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Differential sensitivity of fatty acids and lipid damage on *Microcystis aeruginosa* (Cyanobacteria) exposed to increased temperature

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ABSTRACT

Changes in fatty acid (FA) composition can mean a mechanism of acclimation of Cyanobacteria to climate change. The objective of the present study was to evaluate the effects of increased temperature on *M. aeruginosa* cultures in terms of FA content, lipid damage, biomass and reactive oxygen species (ROS). Unicellular cultures were exposed to high (29°C) and control (26°C) temperature for 12 days. Differential sensitivity of ω3 FAs was observed after 2 days of exposure to elevated temperature (29°C). Also, no significant differences in ROS content at different temperatures were observed although there was a significant decrease compared to the value at the start of the incubation. Thus, low FA peroxidation of selected ω6 PUFAs and potentially increased activation of antioxidant systems, resulting in lower lipid damage (on average 35%), could explain the strong acclimation to high temperature as shown by the increased growth rate (11%) compared to the control conditions. In high temperature conditions we found

a retarded desaturation to 18:3ω3 and 18:4ω3 PUFAs which were 40% lower compared with control at the end of incubation.

Overall, growth rate and omega-6 FA were increased at high temperature as a mechanism of successful acclimation. This is highly relevant for the ecological role of *M. aeruginosa* as food source for grazers. A reduced FA level can have serious implications for the flow of energy and thus the overall functioning of the ecosystem.

Key words: fatty acids, lipid damage, *Microcystis aeruginosa*, temperature, ω6/ω3 ratio

Introduction

The cosmopolitan distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stresses such as heat, cold, desiccation, salinity, nitrogen starvation, photo-oxidation, anaerobiosis and osmotic stress etc. (Fay 1992, Tandeau de Marsac and Houmard 1993, Sinha and Häder 1996). They have developed a number of mechanisms by which cyanobacteria defend themselves against environmental stressors.

The average surface temperature of the planet has increased with about 0.6±0.2°C since the end of the nineteenth century (IPCC 2007). In addition, temperatures are expected to increase globally with larger changes at higher latitudes (IPCC 2013). Consequently, high temperature affects in an indirect and direct way the structure and functioning of aquatic ecosystems (Häder et al. 2007). In this century, global temperatures are expected to increase with about additional 2–5ºC (Houghton et al. 2001). This may have implications for organisms that thrive at higher temperatures. For instance, harmful Cyanobacteria such as *Microcystis* have an optimal temperature for growth at 25ºC or above (Paerl & Huisman 2008).

Phytoplankton are major biomass producers (Häder et al. 2007) that act as a sink of carbon in aquatic ecosystems that may be transferred to other trophic levels of food webs (Sherr & Sherr 1987). However, Cyanobacteria also produce different types of secondary metabolites such as cyanotoxins (e.g. the microcystin, MC). MCs are predominantly produced by freshwater cyanobacteria of the genera *Microcystis*, *Planktothrix* and *Anabaena* (Dittmann et al. 2013), which would have adverse effects on humans and the environment (Babica et al. 2006).

In Cyanobacteria cells, lipids are mainly found in the membranes (Singh et al. 2002), which are structurally made up of large amounts of polyunsaturated fatty acids (PUFAs). Unsaturated FA (UFA) play vital roles to membrane physiology. The ratio of UFAs to saturated FAs (SFA) determines membrane fluidity, which promotes several cellular activities such as membrane fusion and fission (Altabe et al., 2013). The optimal membrane fluidity to maintain physiological homeostasis is achieved by upward or downward regulation of UFAs synthesis ('homeoviscous adaptation', Hazel, 1995). Therefore, Cyanobacterias can survive in diverse and extreme conditions because of their ability to modify the type and quantity of their cellular lipids (Sinensky, 1974). Temperature stress can induce changes in FAs in cell membranes. The ability to modify the type and quantity of cellular lipids allows Cyanobacteria to avoid damage and to be protected against the effects of extreme conditions (Sato et al. 2000). The UFAs are highly susceptible to oxidative stress due to high density of double bonds (Bandyopadhyay et al. 1999). In this case, lipid peroxides produced by reactive oxygen species (ROS), can damage FAs and can be measured by Thiobarbituric Acid Reactive Substances (TBARS) reaction (He et al. 2002). Cellular membranes, made up of large amounts of PUFAs, are therefore highly susceptible to attack by ROS and consequently experience changes in membrane fluidity, permeability, and cellular metabolic functions (Bandyopadhyay et al., 1999; Schuhmann et al., 2011). An increased temperature of water bodies stimulates the metabolic rate of the plankton (Zinser et al. 2007) and consequently could favour the dominance of Cyanobacteria (Paerl & Huisman 2008). This metabolism activation implies an increase in oxygen consumption, which could be the reason of an increased ROS. The oxidative stress is produced when there is a higher ROS concentration compared to antioxidants activity/concentrations (Halliwell 2006). In addition, like all photosynthetic organisms, Cyanobacteria cannot avoid solar exposure and a possible ROS increase, due to the photosynthetic electron transport chain (Helbling & Zagarese 2003).

The chemistry of ROS is well documented (Imlay 2003) and includes singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). These powerful oxidizing agents cause inhibition of Cyanobacterial growth (Dziallas & Grossart 2011), decline in the activity of photosystem II (PSII) (Saison et al. 2010) and consequently a photosynthesis inhibition (Hernando et al. 2002). Using the oxidation of 2´,7´- dichlorofluorescein diacetate (DCFH-DA), Qian et al. (2010) showed

that higher ROS concentration damages the pigment synthesis and membrane integrity, being very harmful to *Microcystis aeruginosa*.

In view of the ecological and toxicological importance of the genus *Microcystis* (Sanchis et al. 2004, Karsten 2008), the objective of the present study was to evaluate the effects of increased temperature on *M. aeruginosa* cultures. Their response was evaluated in terms of biomass, ROS concentration, FA composition and lipid damage. We hypothesize that the increased temperature and the duration of the exposure (i.e. incubation time) exert a strong influence on *M. aeruginosa* responses, driving distinct FA compositions and lipid damage, with physiological consequences. Our results will contribute to a better understanding of possible climate change effects on Cyanobacteria blooms with serious implications for the overall functioning of the ecosystem. Additionally, changes in the cellular chemical composition could modify the toxicity of aquatic ecosystems that are used for drinking water in medium-latitude cities.

Materials and Methods

Experimental set-up

The experiments were performed with the *Microcystis aeruginosa* strain CAAT 2005-3, isolated from a water body located in the town of Pila, Buenos Aires, Argentina (Rosso et al. 2014). The unialgal cultures were grown in Blue-Green (BG-11) medium (Rippka et al. 1979). For the experiments only Cyanobacteria in the exponential growth phase were used. The *M. aeruginosa* culture was pre-adapted at 26°C under artificial light at a photon flux density of 30 μE m^{-2} s^{-1} (monitored daily with an ILT 950 spectroradiometer, International Light Technologies, Inc., USA) under 14:10 h light:dark photocycle. After two weeks of growth and reaching the exponential phase, the unicellular cultures were transferred to 3 L Erlenmeyer flasks with culture medium on a shaker (90 rpm) and grew further at 26°C in the same growth chamber with controlled environment and monitored daily (Model Standard InforsMultitron) prior to start the experiment. Cultures were kept at the control experimental temperature (26°C, referred to as "C" for at least five generations. After that, the cultures were transferred to six Erlenmeyer's with the addition of 100 mL culture medium to each one (500 mL, final volume) and were exposed to two treatments in independent triplicates: 29°C (referred to as 'HT') and 26°C ('C'). These incubations were realised in two independent growth chambers (Model Standard InforsMultitron) with the same

characteristics of controlled daily temperature as described before). The light and shaker conditions were the same as in the previous growth and remained the same during all treatments for both temperatures.

Sampling and samples analyses

Culture samples were collected at days 0, 1, 2, 7 and 12 at 9:00 AM. At each sampling event, culture aliquots for count cells (2 mL) were put into 2 mL vials, kept in the dark and fixed with formalin that was neutralized with sodium borate. Samples for 2-7-dichlorodihydrofluorescein diacetate (DCF-DA) oxidation rate, *in vivo* ROS detection (8 mL), FA (10 mL) and TBARS (8 mL) were filtered through GF/F fibber glass filters and measured *in vivo* for ROS measurements or kept at -80°C until further analysis (FA and TBARS).

