

Physiological response to high-light intensities in two *Chlamydomonas* microalgae, one polar and one tropical

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Abstract

This study investigated the effect of different light intensities (120, 650, 725, 850, 1200, 2100, and 3250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on the growth, biomass productivity, photopigment, and macromolecular composition in two *Chlamydomonas* microalgae: the polar *Chlamydomonas* sp. RCC2488 (*malina*) and the model *C. reinhardtii*. Intermediate light intensities (725 and 850 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) significantly favored growth rate and biomass production in *C. malina*, whereas *C. reinhardtii* arrested growth at 850 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and halted growth at 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Total chlorophyll content decreased from 1200 and 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in *C. malina* and *C. reinhardtii*, respectively. No changes in carotenoid content were observed in *C. reinhardtii*, while the content of this photopigment increased in *C. malina* from 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, suggesting higher photoprotection levels. Energy reserve metabolite accumulation was observed in *C. reinhardtii* from 725 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, above 33% of the DCW. In *C. malina*, lipid accumulation (up to 31% of the DCW), mainly TAG, was promoted from 2100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. These results highlight the adaptability to different light intensities of the polar microalga and its potential for biotechnological applications under high light intensities focused on targeted production of biomass, lipids and photopigments.

INTRODUCTION

Polar microalgae that thrive in cold climates have recently received great attention for biotechnological applications since they obtain relatively high biomass productivity at low temperatures and possess high-value compounds [1–4]. These organisms have successfully colonized severe environments in polar regions, such as low-temperature, strong seasonality in light availability, dehydration, and osmotic and nutritional stress for a significant period using several adaptations in their cell morphology, physiology, and metabolism [4–11]. For example, polar microalgae on snow surface are exposed to high solar irradiance and intensity in summer by increasing concentration of photo-protective pigments and active non-photochemical quenching (NPQ) mechanism [4, 9, 12]. Conversely, some of them have gained light-harvesting complex state transition mechanisms between photosystem (PS) I and PS II to maximize absorption of extremely low light that occurs inside or under ice during winter [13]. Such flexible adaptation mechanisms to harsh environments may benefit up-scale cultivation, where productivity heavily relies on robustness of used strains, and production of valuable compounds [14, 15].

The polar microalgae *Chlamydomonas* sp. RCC2488 (strain MALINA FT89.6 PG5, henceforth referred to as *C. malina*) is a potential strain for high biomass and polyunsaturated fatty acid (PUFA) production for biotechnological applications. In our previous work, this polar strain was cultivated in flat panel photobioreactors at light intensities from 70 to 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, showing similarities in growth rate and macromolecular composition while maintaining high PUFA content, demonstrating high adaptability and metabolic flexibility to different light intensities [16].

In this study, to shed light on the knowledge about the photoprotection mechanisms of polar *C. malina* at light intensities beyond 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, cultivations in bubble column photobioreactors at 120, 650, 725, 850, 1200, 2100, and 3250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensities were assessed and compared to a non-high-light tolerant strain such as the tropical model *C. reinhardtii*. Growth parameters, chlorophyll and carotenoid kinetics, and macromolecular composition, including total, types, and profile of lipids, were analyzed in both strains. Our results shows that *C. malina* is a potential strain candidate for solar-powered cultivation or artificial high light intensities in cold climates. However, understanding its high-light intensity tolerance is needed to improve its adaptive capacities for high-value compound production.

MATERIALS AND METHODS

Strains and culture conditions

The polar marine microalga *Chlamydomonas malina* (strain *Chlamydomonas* sp. RCC2488 [MALINA FT89.6 PG5]), is a strain isolated from the Beaufort Sea, within the Arctic Ocean at latitude 69°48 N and longitude 138°26 E [17]. This strain was obtained from the Roscoff Culture Collection, France (RCC). The strain *C. reinhardtii* CC125 was obtained from the Chlamydomonas Resource Center, USA (CRC). Stock cultures were kept in agar plates containing f/2 medium for *C. malina* [18] under phototrophic conditions and Tris-Acetate-Phosphate (TAP) medium for *C. reinhardtii* [19] under mixotrophic conditions. For all experiments, the inoculum was prepared in 250 mL Erlenmeyer shake-flasks (100 rpm) containing 100 mL of f/2 at 4 °C for *C. malina* and TAP at 22 °C for *C. reinhardtii*, with an irradiance of 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and ambient levels of CO₂ for 5 days. For f/2 medium preparation, artificial sea salts (Millipore, USA) adjusted to a salinity of 35 were used.

The inoculum concentration of all experiments for both strains was around 0.1 g of dry cell weight (DCW) per liter. The experiments at different light intensities were conducted in f/2 medium at 4 °C under phototrophic mode for *C. malina* and in TAP medium at 22 °C under mixotrophic mode for *C. reinhardtii*, both for 6 days at 120, 650, 725, 850, 1200, 2100 and 3250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. After the cultivation period, cells were harvested by centrifugation (3000 x g, t = 5 min), washed with 0.5 M ammonium formate, centrifuged again (3000 x g, t = 5 min), and the pellets were stored at -70 °C for further analyses.

Photobioreactor cultivation setup

Cultivations for all light intensity experiments were done using batch cultures for 6 days in bubble column photobioreactors (Dusher, Mexico City, Mexico) according to the design of Hulatt et al. (2017). The arrangement consisted of glass tubes (Proviplab, Mexico City, Mexico) measuring 35 mm internal diameter with 280 mL of working volume, fitted with sealed silicon stoppers and autoclaved as complete units at 121 °C for 20 min. The medium was autoclaved separately in 1 L flasks. Humidified and filtered air (0.2 m, Millex™-FG, PTFE filters, Millipore, USA) for *C. reinhardtii* cultivations and air enriched with 1% CO₂ (v/v) for *C. malina* cultivations was supplied to each photobioreactor at a flow rate of 280 mL min⁻¹ (1 vvm) using a rotameter (LZT, M-6, Lehrmit Instrument Company, China) for mixing. These photobioreactor systems were placed in a temperature-controlled environment chamber, at 4 °C and 22 °C (Dusher, Mexico City, Mexico), fitted with total three white lamps (LED, MG05607, Majgoo, China): two lamps illuminating vertically from the top and the third

lamp illuminating in 45 ° angle from the left side of the chamber. The light intensities (continuous light) applied were: 120, 650, 725, 850, 1200, 2100 and 3250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

Growth measurement

Growth of *C. malina* and *C. reinhardtii* (DCW) was recorded daily using a correlation made with the optical density and the dry weight using the equations:

$$C. \text{ malina: } \text{DCW} = (0.884 * A_{750}) + 0.0117 \text{ Eq. 1}$$

$$C. \text{ reinhardtii: } \text{DCW} = (0.9232 * A_{750}) - 0.0472 \text{ Eq. 2}$$

Where DCW is the dry cell weight ($\text{g}_{\text{DCW}} \text{ L}^{-1}$) and A_{750} is the absorbance measured at 750 nm. Samples of the culture (0.5 – 1 mL) were collected daily to measure the absorbance at 750 nm in a 1 cm micro-cuvette using a spectrophotometer (model GENESYS™ 5, Spectronics Instruments, Thermo Electron Co. USA). The DCW was evaluated gravimetrically by filtrating 5 – 10 mL of culture broth through a pre-dried and pre-weighted 0.45 μm pore size nitrocellulose membrane filter (Merk, Millipore, MA, USA). The membrane containing the biomass was washed three times with 0.5 M ammonium formate prior to drying in an oven at 60 °C for 24 h or until constant weight. The cell density (cell mL^{-1}) was assessed using a hemacytometer and a microscope at a magnification of 40x.

