Physiological responses and specific fatty acids composition of *Microcystis aeruginosa* exposed to total solar radiation and increased temperature

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Abstract

The combined effects of increased temperature and solar ultraviolet radiation (UVR, 280–400 nm) on *M. aeruginosa* cultures was analyzed in terms of cell abundance, reactive oxygen and nitrogen species (ROS/RNS), antioxidant activity of catalase (CAT), superoxide dismutase (SOD), glutathione *S* transferase (GST), fatty acids (FA) content and lipid damage. After 12 days exposure to high temperature (29 °C), cells were exposed to solar UVR (4 h). Ultraviolet-B radiation (UVBR, 280–315 nm) resulted into low cell abundance, high ROS/RNS and a significant increase in SOD activity with no changes in GST and a decreased CAT activity at control temperature (26 °C). A significant increase in the analyzed enzymatic antioxidants was observed at 29 °C, as a response to avoid ultraviolet-A radiation (UVAR, 315–400 nm) damage. The relative abundance of ω6 FAs was not affected by UVAR while ω3 FA were highly sensitive at 29 °C but unsaturated fatty acids (UFA) peroxidation did not occur. The differential response in FA to high temperature and UVAR results in differences in lipid damage and antioxidants. It was evident that selected UFAs (mostly ω6) play an important role in high temperature adaptation in addition to enzymatic antioxidant increased activity shifting the temperature growth from 26 to 29 °C. Thus, cell death and UFA damage were avoided at high temperature and low solar irradiance thanks to an increased enzymatic antioxidant activity.

Graphic abstract

Keywords Fatty acids · Oxidative stress · *Microcystis aeruginosa* · Temperature · UVR

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1 Introduction

Due to greenhouse gas emissions, an increase of about 0.6 °C in the annual average surface planet temperature was registered in the last 30 years [1]. In addition to the increased temperature, another atmospheric problem is the decrease of stratospheric ozone as a consequence of the chlorinated fluorocarbons (CFCs) [2]. According to Bais et al. [3], this ozone problem will persist for the next decades and will probably spread in different areas of high latitudes [4]. However, according to latest observations, Bais et al. [5] suggested that the combined effects of changes in ozone, aerosols, greenhouse gases, clouds, and reflectivity will determine future levels of solar UVB radiation (UVBR) at the Earth's Surface with ozone values stabilized at the last years and with projections of a soon recovery.

As a consequence of the reduced ozone layer, the amount of solar UVBR that reaches the Earth's surface increased [6]. This decrease in the ozone layer and consequent significant increase in UVBR has been registered mainly in Polar Regions and at mid-latitudes [7].

Future changes in ozone concentration will depend on the changes in solar cycle, temperature dynamics and climate [5, 8]. However, given the stabilizing and recent evidence for recovery of stratospheric ozone, climate change and other environmental variables are now the most important factors changing exposure to UVR in aquatic ecosystems. Both UVBR and UVA cause deleterious effects on the physiological parameters of phytoplankton and other aquatic organisms [9]. Global warming enhances stratification of water column in lakes depends on their morphometry and decreases with larger wind speeds [10]. The organism exposure to solar UVR may be regulated by changing the position in the water column [11]. According to certain variables dependent on climate change, the depth of the mix layer can be variable, modifying the phytoplankton exposure to solar radiation [12]. Furthermore, the presence of gas vesicles in some cyanobacteria species allow them to regulate their position in the water column [13]. Therefore, organisms from shallower depth could be exposed to increased solar UVR doses due to less light attenuation [14]. The organic matter present in the water column can attenuate the solar irradiance reducing the exposure dose of those organisms that are found deeper. The inorganic and organic particulate matter in water depend in part of human activities [15]. Thus, increased discharge of fertilizers in agriculture may contribute to increase the nutrient concentrations in lakes and rivers [16]. Consequently, high nutrients concentrations and high temperature could lead to harmful algal blooms (HABs), including those produced by cyanobacteria [17]. For example, *Microcystis* is a genus forming blooms and often causes serious problems in the management of water

quality due to the production of toxins [18]. Cloud cover is another factor, which may affect UVR doses in surface waters and the phytoplankton occurring in it [5].

In addition to changes in surface mixed layer depth, temperature is another factor that controls the growth and productivity of phytoplankton by modifying the photosynthetic process [19]. Consequently, both atmospheric phenomena (increased temperature and UVR) affect in an indirect and direct way the structure and function of aquatic ecosystems and the physiology of the organisms that live there [20].

To monitor the response of aquatic organisms to environmental changes, fatty acids (FA) have been used as biomarkers [21]. The membrane fluidity is regulated by the FA composition of the membrane phospholipids [22] and as such will change in case of temperature change. Thus, the lipid composition of the membrane could be changed with an increase or decrease of the degree of FA unsaturation (referred to as homeoviscous adaptation, [23]). In cyanobacteria, the highest percentage of lipids is in membranes [24], with a high proportion of unsaturated fatty acids (UFAs) particularly polyunsaturated fatty acids (PUFA). To avoid damage under stress conditions, cyanobacteria modify the type and quantity of their cellular lipids [25]. The high amount of double bonds makes PUFAs highly sensitive to any attack by reactive species [26]. Thus, the lipid peroxides produced by reactive oxygen/nitrogen species (ROS/RNS) results in the formation of Thiobarbituric Acid Reactive Substances (TBARS) [27]. In addition to their use as biomarkers, PUFA are very important to promote human health and because of that, there is an interest in evaluating the environmental factors that favour its production in organisms, especially at the lower levels of the food web [28, 29].

Both microalgae and cyanobacteria need sunlight to carry out the photosynthesis process, increasing the probability of producing high cellular concentrations of ROS due to photosynthetic electron transport chain [30]. The stimulation of the metabolism due to an increased temperature [31] implies an increase in oxygen consumption, which could lead to an increase of ROS. The ROS produced could be singlet oxygen ($^1\text{O}_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\text{OH}^\cdot$) [32]. The presence of ROS in cyanobacteria cells produces inhibition of growth [33], degradation of Chlorophyll-a (Chl-a) and damage of the photosystem II (PSII) [34]. The oxidation of the membrane-permeable non-fluorescent 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) has been used to detect the presence of several ROS and reactive nitrogen species (RNS) in biological media [35], with an increase in DCFHDA oxidation used as an indicator of oxidative stress. DCFH-DA was initially thought to be useful as a specific indicator for hydrogen peroxide, even though the reaction is not direct. However, it was already demonstrated that DCFH

is oxidized by other ROS, including superoxide anion radical, hydroxyl radical, peroxy, alkoxy, hydroperoxy and peroxynitrite that are products of normal metabolism [36]. The use of the oxidation of 2',7'-dichlorofluorescein diacetate (DCFH-DA) was useful to demonstrate the presence of reactive species in different cyanobacteria exposed to UVR or increased temperature, including *Microcystis aeruginosa* [37–39]. In the interpretation of DCFH-DA results, it is important to keep in mind that incubations are performed in the dark so any oxidation that occurs is due to endogenously generated ROS, not those directly induced by UVR. Oxidative stress occurs when the concentration of reactive species exceeds the available antioxidant activity [40].