Fatty Acids

After the filtration of 10 ml of exposure culture, samples were stored at −80°C and freeze dried prior to analysis. Hydrolysis of total lipid extracts and methylation to FAs methyl esters (FAME) in the particulate material collected on the filters were accomplished according to the method described by Abdulkadir & Tsuchiya (2008), modified by De Troch et al. (2012). FAMEs were analyzed using a gas chromatograph (HP 6890 N) coupled to a mass spectrometer (HP 5973) according to the procedures described by Hernando et al. (2018).

Shorth FA notations of the form A:BωX 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 (Guckert et al. 1985).

Biomass determination

For biomass quantitative estimations, cells were analysed in a Sedwick-Rafter counting chamber under a phase contrast Olympus inverted microscope according to the procedures described by Villafañe & Reid (1995). Different aliquot volumes depending on the density of the culture sample were sonicated approximately for 1 min with an ultrasonic homogenizer (US50; Nissei Co., Tokio, Japan) to separate the colonies into single cells. Growth rates (μ; d^{-1}) were estimated as the slope of the regression line of the

natural log of cells biomass versus incubation time for the portion of the growth curve in the exponential phase.

DCFH-DA oxidation rate

Cellular generation of reactive species was determined by *in vivo* measuring of the oxidation of 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA is a fluorogenic probe with the ability of passing through cell walls and membranes. The principle remains in cellular esterases which hydrolize the probe to the non-fluorescent 2´,7´dichlorodihydrofluorescein (H_2DCF). Then this substance is oxidized by ROS and cellular peroxidases to the highly fluorescent compound 2´,7´dichlorofluorescein (DCF).

After the filtration of 8 mL culture, *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 µM DCFH-DA at 27°C as described by Bass et al. (1983), modified by Malanga et al. (2001).The fluorescence of the supernatant (without cells) was monitored 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.

TBARS

Pre-filtered samples were suspended in 0.5 mL of 50 mM potassium phosphate buffer (KPI), sonicated and centrifuged for 10 min. at 3000 rpm. Then 0.5 mL of 50 mM KPI was added and centrifuged for 10 min at 3000 rpm. A 0.5 mL volume aliquot of the supernatant was treated with 0.5 mL 20% (w/v) trichloroacetic acid, stored at 4°C for 30 min and centrifuged for 10 min. at 3000 rpm. A 0.7 mL volume aliquot of the supernatant was treated with 0.7 ml 0.7% (w/v) 2-thiobarbituric acid. The mixture was heated at 100°C in a water bath for 45 min. and centrifuged 10 min at 3000 rpm (Malanga & Puntarulo 1995). The absorbance of the organic layer (upper layer) was measured at 535 nm.

Statistical analyses

One-way ANOVA and Repeated-measurements ANOVA (RMANOVA) were performed (Statistica, version 9) to determine the significance of the differences observed between treatments for each parameter values during the temperature treatment. The Sphericity assumption that concerns variance

homogeneity was verified using the Mauchley's test and Levene's test. When the interaction was significant or the assumptions of sphericity were not satisfied, a one-way ANOVA was performed evaluating the effect of treatment at different days of exposure. Tukey test was additionally performed to determine the differences between treatments (Scheiner 2001).

To test for the effect of exposure time, a multivariate analysis of the overall relative FA composition was conducted with a non-metric multidimensional scaling method (NMDS) based on Bray-Curtis similarity using Primer 6 software (Clarke & Gorley 2006). A one-way analysis of similarity (ANOSIM) was performed to test for significant differences between the groups based on the exposure day. Similarly, a percentage analysis (SIMPER) was calculated to determine the main FAs contributing to the differences found.

Univariate analyses of the effect of the treatments on FAs with a contribution of >15% on all sampling days (i.e. FAs 16:0, 18:0, 18:3ω6, 18:2ω6, 18:4ω3, 18:3ω3 and 18:1ω9) were performed on non-transformed absolute FA concentrations (µg L^{-1}). A two-way multivariate PERMANOVA with repeated measures was performed to determine whether these FAs differed between the treatments during the incubation time (day 0 (D0), day 1 (D1), day2 (D2), day 7 (D7) and day 12 (D12)). All analyses (nMDS, ANOSIM, SIMPER and PERMANOVA) were performed with Primer6.1.11 software (Clarke & Gorley 2006) with PERMANOVA add-on software (Anderson et al., 2008).

Changes in the relative amount of unsaturated FAs (UFAs) in relation to incubation time was further evaluated by RMANOVA analyses based on the concentration ratio of UFAs / saturated FAs (UFAs/SFAs and was called relative abundance). The same FAs as in the PERMANOVA were used based on the same >15% threshold. The SFAs used to calculate the UFAs/SFAs ratio were 16:0 plus 18:0 considering that both of them showed the same increasing trends in their concentrations as a function of incubation time (R=0.94). The other saturated FA found (12:0, iso-15:0, 14:0, 15:0, iso-16:0 and 17:0) decreased to 1% of the initial concentration after day 1. The UFAs used for calculations of the ratio were 16:1ω9, 16:1, 17:1, 18:1ω9, c18:1ω11, 18:2ω6, 18:3ω6, 18:3ω3 and 18:4ω3.

Results

Fatty acid profiling and impact of temperature

The relative PUFA concentration was high in all treatments, with an average of 40-45% at the beginning of the experiment (**Table 1**). The ANOSIM analysis showed that all FAs concentrations were significantly different (global R=0.8) between the time intervals (days) and that all pairwise comparisons between days (except for D0-D1, D1-D2) yielded R > 0.8. The nMDS analysis showed clear differences in FA composition as a function of incubation time (**Fig. 1**). In particular, the initial days of incubation (D0-D2) differed considerably from the later phases (D7–D12) with R=0.82–1.0. All possible combinations of incubation times (days) showed strong differences (R > 0.8) except for D0–D1 and D1–D2. There was on average a high similarity (96%) in the FA profiles between both temperature treatments on D1 and D2, which was followed by changes in FA composition on D7 and D12 (**Table 2**).

On the other hand, the nMDS analysis showed that temperature (26° and 29°C) had no significant effects on the absolute concentrations of individual FAs (R=0.023, **Table 1**). Only incubation time yielded a clear separation in the nMDS plot (2Dstress=0.02) (**Fig. 1**, SIMPER overall R=0.8). The PERMANOVA test with repeated measures on the absolute FA concentration of selected FAs showed the same outcome: only a significant effect of incubation time was found (p (perm)=0.001). From D0 to D12, the UFA/SFA ratio didn't showed any significant difference between the treatments, so no effect of temperature was observed. However, at D7 and D12 the UFA/SFA ratio increased significantly in both temperature treatments (**Fig. 2**, **Table 2)**. The relative concentration of 18:2ω6 and 18:3ω6 increased significantly at both temperatures after a longer incubation i.e. on D7 and D12 compared to the previous days **(Fig. 3A, B)**. The relative abundance of 18:2ω6 increased significantly on both days with temperature rise, while that of 18:3ω6 remained unchanged. The increment of the two ω6 PUFAs on both incubation days was due to the increased PUFAs and decreased SFAs abundance **(Table 2)**. There was a significant increased relative abundance of 18:1ω9 ($P < 0.01$) in cells exposed to 29°C for the entire duration of the experiment (**Fig. 3C**). For both ω3 PUFA, the responses had the same trend with a significant increased ratio with SFA on D7 and D12 when Cyanobacteria were exposed to 26°C ($P < 0.01$). For 18:3ω3, in addition, there was a significant decrease of the ratio on D12 at 29°C in comparison with 26°C. However, there is no significant change in the FA relative abundance on D7 compared to the previous days at 29°C (**Fig. 4A**). For 18:4ω3 there was a significant increase of the ratio on D 7 and D12 at 26°C in comparison with previous days but no significant change in the FA relative abundance was observed on both days when

compared to the previous days at 26°C (**Fig. 4B**). The ratio between ω6 and ω3 didn´t show changes in any incubation time in cells exposed to 26°C. However, it was significantly higher at 29°C compared to 26°C on D2, D7 and D12 ($P < 0.01$) (**Fig. 5**).

