

# The effect of environmental factors on fatty acid composition of *Chromera velia* (Chromeridae)

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**Abstract** The effect of light intensity, light spectral quality, temperature and salt concentration on the fatty acid composition of *Chromera velia* was studied. *Chromera velia* is a unicellular, marine, photosynthetic, eukaryotic alga and a close relative of the apicomplexan parasites. *Chromera velia* was able to grow at light intensities between 20 and 450  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , in the temperature range 17–32 °C and at salinities between 0.2 and 1 M NaCl. The cells responded to variations in the growth regime by modifying fatty acid composition: the ratio of fully saturated palmitic acid (C16:0) and five times unsaturated eicosapentaenoic acid (C20:5n–3) was especially prone to variation. Intermediate fatty acids, namely stearic, linoleic and dihomo- $\gamma$ -linolenic acids, changed minimally and were probably not involved in the response to the growth regimes. The highest proportion of eicosapentaenoic acid was observed when the cultures were maintained at 32 °C, at an irradiance of 80  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , provided

by an incandescent light source, under a 12-/12-h day/night photoperiod.

**Keywords** *Chromera velia* · Chromeridae · Fatty acids · Eicosapentaenoic acid · Light · Temperature · Salinity

## Introduction

The fatty acid (FA) composition of algae is highly dependent on environmental conditions such as light, temperature, salinity, pH and nutrient availability (Guschina and Harwood 2006). Increase in saturation with higher photon flux densities (PFDs) is usually observed since the polyunsaturated FAs of membrane polar lipids under high light often become less abundant than neutral storage lipids, which mainly contain saturated FAs (Khotimchenko and Yakovleva 2005). Polyunsaturated fatty acids (PUFAs) are necessary to maintain photosynthetic membrane activity and play an important role in acclimation to growth at low irradiance (Klyachko-Gurvich et al. 1999). Studies on the impact of light quality on carbon allocation are less numerous. It was demonstrated that in *Nannochloropsis* sp., light quality modified lipid productivity (Das et al. 2011) or growth of *Arthrospira* (*Spirulina*) *platensis* (Wang et al. 2007). Temperature has major effect on the types of FA produced by microalgae (Thompson et al. 1992). Variations in temperature might lead to changes in the membrane lipid order and, in the case of elevated temperatures, may cause protein unfolding and denaturation. When the temperature is lowered, a number of changes in membrane lipids have been observed (see Morgan-Kiss et al. 2006) that corresponded with an attempt to maintain the lipid order at physiologically advantageous values. An increase in FA unsaturation is the most common response to the lowering of the growth temperature, as was shown in work by

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Thompson et al. (1992). The increase in the number of double bonds and the shortening of the FA carbon chains are usually associated with a decrease in phase transition temperature and with a decrease in membrane order (Murata and Wada 1995; Nishida and Murata 1996). However, not all the double bonds in a FA molecule have an equivalent impact on membrane physical properties. For example, phosphatidylcholine (PC), esterified on both the sn1 and sn2 positions with stearic acid (18:0/18:0)-PC, has a melting point ( $T_m$ ) (transition temperature from gel to liquid-crystalline phase) of 55 °C. Replacement of the stearic acid at the sn2 position with oleic acid (18:0/18:1)-PC led to a decrease in  $T_m$  to 6.3 °C (Russel 1989). The insertion of a second double bond to form (18:0/18:2)-PC was found to decrease the  $T_m$  to -16 °C. However, the addition of a third double bond forming (18:0/18:3)-PC did not decrease, but increased the  $T_m$  by 3 °C to -13 °C. For comparison,  $T_m$  values for 16:0/16:1- and 16:0/22:6-PC do not differ significantly (-12 and -10 °C, respectively) (Coolbear et al. 1983). These findings suggest that monounsaturated FAs are more effective than PUFAs in decreasing membrane order (making the membrane more fluid), possibly by interfering with the cooperative liquid-to-gel transition.

Cell lipid composition is also influenced by environmental salinity. The alteration of external salinities can influence the internal homeostasis of cells in several ways (Kirst 1989; Erdmann and Hagemann 2001). Firstly, osmotic stress, caused by a flux of water across the semi-permeable cell membrane, can lead to changes in the cellular water potential. Hyperosmotic conditions lead to shrinkage of the plasmalemma (Bisson and Kirst 1995) while, hypoosmosis causes water influx, potentially resulting in an increased turgor pressure, a stress that is better tolerated by algae which possess a rigid cell wall. Secondly, ionic stress, which is caused by passive loss or uptake of inorganic ions, can lead to the disturbance of the hydration sphere around proteins and other macromolecules, affecting their conformation and charge interaction and thus impeding their function (Xiong and Zhu 2002). One of the survival mechanisms of aquatic algae is to change its FA content to protect from osmotic stress during rapid salinity changes, like those that occur in natural environments such as coastal rock pools (Lee et al. 1989).