Total chlorophyll and carotenoid determination

Total chlorophyll and carotenoids from *C. malina* and *C. reinhardtii* were extracted with acetone in the absence of light. For this, approximately 0.2 mL of microalgal biomass was resuspended in 0.8 mL of 90% acetone with 0.5 g of glass beads (1 mm, Sigma-Aldrich, USA). The suspension was incubated in the dark for 3 hours at 30 °C with agitation at 300 rpm. After incubation, the sample was sonicated for 3 cycles using a sonicator (model VibraCell™, Sonics & Materials Inc, USA). Each cycle consisted of 10 seconds of cell disruption at 50 Hz followed by 30 seconds of rest, for a total of 40 seconds per cycle. Pigment concentrations were measured in a 1 cm glass micro-cuvette using a UV-Vis diode array spectrophotometer (model GENESYS™ 5, Spectronics Instruments, Thermo Electron Co., USA) at 470, 645, and 663 nm. Chlorophyll *a* and *b*, and carotenoid content were calculated according to Eqs. 3 – 6 [20, 21].

$$[\text{Chl } a] = (A_{663} * 0.0127) - (0.00269 * A_{645}) \quad \text{Eq. 3}$$

$$[\text{Chl } b] = (A_{645} * 0.0229) - (0.00468 * A_{663}) \quad \text{Eq. 4}$$

$$\text{Total chlorophyll} = [\text{Chl } a] + [\text{Chl } b] \quad \text{Eq. 5}$$

$$[\text{Carotenoid}] = [(A_{470} * 1000) - (1.82 * [\text{Chl } a]) - (85.02 * [\text{Chl } b])]/198000 \quad \text{Eq. 6}$$

Where [Chl *a*] and [Chl *b*] are the concentrations of chlorophyll *a* and *b* in mg mL^{-1} , respectively, [carotenoid] is the concentration of total carotenoid in mg mL^{-1} . A_{663} , A_{645} , and A_{470} are the absorbance measured at 663, 645, and 470 nm.

Protein, carbohydrate and lipid analyses

Protein content was determined using the Lowry method [22] with a previous alkaline hydrolysis procedure with 1N NaOH.

For carbohydrate determination, samples were hydrolyzed with HCl, and the resulting suspension was quantified using the phenol-sulphuric acid method [23].

Lipids from *C. malina* were extracted using organic solvents, such as chloroform and methanol, according to previous established protocols [16, 24]. These extracts were separated into neutral lipids (triacylglycerols, TAGs) and polar lipids by using solid-phase extraction as described previously [16, 24]. After separation, the lipids of each fraction were derivatized to fatty acid methyl esters (FAMES) with 5% H_2SO_4 in methanol and heated at 70 °C for 3 h. Phases were separated using a mix of hexane: H_2O (1:1). FAMES from both neutral (TAGs) and polar lipids were identified and quantified by gas chromatography (GC) fitted with a Flame Ionization Detector (Scion 436, Bruker, USA) and an Agilent CP-Wax 52 CB column (Agilent Technologies, USA) using a splitless injector [16, 24]. External Supelco® 37-component standards (Sigma-Aldrich, USA) were used to identify the FAMES.

Statistical analysis

The Saphiro-Wilk test was used to verify the normal distribution of the data, and the Brown-Forsythe test to confirm the homogeneity of variance between treatments. One-way analysis of variance (ANOVA) and post-hoc Tukey's multiple comparison test were used. P values smaller than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Polar microalga *C. malina* exhibited significant tolerance to high light intensities, while *C. reinhardtii* did not grow to light intensities greater than 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$

Polar microalga *C. malina* was able to grow in all light intensities tested, while *C. reinhardtii* halted growth from above 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and even experienced strong photoinhibition at 2100 and 3250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, showing cell whitening (data not shown). For *C. malina*, the highest biomass

concentration of 3.4 g_{DCW} L⁻¹ and overall biomass productivity of 545 mg_{DCW} L⁻¹ day⁻¹ were found at 2100 μmol photons m⁻² s⁻¹, which were similar to the ones obtained at 120, 650, 725, and 850 μmol photons m⁻² s⁻¹ ($p < 0.05$, Figure 1A and E, Table 1). In the case of *C. reinhardtii*, the maximum biomass concentration of 1.8 g_{DCW} L⁻¹ and overall biomass productivity of 281 mg_{DCW} L⁻¹ day⁻¹ were achieved at 725 μmol photons m⁻² s⁻¹, also similar to the ones obtained at 120 and 650 μmol photons m⁻² s⁻¹ ($p < 0.05$, Figure 1B and F, Table 1). In both microalgae strains, the maximum cell density was found at 120 μmol photons m⁻² s⁻¹ ($p < 0.05$, Figure 1C and D, Table 1) with a concentration of 8×10^8 cells mL⁻¹ for *C. malina* and 4.4×10^7 cells mL⁻¹ for *C. reinhardtii*. Previous work had demonstrated the ability of *C. malina* to grow between 70 and 500 μmol photons m⁻² s⁻¹ in a similar configuration of photobioreactors, suggesting that this microalga could grow at higher light intensities due to its natural habitat [16]. Results of the present work went beyond and confirm that *C. malina* is a microalga that can grow at higher than 500 μmol photons m⁻² s⁻¹ of light intensity, without significantly reducing its growth rate (μ_{DCW} , Table 1). This ability may be a result of extreme adaptations to polar regions, where *C. malina* cells experience low/lack light intensities in winter and continuous high light levels in summer. But it appears that the response to different light intensities in polar microalgae is not general [1, 4, 5, 25, 26].

For example, the polar Antarctic microalga *C. priscuii* UWO241 cannot tolerate light intensities higher than 250 μmol photons m⁻² s⁻¹ [25, 26]. Some polar diatoms also exhibited low tolerance to high light irradiance, like the diatoms *Chaetoceros neogracilis* and *Thalassiosira gravida*, which sustained growth up to 400 μmol photons m⁻² s⁻¹, the highest irradiance evaluated by [27]. Other examples include *Nitzschia frigida*, *Fragilariopsis cylindrus* and *Chaetoceros gelidus* which ceased growing beyond 200 μmol photons m⁻² s⁻¹ [27]. As suggested before [16], it would be interesting to study the differences in the photoprotection mechanisms between *C. priscuii* UWO241 and *C. malina* to identify the main components, at the molecular level, that make *C. malina* tolerate very high light intensities in contrast to *C. priscuii*, which, interestingly, is the closest relative to *C. malina* [16].

Other polar microalgae have shown tolerance to intermediate light intensities, like the haptophyte *Phaeocystis antarctica* who was able to grow up to 542 μmol photons m⁻² s⁻¹ at 4°C [28], while the prasinophyte *Micromonas commoda* could reach growth at 750 μmol photons m⁻² s⁻¹ [29].

While plants employ protective strategies to high light intensities such as leaf movement and chloroplast phototranslocation [30], microalgae exhibit fewer characterized mechanisms; among them, energy dissipation as heat, measured as NPQ has been demonstrated [31]. Enhanced secondary carotenoid production, such as astaxanthin, has also been reported in some algae from the genera *Chlamydomonas* and *Chloromonas* [9, 32]. Those photopigments act as a shield of the photosystems against excessive irradiation [30, 33], and specifically, enhanced activity of one of the xanthophyll cycles, the violaxanthin cycle (VAZ cycle), which accumulates the major photoprotective compound zeaxanthin to perform as a protective response [34].

On the other hand, *C. reinhardtii* is a tropical microalga that, in its natural habitat, may not be exposed to very high light intensities; therefore, this microalga may not need efficient photoprotective mechanisms such as *C. malina*. Studies of several strains of *C. reinhardtii* cultivated at high light intensities have shown that this microalga cannot survive beyond 850 μmol photons m⁻² s⁻¹, mainly due to the lack of an efficient NPQ mechanism which involves central roles of proteins violaxanthin de-epoxidase (VDE), light harvesting complex stress-related 3 protein (LHCSR3) and photosystem II protein (PsbS) [35, 36].

