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RESEARCH

Analysis of cell growth, photosynthetic behavior and the fatty acid profile in *Tetraselmis subcordiformis* under different lighting scenarios

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Abstract

Tetraselmis has been investigated as a potential source of lipids. This microalga possesses good growth characteristics and can be used to develop viable platforms for fatty acid production. This research aims to evaluate the effect of high photon flux density with light-dark cycles and light wavelength on biomass production and fatty acid profile in *Tetraselmis subcordiformis*. A low light control and treatments with high photon flux density with different light-dark cycles (24:0 h, 12:12 h, 1:1 h, and 15:15 min) and different light wavelength (white, green, red, and blue) were evaluated to determine cell concentration, nutrient consumption, chlorophyll content, photosynthetic yields, lipid content, and fatty acid profile. Significant differences were found in all variables, except for phosphate consumption. High photon flux density promotes cell growth with *T. subcordiformis* reaching biomass productivities of 0.10 g L^{-1} day^{-1} when continuous white light is used. However, no differences were observed in biomass productivities and lipid content for all high photon flux density treatments. On the other hand, red light resulted in higher cell growth, with a productivity of 0.12 g L^{-1} day^{-1}, and the highest lipid content was achieved under white light. There was a significant effect on the fatty acid profile under different light conditions, with palmitic acid, oleic acid, and eicosapentaenoic acid being the most abundant. This study demonstrated that cellular growth and fatty acid profiles in *T. subcordiformis* can be influenced by different lighting schemes in the cultivation.

Keywords *Tetraselmis* · High Light Intensity · Photoperiod · Light spectrum · Fatty acid · Photosynthesis

Introduction

Microalgae are photosynthetic microorganisms that live in a variety of environmental conditions (Lu et al. 2021), they have simple nutritional requirements, do not compete with cultivable land, are capable of fixing carbon dioxide (CO_2), and capture light energy during photosynthetic processes to contribute to the production of oxygen (O_2), biomass conversion, and production of metabolites of interest (Chauton et al. 2015). Part of the biotechnological value of microalgae is based on the compounds they synthesize (Maltsev and Maltseva 2021), including fatty acids, as well as having high nutritional potential as sources of proteins, carbohydrates, other types of lipids, vitamins, and pigments (Schüler et al. 2021). Their synthesis capacity is due to the possibility of obtaining different types of products by changing the cultivation conditions (Lu et al. 2021). Due to this characteristic, improvements can be made in cultivation systems by establishing parameters and controlling growth conditions in the culture medium, temperature, pH, light configuration, carbon source, and agitation, among others (Adarme-Vega et al. 2012). Microalgae are producers of fatty acids, which play a role in metabolic pathways that allow for the formation and conversion of lipids. Their composition determines their properties and uses and their application is being directed towards industries such as bioenergy, food, feed, aquaculture, pharmaceuticals, and cosmetics (Dammak et al. 2016; Maltsev and Maltseva 2021).

Among the reported genera of microalgae for the production of fatty acids is *Tetraselmis*. This marine chlorophyte has the ability to grow under various cultivation conditions and has significant nutritional value without any reported toxicity (Gomez and Perez 2020; Montes-González et al. 2021). They are unicellular and flagellated microorganisms,

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with a cordiform and elliptical cell shape. Their cell size ranges from 10 to 25 µm in length, 7 to 20 µm in width, and 6.5 to 18 µm in thickness. They have four flagella of equal length located at the apical part of the cell, covered with hairs and scales. The cells are often covered by a thin wall called the theca, formed by the extracellular fusion of scales. Each cell typically has a single, large chloroplast that includes an eyespot and a pyrenoid. Notably, these cells exhibit pronounced positive phototaxis (Arora et al. 2013; González et al. 2015; Gomez and Perez 2020).

Currently, *Tetraselmis* species have been used as food for commercial cultivation of fish, mollusks, and crustacean larvae, as well as to complement fertilizers, medicines, and cosmetics. This alga is known for producing a high content of lipids, around 49 % of dry weight, and its composition is influenced by modifications made in the cultivation conditions. Additionally, it has been considered an essential source of fatty acids, especially eicosapentaenoic acid (EPA) (Arora et al. 2013; Gomez and Perez 2020). Several challenges must be overcome to achieve high lipid and cell productivities in microalgae, and new applications are being implemented to increase fatty acid accumulation in biomass, biomass production, and recovery (Chauton et al. 2015; Kothri et al. 2020; Schüler et al. 2021).

Tetraselmis subcordiformis stands out for its lipid content. It has been used for wastewater treatment, starch production, lipid production for biofuels, bioethanol, and biohydrogen, employing different cultivation systems (Wei et al. 2015; Dudek et al. 2022; Pan et al. 2022; Shen et al. 2023). Research conducted on other species of *Tetraselmis*, such as *T. chui*, *T. striata*, *T. suecica*, and *T. tetrathele*, has demonstrated the effect of light intensities ranging from 50 to 2500 µmol photons m^{-2} s^{-1}, as well as different light-dark cycles, on growth, pigment production, nutrient consumption, total lipid content, and fatty acid composition in different cultivation systems (Meseck et al. 2005; Go et al. 2012; Fakhri et al. 2015; Dammak et al. 2016; Schulze et al. 2016; Zevallos Feria 2017; Yusof et al. 2021). Additionally, the quality of the light spectrum has been investigated to determine methods evaluating light wavelengths such as red, blue, violet, white, and green to identify changes in biomass productivity, pigment production, lipid production, and fatty acid profile (Abiusi et al. 2014; Teo et al. 2014; Kim et al. 2017; Diamantopoulou et al. 2021).

These conditions are gaining interest to be applied in different cultivation systems (Arkronrat and Oniam 2019). However, photosynthetic yields, chlorophyll *a* and lipid production, as well as nutrient consumption and fatty acid profile, have not been reported for *T. subcordiformis* when different light schemes such as wavelengths and high light intensity with light-dark cycles are employed. Therefore this study aims to evaluate the effect of high photon flux density with different light-dark cycles and light wavelengths on biomass production and fatty acid accumulation in *T. subcordiformis*.

Materials and methods

Microalgae and culture medium

Tetraselmis subcordiformis was obtained from the culture collection at the Plant Biotechnology and Tissue Culture Laboratory of the University of Antioquia (Colombia). For cell maintenance, 1 L Erlenmeyer flasks were used with a culture volume of 400 mL. The batch cultures were grown in modified HL-29 medium (Bayona et al. 2012), with the following composition (mM): $NaNO_3$ 20.0019, K_2HPO_4 1.0047, $CaCl_2.2H_2O$ 13.2449, $MgSO_4.7H_2O$ 102.1832, KCl 10.0597, NaCl 496.2185, $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$ EDTA 0.1457, $FeSO_4.7H_2O$ 0.1295, H_3BO_3 0.0323, $(NH_4)_6Mo_7O_2.4H_2O$ 0.00002, $ZnSO_4.7H_2O$ 0.0007, $MnCl_2.4H_2O$ 0.0035, $CoCl_2.6H_2O$ 0.0003, $CuSO_4.5H_2O$ 0.0003, V_2O_5 0.0001. Carbon was supplied by daily pulses of 99.9 % pure CO_2 with a feeding rate of 0.4 L min^{-1} through a PTFE membrane with a pore size of 0.45 µm. Lighting was set at 20 µmol photons m^{-2} s^{-1}, with a temperature range of 20-26 °C, with a salinity of 5% and constant agitation of 100 rpm using a DIMAQ orbital shaker.

Experimental design

Cell growth kinetics were assessed over 26 days evaluating different light treatments. Two factors were evaluated, type of light and measurement time. For the type of light, nine treatments were evaluated in two different experiments, consisting of cultivating the algae under controlled (control) conditions in continuous light, compared to an experiment with high photon flux density (considering high light intensity above 1500 µmol photons m^{-2} s^{-1}; Table 1) with different light:dark cycles (24:0 h, 12:12 h, 1:1 h, and 15:15 min), as well as different light wavelengths (white, green, red, and blue), for the specific wavelengths tested (Table 1). The cultures were carried out under the same maintenance conditions (see above). All treatments were evaluated when the culture reached a cell concentration of 1 g L^{-1}. The algae cultures were completely randomized and four repetitions per treatment were conducted in 250 mL containers with 120 mL of culture, resulting in a total of 36 experimental units with independent sampling. Samples (5 mL) were taken at 0, 4, 7, 11, 14, 18, 21, and 26 days to measure cell growth, nutrient consumption, pigments, photosynthetic yields, total lipid content, and fatty acid profile. Based on the *T. subcordiformis* kinetics, the maximum growth rate (μ_{max}, day^{-1}), productivity (P, g L^{-1} day^{-1}), doubling time (Td, day), and

Table 1 Photon Flux Density emitted by each light source and their respective wavelengths

LIGHT SCHEME	POWER (W m^{-2})	WAVELENGTH (nm)	PFD (µmol photons m^{-2} s^{-1})
Control	5.27	420-730	20.00
High photon flux density -Photoperiod			
White	397.97	400- 720	1826.48
Wavelengths			
White	40.65	415-745	187.58
Red	40.25	630	211.97
Blue	40.14	460	153.19
Green	40.22	518	174.19

nutrient consumption rate (NO_3^-, g L^{-1} day^{-1}- PO_4^{3-}, mg L^{-1} day^{-1}) were determined for each condition.