In cyanobacteria, proteins [41] and DNA [42] are the primary targets for UVR exposure and reactive species generation. Further, photosynthesis inhibition [43, 44] and morphological changes [43] will be generated. In comparison with UVBR, UVAR produces the highest damage in photosynthetic organisms due to highest irradiance level reaching earth and it does not depend on the thickness of the ozone layer [45, 46]. It is well known that UVBR is more effective per energy unit [47] and also acts directly on DNA and proteins, meanwhile UVAR is thought to cause long-term photosensitized oxidation [48]. In addition, Hernando et al. [44] showed that different responses were activated in *M. aeruginosa* depending on whether the exposure is to UVAR or UVBR as well as the dose level. However, cyanobacteria have developed a great number of strategies to avoid or mitigate the reactive species damage using antioxidants as scavenger. These might be non-enzymatic such as ascorbic acid, glutathione and tocopherols as well as enzymatic such as superoxide dismutase (SOD), peroxidases, catalases (CAT) [49] and glutathione *S* transferase (GST) [50]. Most of cyanobacteria studies that evaluate the effects of variables associated to climate change such as UVR and increased temperature, have not evaluate the combined effects of both variables. Changes in several external variables may not result in simple additive responses relative to those that occurred with only one variable [51]. Different changes in these variables can produce synergistic or antagonistic effects [52]. So far, few studies evaluated the interactive effects of these variables, which may act in synergistic or antagonistic way [53]. Giordanino et al. [54] found that an increased temperature prevents the trichome/chains reduction in *Anabaena* sp., *Nostoc* sp. and *Arthorspira platensis*. In addition, it also increases the photosynthetic recovery of *Anabaena* sp. and *Nostoc* sp. after UVR exposure. On the other hand, Gao et al. [55] found that increased temperature favours *A. platensis* cells that were previously damaged by UVR exposure.

Due to the potential toxicity of cyanobacteria and their ecological importance [56, 57], the objective of the present

study was to evaluate the combined effects of increased temperature and solar UVR on *M. aeruginosa* cultures. Their response in a lab experiment was evaluated for FA composition and lipid damage, reactive species concentration, enzymatic antioxidant activity (CAT, SOD and GST) and cell abundance. Our results will contribute to a better understanding of possible climate change effects on cyanobacteria and the potential changes in the toxicity of aquatic ecosystems used a source of drinking water in medium-latitude cities.

2 Materials and methods

2.1 Experimental set-up

The *Microcystis aeruginosa* strain CAAT 2005-3, was isolated from a river in the province of Buenos Aires, Argentina [58]. The *M. aeruginosa* culture was pre-adapted for 14 days at 26 °C under artificial light at a photon flux density of 30 $\mu\text{E m}^{-2} \text{s}^{-1}$ (6.6 W m^{-2}) under 14:10 h light:dark photocycle in an incubator shaker (Model Satandard Infors Multitron). This irradiance was achieved with the addition of 2 Philips TL-D 18 W tubes secured by means of a wood to the upper part of the incubator. This irradiance was monitored daily with an ILT 950 spectroradiometer, International Light Technologies, Inc., USA. The cultures grew with shaker conditions and with the addition of BG-11 culture medium [59].

Once in the exponential growth phase (i.e., after 2 weeks), cultures were kept at the control experimental temperature (26 °C, “C”) or exposed, in independent triplicates, to 29 °C (“HT”) in two independent growth chambers.

After 12 days of growth at both temperatures and still in exponential phase, cells were exposed simultaneously to three solar irradiance treatments with initial addition of 10% BG-11 medium in 80 mL quartz tubes (path length: 25 mm; length: 137 mm). Prior to experimental incubations, cultures were pre-adapted to solar radiation in containers covered with UV cut-off filters followed by exposure to only PAR irradiance in an outdoor water bath with running water for control (26 °C $\pm$ 1) and high temperature (29 °C $\pm$ 1) for 1 day before to the irradiance experiments day. After this period, cells were exposed simultaneously to the following treatments:

- (1) Cultures that received full radiation (UVBR, UVAR and PAR) (TUVBR treatment)
- (2) Cultures that received UVAR and PAR—tubes covered with UV cut-off filter foil (Montagefolie No. 10155099, Folex, Germany: 50% transmission at 320 nm) (TUV treatment)

- (3) Cultures that received only PAR—tubes covered with an Ultraphan film (UV Opak, Digefra, Munich, Germany—50% transmission at 395 nm) (PAR treatment).

The spectra of the materials used in our experiments are described in Hernando and Ferreyra [60].

To determine the UVBR effects, the difference between TUVB and TUVA values for each parameter (FAs, TBARS, reactive species, enzymatic antioxidants and cell abundance) was calculated.

The *M. aeruginosa* culture was exposed to natural sunlight at Buenos Aires (34°35'S; 58°22'W) during spring sunny days (November 28 and 30, 2017). The cultures were exposed in two outdoor water bath at the same two pre-exposed temperatures (26 and 29 °C, ± 1 °C), using running tap water at stable controlled temperatures during the entire incubation and monitored every 15 min. The three replicates tubes for each treatment and at each temperature, were placed in a black anodized aluminium racks and in two black plastic water bath that were painted black on the inside to minimize reflectance. Each rack with nine tubes and the appropriate cut-off filters, floated horizontally in both 100-L tanks at a depth of approximately 1 cm below the surface.

M. aeruginosa was exposed to solar irradiances that are normally recorded for temperate latitudes during springtime (October–November). The experimental doses reached were 77.84 kJ m⁻² of UVBR and 13,295 kJ m⁻² of total radiation (UVR + PAR) hereafter referred to as “Run 1”. The other experiment was done with an UVBR dose of 68.23 kJ m⁻² and total radiation (UVR + PAR) of 10,285 kJ m⁻² and will hereafter referred to as “Run 2”. Both days of experimentation were contrasting in terms of cloudiness. During Run 1 experiment, the sky remained mostly clear, the average irradiance during the experiment was 0.201 W m⁻² nm⁻¹ for 320 nm and 503 W m⁻² for PAR. In contrast, during Run 2 experiment the sky remained partially cloudy with an average irradiance during the experiment of 0.179 W m⁻² nm⁻¹ for 320 nm and 478 W m⁻² for PAR.

Incident solar radiation was monitored continuously during the experiment using a radiometer (model BIC 250, Biospherical Instruments, Inc.), which records irradiances at three wavelengths in the ultraviolet region (305, 320 and 380 nm, approx. 10 nm bandwidth). The radiometer was intercalibrated with the reference instrument (RGUV) [61]. Data were recorded every minute at 1 m of the experimental setup. The equation from Orce and Helbling [62] was used to calculate UVBR doses expressed in kJ m⁻². To monitoring UVR and PAR irradiances a spectroradiometer (model ILT 950, International Light Technologies, Inc., USA) was used. Data (provided in $\mu\text{W cm}^{-2}$) were recorded every minute next to the BIC 250 radiometer. The spectroradiometer calibration was done by International Light Technologies some

weeks before the start of the experiment and inter-calibration was done with GUV 511 from INGEBI.