*Chromera velia*, together with *Vitrella brassicaformis*, belongs to a newly discovered group of unicellular algae, the Chromeridae (Moore et al. 2008, Obornik et al. 2011). Chromerids are included in the red lineage, in terms of plastid origin (Janouskovec et al. 2010), in contrast to most organisms in this evolutionary lineage; however, they do not contain chlorophyll *c*. They are also notable as the closest known relatives of the apicomplexan parasites. *Chromera velia* is unique in its mechanism of photoprotection because it uses photorespiration in addition to thermal energy dissipation via non-photochemical quenching (Quigg et al. 2012). The species also has the ability to synthesize large amounts of

eicosapentaenoic acid (EPA), possibly to a similar level as some members of the Eustigmatophyceae group, such as *Nannochloropsis* sp. (Sukenic and Carmeli 1989), *Nannochloropsis oculata* (Seto et al. 1992) or *Monodus subterraneus* (Cohen 1994). So far, *C. velia* has been reported to contain highly unsaturated structural lipids (Botte et al. 2011) and the synthetic pathway of sterols in *C. velia* has been described (Leblond et al. 2012). The effects of environmental factors on the FA composition of *C. velia* cells have not yet been reported. PUFAs, such as arachidonic acid, together with EPA, are highly abundant components of structural polar glycolipids of the photosynthetic membranes in *C. velia* (Botte et al. 2011); these lipids are very important for chloroplast functionality. Depending on their interaction with the proteins embedded in the membrane, these FA can be classified into three main classes (Palsdottir and Hunte 2004; Hunte 2005): (1) bulk lipids, which show only non-specific interactions with membrane proteins and contribute to the membrane fluidity in thylakoids; (2) annular lipids, which constitute a shell around the membrane bound protein through direct contact with the protein; and (3) integral lipids, which are bound to the interior of proteins, often at the interface between two subunits or between transmembrane  $\alpha$ -helices, for example in the photosystem complexes or the cytochrome b6f complex.

In this study, the question of how the FA composition of *C. velia* changes under different environmental conditions has been addressed. Also, it particularly focused on changes in long-chain highly unsaturated fatty acids, which is an interesting topic in terms of its potential for biotechnological application.

## Materials and methods

### Growth conditions

Stock cultures of *Chromera velia* (strain RM12) were maintained in *f*/2 medium for at least ten generations. The stock cultures grew in 2-L Roux bottles, at 28 °C, at 80  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  provided by incandescent 150 W bulbs (Benlux original, Serbia), and were bubbled with filter-sterilized air at rate of 1 L  $\text{min}^{-1}$ . The cultures were weekly diluted to keep the cell concentration at  $1\text{--}2 \times 10^6$  cells  $\text{mL}^{-1}$ . For every subsequent treatment, 100 mL of the culture was transferred to a 250-mL glass tube and diluted to a concentration of  $\sim 1 \times 10^6$  cells  $\text{mL}^{-1}$ . Prior to treatment, cells were concentrated by centrifugation at  $6000 \times g$ , for 10 min, at 25 °C, and resuspended in fresh medium or, for the experiments on the effect of salinity, in media containing different salt concentrations. The cultures were then subjected to the experimental treatment for 7 days. Initially, in order to identify irradiance and temperature limitations on the growth of

*C. velia*, the growth rates were determined in a matrix of different temperatures and light intensities using the crossed gradient of a light and temperature table (Labio Ltd., Czech Republic, described in Kvidarová and Lukavský 2001). The metallic table (100 × 200 cm) allowed us to create gradients of temperature (in the *x*-axis) and light intensity (in the *y*-axis). Temperatures of 12, 17, 23, 28, 32 and 36 °C and irradiances of 20, 80, 200 and 450 μmol photons m<sup>-2</sup> s<sup>-1</sup> of fluorescent (FL). Cells were transferred to 96 flat bottom well plates. In total, 24 plates were monitored. The optical density (OD) was monitored at 750 and 680 nm every 24 h for 14 days, using the well plate reader (Tecan Sunrise, Tecan Group Ltd., Switzerland). Specific growth rates,  $\mu$ , were determined as the slope of the linear regression of semi-logarithmic plot of OD<sub>680</sub> vs. time, during the exponential phase of the growth. All growth experiments were run in seven replicates.