Light characterization

The emission spectrum of the light sources used was measured using an Ocean Optics redtide USB650 spectrometer and the software Ocean View Lite. The following equation was used to calculate the photosynthetic photon flux emitted by the light source $PFD : I_o * \lambda_E/h * c * N_a$, where I_o is the irradiance of the lamp (W m^{-2}), λ_E is the spectral wavelength (m), h is Planck's constant (J s), c is the speed of light (m s^{-1}), and N_a is Avogadro's number (µmol^{-1}). Table 1 shows the photon flux density (PFD) and their respective wavelengths for each treatment. For the wavelength experiment, the lights were adjusted to a power of 40 w m^{-2} and then the emission spectrum was obtained. LED lights were used for each lighting condition, except for the control light where white fluorescent lamps were used. For high-density photon flux light treatments, white light was used.

Growth measurement

Microalgal growth was assessed by measuring absorbance at 540 nm using a plate spectrophotometer (Biotek, power wave XS2).

To determine the dry weight of the biomass, 2 mL of cell culture was taken and deposited into Eppendorf tubes that had been previously dried and weighed. The tubes were then centrifuged at 20,000 × g for 5 min, the supernatant was discarded, and the sample was washed twice with 0.5 M ammonium formate. The samples were then dried at 70 °C for 24 h and the dry weight was quantified using gravimetric analysis. Linear correlations were established between OD_{540} and the corresponding dry weight values obtained ($y = 1.5686x - 0.0956$) ($R^2 = 0.992$).

Nutrient consumption

Samples from each culture were taken and centrifuged at 20,000 × g for 5 min. The supernatant was used for the determination of nitrate (NO_3^-) using the salicylic acid spectrophotometric method of Cataldo et al. (1975), and phosphate (PO_4^{3-}) using the ascorbic acid method of Murphy and Riley (1962).

Chlorophyll *a* fluorescence

Photosynthetic performance was assessed by measuring chlorophyll fluorescence in vivo using a pulse amplitude modulation fluorometer (Junior PAM, Walz GmbH, Germany). Triplicate 300 µL samples were taken the cultures of the control, high photon flux, and wavelength treatments on days 14 and 26 of cultivation. These samples were incubated in darkness for 20 min. This is the minimum estimated time for green microalgae cultures to ensure that the reaction centers of the photosystem are oxidized or 'open' for measurement. For the photosynthetic assessment, the equipment allows the evaluation of 13 irradiance levels (0, 25, 45, 65, 90, 125, 190, 285, 420, 625, 820, 1150, and 1500 µmol photons m^{-2} s^{-1}), each subjected to short pulses of 30 s exposure under high irradiance actinic light (10,000 µmol photons m^{-2} s^{-1}). The maximal quantum yield of photochemical energy conversion in the photosystem II (PSII) was determined as follows: $F_v/F_m = (F_m - F_0)/F_m$, where F_0 is the minimum level of fluorescence emitted due to exposure to measuring light, and F_m is the maximum fluorescence. Fluorescence data were obtained to estimate the effective quantum yield (Y_{II}) using the equation $Y_{II} = (F'_m - F)/F'_m$, where F_m' is the maximum fluorescence in light induced by a saturation pulse and F is the fluorescence under actinic light. Y_{II} was used to calculate the relative electron transport rate of PSII as rETR, using the equation $rETR = Y_{II} \times E_{PAR}$, where E_{PAR} is the incident irradiance of PAR (photosynthetically active radiation, λ=400–700 nm) light. Data were also

obtained to determine the quantum yield of photochemical extinction dependent (Y_{NPQ}) and independent (Y_{NO}) of energy: $Y_{NO} = 1/(NPQ + 1 + qL * ((F_m/F_o) - 1))$ where *NPQ* is non-photochemical fluorescence quenching and *qL* is coefficient of photochemical fluorescence quenching; $Y_{NPQ} = 1 - Y_{II} - Y_{NO}$ (Zhao et al. 2017; Giraldo et al. 2021).

Chlorophyll *a* content

Pignents were exracted from fresh biomass from each culture dimethyl sulfoxide at 60 °C. Samples were incubated in darkness at 60 °C for 15 min with vortex agitation every 3 min. The final extract obtained was centrifuged and used to quantify the pigment content by spectrophotometry at wavelengths of 480 nm, 665 nm, and 649 nm, and the following calculation model expressed in mg g^{-1} biomass was used: Chl*a* = 12.47(OD_{665}) − 3.62(OD_{649}) (Griffiths et al. 2011).

Determination of total lipid content

The total lipid content was estimated gravimetrically. A known amount of previously concentrated and biomass washed with 0.5 M ammonium formate was used. Cell disruption was performed using Misonix ultrasonic liquid processors at an amplitude of 12 dB for 5 min, with 1 min of rest between each disruption time. To the treated biomass, 10 mL of hexane:isopropanol (3:1) was added, and the mixture was homogenized by vortex agitation for 2 min, followed by centrifugation at 20,000 × *g* for 10 min at 4 °C. The organic phase was transferred to a glass flask and three more extractions were performed. The extracts were recovered and dried in a Buchi R215 Labortechnik AG rotary evaporator. The lipid content was calculated by relating the weight of the obtained extract to the residual biomass.

Determination of fatty acid content

The fatty acids were identified by gas chromatography after transesterification of the metabolites using 2 % H_2SO_4 in methanol. A gas chromatograph coupled with a mass spectrometer (Agilent 7890/MSD 5975C) equipped with a low polarity capillary column (HP-5 Agilent) was used for the separation of the compounds of interest. The analyte signals were acquired in SCAN mode. The results were expressed as the average of three independent measurements. Between 10 and 20 mg of sample was weighed and transesterified with 1 mL of the derivatizing reagent. The fatty acid methyl esters were extracted with hexane up to a final volume of 2 mL and were directly injected into the gas chromatograph. Quantification was performed with calibration curves for each analyte and the control used was Supelco 37 Component FAME Mix (reference CRM47885). The results are expressed as mg of fatty acid per g of sample. To express the fatty acid profile, the identification of the signals and compounds was performed by comparison of the fragmentation patterns contained in the NIST17 library. The relative percentage is expressed as the percentage of each fatty acid with respect to the analytes identified as fatty acids.

Statistical analysis

GraphPad Prism 8 was used for data processing. Results were analyzed using one-way and two-way analysis of variance (ANOVA) with the mean ± standard deviation (±SD) of four independent replicates, and two factors, type of light and measurement time. A significance level of $P \leq 0.05$ was considered and Tukey multiple comparison test was used to compare the means. Shapiro-Wilk and Kolmogorov-Smirnov test were used to assess the normal distribution of the data and Brown-Forsythe test was used to evaluate differences between SDs.

Results

Influence of the light scheme on the cell growth of *Tetraselmis subcordiformis*

The cultivation of *T. subcordiformis* was established under high photon flux density conditions with different light and dark cycles in order to evaluate cellular productivity, photosynthetic performance, pigment content and fatty acid profile. The biomass production increased considerably at 1826 µmol photons m^{-2} s^{-1} compared to the control (20 µmol photons m^{-2} s^{-1}) ($P \leq 0.05$). The highest cellular concentrations were observed under high irradiance (Fig 1a). Cultures with the 12:12 h light:dark treatment showed less growth compared to the 1:1 h and 15:15 min cultures. However, no significant differences in growth were found between the two latter treatments ($P=0.79$). Although an increase in growth and biomass production was also observed using continuous light (24:0 h), no significant differences were observed in the yields achieved in high photon flux density treatments, however, but there were significant differences compared to the control ($P \leq 0.05$). These data reflect that regardless of the light and dark cycles used, productivities of around 0.10 ± 0.01 g L^{-1} day^{-1} and a growth rate of 0.07 ± 0.002 day^{-1} were obtained (Table 2). On the other hand, a decrease in