Biomass and growth rate

Total cell number of *M. aeuruginosa* showed a positive and significant correlation with incubation time (**Fig. 6,** $P< 0.01$). Such biomass (cells L^{-1}) increased significantly over time in both temperature treatments between D2 and D12 ($P <0.01$). On D2 and D7 the cell number was significantly higher at 29° than at 26°C ($P< 0.01$). However, there were no significant differences between both temperatures at D1 and D12 ($P=0.06$ and 0.23 respectively) (**Fig. 6**), despite the fact that the cell number was high in the 29°C treatments on both days.

In the exponential phase, the specific growth rate (μ) was significantly higher (11%, $P<0.01$) in cells exposed to 29 °C (0.58 ± 0.01 day^{-1}) compared to control (0.52 ± 0.01 day^{-1}).

Oxidative metabolism

The cellular content of ROS, measured by DCFH-DA oxidation rates, was significantly lower during the entire incubation time at both temperatures compared to day 0 ($P <0.01$). During this period, the maximum value was observed at D2 for cells exposed to 26°C, being significantly higher than those at 29°C ($P <0.01$). No significant differences were found between temperature treatments at D1, D7 and D12. It should be noted that at both temperatures, the content of reactive oxygen species on the 12th day was significantly lower than on all other experimental days (**Fig. 7A**).

The maximum TBARS (used as a measure of lipid damage) content was observed at the start of the incubation (D0) with a value of 0.53 ± 0.12 nmol 10^{-6} cells. During the rest of the incubation, such content decreased significantly for both temperature treatments, being significantly lower ($P < 0.01$) in cells exposed to 29°C (RMANOVA, $F=23.32$) (**Fig. 7B**).

Discussion

It is known that water temperature strongly influences the composition and physiological state of phytoplankton (Reynolds, 1984), in particular by changing the FA metabolism in cells and their membrane lipid composition (Harwood & Jones, 1989). Changes in FA composition at the base of the food web, constitute one significant mechanism by which Cyanobacteria may influence higher trophic levels. Thus, by shifts in FA composition in a food web due to Cyanobacterial blooms could potentially affect zooplankton/fish health by altering their metabolic processes, a pathway that is only recently being explored in trophic ecology.

The total FA content in *M. aeruginosa* cultures, expressed as the UFA/SFA ratio, increased as the experiment progressed. This resulted in a significantly higher FA concentration observed at the end of the incubation (i.e. after 7 days). This increase was in the same range as the one reported for diatom cultures (Siron et al. 1989) and was probably related to the storage of cellular lipids. The latter occurs when the cell division of phytoplankton is blocked due to nutritional deficiency while the cell is still functional. Our results are in line with those observed by Rousch (2003) who noted an increase in total FAs with increasing temperature in *Chaetoceros muelleri* during short and long duration experiments suggesting that elevated temperature, rather than treatment duration, is responsible for changes in FA composition.

The Cyanobacteria contained on average 60% SFA of the total FAs. This could be related to the need for PUFA concentration in membranes in order to sustain an optimal membrane fluidity. We observed no significant differences in the FA composition, keeping the same proportion of UFA and SFA independent of the temperature. These results are opposite to those reported from cold environments. Hernando et al. (2018) demonstrated a higher relative abundance of PUFA (60%) compared to SFAs in coastal phytoplankton assemblages from Antarctic waters in order to be able to tolerate low temperature stress. High levels of PUFAs in cellular membranes preserve membrane fluidity at low temperatures (Lodish et al., 2000) and ensure the functioning of integral membrane proteins.

Changes in the FA composition induced by temperature are believed to be necessary to maintain a definite state of cell membranes (their viscosity). Hence, temperature-dependent changes in the FA composition have an adaptive significance (Somerville & Browse 1991, Sakamoto et al. 1998). Temperature changes of around 5°C or less have been effective in producing a change in the membrane lipid composition of microalgae (Jiang & Gao 2004, Gombos et al. 1997, Chaisutyakorn et al. 2018;

Hernando et al. 2018). In addition, as a result of the temperature rise, there was a significant increase in the UFA/SFA ratio on the 7th and 12th days of incubation, but the increase in UFA was proportional to the increase in SFA regardless of the incubation temperature. However, it was demonstrated the effect of the temperature on the UFA/SFA ratio for diverse organisms: bacteria, animals and plants. In most cases, the relative content of UFA increased with a decreasing temperature (Harwood et al. 1988, Somerville & Browse 1991). Thus, various organisms respond to temperature changes by similar changes in the FA composition. Despite no significant differences were found in the UFA/SFA rate in our experiments, a differential sensitivity of ω3 was observed with respect to ω6 at 29°C. The temperature is known to modify the activity of various desaturases in cyanobacteria and higher plants at the level of gene transcription as well as at the post-transcriptional level (Los 1997). FA desaturases are the enzymes that introduce the double bonds into the hydrocarbon chains of FAs, and thus these enzymes play an important role during the process of cold acclimation of Cyanobacteria (Wada & Murata 1990). Most of the cyanobacterial desaturases are intrinsic membrane proteins that act on acyl-lipid substrates. In our experiment we assume that changes in FA composition were also related to the activity of desaturases although we have no data on that. In our study we demonstrated a differential sensibility of ω3 and ω6 to temperature. Previous studies have shown an effect of temperature on FA composition, and specifically a decrease in ω3 PUFAs (EPA and DHA) with increasing temperature has been reported (Renaud et al. 2002, Guschina & Harwood 2006). As was showed in our experiment, there was a decreased 18:4ω3 and 18:3ω3 relative abundance and an increased relative abundance of 18:3ω6 and 18:2ω6 at high temperature (29°C). Therefore, adaptation of *M. aeruginosa* to temperature rise could be related to a decrease and an increase in the activity of ω3 and ω6-desaturases, respectively. It has been suggested that FA desaturation activity and the availability of potential FA precursors could also explain the variations in FA content (Suutari et al. 1996). Wada and Murata (1990) demonstrated that the Cyanobacteria *Synechocystis* PCC6803 desaturates the fatty acids after the downward shift in temperature by the induction of desaturase activity. The high relative abundance observed in high temperature conditions from day 1 of incubation for 18:1ω9 may explain the significant increase of the PUFA ω6, considering that this MUFA ω9 is a precursor of the ω6 (Akoh & Min 2008). In support of our results, experiments with *Chlorella vulgaris* showed also a decrease in the relative content of 18:3ω3 accompanied by an

accumulation of 18:2ω6 at elevated temperature (Sushchik et al. 2003). In addition, Mayzaud et al. (2013) demonstrated that high temperatures decreased the amount of ω3 PUFAs (such as EPA and 18:4ω3) in natural blooming phytoplankton in an Arctic fjord.

Polyenoic FA, above all those of the ω3 FA group, is known to be an intrinsic component of the galactolipids of the photosynthetic membrane and is important for a successful functioning of the photosynthetic apparatus (Harwood & Jones 1989). An increased photosynthetic rate (not measured) as consequence of a higher relative abundance of both ω6 PUFAs in detriment of both ω3 suggests that high temperature improve the production of necessary cellular products for an increased growth (Schuurmans et al. 2015). There is broad evidence from literature that high temperature increases the biomass of *M. aeruginosa* (e.g. Giannuzzi et al. 2016; 2017) and promotes the growth rate. Our results were in agreement with these previous findings as we observed an increased biomass at increased temperature. The maximal cell number was reached at 29°C demonstrating that temperature had a clear impact on the growth rate. Imai et al. (2009) reported that the effect of temperature on *M. aeruginosa* is proven by a significant higher growth rate at 30°C (0.5 day^{-1}) compared to the one measured at 25°C (0.3 day^{-1}) and 20°C (0.18 day^{-1}). This is in agreement with the present results, considering that the exponential growth rate of cells exposed to 29°C was significantly higher (11% higher, 0.58 day^{-1}) compared to those observed at the control temperature of 26°C (0.52 day^{-1}). In accordance, Tasaka et al. (1996) showed a higher growth rate of *Synechocystis* sp. exposed to 30°C in comparison with 25°C and with a higher relative abundance of PUFAs. Furthermore, a comparison of *Synechocystis* sp. mutated cells revealed that the replacement of PUFAs by MUFAs or SAFAs suppressed the growth of the cells (Tasaka et al. 1996).