Table 1. Kinetic and stoichiometric parameters of the cultivations evaluated in this work.

Light intensity ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	μ_{DCW} (day^{-1})		$\mu_{\text{cell number}}$ (day^{-1})		X_{max} ($\text{g}_{\text{DCW}} \text{ L}^{-1}$)		X_{max} (cell L^{-1})		Q_x ($\text{mg}_{\text{DCW}} \text{ L}^{-1} \text{ day}^{-1}$)		Q_{PROT} ($\text{mg}_{\text{PROT}} \text{ L}^{-1} \text{ day}^{-1}$)		Q_{CARB} ($\text{mg}_{\text{CARB}} \text{ L}^{-1} \text{ day}^{-1}$)		Q_{LIP} ($\text{mg}_{\text{LIP}} \text{ L}^{-1} \text{ day}^{-1}$)		Q_{PI} ($\text{mg}_{\text{PI}} \text{ L}^{-1} \text{ day}^{-1}$)	
	Cma	Cre	Cma	Cre	Cma	Cre	Cma	Cre	Cma	Cre	Cma	Cre	Cma	Cre	Cma	Cre	Cma	Cre
120	0.41 ± 0.04	0.53 ± 0.01	1.17 ± 0.01	0.64 ± 0.04	3.2 ± 0.2	1.7 ± 0.02	8.0×10^8 $\pm 9 \times 10^7$	4.4×10^7 $\pm 1 \times 10^7$	505 ± 29	267 ± 3	68.4 ± 3.8	79.1 ± 2.9	37.7 ± 2.0	41.4 ± 3.9	33.4 ± 0.2	18.6 ± 3.6	23. $\pm 0.$	
650	0.42 ± 0.01	0.44 ± 0.01	1.05 ± 0.02	0.53 ± 0.05	3.3 ± 0.1	1.7 ± 0.1	4.5×10^8 $\pm 5 \times 10^7$	2.0×10^7 $\pm 1 \times 10^6$	535 ± 7	272 ± 16	71.1 ± 5.4	55.7 ± 3.5	36.2 ± 3.7	50.2 ± 4.1	35.8 ± 2.5	42.6 ± 3.7	25. $\pm 0.$	
725	0.41 ± 0.01	0.36 ± 0.03	0.96 ± 0.04	0.51 ± 0.02	3.3 ± 0.04	1.8 ± 0.02	2.8×10^8 $\pm 1 \times 10^8$	1.5×10^7 $\pm 6 \times 10^5$	529 ± 8	281 ± 3	71.1 ± 2.8	55.5 ± 1.6	37.3 ± 3.2	62.1 ± 2.6	36.5 ± 2.2	35.9 ± 5.1	24. $\pm 2.$	
850	0.44 ± 0.05	0.39 ± 0.1	0.95 ± 0.03	0.36 ± 0.02	3.1 ± 0.4	0.6 ± 0.1	2.5×10^8 $\pm 1 \times 10^8$	7×10^6 $\pm 4 \times 10^5$	493 ± 71	89 ± 11	70.8 ± 2.8	33.6 ± 3.0	38.3 ± 2.7	49.6 ± 2.5	36.4 ± 2.4	59.7 ± 4.4	25. $\pm 0.$	
1200	0.47 ± 0.03	ND	1.05 ± 0.05	0.16 ± 0.05	3.4 ± 0.2	0.02 ± 0.002	3.5×10^8 $\pm 6 \times 10^7$	2.5×10^6 $\pm 5 \times 10^5$	545 ± 39	0.0 ± 0	69.1 ± 5.2	ND	37.6 ± 2.8	ND	36.6 ± 3.3	ND	19. $\pm 0.$	
2100	0.44 ± 0.03	ND	0.71 ± 0.02	ND	2.4 ± 0.04	ND	6.4×10^7 $\pm 4 \times 10^6$	ND	390 ± 6	ND	41.2 ± 2.7	ND	46.2 ± 2.8	ND	51.9 ± 3.0	ND	23. $\pm 1.$	
3250	0.37 ± 0.05	ND	0.48 ± 0.04	ND	1.2 ± 0.1	ND	2.3×10^7 $\pm 7 \times 10^6$	ND	192 ± 14	ND	33.1 ± 3.5	ND	53.2 ± 1.8	ND	66.9 ± 1.2	ND	15. $\pm 0.$	

μ_{DCW} , $\mu_{\text{Cell number}}$: dry cell weight or cell number specific growth rate

X_{max} : Maximum cell mas produced

Q_x : Biomass productivity at maximum total cell mas produced

Q_{PROT} , Q_{CARB} , Q_{LIP} , Q_{PUFA} , Q_{TAG} : Protein, carbohydrate, lipid, PUFA or TAG productivity at maximum cell mas produced

Cma: *Chlamydomonas malina*

Cre: *Chlamydomonas reinhardtii*

ND: Not Detecte

At high light intensities, physiological responses were similar in both microalgae

Both microalgae responded similarly to light stress. Nevertheless, *C. malina's* physiological responses were significantly enhanced from 2100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The physiological responses of *C. reinhardtii* were lower in magnitude compared to *C. malina* and from a lower light intensity (650 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

a. Carotenoid synthesis is prioritized over chlorophyll synthesis

Plants and green algae contain chlorophyll *a* and *b* associated with the light-harvesting chlorophyll protein complex (LHC). Chlorophyll *a* is the most abundant pigment in most photosynthetic organisms. It is the main photopigment involved in photosynthesis, acting at the center of the antenna array as the primary electron donor in the photosynthetic electron transport chain [37, 38]. The content of this pigment decreased as light intensity increased, from 2.6 (120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) to 1.1 $\text{mg g}_{\text{DCW}}^{-1}$ (3250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in *C. malina* and from 1.1 (120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) to 0.3 $\text{mg g}_{\text{DCW}}^{-1}$ (850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in *C. reinhardtii* ($p < 0.05$, Figure 2A). Chlorophyll *b*, the second most abundant in plants and green algae, is an accessory pigment that regulates the size of the antenna, and is also essential for the assembly and functioning of most LHC proteins [39, 40], and is responsible for gathering light energy and transfer it to chlorophyll *a* [37, 38]. As light intensity increased, the content of chlorophyll *b* also decreased around 3.8-times in both microalgae ($p < 0.05$, Figure 2B).

In consequence, total chlorophyll content in both microalgae decreased 2.5-times for *C. malina*, and 3.7-times for *C. reinhardtii* ($p < 0.05$, Figure 2C). Several studies also reported a decrease in total chlorophyll content with increased light intensity in cultivations of *Chlorella vulgaris*, *Chlorella sp. Monoraphidium*

dybowskii, and *Pavlova lutheri* [41–44]. Similar phenomena can be observed in plants, which also decrease the size of LHC antenna [45, 46] to avoid photon excess due to high light intensity and consequently, reactive oxygen species (ROS) production [47]. Specifically, the decrease in chlorophyll *a* may reflect unnecessary synthesis of chlorophyll *a*, since the amount of light energy required for growth had already been completely absorbed by the cells; therefore, no more chlorophyll *a* was needed [48]. Another hypothesis could be that oxidative stress may cause decomposition of chloroplasts, which would be unable to synthesize chlorophyll *a*, causing severe damage to microalgal cells [43]. Since chlorophyll *b* is synthesized from chlorophyll *a*, then as chlorophyll *a* decrease, chlorophyll *b* also. Detrimental responses due to low amounts of chlorophyll *b* have been documented, including an inefficient stabilization of most LHC proteins in the thylakoid membranes [49, 50]. Consequently, the LHC proteins decrease partly due to the degradation of unbound LHC apoproteins by proteases [51].