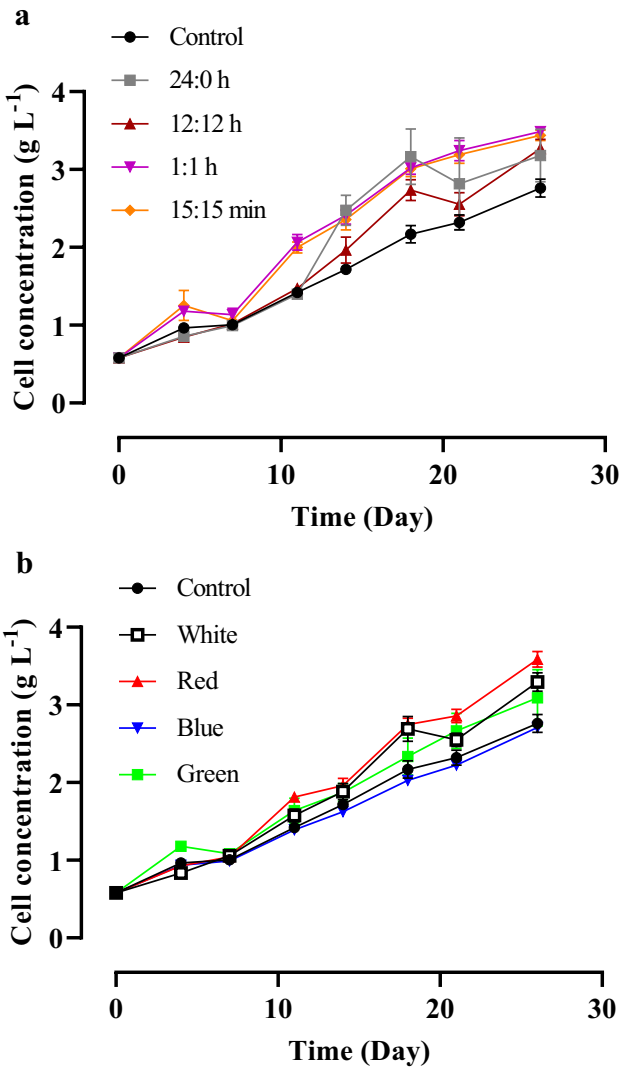

Fig. 1 Growth curve of *T. subcordiformis* under control culture conditions for: (**a**) high photon flux density with light and dark cycles of 24:0 h, 12:12 h, 1:1 h and 15:15 min and (**b**) different light wavelength (white, blue, red and green). Data shown are means ± SD from four independent replicates

cell doubling time was observed when cultured under high irradiance, decreasing from 9.94 day under control conditions to 6.65 day under continuous light 24:0 h, 7.73 day with 12:12 h, 7.80 d with 1:1 h, and 7.29 day with 15:15 min (Table 2).

The effect of light wavelength on biomass production was also determined throughout the cultivation process (Fig 1b). A significant ($P \leq 0.05$) difference was found between the control conditions and the evaluated light wavelengths for the cell growth for every day of cultivation. An increase in the growth was observed under red light, reaching a productivity of 0.12 g L^{-1} day^{-1}, however, a similar productivity of 0.10 g L^{-1} day^{-1} was also obtained using white light (Table 2). On the other hand, cultures in blue and green light showed lower growth, similar to that of the control, compared to white and red light, resulting in longer doubling times (Table 2). The highest cell growth rate (0.09 day-1) was obtained under red and white light conditions , while under a blue and green light regime, a growth rate of 0.07 day^{-1} was obtained. However, even though the culture was subjected to the same power (40 W m^{-2}) at different wavelengths, the PFD values obtained from the light characterization were different. Therefore, the change in the growth of *T. subcordiformis* is not only mediated by the wavelength but also by the light intensity. This is evident under red light, where the culture exhibited greater growth compared to blue light (Fig. 1b), with an intensity of 211.97 µmol photons m^{-2} s^{-1} in red light and 153.38 µmol photons m^{-2} s^{-1} in blue light (Table 1).

When the microalgae were exposed to high photon flux density, nitrate consumption increases compared to control culture conditions ($P \leq 0.05$ (Fig. 2a) reaching a NO_3^- consumption of 0.05 g L^{-1} day^{-1} at all light-dark cycles with high intensity (Table 2). On the other hand, a significant difference was obtained between each treatment for the different wavelengths ($P \leq 0.05$). Figure 2c shows a faster nitrate consumption when using red light, followed by white and green light, and lastly blue and control light, reaching consumption rates ranging from 0.04 g L^{-1} day^{-1} under control conditions to 0.05 g L^{-1} day^{-1} under white and red light. For blue and green light, rates of 0.04 g L^{-1} day^{-1}, were obtained. No significant differences were observed between the control, blue and green light treatments. For all light treatments evaluated in this study, the same PO_4^{3-} consumption rate was observed (P=0.99) (Table 2). This can also be observed in Fig. 2b and d, where the decrease of PO_4^{3-} is similar for all cases.

Photosynthetic yields and chlorophyll *a* production

Comparisons between high photon flux density treatments and he control condition demonstrated a decrease in effective quantum yield of photosystem II (Y_{II}) values ($P \leq 0.02$). A significant difference was found with the control light treatment compared to the other light treatments (24:0 h, 12:12 h, 1:1 h and 15:15 min) (Fig 3a). On the other hand, under different wavelengths an increase in Y_{II} was observed under green and blue light while it decreased under white and red light compared to the control (Fig. 3b). The yields under control conditions, blue light, and green light were not significantly different. A rapid light curve (RLC) was constructed to identify photolimited, photosaturated, and photoinhibited intervals depending on incident irradiance on the culture. The results indicate that rETR was achieved under control light conditions (Fig. 3c and d), significantly

Table 2 Maximum specific growth rate (μ_{max}), doubling time (Td), productivity (P) , and nutrient consumption rate (NO_3^-, g L^{-1} day^{-1}; PO_4^{3-}, mg L^{-1} day^{-1}) in *T. subcordiformis* cultures under control culture conditions, high photon flux density with light and dark cycles of 24:0 h, 12:12 h, 1:1 h and 15:15 min and different light wavelength (white, blue, red and green). Data shown are means ± SD from four independent replicates

LIGHT SCHEME	M_{MAX} (day^{-1})	TD (day)	P (g L^{-1} day^{-1})	NO_3^- (g L^{-1} day^{-1})	PO_4^{3-} (mg L^{-1} day^{-1})
High photon flux density with light and dark cycles					
Control	0.07 ± 0.003^{a}	9.94 ± 0.40^{a}	0.08 ± 0.004^{a}	0.04 ± 0.01^{a}	2.71 ± 0.12^{a}
24:0h	0.11 ± 0.01^{b}	6.65 ± 0.56^{b}	0.10 ± 0.01^{b}	0.05 ± 0.01^{b}	2.72 ± 0.12^{a}
12:12h	0.09 ± 0.01^{c}	7.73 ± 0.44^{c}	0.10 ± 0.005^{b}	0.05 ± 0.01^{b}	2.72 ± 0.12^{a}
1:1h	0.09 ± 0.004^{c}	7.80 ± 0.39^{c}	0.11 ± 0.003^{b}	0.05 ± 0.01^{b}	2.70 ± 0.12^{a}
15:15min	0.10 ± 0.003^{bc}	7.29 ± 0.26^{bc}	0.11 ± 0.001^{b}	0.05 ± 0.01^{b}	2.71 ± 0.12^{a}
Wavelengths					
Control	0.07 ± 0.003^{a}	9.94 ± 0.40^{a}	0.08 ± 0.004^{a}	0.04 ± 0.01^{a}	2.71 ± 0.12^{a}
White	0.09 ± 0.01^{b}	8.18 ± 0.50^{b}	0.10 ± 0.01^{b}	0.05 ± 0.01^{b}	2.72 ± 0.12^{a}
Red	0.09 ± 0.002^{b}	7.87 ± 0.22^{b}	0.12 ± 0.004^{b}	0.05 ± 0.01^{b}	2.71 ± 0.12^{a}
Blue	0.07 ± 0.002^{a}	10.66 ± 0.29^{a}	0.08 ± 0.002^{a}	0.04 ± 0.01^{a}	2.72 ± 0.12^{a}
Green	0.07 ± 0.01^{a}	10.00 ± 0.72^{a}	0.08 ± 0.01^{a}	0.04 ± 0.01^{a}	2.69 ± 0.09^{a}

* Equal letters between each treatment indicate that there are no significant differences