Impact of temperature on oxidative metabolism

For aquatic ecosystems, temperature is one of the most important environmental factors affecting growth of primary producers (Eppley, 1972) and planktonic communities (Graham and Vinebrooke, 2009). As mentioned in the introduction, an increase of environmental temperature, which leads to metabolic activation, combined with an increase in O_2 consumption initiates oxidative stress (González et al., 2015). An increase in lipid damage (measured by TBARS concentration) is thus expected as ROS production

increases. PUFAs are extremely labile to oxidation due to their conjugated double-bond structures. Therefore, a high percentage of lipid unsaturation could exacerbate membrane susceptibility to radical attack (González et al. 2015). Hydroperoxides and malondialdehyde (MDA) were often considered as indicators of membrane damage (Hagege et al. 1990). Thus, MDA and a variety of aldehydes have long been recognized as secondary products derived from the degradation of lipid hydroperoxides. MDA detection with TBARS is the most currently used assay for the determination of lipid oxidation (Simontacchi et al. 2011). There are reports that a decrease in PUFA content coincides with the increased levels of MDA in response to high osmotic stress. These responses, which are temporarily associated with an increase in electrolyte leakage, suggest that in fact water stress induces damage at the cellular and subcellular membrane levels *via* lipid peroxidation (Aziz and Larher 1998). Cell membranes, which are structurally made up of large amounts of PUFA, are then highly susceptible to oxidative attack and consequently changes in membrane fluidity, permeability, and cellular metabolic functions are expected (Bandopadhyay et al. 1999).

Although we observed a decrease in the ROS concentration starting at day 1 and throughout the experiment, probably due to an increase in antioxidant protection (Mittler 2002, Giannuzzi et al., 2016), there were no significant differences between the temperatures evaluated. However, the lipid damage was significantly low under high temperature conditions throughout the experiment. Giannuzzi et al. (2016) showed the same results exposing the same *M. aeruginosa* strain to 29ºC for 7 days. Oxidative stress has been linked to a number of cellular toxic processes, including damages to proteins, membrane lipid peroxidation, enzyme inactivation and DNA breakage (Halliwell & Gutteridge 2007). A lower ROS concentration and lipid damage has allowed an exponential growth after D1 in both 26 and 29°C conditions, being significantly higher at high temperature (11%) probably as a result of greater enzymatic antioxidant protection (Giannuzzi et al., 2016).

Another mechanism by which *M. aeruginosa* can protect itself from the physiological consequences of temperature rise is using toxins (microcystins, MCs) as antioxidants (Malanga et al., 2019). However, MCs have different responses to temperature increase. Giannuzzi et al (2016) determined five MCs of the same *M. aeruginosa* strain by LC-MS/MS analysis. In their experiments, the highest MC concentration, 205 fg [Leu^{1}] MC-LR cell^{-1} was measured at the beginning of the experiment and subsequently declined

to 70 fg cell^{-1} after several days of exposure to 29°C. The same trend was observed for all other MCs except for the least abundant MC-LR which showed a continuous increase during exposure time. Unfortunately, we were not able to measure MC levels in parallel to the FA data that we collected. There is an urgent need to determine some potential relationship between pattern of toxin consumption and PUFA sensitivity as a response to high temperature exposure for several days.

As consequence of increase antioxidant protection, we measured a decrease in FA peroxidation at 29°C throughout the entire experiment as well as a relative increase in 18:2ω6 PUFAs concentration at D7 and D12. The increase in ω6 relative abundance observed at days 7 and 12 under high temperature was probably due to a higher antioxidant protection of desaturase enzymes involved in ω6 production compared to the control conditions. From our results it is evident that in *M. aeruginosa*, the temperature elevation retarded the desaturation of the PUFAs 18:3ω3 and 18:4ω3. An increased relative abundance of both ω3 FAs was observed at days 7 and 12 in control conditions but no significant change or even a decrease at day 12 was observed at high temperature. As far as we know, there is no experimental evidence of a relationship between the antioxidant response and the differential sensitivity of PUFAs under increased temperature.

Conclusions

Differential sensitivity of ω3 fatty acids was observed after 2 days of exposure to elevated temperature (29°C). Also, no significant differences in ROS content at different temperatures were observed although there was a significant decrease compared to the value at the start of the incubation. Thus, low FA peroxidation of selected ω6 PUFAs and potentially increased activation of antioxidant systems, resulting in lower lipid damage, could probably explain the strong acclimation to high temperature as shown by the increased growth rate compared to the control conditions. To our knowledge, this study represents the first response of the ω6/ω3 ratio in relation to lipid damage in conditions of increased temperature in *M. aeruginosa*. The results obtained in this study demonstrate the adaptive response, in terms of FA composition, of *M. aeruginosa* during high-temperature acclimation. Thus, this work provides insights for new strategies that might be used to manipulate the FA content of cyanobacteria in order to control their growth and blooms. Furthermore, this response in terms of changes in PUFA is highly relevant for the

ecological role of *M. aeruginosa* as food source for grazers and can have serious implications for the flow of energy and thus the overall functioning of the ecosystem. New developments in the chemical industries, particularly in the area of converting natural products to industrial feedstocks, will further enhance the range of commercially important products synthesized by Cyanobacteria.

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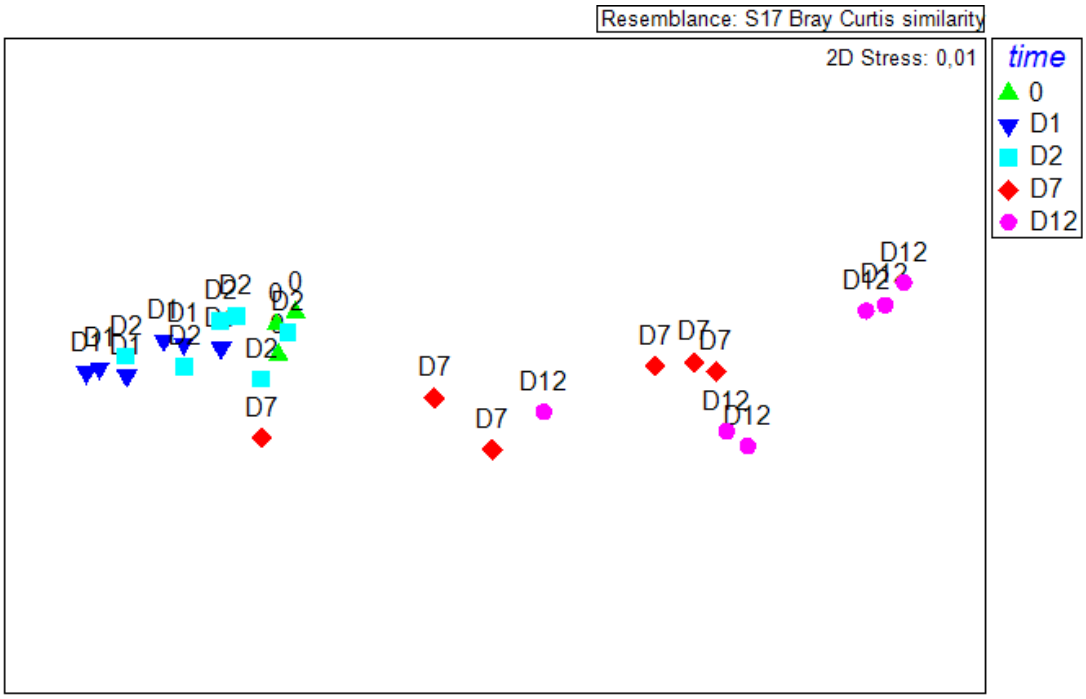