The effect of solar radiation on the cell abundance of *M. aeruginosa* was evaluated at the end of the exposure treatments (4 h long in average).

The variation (%) in FAs, TBARS, ROS/RNS, enzymatic antioxidants and cell abundance were calculated as follows:

$$\text{UVR\%} = (\text{TUVA} - \text{PAR}) \times 100/\text{PAR}.$$

$$\text{UVBR\%} = (\text{TUVB} - \text{TUVA}) \times 100/\text{PAR}.$$

Where PAR, TUVA and TUVB denote the measurements of the respective parameter under each of the irradiance treatments.

2.2 Sampling and sample analyses

The short-term incubations started at 9:40 AM until 3:13 PM for the first experiment (Run 1, November 28th 2017) and from 9:48 until 2:18 PM for the second one (Run 2, November 30th 2017) centred approximately at local noon time (12.30 h).

Culture aliquots for count cells (1.5 mL) were put into vials, kept in the dark and fixed with formalin that was neutralized with sodium borate. Samples (8 mL for all of them except FA with 10 mL) for 2–7-dichlorodihydrofluorescein diacetate (DCF-DA) oxidation rate, for in vivo ROS/RNS detection, CAT/SOD/GST, FA, TBARS and Chl-a were filtered through GF/F fiber glass filters (0.7 μm) and kept at -80 °C until analysis except for the immediately analysis of reactive species production.

2.3 Fatty acids

After the filtration on GF/F filters, the samples were stored at -80 °C before analyses. The method described by Abdulkadir and Tsuchiya [63], modified by De Troch et al. [64] was applied for lipid extraction. FAs methyl esters (FAME) were analyzed using a gas chromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973) according to the procedures described by Hernando et al. [65].

Shorthand FA notations of the form A:B ω X are used, where A represents the number of carbon atoms, B gives the number of double bonds and X gives the position of the double bond closest to the terminal methyl group [66].

2.4 DCFH-DA oxidation rate

The oxidation of 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was used to determine the reactive species cellular content. Such determination was evaluated in vivo and the quantification is proportional to the oxidation of the

non-fluorescent 2',7'-dichlorodihydrofluorescein (H_2DCF) to the highly fluorescent compound 2',7'-dichlorofluorescein (DCF) in presence of ROS/RNS.

According to Bass et al. [67], filters with *M. aeruginosa* cells were incubated in the dark for 30 min in 2 mL of 40 mM Tris-HCl buffer (pH 7.0), in the presence of 5 μ M DCFH-DA at 27 °C. Modifications were made to the technique according to Malanga et al. [68]. The fluorescence of the supernatant was determined in a microplate reader (Beckman counter DTX 880, Multimode Detectors) with excitation (λ_{ex}) at 498 nm and emission (λ_{em}) at 525 nm. In all cases, parallel blank controls were included.

2.5 TBARS

Cellular TBARS content was used as an indicator of lipid peroxidation. TBARS content provides a rough estimate of the presence of aldehydes, yet most reactivity originates from malondialdehyde (MDA), a product of lipid peroxidation that can react with thiobarbituric acid under acidic and hot conditions to allow a colorimetric assay. Samples for TBARS analyses were filtered through 0.7 μ m glass-fiber filters, which were stored for less than 2 months at -80 °C before analysis. These filters were submerged in 2 ml of both 120 mM potassium chloride and 50 mM potassium phosphate buffer (pH 7.0), sonicated, and centrifuged at 600 g for 10 min. An aliquot (0.8 mL) of the supernatant was treated with 0.7 mL 30% (w/v) Trichloroacetic acid (TCA) and 50 mM potassium phosphate buffer (pH 7.0), and brought to a final volume of 2 mL before centrifugation. A volume of 0.2 mL of 3% (w/v) sodium dodecyl sulfate (SDS) and 0.05 mL of 4% (w/v) butylatedhydroxytoluene (BHT) in ethanol were added to 1 mL of the supernatant from the second centrifugation. After mixing, 2 mL of 0.1 N HCl, 0.3 mL of 10% (w/v) phosphotungstic acid and 1 mL of 0.7% (w/v) 2-thiobarbituric acid were added. The mixture was heated at 95 °C in a water bath for 45 min. 5 mL of *n*-butanol were added before the sample was vortexed, and centrifuged at 600g for 10 min. The fluorescence of the supernatant organic layer was measured at λ_{ex} = 15 nm and λ_{em} = 555 nm. The values were expressed as nmol TBARS (malondialdehyde equivalents) per particulate carbon units. Malondialdehyde standards were prepared from 1,1,3,3-tetramethoxypropane [69].

2.6 CAT, SOD and GST activity

CAT activity was evaluated by the decomposition rate of hydrogen peroxide (H_2O_2) at 240 nm at 25 °C as described by Beutler [70], using a phosphate buffered saline tablet (C987D49, Sigma-Aldrich Inc., USA). One unit of CAT was defined as the amount of enzyme catalyzing the elimination of 1 μ M H_2O_2 per minute.

For the SOD and GST activity, cell extracts were obtained using the same process as that used for the CAT assay. The SOD activity was measured using a SOD assay kit (Cayman Chemicals). The amount of enzyme to exhibit 50% of dismutation of the superoxide radical correspond to one unit [71].

The activity of GST was assayed by the method of Habig et al. [72]. The reaction mixture (200 μ L) consisted of 50 mM potassium phosphate buffer (pH 7.0), 200 mM GSH, 100 mM 1-chloro-2,4-dinitrobenzene (CDNB) and an aliquot of sample to be tested. The enzyme activity was calculated using a molar extinction coefficient of 2,4-dinitrophenyl-S-glutathione as 9.6 $nM^{-1} cm^{-1}$ at 340 nm and 25 °C. One unit of GST was defined as the amount of enzyme catalyzing the formation of 1 μ mol of 2,4 dinitrophenyl-S-glutathione per min.

2.7 Cell counts

To quantify cell abundance, the procedure determined by Legresley and McDermott [73] was followed using a Sedwick-Rafter counting chamber with under a phase contrast Olympus inverted microscope. Samples were first sonicated with an ultrasonic homogenizer (US50; Nissei Co., Tokio, Japan) to separate the colonies into single cells.

2.8 Statistical analyses

One-factor ANOVA was performed (Statistica, version 9) to determine the significance of the differences observed between treatments for each parameter values at different solar irradiances/temperatures. A Tukey post hoc test was additionally performed to determine the differences between treatments [74].

Changes in the relative amounts of unsaturated FAs in relation to irradiance treatment/temperature was further evaluated by one-way ANOVA analyses based on the concentration ratio of UFAs/saturated FAs (UFAs/SFAs). The FAs used to calculate a UFAs/SFA ratio were those that contributed > 15% to the total FA concentration. The SFAs used for calculation of the UFAs/SFAs ratio were 16:0 plus 18:0 considering that both of them showed the higher concentrations as compared to the other saturated FA found (12:0, iso-15:0, 14:0, 15:0, iso-16:0 and 17:0).

