of diazotrophic cyanobacteria (Nostocales) and production of neurotoxic anatoxin-a in a Planktothrix (Oscillatoriales) dominated temperate lake. Water Air and Soil Pollution 227: 321.

Utermöhl, M., 1958. Zur Vervollkommung der quantitativen phytoplankton methodik. Mitteilungen Internationale Vereinigung Limnologie 9: 1–38.

Wilk-Woźniak, E., W. Solarz, K. Nabajek & A. Pociecha, 2016. Alien cyanobacteria: an unsolved part of the “expansion and evolution” jigsaw puzzle? Hydrobiologia 764: 65–79.

Xiao, M., A. Willis & M. A. Burford, 2017. Differences in cyanobacterial strain responses to light and temperature reflect species plasticity. Harmful Algae 62: 84–93.

Yamamoto, Y. & H. Nakahara, 2009. Seasonal variations in the morphology of bloom-forming cyanobacteria in a eutrophic pond. Limnology 10: 185–193.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.