Figure 1

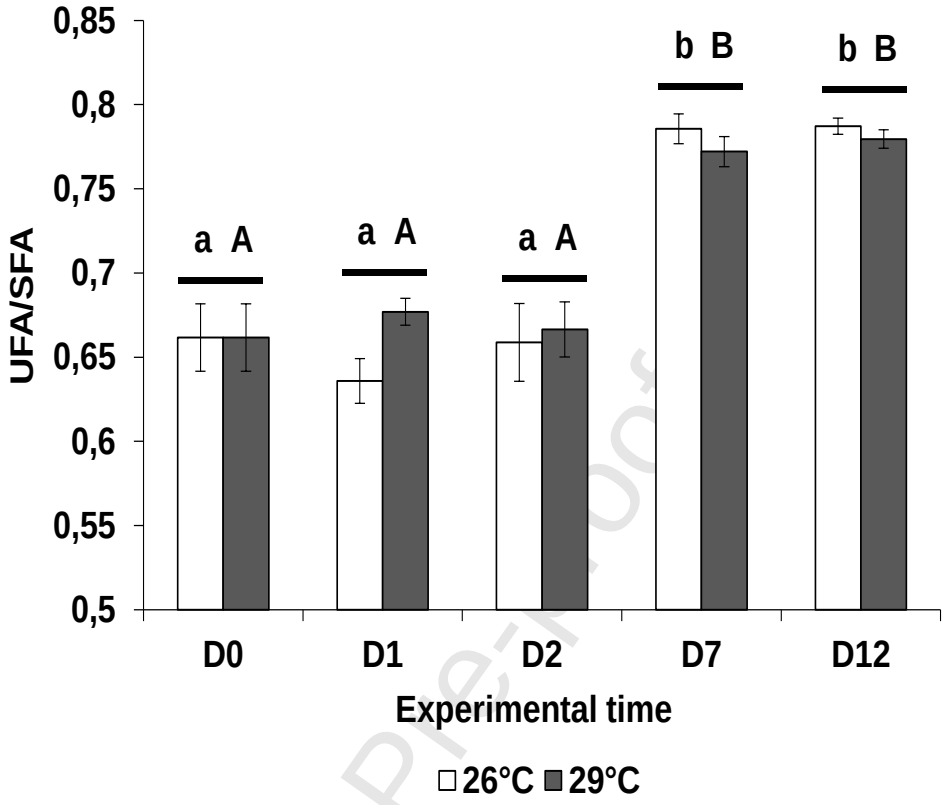
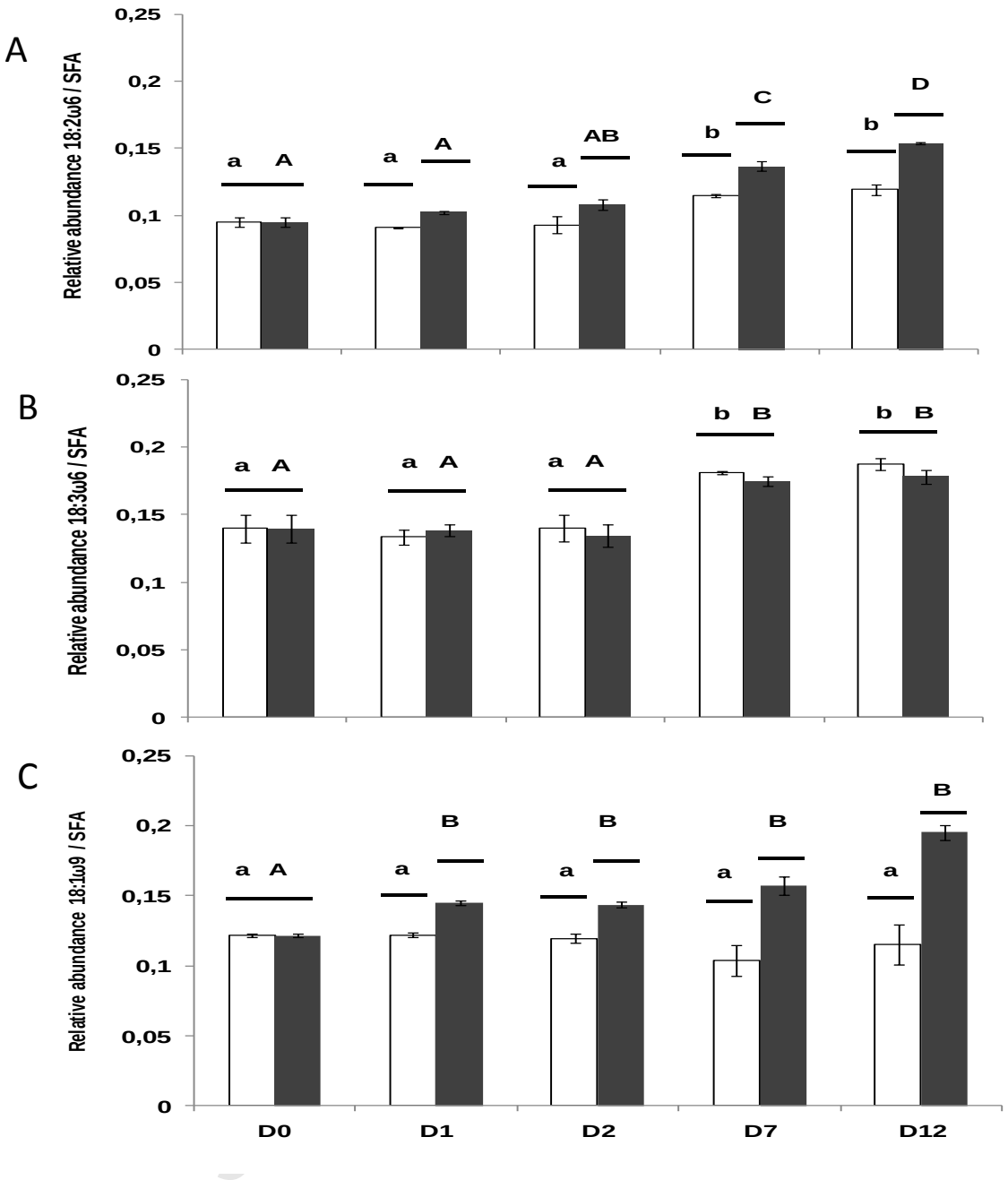


Figure 2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b B
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C
Relative abundance 18:1ω9 / SFA
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Figure 3

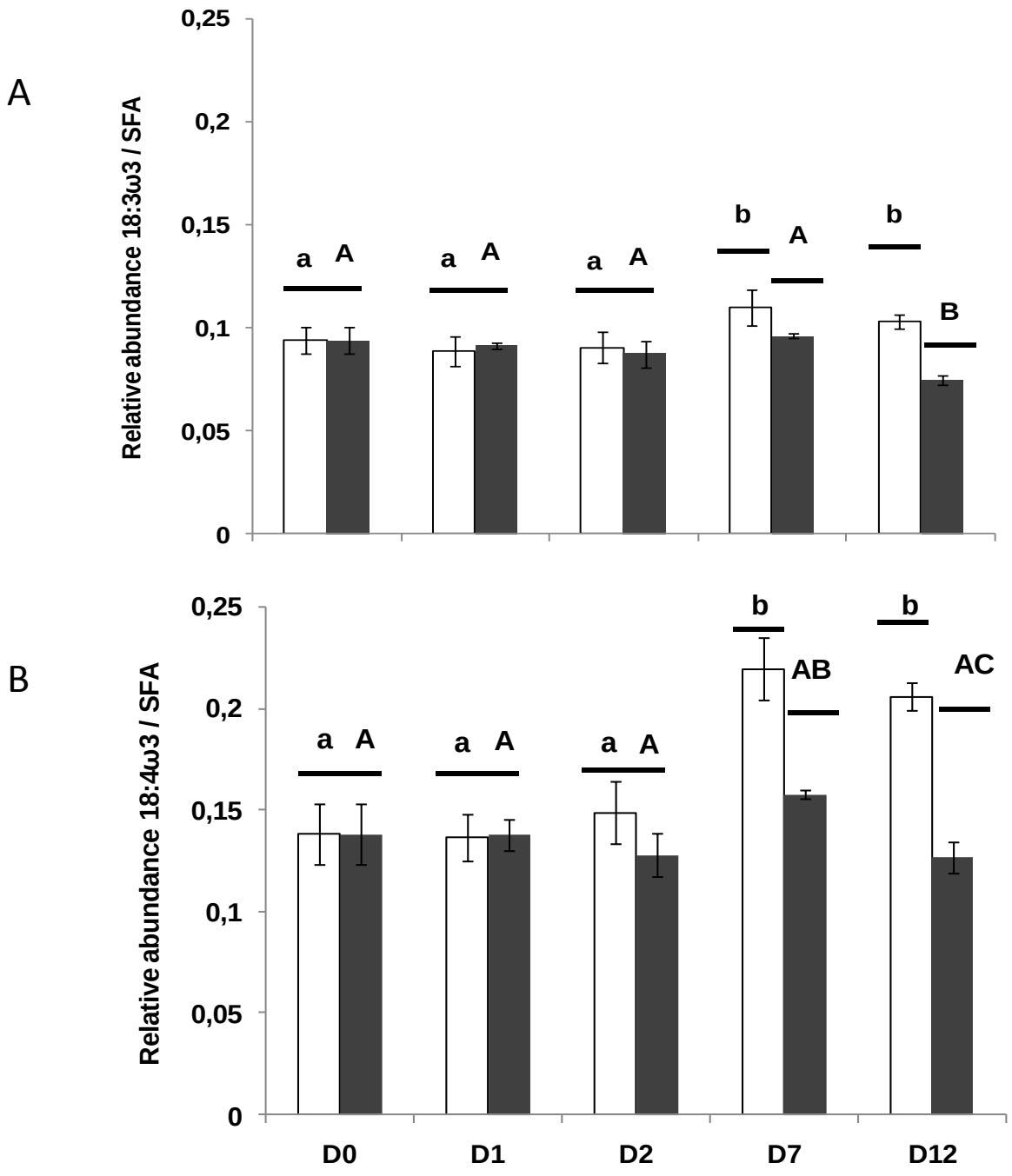
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Figure 4