One of the photoprotection mechanisms of microalgae includes a shift in chlorophyll synthesis toward carotenoid synthesis, which are known as powerful antioxidants associated to the photoprotective function of PSII against oxidation caused by high light [52]. In this context, results reported here are in accordance with the photoprotection role of carotenoids, since the content of such pigments increased 3-times in *C. malina* as light intensity increased ($p < 0.05$, Figure 2D). It should be noted that this content did not change in *C. reinhardtii* ($p \geq 0.05$, Figure 2D). This may suggest that one of the most important photoprotection mechanisms of *C. malina* could be the increase in the carotenoid synthesis, which was absent in *C. reinhardtii* and probably one of the causes of its inability to grow at high light intensities. Carotenoid content also increased twice in *Chlorella* sp. L1 and triple in *Monoraphidium dybowskii* Y2 with the increase in light intensity [43].

Carotenoids not only have an antioxidative role but also participate in the quenching of excessive excitation energy in response to high light stress [31, 43]. For example, the carotenoid zeaxanthin is known as an energy quencher by dissipating excess excitation energy as heat in the antenna of PSII, which protects cells from the oxidative stress that occurs under high-light stress [53]. Since zeaxanthin plays a central role in different photoprotective mechanisms in chloroplasts, in future research, we suggest monitoring the levels of this carotenoid when cells are cultivated at high light intensities.

In summary, the chlorophyll to carotenoid shift may indicate a reduced chloroplast photosynthetic activity and increased photoprotective mechanism against light stress.

b. Energy reserve metabolites over protein synthesis were promoted

Microalgae have been reported to grow on various light intensities exhibiting remarkable changes in their gross chemical composition, pigment content, and photosynthetic activity [54]. The highest protein content was found in the treatments from 120 to 1200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *C. malina* (43% of DCW, $p < 0.05$, Figure 3A) and at 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *C. reinhardtii* (47.5% of DCW, $p < 0.05$, Figure 3B).

In *C. malina*, the content of carbohydrate and lipid had no significant differences ($p > 0.05$, Figure 3A) between 120 and 1200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, suggesting that those light intensities did not affect the synthesis of energy reserve metabolites; therefore, the cells may not be under light stress. However, at 2100 and 3250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ cells increased their carbohydrate content by 9% (no significant differences between 2100 and 3250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, $p > 0.05$, Figure 3A), and its lipid content by 11 and 20% ($p < 0.05$, Figure 3A), respectively, clearly indicating light stress. For *C. reinhardtii*, carbohydrate content was similar at 120, 650, and 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (28.7%, $p > 0.05$, Figure 3B) and interestingly only different at 725 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (37.3%, $p > 0.05$, Figure 3B). But it is clear that the lipid content increased by 12.4% at 650 and 725 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (no significant differences among those light intensities, $p > 0.05$, Figure 3B), reaching a maximum content of 35.8% at 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, suggesting light stress at those light conditions. In other algal species cultivated at high light intensities, it has been observed a diminution in the protein content and, in some cases, also in the carbohydrate content, but an increase in the lipid content [43, 55–58]. For example, *Scenedesmus* sp. 11-1 maximized its lipid content at a high light intensity of 400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ [55]. In cultivations of *Nannochloropsis* sp. at a high light intensity of 700 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and a salinity of 40, protein was shifted to lipid synthesis [59].

Both microalgae can accumulate high amounts of carbohydrate (32.0 and 37.3% of DCW for *C. malina* and *C. reinhardtii*, respectively) and lipid (40.2 and 35.8% of DCW for *C. malina* and *C. reinhardtii*, respectively) which can be also noted in their similar growth rate in terms of DCW (0.37 and 0.39 day^{-1} , Table 1) at maximum energy reserve metabolite production conditions. Maximum carbohydrate productivities achieved were 53.2 $\text{mg L}^{-1} \text{ day}^{-1}$ at 3250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 62.1 $\text{mg L}^{-1} \text{ day}^{-1}$ at 725 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in *C. malina* and *C. reinhardtii*, respectively (Table 1). High lipid productivities were also obtained at 3250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *C. malina* (66.9 $\text{mg L}^{-1} \text{ day}^{-1}$) and at 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *C. reinhardtii* (59.7 $\text{mg L}^{-1} \text{ day}^{-1}$) (Table 1). However, these values remain well below those reported for strains recognized as high lipid producers, such as *Nannochloropsis* sp., which achieved 410 $\text{mg L}^{-1} \text{ day}^{-1}$ at 700 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ [59]. This suggests significant potential for further enhancing lipid productivity, particularly in *C. malina*, for biotechnological applications.

c. Polar lipid content was decreased while triacylglycerol synthesis was increased as a light stress indicator

Different light intensities have been reported as an efficient strategy to increase the lipid content in microalgae, altering also the lipid profile [54]. Typically, low-light or optimal-light intensity induces the formation of polar lipids associated with the chloroplast membranes [60–63]. In our previous work with *C. malina*, polar lipids were induced at the highest light intensity tested (500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) [16]. Here, we confirm that the polar lipid fraction is maintained until 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (172 $\text{mg g}_{\text{DCW}}^{-1}$, $p > 0.05$, Figure 4A), probably as a “soft” mechanism to remodel or relocate membrane lipids to cope up to that light intensity, or simply *C. malina* cells rather maintain its membrane fluidity and flexibility at low temperature (4 °C), since apparently there are no light stress from 120 to 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. For *C. reinhardtii*, the polar lipid fraction decreased with increasing light intensity from 99.5 $\text{mg g}_{\text{DCW}}^{-1}$ at 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ to 31.1 $\text{mg g}_{\text{DCW}}^{-1}$ at 850 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ($p < 0.05$, Figure 4B).

On the other hand, excess light intensity promotes oxidative damage of PUFA [54]. This light condition shifts the polar lipid content to an increase in the amount of neutral storage lipids, mainly TAGs [60–63]. Here, highest light intensities (1200, 2100 and 3250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) induced higher content of TAG in *C. malina* (up to 7-times, $p < 0.05$, Figure 4A), while in cultivations of *C. reinhardtii* the same was observed but from 650 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (11-times, $p < 0.05$, Figure 4B). In general, TAGs are primarily synthesized in the presence of light, stored in cytosolic lipid bodies, and then reutilized for polar lipid synthesis in the dark [64]. Therefore, at higher light intensities, TAG was induced in both Chlamydomonas as in the case of other algae cultivated at excess light intensities, such as the filamentous green alga *Cladophora* sp. [62], the red microalga *Tichocarpus crinitus* [61], and the haptophyte *Pavlova lutheri* [65]. TAG production under high-light conditions might serve as a protective mechanism for the cell. Additionally, these neutral lipids may help the cell to regenerate its electron acceptor pool, which may be depleted under high-light stress [66].

d. PUFA content decreased in *C. malina* but enhanced in *C. reinhardtii*

In *C. malina* PUFA content was maintained at a similar concentration, from 120 to 850 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($147.6 \text{ mg}_{\text{PUFA}} \text{ g}_{\text{DCW}}^{-1}$, $p > 0.05$, Figure 4C), then it gradually decreased to a value of $89.8 \text{ mg}_{\text{PUFA}} \text{ g}_{\text{DCW}}^{-1}$ as the light intensity increased to 3250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Under those light conditions, the fatty acid profile in the polar fraction was mainly composed of monounsaturated fatty acid (MUFA) and PUFA, with major content of C16:3, C16:4 *n*-3, C18:3 *n*-6 and *n*-3, and C20:3 *n*-6 and *n*-3 (Table 3). Table 3 shows the fatty acid profile contained in the polar and TAG fractions in *C. reinhardtii*. The lipid profile in the TAG fraction at light intensities from 650 to 850 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was mainly composed of C16:0, C18:1 *n*-9, C18:2 *n*-6, C18:3 *n*-3, and C16:1 *n*-9.