To test the effect of light intensity and quality on FA composition, *C. velia* cells were grown at a constant temperature of 28 °C. The cells were exposed to six different polychromatic light conditions created by two spectrally different light sources adjusted to three light intensities each (20 μmol photons m<sup>-2</sup> s<sup>-1</sup> = low light, LL; 200 μmol photons m<sup>-2</sup> s<sup>-1</sup> = high light, HL; 80 μmol photons m<sup>-2</sup> s<sup>-1</sup> = control). The difference in spectral quality was obtained using an FL tube (Osram Dulux-L, 950) or incandescent bulbs (IL, Benlux original, 150W). Experiments were also performed using blue, green and red monochromatic LED light sources (peak maxima of ~462, ~514 and ~630 nm, respectively). The irradiance of LED illumination was set to 20 μmol photons m<sup>-2</sup> s<sup>-1</sup>. Light intensities were measured using a calibrated quantum meter (Li-189, Li-Cor, USA) equipped with a 2π quantum sensor. In order to study the effects of temperature, *C. velia* was cultured at 17, 23, 28 and 32 °C, at an irradiance of 80 μmol photons m<sup>-2</sup> s<sup>-1</sup> supplied by an incandescent light source. To study the effect of salinity, cells were resuspended in *f*/2 medium modified in terms of NaCl concentration to obtain 0.2, 0.6, 0.8 and 1 M NaCl; the cultures were maintained on an orbital shaker, at 28 °C and 80 μmol photons m<sup>-2</sup> s<sup>-1</sup> using an incandescent light source. Cultures resuspended in *f*/2 medium containing 0.4 M NaCl were used as control.

### Absorption spectra of whole cells

Whole-cell absorption spectra were measured with a UV500 spectrophotometer (Thermo Spectronics, USA) using an integration sphere. The measurement settings were as follows: the bandwidth was 4 nm, and the data interval was 0.5 nm in the range 350–800 nm. The absorption spectrum of *C. velia* cells is shown in the supplementary material (Fig. S1).

### Emission spectra of light sources

Emission spectra were recorded with an Optical spectrometer (SM 9000, PSI, Czech Republic) and normalized to the

respective maxima in the absorption range of *C. velia* cells. The emission spectra of the light sources used for this study are shown in the supplementary material (Fig. S1).

### Cell harvesting, lipid extraction, derivatization and fatty acid methyl ester analysis

Cells were harvested by centrifugation at 6000×*g* for 10 min at 4 °C. Cells were washed two times with distilled water and then resuspended in 800 μL of distilled water. Two millilitres of methanol and 1 mL of dichloromethane were then added; following this, the suspension was sonicated for 10 min in a cooled sonication bath (Kraintek 6, Kraintek Czech s.r.o., Czech Republic). After sonication, 1 mL of distilled water and 1 mL of dichloromethane were added, and the suspension was briefly vortexed. Following centrifugation at 500×*g* for 10 min at 4 °C, the organic phase was separated. The lower dichloromethane layer was transferred to clean evaporation vials; 2 mL of dichloromethane was added, the mixture was vortexed and centrifuged. The lower phases were pooled and dried on a rotary evaporator. Dried extracts were diluted in dichloromethane/methanol (2:1 *v/v*) at a concentration of 1 mg of lipid extract in 50 μL of solvent and stored at -70 °C until further analysis. Methyl esters of fatty acids (FAME) were prepared following the method described by Kainz et al. (2002). For the analysis, 1 μL of methylated sample was used. Quantitative and qualitative analysis of the FA complement were performed by means of a GC-FID (HRGC 5300 Megaserie, Carlo Erba, Italy) equipped with a flame ionization detector (FID). A TR-FAME column (60 m × 0.32 mm, df 0.25 μm) was used. Helium was used as a carrier gas, at a pressure of 200 kPa. The temperature ramp was the following: the starting temperature was 140 °C; it was increased to 240 °C at rate of 4.5 °C min<sup>-1</sup> and then maintained at 240 °C for 10 min. The injector was kept at 260 °C and the detector at 250 °C. The retention times of FAMES were compared to known standards (Supelco 37 Component FAME Mix; PUFA no. 3 Supelco (from menhaden oil)). The amount of individual fatty acids was calculated using internal standards with a known heptadecanoic acid (C17:0) content, and corrected by multiplying the integrated peak areas by the correction factors of the FID response. The double bond index (DBI) was calculated using the formula:  $DBI = \sum (\% \text{ of fatty acid} \times \text{no. of double bonds})/100$  (Skoczowski et al. 1994).