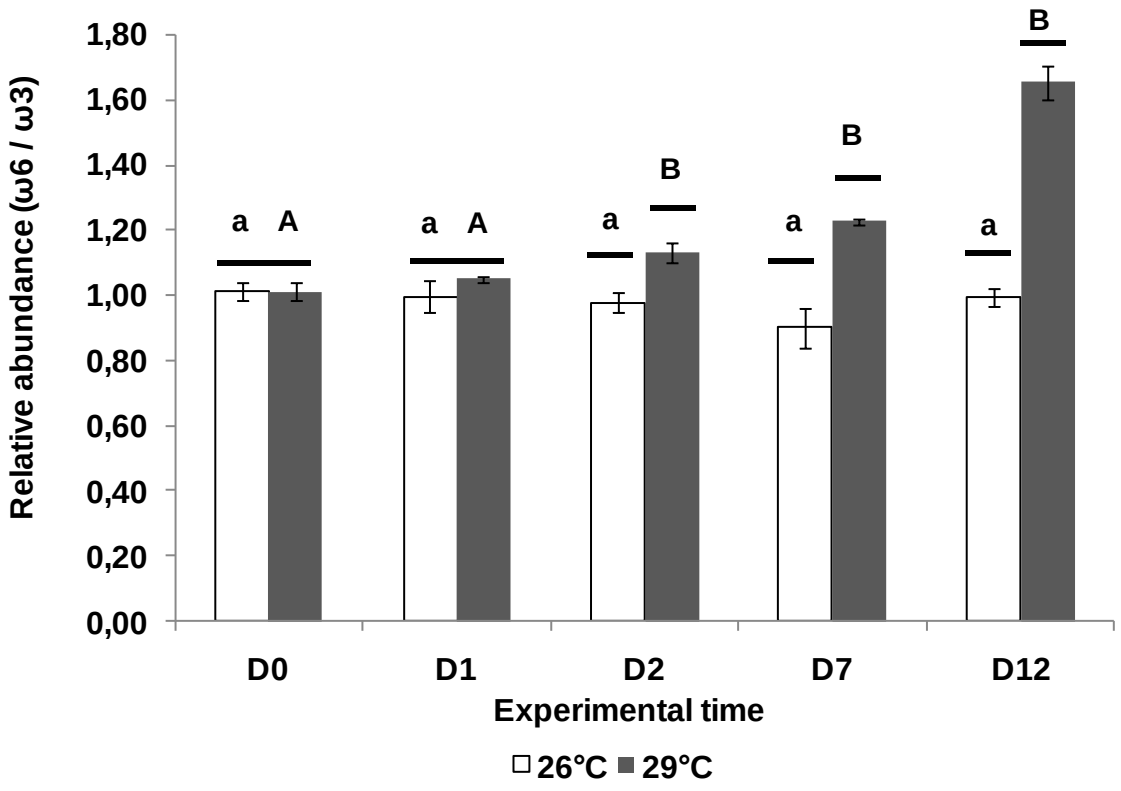

Figure 5

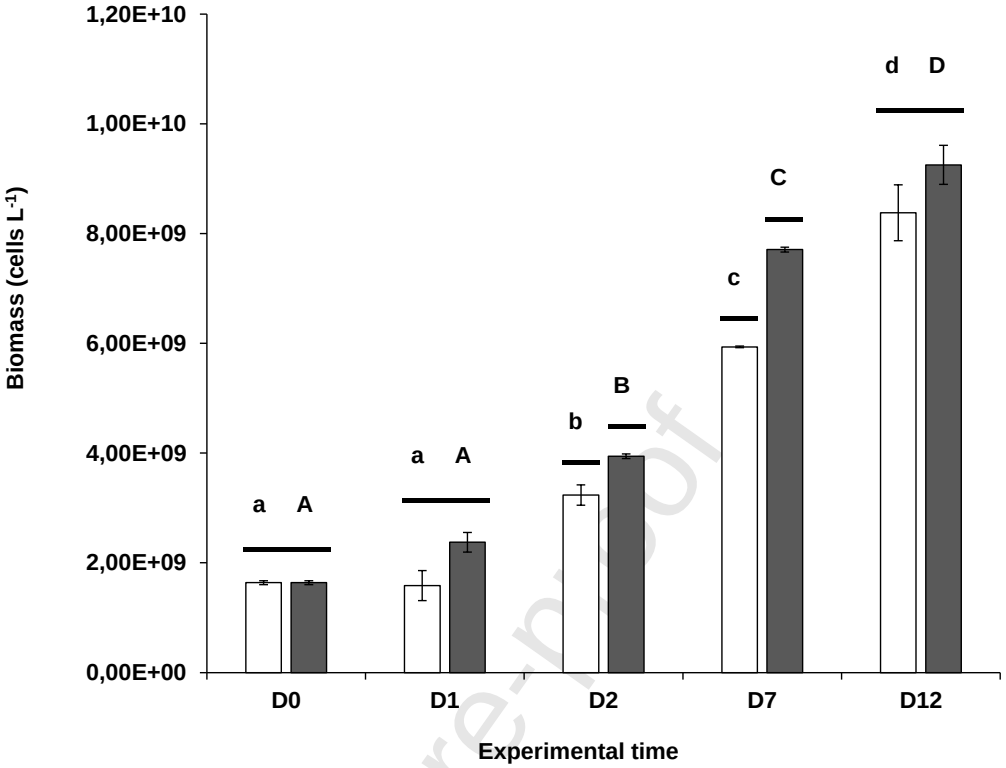

Figure 6

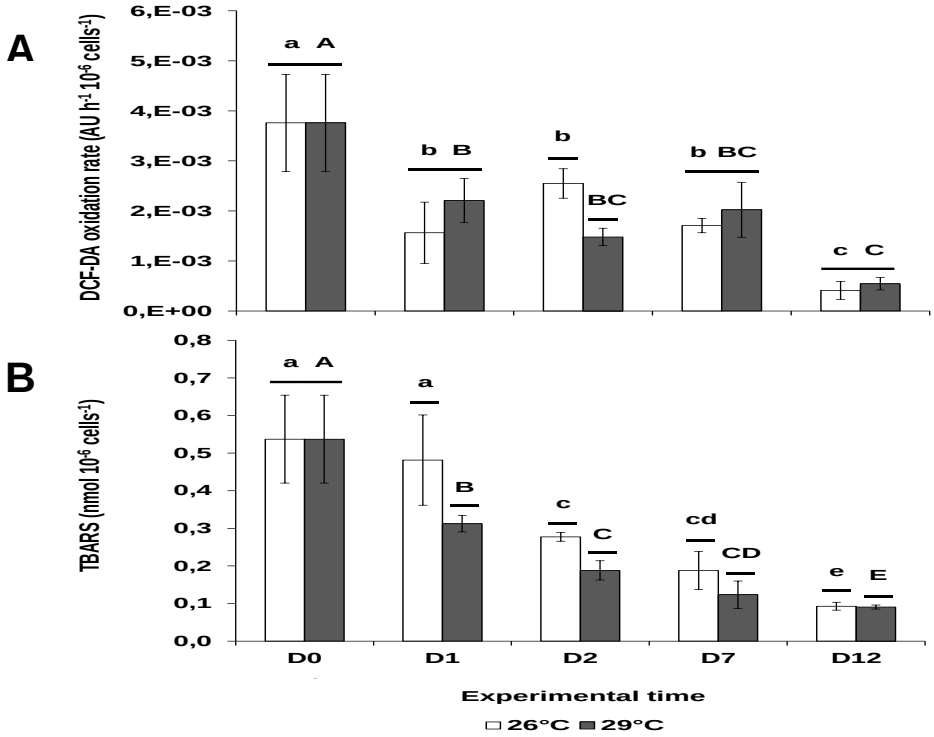

Figure 7

Tables and Figure legends

Figure 1: Multidimensional scale plot (NMDS) based on Bray-Curtis similarity between total Fatty Acids (FAs) composition of *M. aeruginosa* for both experimental temperature at different incubation time (time factor). Data is presented for 19 FAs.

Figure 2: Relative abundance of the total unsaturated FAs in relation to saturated FAs (16.0+18.0) as a function of experimental time when exposed cultures at 26°C (white bars) and 29°C (grey bars). Each bar represents the mean +/- SD. Different letters correspond to significant differences (Tukey test) between experimental days (normal for 26°C and capital letters for 29°C). Horizontal line at same level is showing no significant differences between temperature treatments for the same day.

Figure 3: Relative abundance of the main unsaturated FAs (A) 18:2ω6, (B) 18:3ω6, (C) 18:1ω9 in relation to saturated FAs (16.0+18.0) as a function of experimental time when exposed cultures at 26°C (white bars) and 29°C (grey bars). Each bar represents the mean +/- SD. Different letters correspond to significant differences (Tukey test) between experimental days (normal for 26°C and capital letters for 29°C). Horizontal line at same level is showing no significant differences between temperature treatments for the same day.

Figure 4: Relative abundance of the main unsaturated FAs (A) 18:3ω3, (B) 18:4ω3in relation to saturated FAs (16.0+18.0) as a function of experimental time when exposed cultures at 26°C (white bars) and 29°C (grey bars). Each bar represents the mean +/- SD. Different letters correspond to significant differences (Tukey test) between experimental days (normal for 26°C and capital letters for 29°C). Horizontal line at same level is showing no significant differences between temperature treatments for the same day.

Figure 5: Relation between the relative abundance of all ω6 PUFA (18:2ω6/SFA + 18:3ω6 / SFA) vs. all ω3 PUFA (18:3ω3 / SFA + 18:4ω3 / SFA)as a function of experimental time when exposed cultures at 26°C (white bars) and 29°C (grey bars). Each bar represents the mean +/- SD. Different letters correspond to significant differences (Tukey test) between experimental days (normal for 26°C and capital letters for

29°C). Horizontal line at same level is showing no significant differences between temperature treatments for the same day.

Figure 6: *M. aeruginosa* biomass (cells per L) as a function of experimental time when exposed cultures at 26°C (white bars) and 29°C (grey bars). Each bar represents the mean ± SD. Different letters correspond to significant differences (Tukey test) between experimental days (normal for 26°C and capital letters for 29°C). Horizontal line at same level is showing no significant differences between temperature treatments for the same day.

Figure 7: Oxidative stress parameters in *M. aeruginosa* cultures exposed to 26°C (white bars) and 29°C (gray bars) as a function of experimental time. (**A**) DCF-DA oxidation rate, (**B**) lipid peroxidation (TBARS cell content). Each bar represents the mean +/- SD. Different letters correspond to significant differences (Tukey test) between experimental days (normal for 26°C and capital letters for 29°C). Horizontal line at same level is showing no significant differences between temperature treatments for the same day.