Since the polar *C. malina* maintains its levels of the PUFA C16:4 *n*-3 independently of the cultivation condition (Table 3), it suggests that, besides its crucial role in keeping the fluidity and flexibility of the cellular membrane at low temperatures [67], this fatty acid is involved in reorganizing the thylakoid membrane for photoprotection purposes. The fatty acid C16:4 *n*-3 acyl group is nearly exclusively present in the monogalactosyldiacylglycerol (MGDG) – the most abundant lipid in the thylakoid membrane of the chloroplasts of Chlamydomonas [67–69]. This galactolipid forms inverted hexagonal phases in the thylakoidal membrane and the luminal side of the membrane, strongly increasing the efficiency of de-epoxidation of violaxanthin to yield zeaxanthin [31].

On the contrary, the synthesis of saturated fatty acid (SFA) and MUFA increased from $31.2 \text{ mg}_{\text{SFA}} \text{ g}_{\text{DCW}}^{-1}$ and $40.5 \text{ mg}_{\text{MUFA}} \text{ g}_{\text{DCW}}^{-1}$, at 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to $170.1 \text{ mg}_{\text{SFA}} \text{ g}_{\text{DCW}}^{-1}$ and $143.2 \text{ mg}_{\text{MUFA}} \text{ g}_{\text{DCW}}^{-1}$ at 3250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($p < 0.05$, Figure 4C). This increase in SFA and MUFA was in accordance with the rise in TAGs, which are generally characterized by mainly containing SFA and MUFA [66]. The fatty acid profile in that TAG fraction at the highest light intensity was composed predominantly of C18:0, C16:0, C18:1 *n*-9 and *n*-7, and C16:1 *n*-9 and *n*-7. In *Nannochloropsis* sp., the degree of unsaturation of fatty acids was lower with increasing light irradiance, with a significant decrease in omega-3 fatty acids (29 to 8% of total fatty acids), caused primarily by a reduction in EPA (C20:5 *n*-3) [70].

Some microalgal species have demonstrated a capacity to accumulate high levels of PUFAs as TAGs [71, 72]. Similar findings were observed in *C. reinhardtii*, where the PUFA content increased from $15.4 \text{ mg}_{\text{PUFA}} \text{ g}_{\text{DCW}}^{-1}$ at 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to $85.5 \text{ mg}_{\text{PUFA}} \text{ g}_{\text{DCW}}^{-1}$ at a light intensity of 650 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($p < 0.05$, Figure 4D). The same observations were made for the microalga *Parietochloris incisa* when it accumulated TAGs full of PUFA and stored as chloroplast lipids [71, 73].

It was suggested that PUFA-rich TAGs may play an additional role beyond being an energy storage product in this alga and rather be metabolically active, acting as a reservoir for specific fatty acids [71, 73]. In response to a sudden change in the environmental condition, when the *de novo* synthesis of PUFA may be slower, PUFA-rich TAG may donate specific acyl groups to MGDG and other polar lipids to enable rapid adaptive membrane reorganization [73, 74]. As mentioned earlier, MGDG plays an essential structural role in the thylakoid membrane, especially at excessive light intensities. The presence of this galactolipid is necessary for the efficient de-epoxidation of violaxanthin to zeaxanthin catalyzed by the enzyme VDE in the violaxanthin cycle (VAZ cycle). VDE is regulated depending on the light intensity; thus, when the light intensity is high, this enzyme is activated to synthesize zeaxanthin, a carotenoid known for its contribution to efficiently activate an efficient NPQ [31]. In this mechanism, MGDG plays two main roles; first, it serves as a solubilizing agent for violaxanthin, thus making the substrate accessible for the enzyme VDE. In addition, due to its structure, MGDG molecule forms an inverted hexagonal phase in aqueous surroundings that acts as a shield, together with the VAZ cycle pigment pool, around the main light-harvesting complex of PSII, the LHCII. This shield forms an attraction site for the VDE in the thylakoid membrane where upon its activation, the mechanism of NPQ is induced; therefore, an efficient photoprotection effect can be observed [31]. Taking into consideration the importance of MGDG, the VAZ pigments, the VDE enzyme, and the NPQ, we suggest analyzing these components in *C. malina* and *C. reinhardtii* under high light intensity conditions for future research.

Our results indicate that *C. malina* is a microalga that is metabolically flexible and able to grow at very high light intensities, producing high-value lipids, such as PUFAs and TAGs. Taken together, *C. malina* can be potentially employed for outdoors cultivations, preferentially in cold areas, either in open raceways or closed photobioreactors aiming to obtain high biomass, carotenoids, and lipid productivities.

Table 2. Fatty acid profile of *C. malina* and *C. reinhardtii* cultivated at different light intensities in tubular photobioreactors.