3 Results

3.1 Impact of temperature and UVR on FA profiles

A decrease in the UFAs/SFA ratio of 11% was observed under UVAR at 26 °C for the experimental high solar irradiance (Run 1) (Table 1). Under an increased temperature, this UVAR decrease as of 44% compared to the ratio

Table 1 Average ($\pm$ SE) total FAs concentration ($\mu\text{g L}^{-1}$) of *M. aeruginosa* exposed to 26 and 29 °C for each experimental irradiance and according to UVR treatment or PAR (control)

Type of FA	Run 1						Run 2					
	UVR + PAR			UVA + PAR			PAR			UVR + PAR		
	29 °C	26 °C	26 °C	29 °C	26 °C	26 °C	29 °C	26 °C	26 °C	29 °C	26 °C	26 °C
12:0	0.12 $\pm$ 0.08	0.12 $\pm$ 0.06	0.08 $\pm$ 0.06	0.08 $\pm$ 0.06	0.11 $\pm$ 0.01	0.08 $\pm$ 0.06	0.16 $\pm$ 0.13	0.11 $\pm$ 0.03	0.11 $\pm$ 0.02	0.08 $\pm$ 0.01	0.10 $\pm$ 0.01	0.13 $\pm$ 0.03
14:0	0.62 $\pm$ 0.08	0.54 $\pm$ 0.08	0.53 $\pm$ 0.18	0.53 $\pm$ 0.18	0.81 $\pm$ 0.24	0.49 $\pm$ 0.16	0.72 $\pm$ 0.54	0.56 $\pm$ 0.05	0.45 $\pm$ 0.03	0.51 $\pm$ 0.05	0.43 $\pm$ 0.03	0.58 $\pm$ 0.04
Iso-15:0	0.12 $\pm$ 0.02	0.08 $\pm$ 0.01	0.17 $\pm$ 0.02	0.17 $\pm$ 0.02	0.10 $\pm$ 0.003	0.19 $\pm$ 0.04	0.10 $\pm$ 0.04	0.24 $\pm$ 0.02	0.08 $\pm$ 0.01	0.50 $\pm$ 0.21	0.12 $\pm$ 0.01	0.68 $\pm$ 0.05
Anteiso-15:0	0.06 $\pm$ 0.01	0.03 $\pm$ 0.001	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.04 $\pm$ 0.01	0.10 $\pm$ 0.02	0.04 $\pm$ 0.005	0.11 $\pm$ 0.01	0.05 $\pm$ 0.002	0.20 $\pm$ 0.07	0.06 $\pm$ 0.002	0.26 $\pm$ 0.02
15:0	0.08 $\pm$ 0.01	0.07 $\pm$ 0.003	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.08 $\pm$ 0.005	0.08 $\pm$ 0.02	0.08 $\pm$ 0.01	0.08 $\pm$ 0.004	0.08 $\pm$ 0.001	0.09 $\pm$ 0.02	0.08 $\pm$ 0.004	0.12 $\pm$ 0.01
Iso-16:0	0.09 $\pm$ 0.01	0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.02	0.09 $\pm$ 0.02	0.07 $\pm$ 0.02	0.12 $\pm$ 0.01	0.07 $\pm$ 0.01	0.13 $\pm$ 0.005	0.09 $\pm$ 0.02	0.14 $\pm$ 0.01
16:0	11.93 $\pm$ 1.09	9.77 $\pm$ 0.39	11.88 $\pm$ 0.88	11.88 $\pm$ 0.88	10.08 $\pm$ 0.75	12.70 $\pm$ 1.80	10.47 $\pm$ 1.15	23.73 $\pm$ 1.93	25.49 $\pm$ 1.59	27.70 $\pm$ 1.62	30.03 $\pm$ 0.74	29.08 $\pm$ 1.52
16:1 + iso-17:0	0.18 $\pm$ 0.02	0.22 $\pm$ 0.01	0.20 $\pm$ 0.02	0.20 $\pm$ 0.02	0.21 $\pm$ 0.02	0.18 $\pm$ 0.04	0.19 $\pm$ 0.03	0.35 $\pm$ 0.06	0.37 $\pm$ 0.03	0.33 $\pm$ 0.004	0.41 $\pm$ 0.01	0.35 $\pm$ 0.03
Cis 9–16:1	0.28 $\pm$ 0.12	0.27 $\pm$ 0.03	0.33 $\pm$ 0.03	0.33 $\pm$ 0.03	0.36 $\pm$ 0.07	0.44 $\pm$ 0.06	0.33 $\pm$ 0.06	0.67 $\pm$ 0.05	0.70 $\pm$ 0.04	0.94 $\pm$ 0.06	0.94 $\pm$ 0.02	1.10 $\pm$ 0.04
Anteiso 17:0 + 16:1	0.15 $\pm$ 0.02	0.13 $\pm$ 0.03	0.16 $\pm$ 0.04	0.16 $\pm$ 0.04	0.18 $\pm$ 0.03	0.16 $\pm$ 0.04	0.23 $\pm$ 0.08	0.16 $\pm$ 0.02	0.14 $\pm$ 0.01	0.16 $\pm$ 0.02	0.16 $\pm$ 0.005	0.19 $\pm$ 0.01
17:0	0.14 $\pm$ 0.01	0.10 $\pm$ 0.02	0.16 $\pm$ 0.02	0.16 $\pm$ 0.02	0.16 $\pm$ 0.04	0.19 $\pm$ 0.05	0.12 $\pm$ 0.01	0.12 $\pm$ 0.004	0.12 $\pm$ 0.01	0.14 $\pm$ 0.02	0.15 $\pm$ 0.003	0.16 $\pm$ 0.004
17:1	0.03 $\pm$ 0.02	0.03 $\pm$ 0.002	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.07 $\pm$ 0.01	0.07 $\pm$ 0.01	0.11 $\pm$ 0.004	0.11 $\pm$ 0.004	0.12 $\pm$ 0.01
18:0	1.45 $\pm$ 0.13	1.16 $\pm$ 0.13	1.58 $\pm$ 0.12	1.58 $\pm$ 0.12	1.45 $\pm$ 0.22	2.08 $\pm$ 0.26	1.52 $\pm$ 0.08	1.85 $\pm$ 0.06	2.07 $\pm$ 0.23	2.07 $\pm$ 0.31	2.13 $\pm$ 0.08	2.62 $\pm$ 0.20
Cis 9–18:1	1.68 $\pm$ 0.64	0.82 $\pm$ 0.33	2.03 $\pm$ 0.22	2.03 $\pm$ 0.22	1.11 $\pm$ 0.35	2.62 $\pm$ 0.35	1.99 $\pm$ 0.39	3.50 $\pm$ 0.16	2.65 $\pm$ 0.13	4.59 $\pm$ 0.43	3.44 $\pm$ 0.11	5.31 $\pm$ 0.22
Cis 11–18:1	0.33 $\pm$ 0.14	0.16 $\pm$ 0.03	0.41 $\pm$ 0.02	0.41 $\pm$ 0.02	0.25 $\pm$ 0.08	0.50 $\pm$ 0.05	0.23 $\pm$ 0.01	0.60 $\pm$ 0.06	0.30 $\pm$ 0.02	0.79 $\pm$ 0.03	0.46 $\pm$ 0.01	0.94 $\pm$ 0.04
18:2 ω -6	0.96 $\pm$ 0.33	0.66 $\pm$ 0.04	0.95 $\pm$ 0.13	0.95 $\pm$ 0.13	0.91 $\pm$ 0.06	1.45 $\pm$ 0.16	1.06 $\pm$ 0.21	1.78 $\pm$ 0.06	1.91 $\pm$ 0.07	2.65 $\pm$ 0.45	2.58 $\pm$ 0.04	3.18 $\pm$ 0.18
18:3 ω -6	0.68 $\pm$ 0.45	0.45 $\pm$ 0.04	0.62 $\pm$ 0.10	0.62 $\pm$ 0.10	0.59 $\pm$ 0.11	1.32 $\pm$ 0.14	0.69 $\pm$ 0.14	1.79 $\pm$ 0.10	1.98 $\pm$ 0.06	3.19 $\pm$ 0.62	2.92 $\pm$ 0.08	3.76 $\pm$ 0.22
18:3 ω -3	0.42 $\pm$ 0.28	0.36 $\pm$ 0.03	0.39 $\pm$ 0.07	0.39 $\pm$ 0.07	0.48 $\pm$ 0.08	0.87 $\pm$ 0.11	0.61 $\pm$ 0.12	1.33 $\pm$ 0.06	1.72 $\pm$ 0.05	2.36 $\pm$ 0.45	2.52 $\pm$ 0.08	2.88 $\pm$ 0.16
18:4 ω -3	0.43 $\pm$ 0.29	0.33 $\pm$ 0.04	0.36 $\pm$ 0.06	0.36 $\pm$ 0.06	0.45 $\pm$ 0.06	0.87 $\pm$ 0.09	0.52 $\pm$ 0.11	1.18 $\pm$ 0.09	1.89 $\pm$ 0.05	2.35 $\pm$ 0.56	2.90 $\pm$ 0.08	2.89 $\pm$ 0.18
SFA	13.37 $\pm$ 1.09	10.93 $\pm$ 0.32	13.45 $\pm$ 1.01	13.45 $\pm$ 1.01	11.54 $\pm$ 0.54	14.79 $\pm$ 2.03	11.99 $\pm$ 1.22	25.58 $\pm$ 1.91	27.56 $\pm$ 1.41	29.78 $\pm$ 1.92	32.16 $\pm$ 0.82	31.71 $\pm$ 1.64
UFA	2.49 $\pm$ 1.34	1.80 $\pm$ 0.16	2.32 $\pm$ 0.35	2.32 $\pm$ 0.35	2.44 $\pm$ 0.19	4.52 $\pm$ 0.49	2.89 $\pm$ 0.58	6.09 $\pm$ 0.30	7.49 $\pm$ 0.22	10.55 $\pm$ 2.08	10.93 $\pm$ 0.25	12.71 $\pm$ 0.74
ω 6	1.64 $\pm$ 0.78	1.11 $\pm$ 0.09	1.58 $\pm$ 0.23	1.58 $\pm$ 0.23	1.50 $\pm$ 0.05	2.77 $\pm$ 0.29	1.76 $\pm$ 0.35	3.57 $\pm$ 0.16	3.89 $\pm$ 0.13	5.85 $\pm$ 1.08	5.50 $\pm$ 0.09	6.93 $\pm$ 0.40
ω 3	0.85 $\pm$ 0.57	0.69 $\pm$ 0.07	0.74 $\pm$ 0.13	0.74 $\pm$ 0.13	0.94 $\pm$ 0.14	1.74 $\pm$ 0.20	1.13 $\pm$ 0.23	2.52 $\pm$ 0.15	3.61 $\pm$ 0.10	4.71 $\pm$ 1.01	5.43 $\pm$ 0.15	5.78 $\pm$ 0.34
Total FA	19.75 $\pm$ 3.40	15.38 $\pm$ 0.72	20.15 $\pm$ 1.88	20.15 $\pm$ 1.88	17.51 $\pm$ 0.95	24.48 $\pm$ 3.38	19.19 $\pm$ 3.12	38.35 $\pm$ 2.32	40.25 $\pm$ 1.78	48.90 $\pm$ 4.93	49.64 $\pm$ 1.14	54.48 $\pm$ 2.82