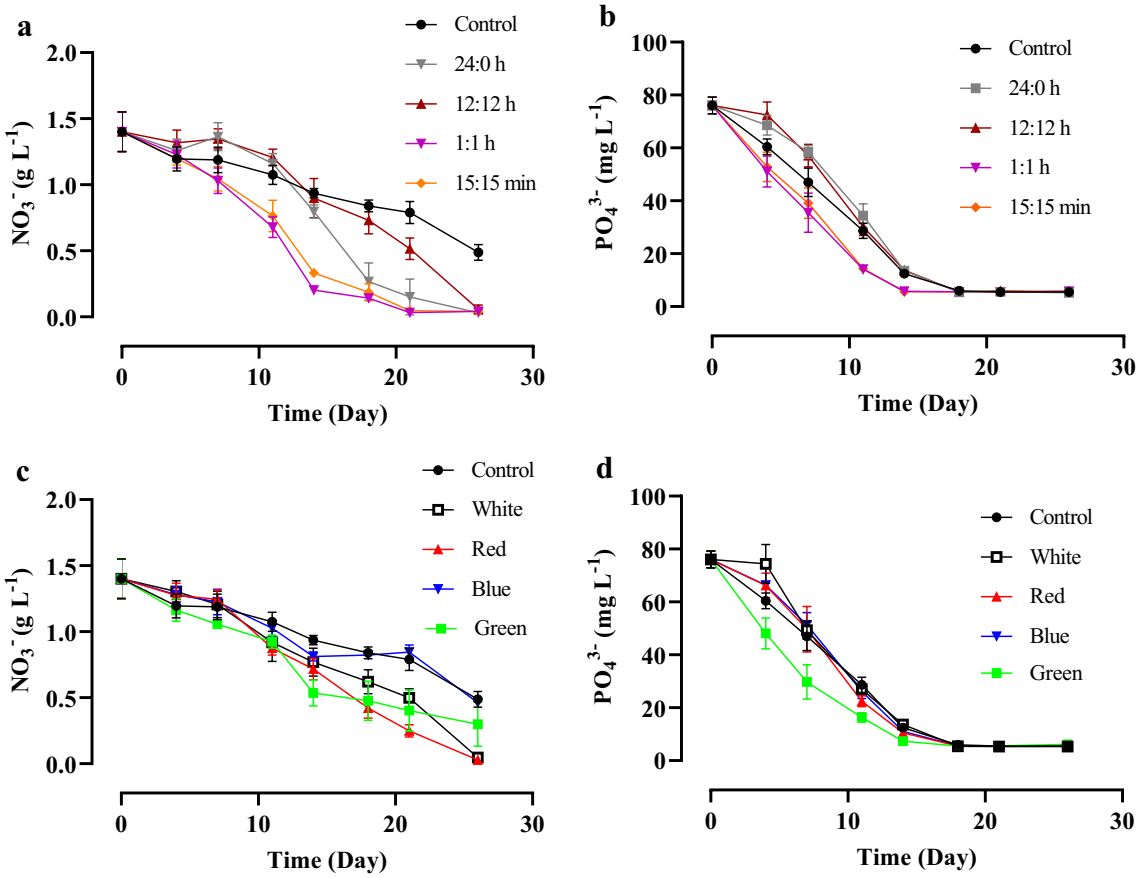

Fig. 2 Nutrient consumption in *T. subcordiformis*. Nitrate (NO_3^-) (**a** and **c**) and phosphate (PO_4^{3-}) (**b** and **d**) under control culture conditions and high photon flux density with light-dark cycles of 24:0 h and 12:12 h, 1:1 h and 15:15 min and different light wavelength (white, blue, red and green). Data shown are means ± SD from four independent replicates

reducing at the higher irradiances (Fig. 3c). However, among the evaluated high-density photon flux light treatments, there was an increase in the performance of *T. subcordiformis* cells when 1:1 h and 15:15 min light conditions were employed ($P \leq 0.05$), as well as under green and blue light conditions (Fig. 3d). *Tetrsaelmis subcordiformis* exhibits characteristics

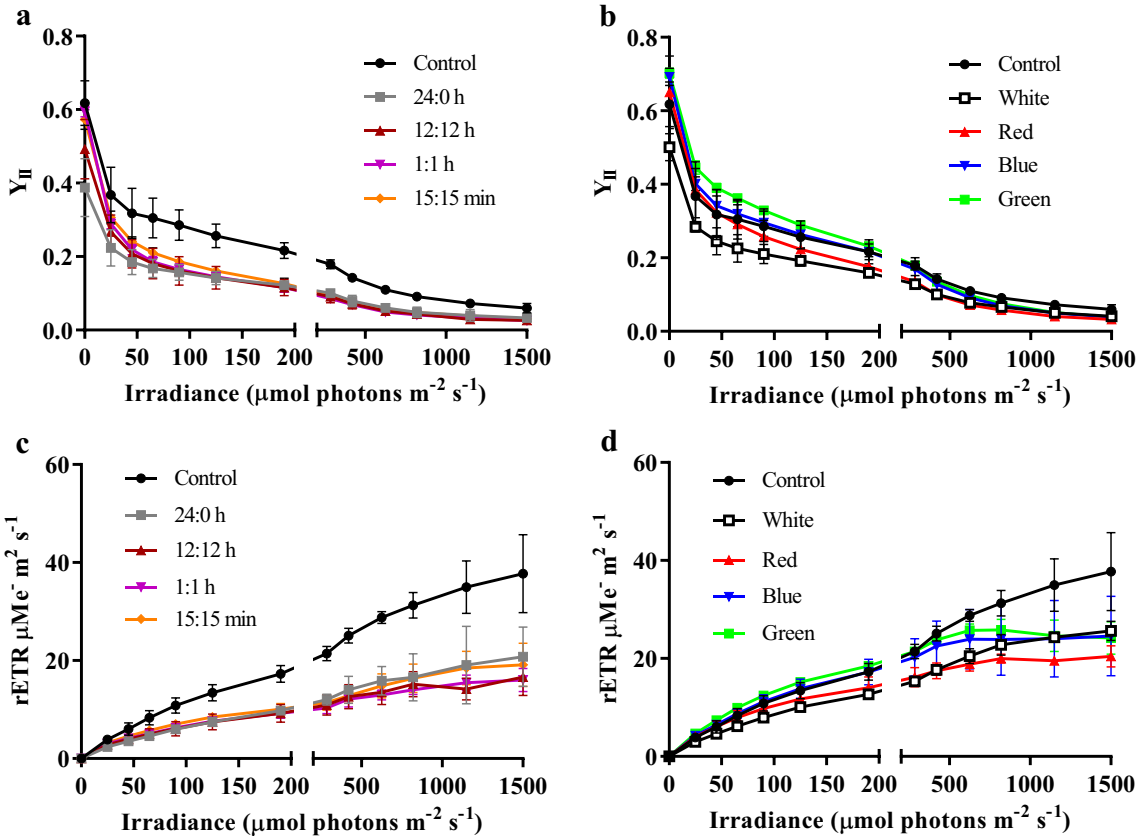

Fig. 3 Effective quantum yield of photosystem II (Y_{II}) and relative electron transfer rate between photosystem II and photosystem I (rETR) of *T. subcordiformis* under control culture conditions and (**a and c**) high photon flux density with light-dark cycles of 24:0 h and 12:12 h, 1:1 h and 15:15 min, and (**b and d**) different light wavelength (white, blue, red and green) on day 14 of the cell growth kinetics. Each point corresponds to the mean ± SD computed from four independent replicates

of photolimitation and photosaturation under the evaluated irradiances, therefore it was found that for both control, high photon flux density, and wavelength treatments, the maximum rETR was observed at 1500 µmol photons m^{-2} s^{-1}, (Fig. 3c and d), indicating that photon flux density did not generate cellular photoinhibition and therefore did not decrease photosynthetic activity in the microalga.

In addition to the previously measured yields, an analysis of the yield of energy-dependent and energy-independent photochemical quenching was performed (Fig. 4). Under different high-intensity light and dark cycles there was an increase in Y_{NO} for longer light cycles (24:0 h and 12:12 h) and a decrease for shorter cycles (1:1 h and 15:15 min) measured at the same culture time (day 14), while for Y_{NPQ}, the opposite effect was observed (Fig. 4a and b). This result shows the switching on and off of different photoprotection mechanisms that occur at the cellular level and the effect that occurs when the culture is subjected to shorter or longer periods of light, where the increase in one mechanism will lead to the drop of the other (Huang et al. 2012; Cinq-Mars and Samson 2021). Higher values of Y_{NO} were reached when green light was used (Fig. 4c), while when white and blue light were used, the yields were much higher for Y_{NPQ} (Fig. 4d). The data obtained so far has allowed us to understand the cellular-level behavior of photosynthetic yield and photoprotection mechanisms under different light conditions. The evaluation of these parameters is essential since they determine how incident light can be used for photosynthetic processes, as well as the activation of various energy dissipation mechanisms that lead to improvements in productivity within the culture.

F_v/F_m of *T. subcordiformis* for all high photon flux density conditions was evaluated on days 14 and 26 (Fig. 5a). Significant differences were obtained between the measurements taken at the two sampling times ($P \leq 0.05$), where a higher F_v/F_m was observed on day 14 compared to day 26 under continuous light, 1:1 h, and 15:15 min light treatments (Fig. 5a). With the continuous light treatment (24:0), there was a clear decrease in F_v/F_m compared to the other treatments, decreasing from 0.38 to 0.23 on the last day of culture, while under 12:12 h conditions, there was an increase from 0.49 to 0.58. No substantial differences were found