Fatty acid	<i>C. malina</i> (mg _{FAME} g ⁻¹)															<i>C. reinhardtii</i> (mg _{FAME} g ⁻¹)		
	120		650		725		850		1200		2100		3250		120		650	
	μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹		μmol m ⁻² s ⁻¹	
	Polar	TAG	Polar	TAG	Polar	TAG	Polar	TAG	Polar	TAG	Polar	TAG	Polar	TAG	Polar	TAG	Polar	TAG
C14:0	4.8	2.01	4.6	1.6	4.1	1.6	4.5	2.1	3.3	5.7	1.3	15.4	ND	34.2	1.3	1.9	ND	
	±0.4	±0.1	±0.5	±0.2	±0.2	±0.2	±0.3	±0.4	±0.3	±0.2	±0.3	±2		±3.5	±0.3	±0.1		
C16:0	0.7	5	1.2	6.3	0.7	7.1	0.6	6.1	3.7	11.8	1.8	31.8	ND	51.7	20.3	2.9	10.2	
	±0.2	±0.3	±0.2	±0.5	±0.2	±0.7	±0.2	±0.9	±0.7	±1.4	±0.4	±2.6		±3	±1.9	±0.2	±1.0	
C16:1 (<i>n</i> -9)	1.2	2.3	1.4	1.7	1.2	1.7	1.1	2.4	1.2	3.5	1.2	13.1	0.3	33.3	1.5	1.1	ND	
	±0.1	±0.5	±0.2	±0.4	±0.4	±0.4	±0.3	±0.4	±0.2	±0.7	±0.2	±1.7	±0.1	±3	±0.3	±0.1		
C16:1 (<i>n</i> -7)	0.79	0.3	1.3	0.4	0.8	0.4	0.8	0.4	0.8	1.4	0.8	9.5	0.8	18.5	2.0	ND	1.3	
	±0.2	±0.1	±0.3	±0.1	±0.2	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.8	±0.03	±1.2	±0.4		±0.2
C16:2	2.3	1.5	3.4	1.4	2.2	1.2	2.2	1.1	2.4	3.4	2.5	3.4	ND	3.31	1.7	ND	ND	
	±0.3	±0.2	±0.2	±0.3	±0.2	±0.2	±0.3	±0.3	±0.4	±0.5	±0.5	±0.3		±0.1	±0.2			
C16:3	11.9	2.2	16.1	2.0	11.2	2.1	11.8	2.3	7.7	2.2	7.4	12.1	3.1	12.5	3.3	ND	3.1	
	±0.6	±0.4	±3	±0.3	±0.8	±0.4	±1.5	±0.3	±0.2	±0.2	±0.6	±0.5	±0.4	±1.2	±0.4		±0.3	
C16:4	23.1	9.2	25.3	9.4	21.9	9.6	25.2	11.0	12.2	9.7	13.0	19.0	3.3	19.4	15.7	ND	7.3	
	±2.7	±0.9	±1.6	±0.7	±1.6	±0.5	±1.0	±1.0	±1.3	±0.7	±1.3	±1.7	±0.2	±1.9	±1.3		±0.5	
C18:0	0.7	3.3	0.5	4.4	0.7	5.5	0.7	3.2	5.3	14.7	3.3	32.8	ND	64.7	3.5	3.0	1.4	
	±0.1	±0.5	±0.03	±0.5	±0.04	±0.6	±0.04	±0.3	±0.4	±1.3	±0.4	±2.5		±3.2	±0.5	±0.3	±0.3	
C18:1 (<i>n</i> -9)	3.4	5.3	3.2	4.4	3.2	4.6	3.6	5.3	3.5	14.3	3.2	15.0	3.5	33.6	8.3	ND	5.7	
	±0.3	±0.3	±0.2	±0.5	±0.2	0.6	±0.7	±0.4	±0.3	±0.7	±0.2	±1.0	±0.4	±3.2	±1.2		±0.7	
C18:1 (<i>n</i> -7)	3.5	5.2	3.5	5.6	3.4	5.7	3.3	6.2	3.4	7.6	3.4	21.1	3.3	40.7	1.2	ND	1.2	
	±0.5	±0.2	±0.4	±0.5	±0.2	±0.4	±0.3	±0.2	±0.4	±0.5	±0.6	±1.3	±0.4	±2.0	±0.2		±0.2	
C18:2 (<i>n</i> -6)	6.5	4.6	7.4	4.9	6.5	4.8	6.9	5.0	5.9	4.8	5.3	4.5	1.4	4.7	5.5	ND	2.9	
	±0.5	±0.5	±0.6	±0.4	±0.4	±0.5	±0.3	±0.3	±0.3	±0.4	±0.3	±0.4	±0.2	±0.5	±0.3		±0.4	
C18:3 (<i>n</i> -6)	16.7	ND	18.8	ND	18.1	ND	17.3	ND	16.4	ND	15.4	ND	2.7	ND	4.4	ND	2.2	
	±2.1		±0.5		±2.5		±1.0		±0.6		±1.2		±0.6		±0.4		±0.3	
C18:3 (<i>n</i> -3)	15.5	3.6	15.8	4.5	13.2	4.4	14.3	4.3	11.0	4.6	11.0	6.3	1.2	24.1	24.6	3.8	15.8	
	±2.0	±0.6	±1.8	±0.3	±1.0	±0.4	±1.2	±0.4	±0.6	±0.3	±0.6	±0.4	±0.3	±2.4	±1.2	±0.4	±1.7	
C18:4	6.9	ND	5.0	2.1	5.2	ND	7.0	ND	7.5	ND	4.33	ND	ND	ND	2.9	ND	ND	
	±0.8		±0.7	±0.1	±0.3		±0.6		±0.2		±0.25				±0.3			
C20:0	9.3	2.1	8.0	3.0	8.3	1.2	11.1	ND	9.3	2.0	4.8	2.0	ND	11.1	ND	ND	ND	
	±0.4	±0.1	±0.3	±0.3	±0.2	±0.1	±0.5		±0.3	±0.2	±0.8	±0.6		±1.1				
C20:1 (<i>n</i> -9)	6.0	1.3	7.0	1.5	7.2	1.6	6.9	1.8	6.8	1.6	6.2	1.6	1.5	8.0	ND	ND	ND	
	±1.0	±0.6	±1.1	±0.5	±0.9	±0.5	±0.1	±0.7	±0.4	±0.5	±0.7	±0.6	±0.5	±0.7				
C20:2 (<i>n</i> -6)	4.5	ND	5.2	ND	4.8	ND	4.4	ND	4.3	ND	4.8	ND	1.4	ND	ND	ND	ND	
	±0.5		±0.3		±0.5		±0.3		±0.3		±0.3		±0.4					
C20:3 (<i>n</i> -6)	9.8	ND	8.8	ND	9.1	ND	12.1	ND	7.7	ND	7.8	ND	2.7	ND	ND	ND	ND	
	±1.0		±1.0		±0.8		±0.8		±0.6		±0.7		±0.5					
C20:3 (<i>n</i> -3)	11.3	ND	14.9	ND	10.5	ND	11.3	ND	10.6	ND	7.4	ND	1.8	ND	ND	ND	ND	
	±1.5		±0.9		±1.3		±1.5		±0.6		±0.6		±0.3					
C22:6	5.4	ND	6.1	ND	6.2	ND	5.9	ND	3.0	ND	2.9	ND	ND	ND	ND	ND	ND	

(n-6)	±0.5		±0.3		±0.2		±0.1		±0.15		±0.2						
C24:0	5.77	ND	6.1	ND	6.0	ND	6.0	ND	3.4	ND	3.9	ND	ND	ND	ND	ND	ND
	±0.4		±0.1		±0.1		±0.1		±0.7		±0.6						
C24:1	7.6	ND	4.6	ND	4.7	ND	7.6	ND	4.8	ND	3.2	ND	ND	ND	ND	ND	ND
	±0.5		±0.5		±0.4		±0.5		±0.8		±0.3						

CONCLUSIONS

The polar microalga *C. malina* exhibited significant tolerance to high light intensities, while *C. reinhardtii* did not grow to light intensities higher than 850 μmol photons m⁻² s⁻¹. At the highest light intensity that could still sustain growth in each strain, physiological responses were similar in both microalgae: a) carotenoid synthesis was prioritized over chlorophyll synthesis as a probable mechanism of photoprotection, b) energy reserve metabolites accumulation was promoted over protein synthesis as an indicator of light stress, c) the polar lipid content was decreased, and in contrast d) triacylglycerol synthesis was increased to probably fulfill MGDG requirements for efficient VAZ cycle as another photoprotection mechanism. PUFA production from *C. malina* remained stable even at 1200 μmol photons m⁻² s⁻¹, demonstrating that this polar microalga has a flexible and robust metabolism capable of maintaining high PUFA production even under high light exposure levels.

Declarations

DATA AVAILABILITY

Data will be made available on request.

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Contributions

DM-S designed the research that resulted in this manuscript. NAA-R, EP-M and MAC-B collected the data of the cultivation and performed the biochemical composition analysis. MMM and DM-S conducted the pigment analysis. GAS-M and DM-S performed the lipid analysis. NAA-R, EP-M, GAS-M, MMM, AM and DM-S contributed to the manuscript drafting, discussion, critical revision of the article for intellectual content, and financial support. All authors contributed to the article and approved the submitted version.

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ETHICS DECLARATIONS

Conflict of interest

The authors declare no competing interests.