The sum of the SFA, PUFA, ω 3 and ω 6 fatty acids total concentration is also shown

Fig. 1 Quotient UFAs (sum of all of them) over the SFAs (16:0 + 18:0) (relative abundance) as a function of high or low solar irradiances when exposed cultures at 26 °C (white bars) and 29 °C (black bars). Each bar represents the mean relative abundance $\pm$ SE. Different letters correspond to significant differences (Tukey test) between treatments inducing a percentage of change (“–” refers to relative decrease) by UVBR or UVA in relation to PAR considered as 100% of the value for each parameter

Fig. 2 Quotient between the main PUFAs (A) 18:2 ω 6, (B) 18:3 ω 6 and (C) 18:1-cis9 over SFAs (16:0 + 18:0) (relative abundance) as a function of high or low solar irradiance when exposed cultures at 26 °C (white bars) and 29 °C (black bars). Each bar represents the mean relative abundance $\pm$ SE. Different letters correspond to significant differences (Tukey test) between treatments inducing a percentage of change (“–” refers to relative decrease) by UVBR or UVA in relation to PAR considered as 100% of the value for each parameter

Fig. 3 Quotient between of the main PUFAs (A) 18:3 ω 3 and (B) 18:4 ω 3 over SFAs (16.0+18.0) (relative abundance) as a function of high or low solar irradiance when cultures were exposed at 26 °C (white bars) and 29 °C (black bars). Each bar represents the mean $\pm$ SE. Different letters correspond to significant differences (Tukey test) between treatments inducing a percentage of change (“-” refers to relative decrease) by UVBR or UVA in relation to PAR considered as 100% of the value for each parameter

values without UVR radiation. No decrease by UVBR was observed at this high irradiance (Run 1). However, for a low irradiance (Run 2), it was produced a ratio decrease of 12% on cultures exposed at 29 °C and 31% due to UVA at 26 °C (Fig. 1).

Changes in the relative abundance of PUFAs were evident in the different UVR treatments (Fig. 2, 3). The high solar irradiance (Run 1) produced a significant decrease (21%) of the ratio 18:2 ω 6/SFA at 26 °C by UVBR while this was not the case for cultures exposed to only UVA comparing with the PAR treatment. At 29 °C, the UVBR decreased the ratio 18:2 ω 6/SFA to 18%. Whereas with a low solar irradiance (Run 2) the decreased ratio was observed in both UVBR (10%) and UVA (22%) exposure at 26 °C. At 29 °C, the ratio 18:2 ω 6/SFA decreased only as consequence of UVBR exposure (18%) (Fig. 2A).