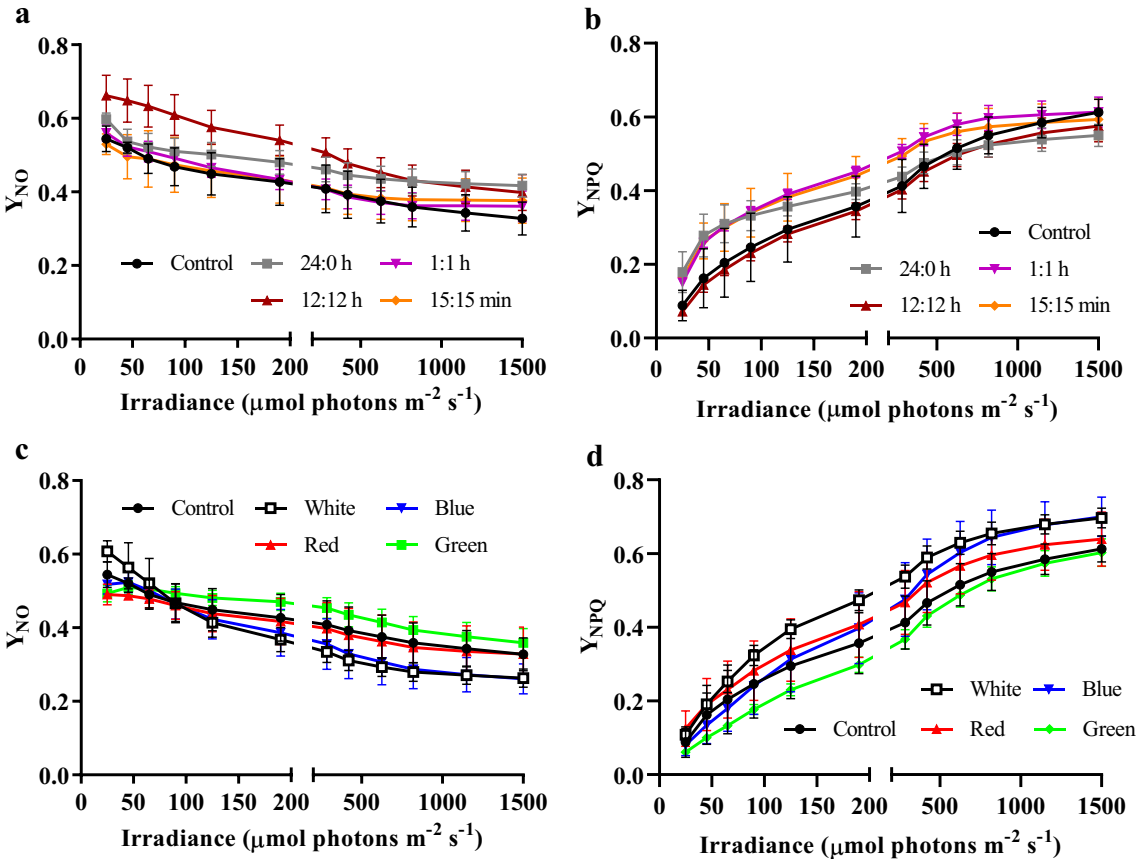

Fig. 4 Quantum yield of photochemical quenching independent (Y_{NO}) and dependent (Y_{NPQ}) of *T. subcordiformis* energy under control culture conditions and (**a and b**) high photon flux density with 24:0 h and 12:12 h light and dark cycles, 1:1 h and 15:15 min, and (**c and d**) different light wavelength (white, blue, red and green) on day 14 of the cell growth kinetics. Each point corresponds to the mean ± SD computed from four independent replicates

between the 1:1 h and 15:15 min light treatments for the two measurement times. On day 14, cells were more receptive to light due to their active exponential growth (Fig. 1a) and greater availability of nutrients in the medium, making them more light-tolerant and less sensitive to photoinhibition (Fig. 3c). In the control culture, F_v/F_m remained above 0.64.

No significant differences were obtained in Chl_a production on day 14 in all high photon flux density treatments. For 24:0 h, 1:1 h, and 15:15 min treatments, a decrease in Chl_a was observed on day 26 compared to day 14, while an increase was observed for the control, and it remained constant for 12:12 h. The F_v/F_m on day 26 compared to day 14 with red, blue, and green lig ht wavelengths did not show significant differences remainng above 0.72 ± 0.01 (Fig. 5c). For the control and under white light, a lower F_v/F_m was observed on day 14 compared to day 26, with values of 0.64 and 0.5, respectively, but the values increased for day 26 (0.70). Under red, blue, and green light, F_v/F_m remained constant on both days. Regarding Chl_a production on day 14, no difference was observed between the control and the evaluated light wavelengths, while on day 26, a decrease in Chl_a was observed when red and blue light were used and the opposite occurred for white, green, and control light, where higher values were obtained for this day.

Lipid content and fatty acid profile

The content of total lipids was evaluated in the final biomass achieved on day 26 for each light condition. Significant differences were found for both highly irradiated cultures, and different light wavelengths in terms of the total lipids were found ($P \leq 0.05$ and $P \leq 0.01$, respectively (Fig. 6). For the high irradiance cultures and under control conditions, significant differences were found ($P \leq 0.05$) (Fig. 6a). The lipid content in the high irradiance treatments was very similar, reaching a lipid productivity of 0.02 ± 0.001 g L^{-1} day^{-1}. However, the 12:12 h cycle had a cell lipid content of 0.179 ± 0.014 g g^{-1}, while an increase was noted in the control samples (0.199 ± 0.003 g g^{-1}). Continuous light treatments 24:0 h, 1:1 h and 15:15 min resulted in lipid contents of 0.156 ± 0.017 g g^{-1}, 0.163 ± 0.007 g g^{-1}, and 0.164 ± 0.004

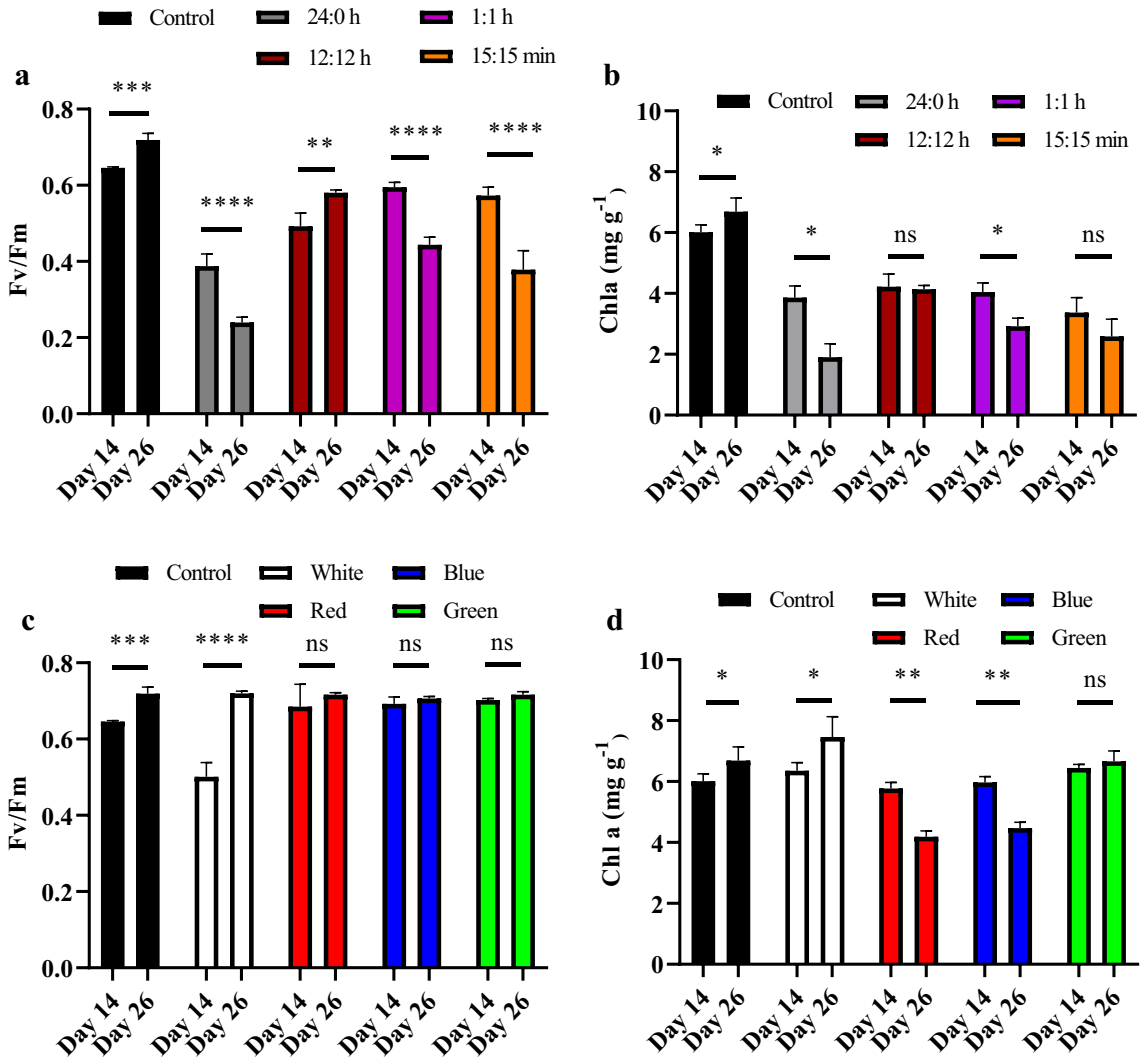

Fig. 5 Optimal and maximum quantum yield of PSII (F_v/F_m) and chlorophyll (Chl a) in *T. subcordiformis* cultivated under control cultivation conditions and (**a and b**) high photon flux density with light and dark cycles of 24:0 h and 12:12 h, 1:1 h and 15: 15 min and (**c and d**) different light wavelength (white, blue, red and green) on day 14 of the growth kinetics. Data shown are means ± SD from four independent replicates

g g^{-1}, respectively. On the other hand, significant differences were achieved with respect to lipid content under white and green light ($P \leq 0.05$) (Fig. 6b). A content of 0.214 ± 0.02 g g^{-1} was observed under white light, 0.189 ± 0.005 g g^{-1} for red light, 0.197 ± 0.01 g g^{-1} for blue light, and 0.180 ± 0.001 g g^{-1} for green light. Significant differences were only obtained between white light and green light, with white light obtaining to higher lipid content compared to the other treatments ($P \leq 0.05$).