### Statistics

All experiments were done as independent triplicates and data were shown as mean ± standard deviation.

Significant differences in fatty acid composition and unsaturation (expressed as double bond index) were compared using one-way analysis of variance (ANOVA) followed by the

post hoc test of Holm-Sidak. Significance was based on  $p < 0.05$ . All statistical analysis was performed using SigmaPlot version 12.5.

## Results

### Light intensity and temperature dependence of growth

The growth of *C. velia* was initially followed using the crossed gradient table of temperature and light, at the irradiances of 20, 80, 200 and 450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and at temperatures between 12 and 36 °C. The cells grew well in the 17 to 32 °C range, with the maximum specific growth rate at 28 °C ( $\mu$  in the range of 0.128 to 0.148  $\text{day}^{-1}$ ; Fig. 1). Below and above these temperatures, no growth occurred ( $\mu < 0.0005 \text{ day}^{-1}$ ). Only at 23 °C, the growth rate was substantially lower at 450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (0.017  $\text{day}^{-1}$ ) than at 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (0.109  $\text{day}^{-1}$ ). The 23 °C treatment also resulted in a higher number of tetrad cells (resting stages) than all the other temperature treatments, at all photon flux densities (PFDs). The ratio of tetrad cells to single or dividing cells may be responsible for the observed variation of  $\mu$ .

### Fatty acid composition of *C. velia*

The fatty acid composition of *C. velia* grown in controlled conditions (e.g. 28 °C, at an PFD of 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was as follows: The dominant fatty acids of *C. velia* were the fully saturated palmitic acid (C16:0) and the five times unsaturated 20 carbon atom EPA (C20:5n-3). Less abundant were once unsaturated oleic (C18:1n-9), linoleic (C18:2n-6) and arachidonic acid (C20:4n-6). Minor fatty acids (less than 5% of total fatty

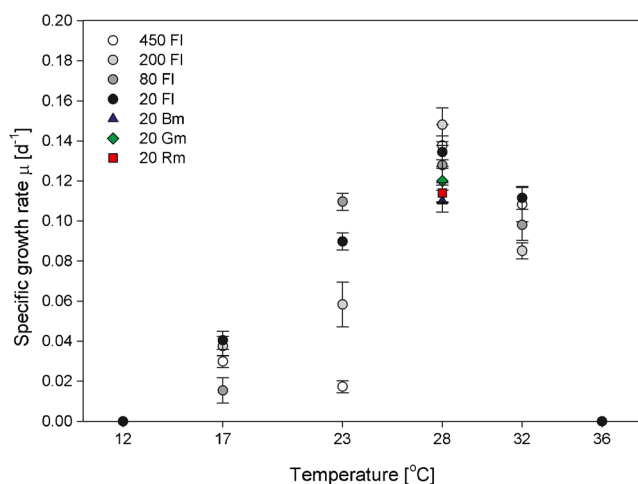
acids in abundance) were fully saturated myristic acid (C14:0) and stearic acid (C18:0), palmitoleic (C16:1), *cis*-vaccenic acid (C18:1n-7),  $\gamma$ -linolenic (C18:3n-6),  $\alpha$ -linolenic (C18:3n-3) and dihomo- $\gamma$ -linolenic (C20:3n-6). EPA and  $\alpha$ -linolenic acid were the only fatty acids of the n-3 family. Under these conditions, >95% of the cells were in the vegetative stage, ~5% of cells were in tetrads and no flagellate cells were observed.

### Dependence of fatty acid composition on light quality

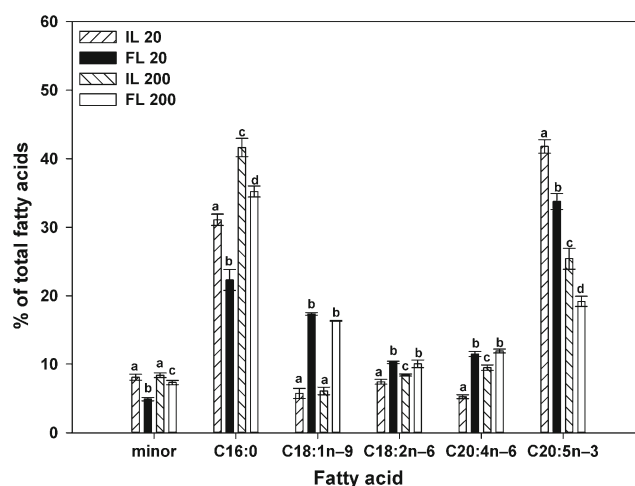
Cells that were grown at higher irradiances of both light sources (IL and FL) showed 10 and 13% higher palmitate levels, respectively, than cells grown at lower irradiance (see Fig. 2). At lower irradiance, EPA levels were higher than at high light, at the expense of palmitate. This difference resulted in a higher (2.6) DBI in comparison to that of high light treated cells (1.9 DBI). At higher irradiances, accumulation of shorter chain FA (C16) was observed, whereas the abundance of C18 FA was unchanged.