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Figures

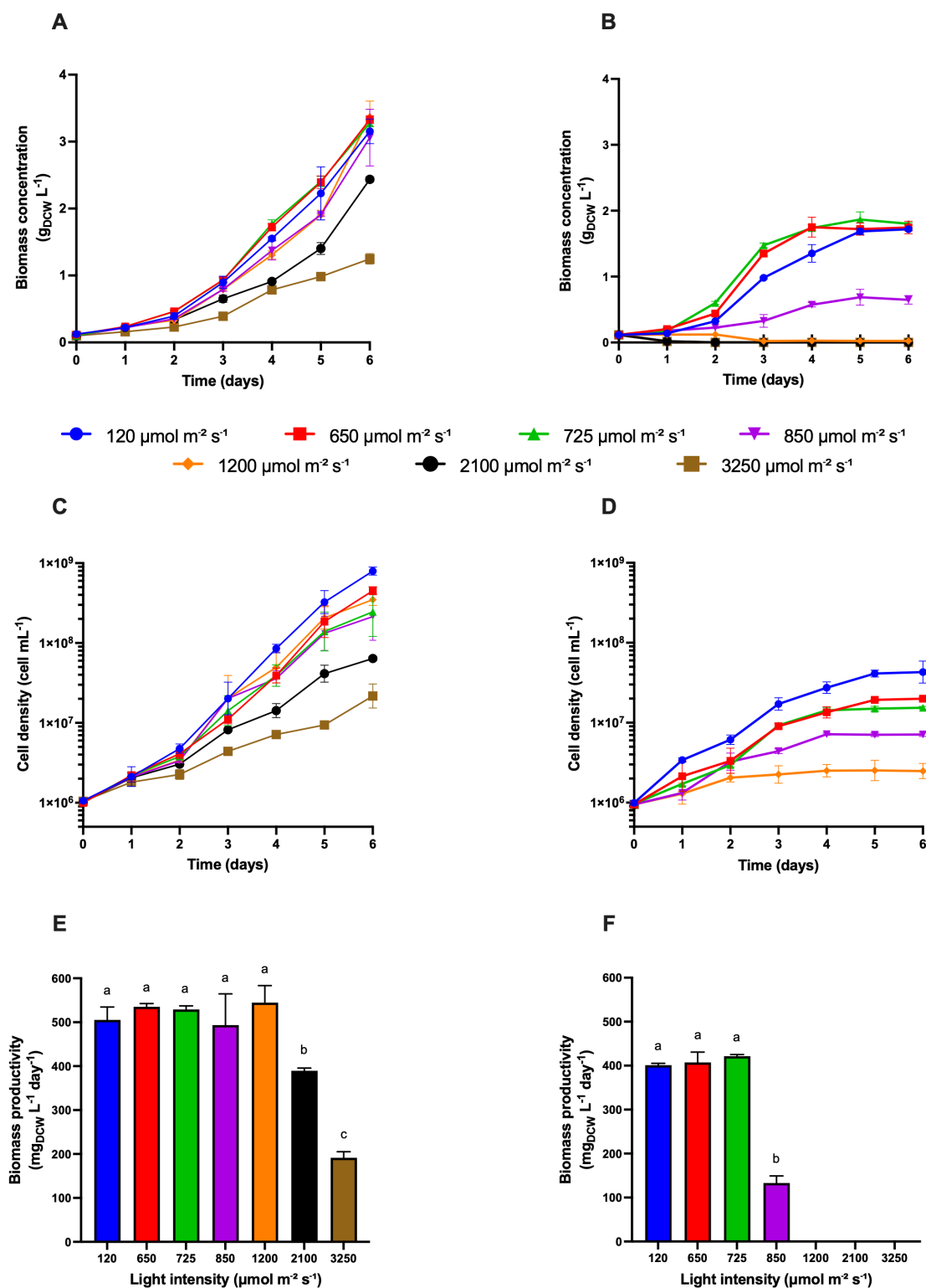


Figure 1

Growth kinetics and biomass productivity. Effect of light intensities on the growth kinetics measured as biomass (A, B) and as cell density (C, D) and the maximum productivities (E, F) of *C. malina* (left side) and *C. reinhardtii* (right side) after 6 days of batch cultivation in tubular photobioreactors. Data are presented as means $\pm$ SD of three independent experiments. Different lowercase letters indicate a significant difference among means of groups (one-way ANOVA with post hoc Tukey HSD test, $p < 0.05$).

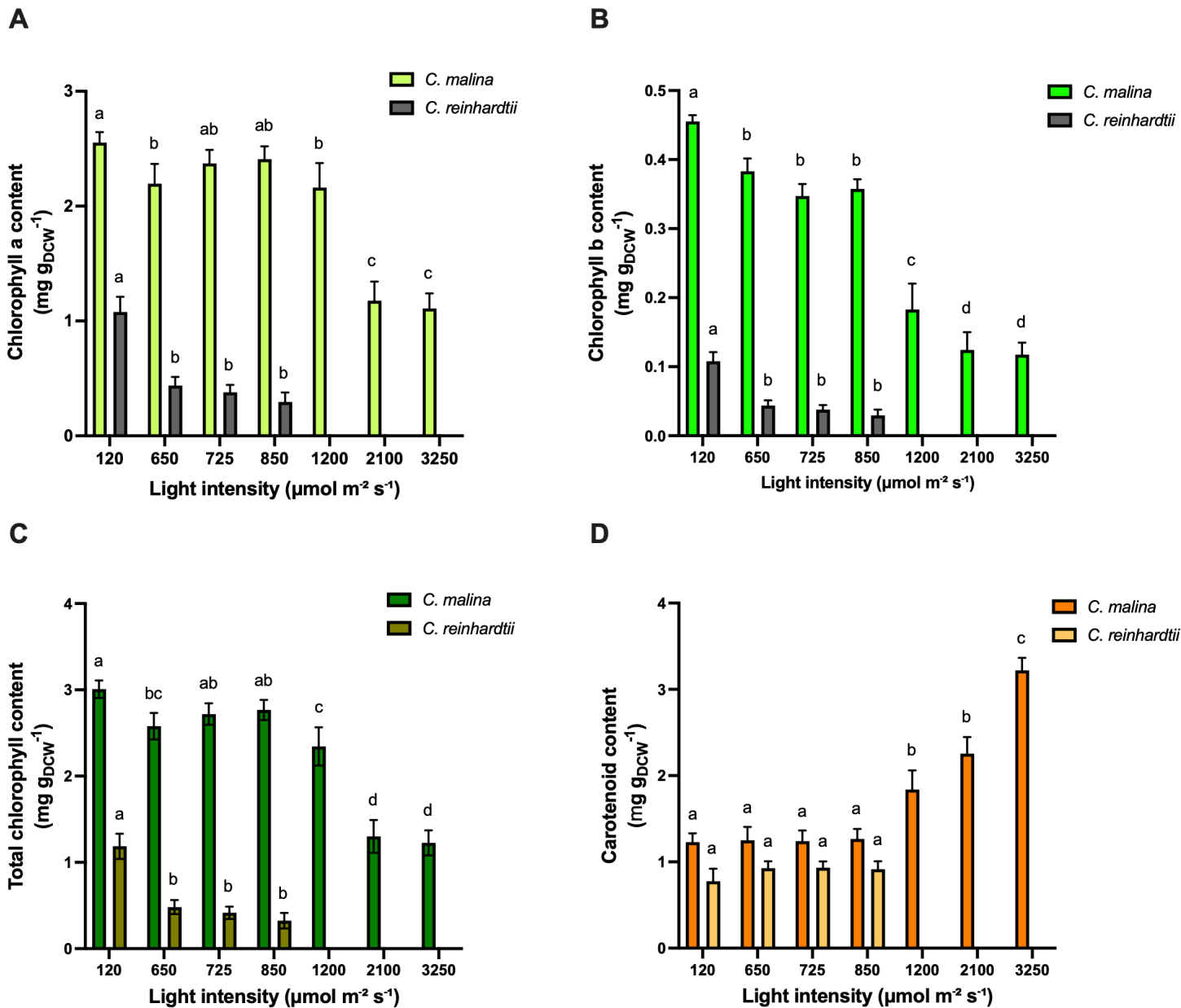


Figure 2

Chlorophyll and carotenoid content. Effect of light intensities on the chlorophyll *a* (A), *b* (B), and total chlorophyll content (C) and total carotenoid content (D) of *C. malina* and *C. reinhardtii* after 6 days of batch cultivation in tubular photobioreactors. Data are presented as means $\pm$ SD of three independent experiments. Different lowercase letters indicate a significant difference among means of groups (one-way ANOVA with post hoc Tukey HSD test, $p < 0.05$).