Tables 3 and 4 show the percentage of fatty acids obtained from the high photon flux density experiment with different light/dark cycles and light wavelengths, respectively. The fatty acid content showed a predominance of saturated fatty acids, followed by monounsaturated and finally polyunsaturated fatty acids. The total lipid and fatty acid profile (Fig. 6a, Table 3) of *T. subcordiformis* on day 26 showed different cellular responses, allowing allowing the determination of which conditions have a significant effect on the fatty acids. For the 12:12 h treatment, where dark periods were longer, the highest amount of saturated and monounsaturated fatty acids was obtained, while shorter light and dark periods, such as those in the 1:1 h and 15:15 min treatments, resulted in higher percentages of polyunsaturated fatty acids. Oleic and palmitic acid were the predominant fatty acids. For palmitic acid (C16:0), similar amounts were obtained for all high photon flux density treatments, and it was higher than control conditions (Table 3). For oleic acid (C18:1n9c), the 1:1h treatment represented 31.56 ± 0.75 %, while under control conditions it reached 25.24 ± 1.30 %. Among the polyunsaturated fatty acids linoleic acid was higher under control conditions compared to

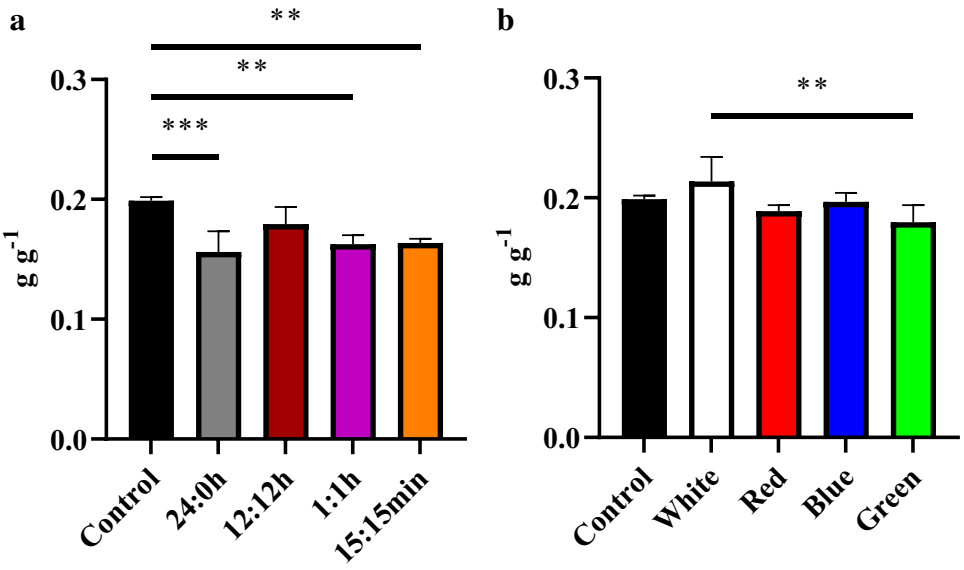

Fig. 6 Total lipid content in *T. subcordiformis* cultivated under control cultivation conditions and (**a**) high photon flux density with light and dark cycles of 24:0 h and 12:12 h, 1:1 h and 15:15 min and (**b**) different light wavelength (white, blue, red and green) on the final day of growth kinetics. Data shown are means ± SD from four independent replicates

Table 3 Fatty acid of *T. subcordiformis* under control conditions, high photon flux density and different light-dark cycles. Data shown are means ± SD from four independent replicates

Fatty acid (%)		Control	24:0 h	12:12 h	1:1 h	15:15 min
Myristic	C14:0	0.37 ± 0.01^{d}	0.64 ± 0.02^{b}	0.49 ± 0.04^{c}	0.72 ± 0.01^{a}	0.77 ± 0.04^{a}
Pentadecyl	C15:0	0.03 ± 0.00^{a}	0.05 ± 0.003^{b}	0.10 ± 0.02^{ac}	0.03 ± 0.00^{ac}	0.04 ± 0.002^{ac}
Palmitic	C16:0	18.71 ± 0.19^{b}	22.56 ± 0.59^{a}	21.22 ± 1.12^{ab}	21.92 ± 0.40^{a}	22.05 ± 0.38^{a}
	---	0.48 ± 0.01^{b}	0.40 ± 0.02^{c}	0.63 ± 0.05^{b}	0.83 ± 0.03^{a}	0.84 ± 0.02^{a}
	---	0.66 ± 0.02^{b}	0.81 ± 0.06^{b}	1.21 ± 0.04^{a}	1.20 ± 0.09^{a}	1.30 ± 0.07^{a}
Palmitoleic	C16:1	1.26 ± 0.05^{a}	1.39 ± 0.01^{ab}	1.16 ± 0.09^{abc}	1.09 ± 0.002^{acd}	1.27 ± 0.03^{ace}
	C16:2	1.04 ± 0.02^{a}	0.75 ± 0.02^{b}	0.72 ± 0.04^{b}	0.77 ± 0.003^{b}	0.72 ± 0.02^{b}
	C16:3	1.98 ± 0.04^{a}	1.40 ± 0.06^{b}	1.37 ± 0.08^{b}	1.19 ± 0.05^{c}	1.04 ± 0.03^{d}
	C16:3	2.00 ± 0.12^{a}	0.98 ± 0.03^{d}	0.82 ± 0.23^{d}	1.46 ± 0.07^{b}	1.41 ± 0.07^{c}
	C16:4	16.84 ± 0.32^{a}	12.26 ± 0.03^{c}	15.05 ± 0.61^{b}	13.40 ± 0.43^{c}	12.99 ± 0.35^{c}
Stearic	C18:0	0.43 ± 0.01^{c}	0.91 ± 0.01^{b}	0.88 ± 0.04^{b}	0.91 ± 0.01^{b}	1.02 ± 0.01^{a}
Oleic	C18:1n9c	25.24 ± 1.30^{d}	26.59 ± 1.84^{cde}	29.66 ± 1.08^{bce}	31.56 ± 0.75^{a}	28.71 ± 0.32^{bcd}
Elaidic	C18:1n9t	5.10 ± 0.25^{a}	6.77 ± 0.14^{a}	4.17 ± 0.06^{b}	3.64 ± 0.34^{b}	4.17 ± 0.20^{b}
Linoleic	C18:2n6c	5.64 ± 0.20^{a}	1.99 ± 0.16^{d}	1.50 ± 0.09^{cd}	2.36 ± 0.32^{bc}	2.36 ± 0.16^{b}
Linolenic	C18:3	0.56 ± 0.03^{a}	1.03 ± 0.02^{b}	0.68 ± 0.12^{ab}	1.02 ± 0.04^{bc}	1.03 ± 0.05^{bc}
Stearidonic	C18:4	10.51 ± 0.06^{a}	4.79 ± 0.13^{d}	6.65 ± 0.26^{b}	4.21 ± 0.20^{e}	5.23 ± 0.19^{c}
	C20:1	1.87 ± 0.06^{a}	1.99 ± 0.12^{a}	1.69 ± 0.09^{b}	1.88 ± 0.13^{a}	1.65 ± 0.14^{ab}
	C20:2	0.22 ± 0.02^{a}	0.13 ± 0.001^{ab}	0.09 ± 0.005^{c}	0.21 ± 0.008^{a}	0.12 ± 0.004^{b}
Arachidonic	C20:4	1.06 ± 0.02^{d}	2.67 ± 0.04^{a}	1.79 ± 0.03^{c}	2.87 ± 0.10^{ab}	2.93 ± 0.02^{b}
	C20:4	0.34 ± 0.04^{c}	0.39 ± 0.018^{bc}	0.36 ± 0.03^{c}	0.50 ± 0.03^{a}	0.46 ± 0.04^{ab}
Eicosapentaenoic	C20:5	6.46 ± 0.09^{d}	10.23 ± 0.20^{a}	8.69 ± 0.13^{c}	8.66 ± 0.06^{c}	9.59 ± 0.17^{b}
	C24:0	0.09 ± 0.003^{c}	0.32 ± 0.03^{b}	0.42 ± 0.01^{a}	0.38 ± 0.004^{b}	0.37 ± 0.004^{b}

* Equal letters between each treatment indicate that there are no significant differences

high photon flux density treatments, while the highest amount recorded for eicosapentaenoic acid (C20:5) was obtained in continuous light treatments (24:0 h), followed by shorter light and dark cycles (15:15 min), representing 10.23 ± 0.20 % and 9.59 ± 0.17 %. However, when a lower light intensity (control) and longer dark cycle (12:12 h) were used, a substantial decrease in EPA content was observed, representing amounts of 6.46 ± 0.09 % and 8.69 ± 0.13 %, respectively.