### Fatty acid composition under monochromatic light

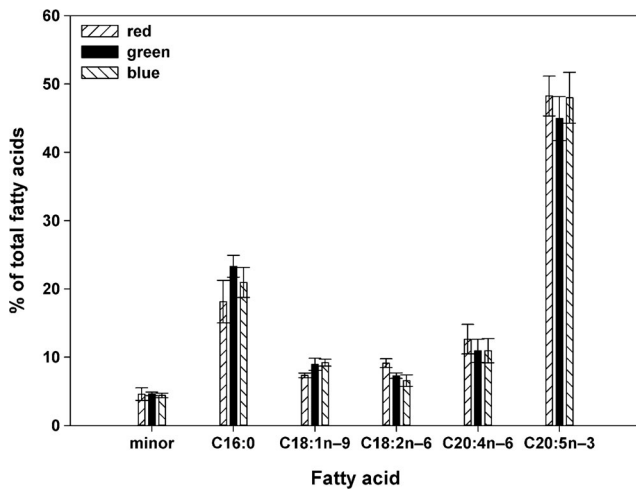
The effect of monochromatic light on FA composition was monitored at three different wavelengths (see Fig. S1 in the supplementary material), at an irradiance of 20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The most abundant FAs were palmitic acid (18 to 23%) and EPA (approx. 48%). No significant difference ( $p > 0.05$ ) was observed between individual FAs or total unsaturation of FAs of cells grown under spectrally different monochromatic lights (Fig. 3, Table S1 in the supplementary material). Cells grown under monochromatic lights were more unsaturated than cells grown at full spectrum IL or FL lights.



**Fig. 1** Effect of temperature and light intensity on growth, expressed as specific growth rate  $\mu$  ( $\text{day}^{-1}$ ), (mean  $\pm$  SD,  $n = 3$ ). Numbers designate light intensity 20 (black circle), 80 (dark grey circle), 200 (light grey circle) and 450 (white circle) in  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . FI fluorescent, Bm blue monochromatic (triangle), Gm green monochromatic (diamond), Rm red monochromatic light (square)



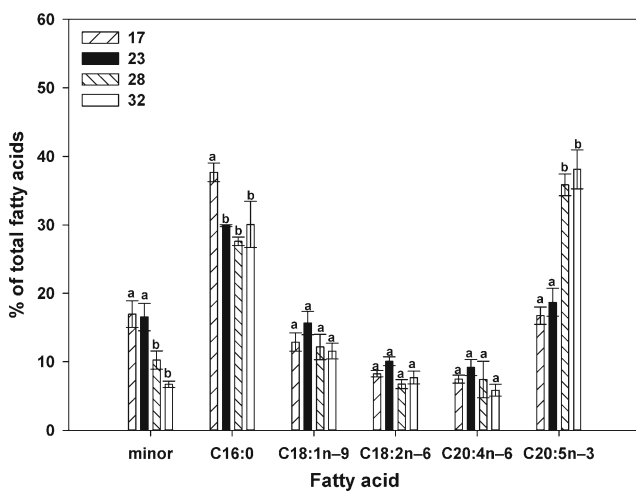
**Fig. 2** Effect of light quantity and quality on fatty acid composition of cells (mean  $\pm$  SD,  $n = 3$ ). Numbers designate light intensity in  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . FL fluorescent, IL incandescent light. Different letters above each column indicate significant differences (one-way ANOVA,  $p < 0.05$ )



**Fig. 3** Effect of blue, green and red light on fatty acid composition of cells (mean ± SD,  $n = 3$ ). No significant differences were observed between different lights (one-way ANOVA,  $p > 0.005$ )

**Influence of growth temperature on fatty acid composition**

The effect of temperature on FA synthesis was monitored at 17, 23, 28 and 32 °C. Minor FAs (C14:0, C16:1, C18:1n-7, C18:3n-3, C20:3n-6) were significantly higher ( $p < 0.05$ ) at 17 and 23 °C mainly due to higher C18:1n-7 content, than at 28 and 32 °C. At 17 °C, palmitic acid was the most abundant FA and constituted 40% of total FAs. Above 23 °C, lower level of palmitate was observed. The amount of EPA was significantly higher ( $p < 0.05$ ) 23 and 32 °C then at 17 and 23 °C. No significant difference in oleic and arachidonic acid was observed ( $p > 0.05$ ) (Fig. 4). At lower temperatures, FAs were less unsaturated, resulting in DBIs of 1.6 at 17 °C and 1.8 at 23 °C. The highest unsaturation of FAs was recorded at 28 or 32 °C, when DBI reached 2.48 and 2.49, respectively. The high temperature treated cells had similar DBI as cells