Table 1: Average (±S.D.) concentration (µg L^{-1}) of the fatty acids of *M. aeruginosa* exposed to26°C and 29°C for each experimental day.

Type of FA (µg l^{-1})	Day 0		Day 1		Day 2		Day 7		Day 12	
	29°C	26°C	29°C	26°C	29°C	26°C	29°C	26°C	29°C	26°C
12:0	0,09 ± 0,02	0,09 ± 0,02	0,10 ± 0,02	0,09 ± 0,02	0,05 ± 0,04	0,07 ± 0,05	0,08 ± 0,04	0,10 ± 0,05	0,09 ± 0,04	0,09 ± 0,04
14:0	0,45 ± 0,05	0,45 ± 0,05	0,42 ± 0,03	0,43 ± 0,03	0,37 ± 0,04	0,44 ± 0,09	0,52 ± 0,05	0,53 ± 0,07	0,71 ± 0,12	0,58 ± 0,05
iso-15:0	0,36 ± 0,02	0,36 ± 0,02	0,34 ± 0,02	0,28 ± 0,02	0,45 ± 0,01	0,35 ± 0,03	0,22 ± 0,02	0,19 ± 0,03	0,14 ± 0,03	0,18 ± 0,02
anteiso-15:0	0,08 ± 0,01	0,08 ± 0,01	0,06 ± 0,00	0,05 ± 0,01	0,06 ± 0,00	0,07 ± 0,03	0,07 ± 0,00	0,05 ± 0,00	0,10 ± 0,02	0,08 ± 0,01
15:0	0,14 ± 0,01	0,14 ± 0,01	0,09 ± 0,00	0,09 ± 0,01	0,11 ± 0,03	0,17 ± 0,14	0,12 ± 0,01	0,10 ± 0,00	0,13 ± 0,02	0,19 ± 0,03
iso-16:0	0,09 ± 0,01	0,09 ± 0,01	0,07 ± 0,01	0,06 ± 0,01	0,10 ± 0,01	0,09 ± 0,04	0,09 ± 0,00	0,08 ± 0,02	0,11 ± 0,01	0,10 ± 0,02
16:0	28,16 ± 0,81	28,16 ± 0,81	24,94 ± 0,73	22,84 ± 0,32	27,07 ± 0,75	25,21 ± 1,34	43,51 ± 2,04	30,39 ± 4,00	55,30 ± 1,58	42,50 ± 5,57
16:1 + iso-17:0	0,45 ± 0,04	0,45 ± 0,04	0,36 ± 0,02	0,38 ± 0,04	0,41 ± 0,05	0,57 ± 0,34	0,46 ± 0,02	0,42 ± 0,03	0,58 ± 0,04	0,77 ± 0,15
cis-9-16:1	1,12 ± 0,03	1,12 ± 0,03	0,90 ± 0,04	0,86 ± 0,01	0,99 ± 0,04	0,98 ± 0,04	1,48 ± 0,01	1,21 ± 0,13	1,95 ± 0,08	1,74 ± 0,22
anteiso-17:0 + 16:1	0,19 ± 0,02	0,19 ± 0,02	0,17 ± 0,00	0,16 ± 0,01	0,16 ± 0,00	0,16 ± 0,01	0,19 ± 0,01	0,19 ± 0,03	0,24 ± 0,04	0,20 ± 0,03
17:0	0,15 ± 0,01	0,15 ± 0,01	0,12 ± 0,00	0,10 ± 0,01	0,14 ± 0,01	0,14 ± 0,07	0,21 ± 0,03	0,14 ± 0,02	0,23 ± 0,02	0,21 ± 0,03
17:1	0,11 ± 0,03	0,11 ± 0,03	0,12 ± 0,01	0,10 ± 0,01	0,13 ± 0,02	0,12 ± 0,01	0,26 ± 0,01	0,18 ± 0,04	0,31 ± 0,02	0,27 ± 0,03
18:0	1,52 ± 0,12	1,52 ± 0,12	1,38 ± 0,04	1,25 ± 0,07	1,42 ± 0,03	1,28 ± 0,19	1,66 ± 0,10	1,26 ± 0,16	2,35 ± 0,08	1,78 ± 0,19
cis-9-18:1	3,61 ± 0,14	3,61 ± 0,14	3,81 ± 0,07	2,94 ± 0,03	4,09 ± 0,05	3,17 ± 0,26	7,10 ± 0,11	3,27 ± 0,40	11,27 ± 0,62	5,14 ± 1,14
cis-11-18:1	0,95 ± 0,04	0,95 ± 0,04	0,62 ± 0,04	0,59 ± 0,04	0,73 ± 0,02	0,67 ± 0,02	0,50 ± 0,04	0,39 ± 0,04	0,67 ± 0,16	0,46 ± 0,07
18:2ω-6	2,81 ± 0,05	2,81 ± 0,05	2,69 ± 0,10	2,19 ± 0,04	3,09 ± 0,19	2,47 ± 0,31	6,18 ± 0,25	3,63 ± 0,46	8,88 ± 0,20	5,28 ± 0,64
18:3ω-6	4,14 ± 0,20	4,14 ± 0,20	3,64 ± 0,23	3,21 ± 0,18	3,83 ± 0,33	3,72 ± 0,45	7,88 ± 0,24	5,73 ± 0,75	10,25 ± 0,10	8,29 ± 0,93
18:3ω-3	2,78 ± 0,13	2,78 ± 0,13	2,40 ± 0,11	2,14 ± 0,20	2,49 ± 0,25	2,40 ± 0,34	4,35 ± 0,24	3,50 ± 0,72	4,30 ± 0,04	4,57 ± 0,65
18:4ω-3	4,09 ± 0,32	4,09 ± 0,32	3,64 ± 0,31	3,29 ± 0,32	3,64 ± 0,39	3,96 ± 0,64	7,11 ± 0,27	6,99 ± 1,39	7,29 ± 0,26	9,13 ± 1,37
Total SFA	26,69 ± 0,52	26,69 ± 0,52	26,32 ± 0,44	24,09 ± 0,22	28,49 ± 0,42	26,50 ± 0,88	45,17 ± 1,24	31,64 ± 2,41	57,64 ± 0,95	44,28 ± 3,32
Total PUFA	19,62 ± 0,30	19,62 ± 0,30	17,82 ± 0,51	15,32 ± 0,44	19,00 ± 0,74	17,49 ± 1,18	34,86 ± 0,64	24,90 ± 2,15	44,92 ± 0,45	34,89 ± 2,80
Total FA	51,31 ± 0,65	51,31 ± 0,65	45,87 ± 1,61	41,05 ± 1,08	49,35 ± 1,95	46,04 ± 4,15	81,97 ± 3,18	58,34 ± 8,12	104,88 ± 2,69	81,56 ± 10,88
PUFA/SFA	0,66 ± 0.02	0,66 ± 0,02	0,68 ± 0,01	0,64 ± 0,01	0,67 ± 0,02	0,66 ± 0,02	0,77 ± 0,01	0,79 ± 0,01	0,78 ± 0,01	0,79 ± 0,00