Significant differences in the fatty acid profile under different light wavelengths ($P \leq 0.05$) were observed. The main

Table 4 Fatty acid of *T. subcordiformis* under control conditions and different light wavelength. Data shown are means ± SD from four independent replicates

Fatty acid (%)		Control	White	Red	Blue	Green
Myristic	C14:0	0.37 ± 0.01^{b}	0.37 ± 0.04^{b}	0.43 ± 0.02^{a}	0.38 ± 0.006^{b}	0.20 ± 0.007^{c}
Pentadecyl	C15:0	0.03 ± 0.00^{b}	0.03 ± 0.001^{b}	0.03 ± 0.001^{b}	0.04 ± 0.003^{a}	0.02 ± 0.002^{c}
Palmitic	C16:0	18.71 ± 0.19^{a}	17.41 ± 0.51^{ab}	19.52 ± 0.39^{ac}	18.90 ± 0.12^{ac}	18.36 ± 0.18^{b}
	---	0.48 ± 0.01^{c}	0.52 ± 0.02^{bc}	0.61 ± 0.02^{a}	0.53 ± 0.007^{b}	0.59 ± 0.005^{a}
	---	0.66 ± 0.02^{b}	0.90 ± 0.02^{a}	0.65 ± 0.03^{bc}	0.61 ± 0.02^{cd}	0.58 ± 0.03^{d}
Palmitoleic	C16:1	1.26 ± 0.05^{b}	1.03 ± 0.07^{ce}	1.01 ± 0.06^{de}	1.14 ± 0.01^{bcd}	1.62 ± 0.02^{a}
	C16:2	1.04 ± 0.02^{d}	0.83 ± 0.07^{d}	1.16 ± 0.01^{c}	1.21 ± 0.004^{b}	1.56 ± 0.05^{a}
	C16:3	2.00 ± 0.121^{c}	1.27 ± 0.11^{d}	2.09 ± 0.19^{ab}	2.32 ± 0.12^{ab}	2.43 ± 0.08^{a}
	C16:3	1.98 ± 0.04^{a}	2.12 ± 0.15^{a}	1.94 ± 0.04^{b}	2.03 ± 0.02^{a}	1.96 ± 0.06^{a}
	C16:4	16.84 ± 0.32^{b}	19.64 ± 0.72^{a}	15.56 ± 0.51^{c}	15.98 ± 0.11^{bc}	15.33 ± 0.12^{c}
Stearic	C18:0	0.43 ± 0.006^{b}	0.41 ± 0.02^{b}	0.40 ± 0.02^{b}	0.41 ± 0.02^{b}	0.47 ± 0.003^{a}
Oleic	C18:1n9c	25.24 ± 1.30^{a}	26.34 ± 0.45^{ab}	28.95 ± 0.35^{ac}	26.87 ± 0.19^{abc}	23.79 ± 1.82^{abc}
Elaidic	C18:1n9t	5.10 ± 0.25^{a}	4.55 ± 0.07^{ab}	4.74 ± 0.31^{bc}	4.70 ± 0.13^{abc}	5.45 ± 0.17^{a}
Linoleic	C18:2n6c	5.64 ± 0.20^{b}	2.99 ± 0.16^{d}	3.21 ± 0.44^{d}	4.27 ± 0.22^{c}	8.44 ± 0.51^{a}
Linolenic	C18:3	0.56 ± 0.03^{c}	0.42 ± 0.01^{d}	0.71 ± 0.03^{a}	0.67 ± 0.007^{ab}	0.67 ± 0.03^{b}
Stearidonic	C18:4	10.51 ± 0.06^{b}	11.71 ± 0.23^{a}	8.13 ± 0.43^{d}	9.50 ± 0.06^{cd}	9.26 ± 0.25^{c}
	C20:1	1.87 ± 0.06^{b}	1.62 ± 0.03^{d}	1.76 ± 0.01^{bc}	1.76 ± 0.06^{c}	2.07 ± 0.06^{a}
	C20:2	0.22 ± 0.02^{a}	0.14 ± 0.00^{ab}	0.15 ± 0.004^{ac}	0.23 ± 0.003^{ad}	0.20 ± 0.03^{abcd}
Arachidonic	C20:4	1.06 ± 0.02^{c}	1.03 ± 0.03^{c}	1.60 ± 0.02^{a}	1.10 ± 0.009^{b}	1.27 ± 0.05^{b}
	C20:4	0.34 ± 0.04^{a}	0.28 ± 0.01^{a}	0.30 ± 0.03^{ab}	0.39 ± 0.003^{ab}	0.32 ± 0.02^{ab}
Eicosapentaenoic	C20:5	6.46 ± 0.09^{d}	7.41 ± 0.07^{a}	7.12 ± 0.08^{b}	6.88 ± 0.03^{c}	6.43 ± 0.10^{d}
	C24:0	0.09 ± 0.003^{c}	0.15 ± 0.01^{b}	0.17 ± 0.01^{a}	0.16 ± 0.004^{ab}	0.19 ± 0.02^{a}

* Equal letters between each treatment indicate that there are no significant differences

fatty acids in all treatments were palmitic acid (C16:0), followed by oleic acid (C18:1) and eicosapentaenoic acid (EPA, C20:5). However, each fatty acid varied depending on the cultivation light color. For instance, oleic acid was 28.95 ± 0.35 % of total fatty acids under red light, followed by white and blue light with 26.34 ± 0.45 % and 26.87 ± 0.19 %, respectively. Under control conditions and green light, it was 25.24 ± 1.30 % and 23.79 ± 1.82 %, respectively. Significant differences were only found between treatments with white and red light and between red and blue light (Table 4). In the case of palmitic acid, the highest percentage was obtained under red light with 19.52 ± 0.39 %, showing significant differences with white light (17.41 ± 0.50 %). However, similar amounts were obtained for treatments under control conditions and blue light, while green light had a lower amount compared to the others (18.36 ± 0.18 %). For PUFAs, the highest content of EPA was under white light with 7.41 ± 0.07 %, showing significant differences compared to other treatments ($P \leq 0.05$). There were no significant differences in EPA content between the control, red light and blue light (Table 4).

Discussion

Influence of the light scheme on the cell growth of *Tetraselmis subcordiformis*

Light is a limiting and critical parameter in the cultivation of autotrophic microalgae, determining the energy flow required for cell growth. Therefore, an increase in biomass could be expected to be influenced by an increase in energy flow through irradiance, as it helps improve photosynthetic yields in CO_2 fixation, growth, and changes in biochemical and morphological composition (Arora et al. 2013; Schulze et al. 2016; Yusof et al. 2021). Currently, both natural and artificial light sources are used for the production of photoautotrophic microalgae. The commonly used artificial light source for cultivation systems are Light Emitting Diodes (LEDs) due to their specific characteristics that allow microalgae to achieve greater efficiency in converting light energy for photosynthetic processes. LEDs have high durability, have low energy consumption, and provide a high output of monochromatic or multichromatic light at different

wavelengths (Abiusi et al. 2014; Lima et al. 2021). On the other hand, it has been found that microalgae have different responses to the intensity, photoperiod, and intermittence of light in terms of growth and biochemical composition (Lima et al. 2021; Lu et al. 2021). In the present study we observed that high photon flux density influenced the growth of *T. subcordiformis* when exposed to 1826 µmol photons $m^{-2}s^{-1}$ (Fig. 1a). To date, different light intensities in the range of 50-2500 µmol photons m^{-2} s^{-1} have been evaluated in the genus *Tetraselmis* to assess growth, pigment production, nutrient consumption and total lipid content.

Meseck et al. (2005), determined that in a culture of *T. chui* at 27 days, the division rate and biomass production were affected by the intensity and duration of light, generating a maximum division rate of 0.58 day^{-1} when exposed to 220 µmol photons m^{-2} s^{-1} and continuous light (24:0) with 0.61 day^{-1}; in addition, complete utilization of nitrate and phosphate was obtained in less time under these conditions. These results agree with those obtained in our study, suggesting that prolonged light duration is important in determining growth and nutrient uptake (Fig. 1a and 2a), however, in our case, a higher light intensity was used. Other studies also suggest that longer exposure times to light help growth in *Tetraselmis* sp cultures, as continuous light results in greater biomass production, reaching maximum growth rates of 0.63 day^{-1} and 0.55 day^{-1} when using a 24:0 and 12:12 photoperiod, respectively (Fakhri et al. 2015), in contrast to our results where μ_{max} of 0.11 d^{-1} and 0.09 d^{-1} was achieved under the same photoperiod. The light intensities for both studies were different. An increase in continuous energy flow can affect cell growth due to the high energy density required by cells. However, periods of darkness are necessary to allow photo-acclimation within the culture, enabling metabolic functions that help decrease cell photoinhibition and facilitate molecule synthesis (Farahin et al. 2021a).