**Fig. 4** Effect of four different temperatures on the fatty acid composition of cells (mean ± SD,  $n = 3$ ) Numbers designate temperatures in degrees Celsius used. Different letters above each column indicate significant differences (one-way ANOVA,  $p < 0.05$ )

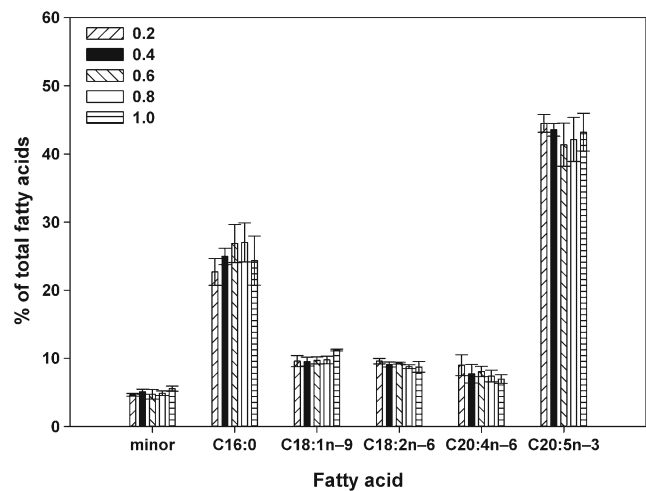
grown at low light, while low temperature treated cells had similar unsaturation as cells grown at high light. Under these growth regimes, lipids showed a lower unsaturation than under the monochromatic light treatments.

**Dependence of fatty acid composition on salinity**

*Chromera velia* grew well in a salinity range between 0.2 and 1.0 M with no significant differences in growth rate (Fig. S2). The minor FAs constituted 5% of total FA. The most abundant FAs were again palmitic acid (25–27%) and EPA (44–42%). In the salinity treatment, there were no significant differences in the contents of all fatty acids ( $p > 0.05$ ) (Fig. 5). Cells maintained high unsaturation of lipids at all salinities (DBI 2.8–2.9). FA unsaturation reached higher levels as a result of the salinity treatment as compared to light source treatments (DBIs 1.9–2.5) or temperature (DBIs 1.8–2.5); however, the highest levels of unsaturation were observed in the cells treated with monochromatic light (DBIs up to 3.24) (Table S1 in the supplementary material).

**Discussion**

Although the literature regarding impacts of environmental variables on algal lipid composition is extensive, very little is known about such impacts on photosynthetic apicomplexans. The response of the lipid complement of *C. velia* to a number of environmental factors, including salinity and temperature, was investigated. The aims were to determine the optimal conditions for potential biotechnological applications and to clarify the ecology and physiology of *C. velia*. Since photosynthesis in *C. velia* shows several



**Fig. 5** Effect of different salinities of growth medium on the fatty acid composition of cells (mean ± SD,  $n = 3$ ). Salinities are expressed in moles per millilitre. No significant differences were observed (one-way ANOVA,  $p > 0.005$ )

unusual features, this study attempted to assess how lipid composition changes as a function of light intensity and quality.

### Light intensity

Our results show that when light intensity increased, the EPA 20:5(n-3) content of *C. velia* decreased (Fig. 2). This is not unusual and similar observations were made for *Nannochloropsis* sp. (Fabregas et al. 2004). In contrast to *Nannochloropsis*, however, the decrease in the EPA content was not accompanied by a decrease in other PUFAs: palmitic acid, for instance, was appreciably more abundant under high light conditions. Overall, a substantially higher level of saturation in *C. velia* FAs was observed, when the cells were exposed to high PFD. This increase in saturation with higher PFD also does not come as a surprise, since the polyunsaturated FAs of membrane polar lipids (such as galactolipids, in which EPA is often a major component in *C. velia*; Botte et al. 2011), under high light, often become less abundant than neutral storage lipids, which mainly contain saturated FAs (Khotimchenko and Yakovleva 2005). The fact that the content of other PUFAs did not decline with exposure to high light is also interesting: Klyachko-Gurvich et al. (1999) suggested that PUFAs are necessary to maintain photosynthetic membrane activity and play an important role in acclimation to growth at low irradiance. This does not seem to be the case for *C. velia* which had increased growth under low light ( $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) compared at high light ( $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) (Quigg et al. 2012).