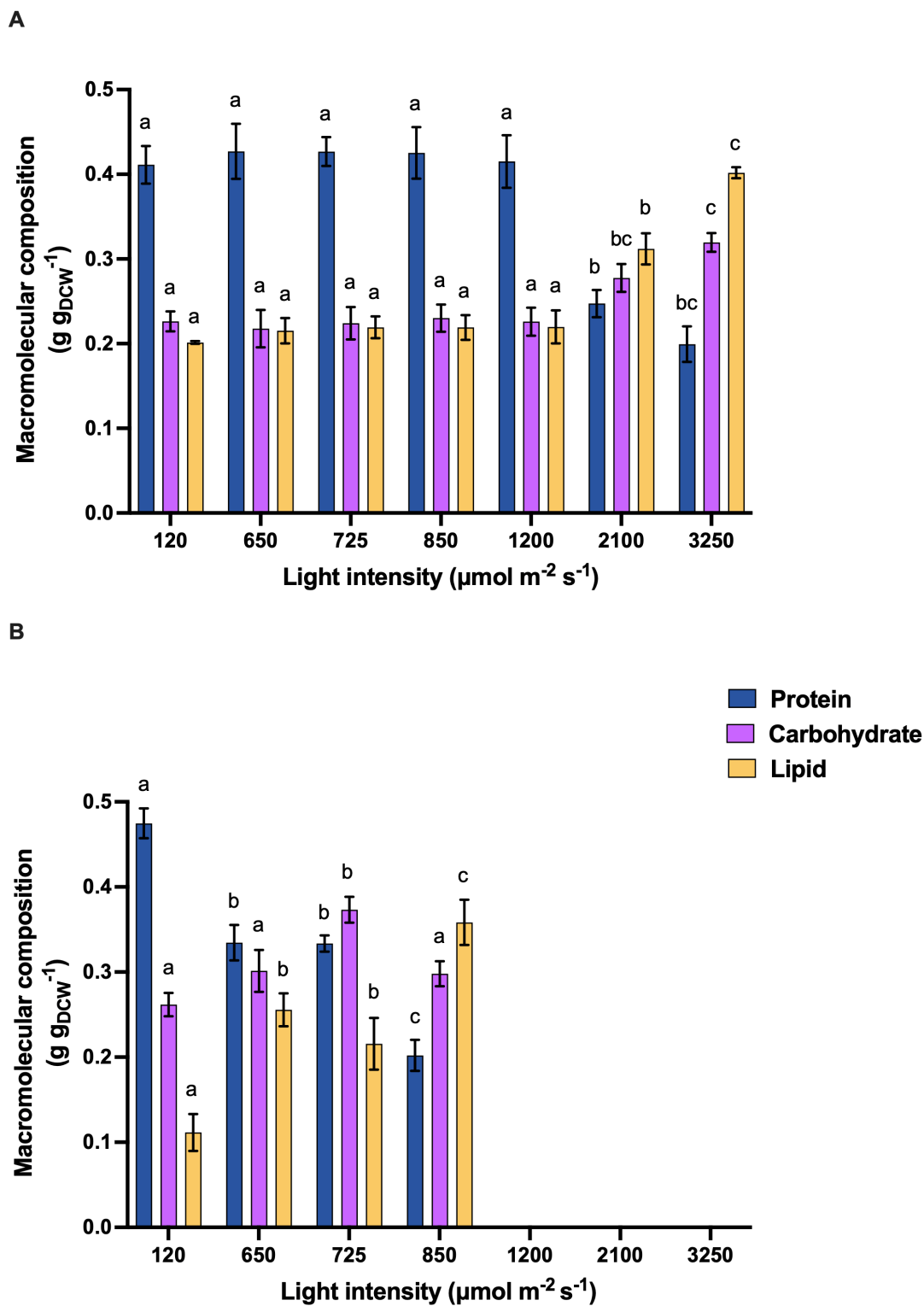


Figure 3

Macromolecular composition. Effect of light intensities on the protein, carbohydrate, and lipid content of *C. malina* (A) and *C. reinhardtii* (B) after 6 days of batch cultivation in tubular photobioreactors. Data are presented as means $\pm$ SD of three independent experiments. Different lowercase letters indicate a significant difference among means of groups (one-way ANOVA with post hoc Tukey HSD test, $p < 0.05$).

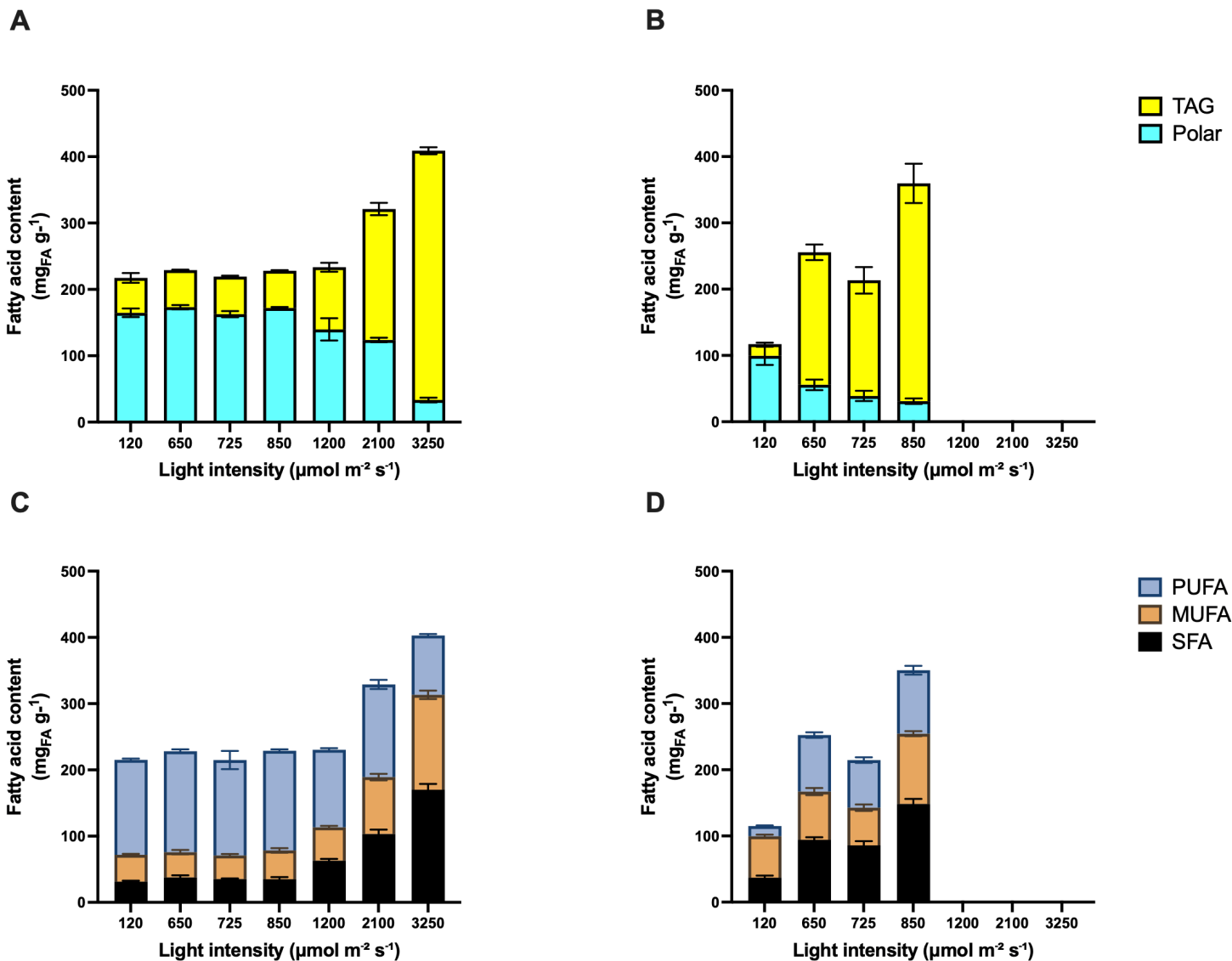


Figure 4

Fatty acid content and types. Effect of light intensities on the fatty acid content in the polar lipids and TAG pools of *C. malina*(A) and *C. reinhardtii* (B), including fatty acids in SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, and PUFA: polyunsaturated fatty acid of *C. malina* (C) and *C. reinhardtii* (D), after 6 days of batch cultivation in tubular photobioreactors. Data are presented as means $\pm$ SD of three independent experiments. Different lowercase letters indicate a significant difference among means of groups (one-way ANOVA with post hoc Tukey HSD test, $p < 0.05$).