Table 2: SIMPER results for the "time" factor, i.e. samples grouped per sampling day. Average similarities of fatty acids within each group are listed as well as the FAs contributing the most to the similarity within the samples. For each FA, it's percent contribution to the within-group similarity is indicates between brackets.

	Day 0	**Day 1**	**Day 2**	**Day 7**	**Day 12**
Average similarity	97.45	97.67	95.47	93.84	94.51
FA	16:0 (53.85)	16:0 (54.22)	16:0 (41.76)	16:0 (18.8)	16:0 (17.14)
FA	18:3ω6 (7.85)	18:3ω6 (7.59)	18:3ω6 (7.39)	18:3ω6 (25.52)	18:3ω6 (34.62)
FA	18:4ω3 (7.58)	18:4ω3 (7.51)	18:4ω3 (7.29)	18:4ω3 (7.78)	18:4ω3 (4.57)
FA	Cis9-18:ω1 (6.87)	Cis9-18:ω1 (7.65)	Cis9-18:ω1 (7.37)	Cis9-18:ω1 (3.66)	Cis9-18:ω1 (2.83)
FA	18:2ω6 (5.42)	18:2ω6 (5.49)	18:2ω6 (5.47)	18:2ω6 (7.09)	18:2ω6 (5.45)
FA	18:3ω3 (5.27)	18:3ω3 (5)	18:3ω3 (4.72)	18:3ω3 (20.19)	18:3ω3 (8.05)
FA	18:0 (2.82)	18:0 (2.95)	18:0 (2.68)		

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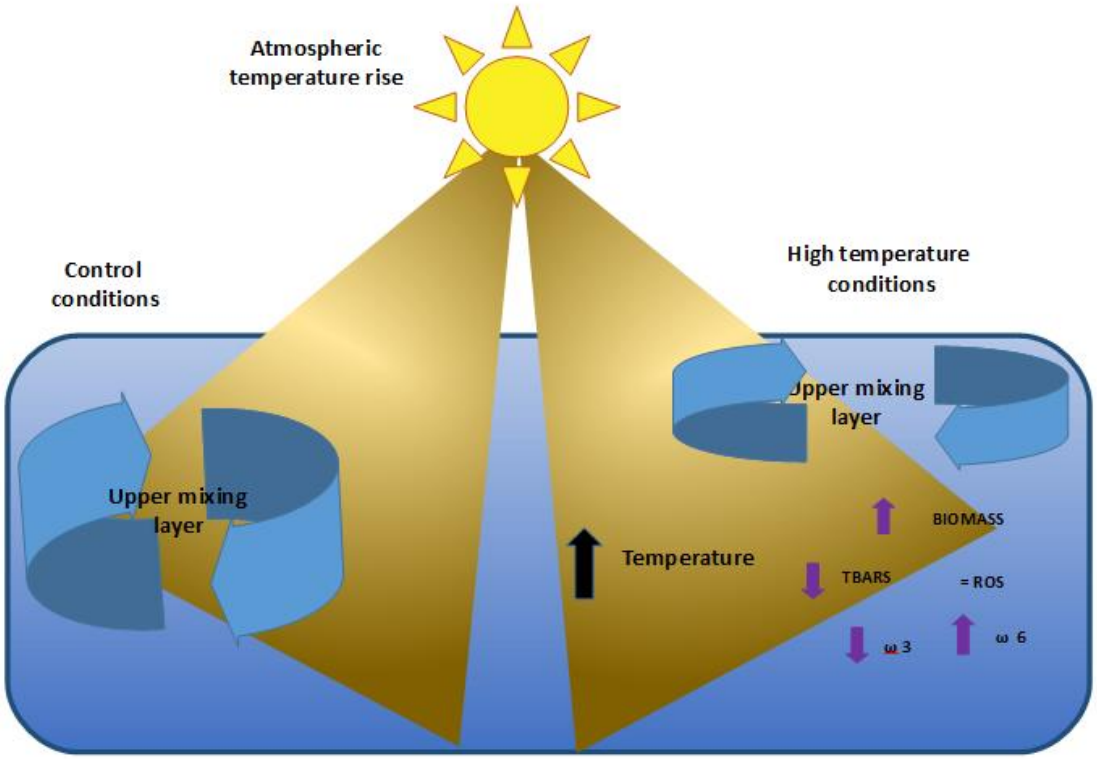

Graphical abstract

Highlights

Cyanobacteria are a group of phototrophic organisms that have a great ecological and economical importance. Variations in environmental factors caused by climate change generate a situation of oxidative damage in *Microcystis aeruginosa* as a direct or indirect consequence. In this study we evaluated the effects of increased temperature on *M. aeruginosa* cultures. Their response was evaluated for biomass, ROS concentration, FA composition and lipid damage.

Significant differences in sensibility of the omega fatty acids to increased temperature and lipid damage resulting in significant changes in growth rate of *M. aeruginosa*.

The originality of our contribution is based on the understanding of relation between lipid damage and differential sensitivity of ω6 as a function of the changes in the fatty acids composition.