### Light quality

No major difference in growth rates in *C. velia* grown under different monochromatic lights of low, non-saturating intensity of  $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  was observed (Fig. S2). This is in contrast to observations in other algae (Atta et al. 2013; Hultberg et al. 2014). It is interesting that in the cited works, growth of organisms of the same genus and even of the same species appear to be stimulated by different light quality. The *C. velia* pigment complement is somewhat unusual because of the high content of isofucoxanthin-like carotenoid, which can represent up to 25% of all pigments (Kotabová et al. 2011). Because of this, cells are very effective in absorbing radiations in the green region of the light spectrum. This explains why *C. velia* cultured under light-limiting intensity of monochromatic green light is capable of attaining growth rates that are not very different from those grown under red or blue light of similar intensity.

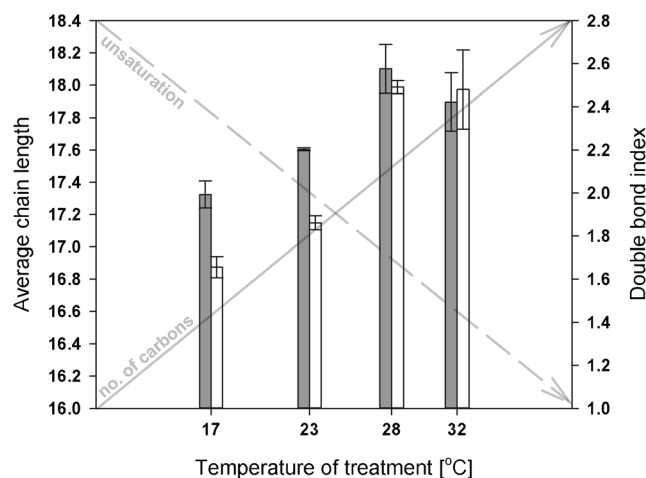
Similarly, the FA profiles of cells grown at different monochromatic lights were not significantly altered. However, cells grown in the monochromatic light regimes had higher EPA content than the cells grown under a low intensity full

spectrum light. In *Chlorella vulgaris*, growth under different light qualities resulted in the same FA profiles, with the exception for green light, where C16:3 and C18:3 increased (Hultberg et al. 2014). Again, the fact that such an effect of green light was not observed in *C. velia* is most likely the consequence of the high absorptivity of *C. velia* cells in this spectral range due to the presence of isofucoxanthin-like carotenoids. The ability of *C. velia* to absorb and effectively utilize green light (and also far-red light, see Kotabová et al. 2014) suggests that it is well equipped to maximize light capture under conditions of overlaying, dense algal communities in coral reefs where chlorophyll containing members of the coral-associated community absorb the blue and red portions of sunlight. In this study, it was shown that *C. velia* isolated from scleractinian corals (Moore et al. 2008; Janouskovec et al. 2012) was capable of maintaining not only high light harvesting potential but also optimal membrane lipid composition under low intensity monochromatic light.

### Temperature

*Chromera velia*, like other poikilotherms, must adapt to variations in external temperatures in order to survive. Although on tropical coral reefs, the water temperature is almost constant and the amplitude of diurnal and annual temperature variations is smaller than those experienced by freshwater algae in the middle latitudes, coral-associated algae are currently subject to stress from increasing temperatures due to global change.

Growth of *C. velia* was strongly dependent on temperature, with the highest growth rate at 28 °C. *Chromera velia* shows an increase both of double bond index and of average chain length when cells were grown at higher temperature (Fig. 6). It has been demonstrated in several studies that high EPA



**Fig. 6** Average chain length (grey bars) and double bond index (white bars) (mean  $\pm$  SD,  $n = 3$ ). Grey arrows designate the common trends in fatty acid unsaturation and chain length upon temperature shift