The ratio 18:3 ω 6/SFA changed in a similar way compared to 18:2 ω 6 but with a high percentage of decrease for both UVBR and UVA. Thus, the ratio decreased with 18% at 26 °C and 30% at 29 °C for UVBR when the irradiance was high (Run 1). The UVA did not generate any effect in any temperature treatment. Simultaneously, cultures exposed to a low irradiance (Run 2) at 26 °C showed a decrease in the ratio by UVB (14%) due to significant differences between UVBR and UVA treatments ($P < 0.01$), as well

as by exposure to UVA (31%) due to significant differences between UVA and PAR treatments ($P < 0.01$). At 29 °C, the 18:3 ω 6/SFA ratio was significantly lower only for UVBR exposure (30%) but not by UVA considering that there were no significant differences between UVA and PAR ($P > 0.05$) (Fig. 2B).

The 18:1-cis9/SFA ratio showed a significant decrease of 42% and 15% for high (Run 1) and low (Run 2) irradiance of UVA at 26 °C, respectively. For a high irradiance, no significant decrease due to UVR was observed in cells at 29 or 26 °C. Meanwhile, for a low irradiance (Run 2) there was a significant decrease of the ratio due to UVBR (8%) at high temperature (Fig. 2C).

The ratio of both ω 3 PUFAs to SFA changed similarly with a decrease of 18% and 20% for 18:3 ω 3 and 18:4 ω 3, respectively due to a UVBR exposed to a high irradiance (Run 1) at 26 °C and no decrease was observed at the UVA exposure (Fig. 3A, B). Opposite to these results, at 29 °C, no decreased ratio values were observed at exposure to UVBR, however values decreased because of UVA (52 and 56% for 18:3 ω 3 and 18:4 ω 3, respectively). When UVBR irradiance was low (Run 2) at 26 °C there was a decreased ratio by exposure to UVBR (14 and 15% for 18:3 ω 3 and 18:4 ω 3, respectively) as well as for UVA (33 and 36% for 18:3 ω 3 and 18:4 ω 3, respectively). The same Run showed

no ratio changes at 29 °C by UVAR exposure considering that TUVA and PAR treatments were not significantly different ($P > 0.05$). However, the ratio decreased after the exposure to UVBR (28 and 34% for 18:3 ω 3 and 18:4 ω 3, respectively) (Fig. 3A,B). When the ratio between ω 6 and ω 3 FAs was evaluated, we determined a significant increase due to UVAR of 13% and 34% when cultures were incubated at 26 and 29 °C, respectively, at a high irradiance (Run 1). With the exposure to a low irradiance (Run 2), the UVAR produced an increased ratio of 11% at 26 °C. In addition, the UVBR produced an increase of 7 and 15% when cultures were exposure at 26 and 29 °C, respectively (Table 1, Fig. 4).

Fig. 4 ω 3/ ω 6 FAs ratio as a function of high or low solar irradiance when exposed cultures at 26 °C (white bars) and 29 °C (black bars). Each bar represents the mean $\pm$ SE. Different letters correspond to significant differences (Tukey test) between treatments inducing a percentage of change (“+” refers to relative increase) by UVBR or UVAR in relation to PAR considered as 100% of the value for each parameter

Fig. 5 Oxidative stress parameters in *M. aeruginosa* cultures exposed to 26 °C (white bars) and 29 °C (black bars) and UVR treatment as a function of high or low solar irradiances. (A) DCF-DA oxidation rate, (B) lipid peroxidation (TBARS cell content). Each bar represents the mean $\pm$ standard error (SE). Different letters correspond to significant differences (Tukey test) between treatments inducing a percentage of change (“+” refers to relative increase) by UVBR or UVAR in relation to PAR considered as 100% of the value for each parameter. Initial time refers to cell values at the beginning of exposure to sunlight

($P > 0.05$). With the exposure to the low irradiance (Run 2) at 26 °C, a similar trend was observed to the one observed for the high irradiance (Run 1) at 26 °C, although with this irradiance, the increase in ROS/RNS concentration due to exposure to UVBR reached only 66%. No significant differences were observed between any treatment ($P > 0.05$) when exposing the cells at 29 °C (Fig. 5A).

In the initial time, the TBARS values were not significantly different between temperatures in both experiments. The TBARS content was significantly higher in TUVA (49%) compared to the PAR treatment ($P < 0.01$) but no significant differences were found for the TUVB ($P > 0.05$) at 26 °C and the UVBR high irradiance (Run 1). A high increased lipid damage by UVA (93%) was also observed in the low exposure irradiance (Run 2) (Fig. 5B).

3.3 Antioxidant protection

At the initial time, no differences in CAT activity were found between temperatures in both experiments. However, for SOD and GST the activity was significantly higher at 29 °C compared to 26 °C for both experiments. The CAT activity was significantly reduced only due to exposure to UVBR (18%) at 26 °C with a high irradiance (Run 1). Meanwhile, at an incubation temperature of 29 °C, no significant differences in CAT activity were found between TUVB and PAR ($P > 0.05$), however a significantly increased activity due the exposure to UVA (132%) was observed. In a low irradiance (Run 2) no significant differences between treatments was observed ($P > 0.05$) at 26 °C but an increased CAT due to UVA (93%) was determined at 29 °C in coincidence with high irradiance (Fig. 6A).

SOD activity significantly increased due to both UVBR (172%) and UVA (133%) at 26 °C with the high irradiance

Fig. 6 Antioxidants activity **A** CAT, **B** SOD and **C** GST as a function of high or low solar irradiance when cultures were exposed at 26 °C (white bars) and 29 °C (black bars). Each bar represents the mean antioxidant activity $\pm$ SE. Different letters correspond to significant differences (Tukey test) between treatments inducing a percentage of change (“+ and –” refers to relative increase or decrease respectively) by UVBR or UVA in relation to PAR considered as 100% of the value for each parameter. Initial time refers to cell values at the beginning of each experiment of exposure to sunlight

(Run 1). At 29 °C, there was a significantly increased SOD activity due only to UVAR (102%). While at the low irradiance (Run 2), no significant differences were found between treatments at 26 °C but an increased activity was observed due to UVAR (54%) at 29 °C (Fig. 6B).

GST activity was the same for all treatments ($P > 0.05$) at 26 °C for the high irradiance (Run 1). At 29 °C, there was an increased activity due to UVAR (165%) with respect to the PAR treatment. At the low irradiance (Run 2), no significant differences were registered at any temperature ($P > 0.05$) (Fig. 6C).

3.4 Impact of temperature and UVR on cell abundance

The initial cell abundance was not significantly different between temperatures; however, the values were higher in Run 2 (in average 4.6×10^9 cell L⁻¹) compared to Run 1 (in average 2.1×10^9 cell L⁻¹). No significant differences were found between such initial values and PAR values after UVR treatments at any temperature but there was a decrease of around 10% and 5% for Run 1 and 2, respectively (Fig. 7). For the high experimental UVBR irradiance (Run 1) at 26 °C, there were a significant decrease in cell abundance due to UVBR (18%) and for UVAR (20%) considering the significant differences ($P < 0.01$) between TUVBR and PAR as well as between TUVA and PAR, respectively. However, when cells were exposed to the same irradiance but in a batch temperature of 29 °C, no significant differences between TUVBR and TUVA ($P > 0.05$) were found, despite the significant differences of both treatments with PAR. These results indicate a cell abundance inhibition (reduced by 20%) only due to UVAR (Fig. 7).

For low experimental irradiance (Run 2) at 26 and 29 °C, there were a significant decrease in cell abundance only for UVAR (17 and 13%, respectively), considering that any differences were found between cell numbers in TUVBR compared with TUVA treatment. The cell number in the PAR treatment was significantly higher ($P < 0.01$) compared with the other two treatments (Fig. 7).

4 Discussion

M. aeruginosa is one of the major bloom-forming cyanobacterial species, which forms noxious blooms in La Plata River basin every year, causing negative impacts on the ecosystem and human health. It is, therefore, important to assess the manner in which these cyanobacteria may adapt to climate change. The experimental conditions of this study simulate their response to high temperatures and then an exposure to high (Run 1) or low (Run 2) solar irradiance depending on the cloudiness of the sky and with different cells abundance as normally occurs.

The cell abundance in Run 2 reached around 4×10^9 L⁻¹ cells, doubling the abundance of Run 1 as usually occurs over time in the La Plata basin blooms. It can produce self-shading [75–77]. Thus, lower irradiance in Run 2 and high abundance were also considered for the interpretation of our results.

4.1 Impact of temperature and UVR on FA profiles

Previous studies have shown that UVR can affect FAs in cyanobacteria by damaging cell integrity including oxidative damage over unsaturated or polyunsaturated lipids [78]. He

Fig. 7 *M. aeruginosa* abundance (cell L⁻¹) as a function of high or low solar irradiance when cultures were exposed at 26 °C (white bars) and 29 °C (black bars). Each bar represents the average abundance ($\pm$ SE). Different letters correspond to significant differences (Tukey test) between treatments inducing a percentage of change

(“–” refers to relative decrease) by UVBR or UVAR in relation to PAR treatment considered as 100% of the value for each parameter. Initial time means cell values at the beginning of each experiment of exposure to sunlight

and Häder [27] have shown PUFA peroxidation in the cyanobacteria *Anabaena* sp. due to UVBR exposure. In addition, Zeeshan and Prasad [79] demonstrated differential response of growth, photosynthesis, antioxidant enzymes and lipid peroxidation to UVBR in three cyanobacteria species (*Nostoc muscorum*, *Plectonema boryanum*, and *Aphanothece* sp.). When the change in the general composition of FAs was evaluated by the UFA/SFA ratio (relative abundance), a decrease in UFA (with a > 10% threshold in the total FAs concentration) was determined due to UVAR, mainly at 26 °C for both irradiance Runs, independently of cell abundance and coinciding with the lipid damage (TBARS). In coincidence, Chris et al. [80] found an increased lipid damage in *Cylindrospermum* sp. exposed to UVBR and by exposure to UVBR an increase in TBARS was determined for *P. corium* [81]. This increase in lipid damage, with a MDA increase, is the result of oxidative damage of PUFA in the cell membranes [82]. In other cyanobacteria, the UVR exposure damaged the SFA [83]. Thus, the SFA composition of *Spirulina platensis* after UVBR decreased 50% while PUFA increased 25%. Bhandari and Sharma [81] reported a SFA decrease after UVBR exposure in *N. spongiaeforme*. In our study, an evident decrease of UFA was found mainly as consequence of UVAR exposure. The lipid damage observed at 26 °C under high irradiance was only registered by a decreased in ratio 18:1-cis9/SFA. However, with high temperature, the PUFA evaluated decreased as consequences of UVAR (in coincidence with TBARS increased, see above). Despite these results, at 29 °C the UVAR decreased the ω 3 PUFA with about 50% for high irradiance but with no effects at low irradiance plus self-shading. Clearly the ω 3 FA were highly sensitive to high UVAR irradiance at both exposure temperatures. Regarding the UVBR effects, ω 3 and ω 6 FA were highly sensitive at 26 °C independently of irradiance level or cell abundance. These results showed insufficient protection by antioxidant activity/consumption and some damage probably caused by H_2O_2 production [44]. However, the ω 3 were not affected by UVBR in high irradiance conditions at 29 °C with a lower SOD activity compared with 26 °C but with a similar CAT activity compared with the PAR treatment. Babel et al. [37] reported an increased enzymatic antioxidant level due to UVBR and temperature increase exposure with a protective role avoiding lipid damage. This is also evident from the present findings as it was noted that the exposure of cultures to high UVBR irradiance at 26 °C (plus previous exposure period at such temperature) resulted in a significant increase in the SOD activity. It was previously reported for *Anabaena* sp [84] and also for *M. aeruginosa* [37]. Interestingly when cells were exposed to UVAR at 29 °C, the increased activity of SOD, CAT and GST was not enough to avoid UFA damage (see below). As previously mentioned, the energy level of UVAR is very high in high solar irradiance. However, when

solar irradiance was low and plus high cell abundance, no UFA damage was observed in ω 3 or ω 6 FA or 18:1-cis9 with a CAT activity increased in UVAR at 29 °C (see below).

It has been shown that under stress conditions, the fluidity of the plasma membrane can be regulated through changes in the composition of UFAs, thus modifying the physiological response [84]. In addition, it is known that an increase in membrane lipid unsaturation increases the tolerance of cyanobacteria to UVR [85, 86]. PUFAs are very important components in the membrane-chlorophyll complex in cyanobacteria [87, 88]. Thus, our results suggest that the high antioxidant protection of UFA due to temperature increase supported the resistance of *Microcystis* to the UVR effects [83]. Another possible adaptation mechanism for an increased membrane lipid unsaturation is the protection of these enzymes from inactivation at high UVBR [89]. It was proved to be a covalent modification with membrane lipids associated proteins [90]. No inhibition was observed by UVAR in high irradiance in any ω 6 FAs at 26 or 29 °C, although the relative abundance of both ω 6 has decreased with a low irradiance plus self-shading between 22 and 31% only in at 26 °C. However, increasing the exposure temperature does not show any decrease in ω 6 FAs relative abundance probably due to an increased CAT and GST activity (see below).

To determine if there is a differential sensitivity of the different FAs to RUV, the ratio ω 6/ ω 3 FA was analyzed. Thus, the concentration of ω 6 was significantly higher at both temperatures due to exposure to high UVAR irradiance and doubling the concentration under conditions of high temperature with greater antioxidant activity (see below). In exposure to a lower irradiance plus self-shading, the increase of ω 6 FAs was due to UVAR and UVBR. With these results we can demonstrate a higher UVR sensitivity of ω 3 FAs.