content occurs under optimal growth conditions (Cohen et al. 1988; Klyachko-Gurvich et al. 1999). *Chromera velia* cells had an increased proportion of EPA (50% at 32 °C). Usui et al. (2012) showed that *Shewanella violacea* had a substantial level of EPA in its membranes, and the membranes appeared highly rigid at its nearly optimal growth temperature. This result countered the generally accepted concept that greater fluidity is a membrane characteristic of microorganisms that inhabit cold environments. Usui et al. (2012) suggested that retaining a fixed level of membrane physical property under a wide range of environmental conditions is more important than simply making the membrane more fluid. EPA, however, because of its five *cis* double bond, is strongly bent and may operate as a rigidifying agent of the membrane, possibly mitigating the effect of the increase in FA unsaturation. Both EPA and docosahexaenoic acid (DHA) are constituents of several types of cellular membranes and are thought to be involved in specific biological functions that require conformational changes of membrane components. The work of Fernandes et al. (2002) indicates that docosahexaenoic acid is minimally influenced by temperature change and that it exhibits great conformational variability. The molecular dynamics simulation studies of aforementioned work on DHA and oleic acid (Fernandes et al. 2002) show that DHA is shorter and more compact than more saturated FAs. The average end-to-end distance of DHA, at 41 °C, is 0.82 nm, somewhat shorter than oleic acid which has a chain length of 1.42 nm. The shortness is even more remarkable when it is considered that it is a 22-atom molecule. The DHA chain conformation has pronounced bends and twists which reduces the separation distance between both ends (a stretched chain would have a greater end-to-end distance). As can be seen in Fernandes et al. (2002), the conformation of DHA forms a right stranded helix along the simulated trajectory, the end-to-end distance of the DHA molecule assumes values ranging from as low as 0.42 nm to as high as 1.7 nm, indicating a great structural flexibility in the molecule. The conformation of EPA is expected to be analogous to that of DHA in the lipid bilayer, although the structure of EPA in the membrane of *C. velia* is still to be elucidated. Acclimation to different temperatures in *C. velia* seems to depend not only on FA composition but also on the distribution of lipid polar head groups within the membranes or adjustment of the protein/lipid ratio (Morgan-Kiss et al. 2006).

### Salinity

The ability to adapt to changing osmotic conditions is a prerequisite for all cellular life. Lee et al. (1989) proposed that high FA content may interfere with the movement of solvent molecules across the cell membrane and more PUFA in higher salinity environments would produce more fluid membranes and thus could assist in the prevention of water loss from the

cell. Renaud and Parry (1994) showed that this indeed occurs in *Isochrysis* sp. and *N. oculata*, but is not true for *Nitzschia frustulum*. In *C. velia*, no significant difference in growth, in FA composition or in the unsaturation of FA over our experimental salinity range was observed. Useful comparisons between our findings and published data are difficult due to the sparsity of studies and the variability in experimental conditions between studies, as well as some contradictory results. In *Isochrysis* sp. grown under elevated salt concentration, the amount of C18 and C20 PUFAs increased (Ben-Amotz et al. 1985); in contrast, Renaud and Parry (1994), also using *Isochrysis* sp. and under the same conditions, reported a decrease in C18:5 and DHA. A lower degree of unsaturation was reported for *Dunaliella* sp., *Nannochloropsis* sp. and *N. frustulum* grown at high NaCl (Renaud and Parry 1994; Xu and Berdall 1997; Hu and Gao 2006). The effect of NaCl concentration on EPA synthesis is species-specific and even strain-specific (Sukenic 1991). Lee et al. (1989) stated that, at 0.7 M NaCl, *Porphyridium purpureum* grew better and had a higher EPA content than at 0.45 M. On the other hand, Pal et al. (2011) showed that *Nannochloropsis salina* contained more EPA at lower salinity of 13 g L<sup>-1</sup> than at 40 g L<sup>-1</sup>. Hu and Gao (2006) observed that, in *Nannochloropsis* sp., an increase of salinity led to a decrease of EPA and linolenic acid, while arachidonic acid was kept constant. Similarly, when *Dunaliella salina* was transferred from 0.4 M up to 4 M NaCl, monounsaturated and saturated FAs increased, with a concomitant decrease of PUFAs (Takagi and Yoshida 2006). A salinity increase led to a higher biomass of *Botryococcus braunii* oleic acid content, while both linolenic and docosaenoic acids decreased (Rao et al. 2007). Observations made from *Phaeodactylum tricorutum* (Yongmanitchai and Ward 1991), where the EPA content was constant in cells grown at 0–5‰ salt and which were slightly decreased when salt increased above 12‰, are in agreement with our results.

In conclusion, our study provides the first detailed analysis of the FA composition of membrane lipids in alga *C. velia*, a representative of the recently discovered division of red algal secondary endosymbionts the Chromeridae. The adaptation of membrane lipids to long-term environmental changes in light intensity, light quality, temperature and salinity was studied. The results show that in several aspects, the response of *C. velia* to these variations is different from standard algal models studied so far. Since *C. velia* accumulates significant amounts of potentially commercially interesting fatty acids, these data help in setting up optimal growth conditions for possible biotechnological applications.

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