4.2 Enzymatic protection by temperature and UVR effects

Clear differences were found between the initial values of reactive species when comparing high with low irradiance, being higher in Run 2, probably due to the high cell abundance. Although the cultures exposed to low irradiance continued to grow exponentially, the increase in cell abundance was around 100% because Run 2 experiment was carried out 2 days after the experiment with high irradiance and pre-exposed cultures continued to grow. The consequent low nutrient concentration and an increase in biomass could produce an increase in the reactive species concentration at the beginning of Run 2. Diverse stress factors such as temperature, excessive light forces and nutritional constraint can generate reactive species [91]. Despite the higher reactive species concentration at the start of the incubation, no significant differences were observed in relation to PAR after the

incubation period, with the exception of the cells exposed to UVBR at 26 °C. However, this difference was about five times larger in the high than in the low irradiance experiment with the consequences that will be explained below. Reactive oxygen species are mainly produced by photosystem I; however, under certain circumstances, PSII contributes to the overall formation of ROS in the thylakoid membrane [49]. The initial basal level of reactive species in the PAR treatment of both experiments can also be explained by the fact that the cells were adapted to a lower PAR level in our culture chamber and despite the pre-adaptation to solar PAR irradiance, it resulted in additional reactive species generation. Previously, it was argued that greater lipid damage by UVAR diminishes or disappears with increasing exposure temperature in response to a greater antioxidant activity. Thus, both SOD and GST have significantly increased their activity due to UVAR exposure at 29 °C. At the initial time of both experiments, these two enzymes already had high concentrations as consequences of pre-adaptation at 29 °C and continued high during the day of pre-adaptation to solar PAR irradiance. Despite that, their activity was significantly higher at the initial time, after 4 h of UVAR exposure these cells doubled their SOD activity or even tripled it for GST under high irradiance conditions at 29 °C.

After the UVBR exposure, SOD activity in *M. aeruginosa* was increased by about 172% when exposed to high solar irradiance at 26 °C. Such increase in SOD activity could be enough to avoid damage to both $\omega 6$ FAs. Castenholz and Garcia-Pichel [92] registered increased peroxide radical anions in cyanobacteria with high light intensity and the consequent increased synthesis of SOD. Furthermore, Latifi et al. [49] demonstrated that H_2O_2 , a product of SOD activity, has detrimental effects on cyanobacteria. Previous studies reported that SOD activity in plants can increase or diminish according to the species, UVBR dose and the presence or not of concomitant PAR or UVAR [93].

As was previously mentioned, CAT is an enzymatic antioxidant, which exclusively dismutate H_2O_2 . It also doubled its activity cells in both solar irradiance, independently of cell abundance when exposed to 29 °C, despite the fact that at the initial incubation time the CAT concentration was the same in cultures grown at 26 or 29 °C and without significant differences with PAR values after the exposure to Run 1 and 2. Furthermore, CAT activity had a significant decrease at 26 °C when cells were exposed at high UVBR irradiance. This result has been reported before [41, 94] and inactivation of CAT due to UVBR is a consequence of photo inactivation and degradation [95] of the haem group in CAT. A high reactive species concentration (statistically not significant) was produced in cells exposed to UVAR at 26 °C with a high solar irradiance, with consequent increased lipid damage despite of an increased SOD activity. For cells exposed at 29 °C and high UVBR irradiance, no lipid damage was

registered probably due to a significant higher CAT (132%), SOD (102%) and GST (165%) activity. An increased temperature may increase the activity of enzymatic antioxidants as was previously mentioned.

In UVBR exposure, the significant increase in reactive species did not impact the general lipid damage index (TBARS), probably due to a significant increase in CAT and SOD at 26 °C. This protection has been more effective for $\omega 3$ compared to the decrease in relative abundance of $\omega 6$. Unlike the effect produced by the increase in temperature on enzymatic antioxidants when exposed to UVAR, no changes in the levels of antioxidants were observed when exposed to UVBR due to temperature increases in high solar irradiance. The increase in enzymatic antioxidant activity noted at 29 °C was not present in exposure to UVBR in high or low irradiance, probably due to the lack of an increase in the concentration of reactive species due to the previous adaptation to 29 °C. Instead, by exposing the cells to UVAR, the high SOD and GST activity by previous exposure to 29 °C and the increase of CAT, SOD and GST during the exposure to high irradiance, avoided an increase in the reactive species concentration and the consequent lipid damage. The decrease in CAT activity after UVBR exposure to high irradiance was probably due to enzymatic damage at high UVBR dose as previously explained. However, an increase in SOD was observed by UVBR exposure at 29 °C when the cultures were exposed to a low solar irradiance plus high cell abundance.

4.3 Impact of temperature and UVR on cell abundance

Decreases in abundance and growth under UVBR have been reported in several cyanobacterial strains such as *Anabaena* sp. [27], *M. aeruginosa* [37, 44]. However, in our experimental conditions, we found some differences between temperature incubations. Thus, at 26 °C and higher experimental solar irradiance, we found a decreased cell abundance by exposure to UVBR and UVAR. When the incubations temperature was 29 °C at high irradiance, only the UVAR produced a decrease in cell abundance. According to our results, such UVBR protection cannot be explained by an increase in CAT, SOD or GST activity. Probably the absence of an increase in the concentration of reactive species and cell protection at 29 °C may be due to a high consumption of non-enzymatic antioxidants under high temperature conditions (not determined). Regarding to UVAR, as previously demonstrated, some antioxidants protections are induced as consequences of high temperature, principally enzymatic antioxidants that avoid the cellular damage by a significant reactive species increase. With a low solar irradiance plus self-shading, no UVBR inhibition was present but the UVAR produced a slight

decrease of cell abundance at 26 °C, being even low at 29 °C in comparison to the high solar irradiance. Thus, such minimum percentage of cell abundance by UVAR at 29 °C could be due to a significantly increased protection by CAT activity.

5 Conclusions

Global warming enhances water temperatures and thus stratification of water bodies depending on their morphometry and wind speed. By reducing the thickness of the upper mixing layer, organisms are more exposed to high UVR doses. According to our results, lower lipid peroxidation of selected UFAs and high capacity of scavenging systems could probably explain the high adaptability of *M. aeruginosa* to high UVR stress in high temperature conditions. It was evident that selected PUFAs (mostly $\omega 6$) play an important role in high temperature adaptation in addition to enzymatic antioxidant increased activity shifting the temperature growth from 26 to 29 °C.

Cell death by UVBR and lipid damage by UVAR were avoided under high temperature acclimation and at high temperature and low solar irradiance due to an increased enzymatic antioxidant activity. Additionally, self-shading may produce a lower SOD, GST and CAT activities compared with decreased abundance.

Extrapolating our results to nature would mean that low irradiance and self-shading may result in lower oxidative stress in *M. aeruginosa* blooms by reducing effective-light exposure. Further, the increasing global temperature might counteract some of the negative effects of UVBR.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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