On the other hand, Montes-González et al. (2021) suggest that *T. suecica* can be cultivated at light intensities higher than 750 µmol photons m^{-2} s^{-1} to promote growth, nutrient consumption, and pigment production. They found that higher nutrient concentrations and light intensities favored the growth rate in *T. suecica*, reaching a specific growth rate of 1.3 day^{-1}. This demonstrates that light must be adjusted to promote the required cell growth. In contrast, Go et al. (2012).found that the maximum cultivation irradiance for *T. suecica* was 108.7 µmol photons m^{-2} s^{-1}, as higher light intensities led to photoinhibition In our case, a maximum growth rate of 0.11 day^{-1} was achieved when continuous light was used in *T. subcordiformis*. Other authors have achieved a µ of 0.67 day^{-1} for *T. suecica* cultivated at 108.7 µmol photons m^{-2} s^{-1} (Go et al. 2012), 0.6 day^{-1} for *T. marina* cultivated at 60 µmol photons m^{-2} s^{-1} (Dahmen-Ben Moussa et al. 2017), and 0.36 day^{-1} under 220 µmol photons m^{-2} s^{-1} for *T. suecica* (Ulloa et al. 2012). All previously reported growth rates for the *Tetraselmis* genus are higher than those achieved in our study. This may have an effect on the amount of light causing significant variations in the growth rate, lipid and pigment composition in the microalgae under study (Fig. 5b and 6a) (Arkronrat and Oniam 2019).

Controlling the quality and intensity of light will help to reduce variability and increase control over biomass productivity and biochemical composition (Schulze et al. 2016). To achieve this, the effect of light and dark exposure time was evaluated, as they affect cell yield during cultivation due to changes in metabolic activities and cell life cycles (Gomez and Perez 2020; Yusof et al. 2021). Gomez and Perez (2020), Zevallos Feria (2017) and Meseck et al. (2005), report significant differences in the growth rates of *T. gracilis*, *T. striata*, and *T. chui* when cultured under different photoperiods, however, the light intensitie used in these studies were lower than that employed in our research. In our case, no significant differences were observed in biomass productivity, growth rate, doubling time, and nutrient consumption when different light and dark cycles were employed (Table 2 and Fig. 2a).

Research has also been conducted on different regions of the light spectrum to determine methods of improvement and optimization in culture. Different light wavelengths have been evaluated, including red, blue, violet, white, and green light, to determine changes in biomass productivity, lipid productivity, and fatty acid profile in *Tetraselmis* (Teo et al. 2014; Schulze et al. 2016). Similar results to our study were found with higher productivity in *Tetraselmis* sp when grown under red light compared to blue light, determining that red light improves culture yields (Kim et al. 2017). A study conducted on a 9-day culture of *T. suecica* demonstrated better growth under white and red light compared to blue and green light, where they achieved a µ of 0.07, 0.12, 0.06, and 0.06 day^{-1} under white, red, blue, and green light, respectively, presenting similar results to our study (Table 2).

Likewise, Teo et al. (2014) found that *Tetraselmis* sp and *Nannochloropsis* sp grow better under blue light compared to red light. However, it is not generally feasible to provide a specific wavelength of light for all microalgal species, as each strain will have its specific range (Kim et al. 2017). On the other hand, it has been indicated that dichromatic light composed of blue and red photons contributes to the growth of *Nannochloropsis oculata* and *T. chui*, as they can stimulate carbon fixation and nutrient absorption (Schulze et al. 2016). Our results also demonstrate that the growth rate and biomass production of the microalgae are related to the light wavelength (Teo et al. 2014). The change in wavelength and light intensity can affect the photosynthetic rate and consequently influence cellular growth in microalgae cultures. Some wavelengths may be more efficient for photosynthesis than others, and photon flux density regulates

cellular growth. This is why these two factors are important in optimizing cultivation conditions to achieve optimal biomass production (Guedes et al. 2023).

Concentrations of nitrate and phosphate decreased over time under high intensity conditions (Meseck et al. 2005; Go et al. 2012; Montes-González et al. 2021). Nitrogen utilization is affected by light, and for our case, it was found that the consumption rate of NO_3^- and PO_4^{3-} (g L^{-1} day^{-1}) in *T. subcordiformis*, did not depend on light and dark cycles, but rather on the intensity of light (Fig. 2a). Meseck et al. (2005), determined that phosphorus uptake by *T. chui* was not sensitive to light intensity and duration, while light duration did influence nitrogen uptake. Nitrogen assimilation during the dark stage is slower than during the hours of light (Meseck et al. 2005). In a 12:12 h photoperiod, nitrate would have to be assimilated in the dark, which decreases its absorption compared to longer light cycles (Fig. 2a).

Photosynthetic yields and chlorophyll *a* production

The photosynthetic efficiency can vary depending on the spectral composition and intensity of the incident radiation, according to the nature of the constitutive pigments, as well as in the content and proportion of these pigments in microalgal strains (Teo et al. 2014; Kim et al. 2017). Different types of pigments widen the range of light absorption and activate photo-acclimation and photoinhibition mechanisms at the cellular level. Understanding the cellular response under different light conditions can be useful and effective for comprehending the photosynthetic response and the photoprotection mechanisms that may be occurring within the cells (Borghini et al. 2009; Fu et al. 2013; Abiusi et al. 2014).

The use of chlorophyll fluorescence allows monitoring the functioning and influence of stress on the physiological state of the photosynthetic apparatus in microalgae, reflecting the performance of photochemical and non-photochemical processes (Baker 2008; Masojıdek et al. 2013). Understanding photosynthesis through fluorescence measurements and the understanding of different photosynthetic parameters can provide tools that allow maximizing the physiological potential in photosynthetic organisms and thus achieving higher productivities in cultivation systems (Maxwell and Johnson 2000). Y_{II} measurements shown in Fig. 3a and b, determine the fraction of energy absorbed by chlorophyll at increasing irradiances and converted to photosynthesis and reflects the number of electrons that are transferred through the electron transport chain (Cosgrove and Borowitzka 2010). The determining factor of this efficiency is the ability to remove electrons from the PSII-receptor plastoquinone, which is directly related to the rate of ATP and NADPH generation (Jimnez-Suancha et al. 2015).

Excess energy can be reduced by different cellular photoprotection mechanisms. Non-photochemical quenching quantifies the regulated (Y_{NPQ}) and consecutive (Y_{NO}) quenching of thermal dissipation processes (Masojıdek et al. 2013; Giraldo et al. 2021). Excitation energy dissipation dependent on energy (NPQ) is activated by accessory pigments such as carotenoids, which can dissipate excess energy through the xanthophyll cycle during photon transport from the light harvesting antenna to the PSII reaction centers (Borghini et al. 2009; Malapascua et al. 2014). At high light intensities, an increase in electrons in the PSII causes acidification in the thylakoids, which converts violaxanthin to zeaxanthin, which is associated with the PSII, serving as an efficient extinguisher of excess energy and increasing the rate of heat loss from the PSII (Baker 2008). When microalgae are exposed to high light levels, reactive oxygen species that can damage photosystems are produced. To counteract this, NPQ is activated and photoprotection mechanisms begin through the xanthophyll cycle. These adaptive strategies allow microalgae to protect themselves against damage induced by intense light, thereby preventing growth inhibition (Fig. 1a) (Granata et al. 2019; Guedes et al. 2023). Other dissipation mechanisms are also activated at the cellular level that do not depend solely on energy (Y_{NO}) (Fig. 4a and c). This is the first energy dissipation mechanism that is activated through fluorescence and is activated at the cellular level in response to the photoacclimation of excess energy trapped in the PSII reaction center. During different light configurations, there is a tendency to decrease this mechanism as Y_{NPQ} is activated (Masojıdek et al. 2013).

The F_v/F_m ratio is an estimate of the maximum and optimal quantum efficiency of photosystem II (PSII) photochemical activity in cells adapted to darkness when the reaction centers are fully oxidized or open (Baker 2008). A decrease in this ratio indicates a reduction, damage, or disturbance in the photochemical efficiency of PSII. Therefore, these measurements often provide a simple and fast way to monitor light stress (Kalaji et al. 2014). Fv/Fm values ranging from 0.6 to 0.8 indicate high photosynthetic performance (Malapascua et al. 2014; Farahin et al. 2021a). Each alga has a specific point at which it thrives and functions optimally under specific environmental conditions (Jimnez-Suancha et al. 2015). In the present study, F_v/F_m of *T. subcordiformis* under control conditions ranged from 0.65 ± 0.003 on day 14 to 0.719 ± 0.017 on day 26 and similar results were obtained under different light wavelengths, where the ratio remained above 0.6 (Fig. 5c). However, under stressful conditions of high irradiance and continuous light, this ratio decreases considerably (Fig. 5a). A F_v/F_m ratio above 0.6 has been found in *T. tetrathele* when cultured at 300 and 1500 µmol photons $m^{-2}s^{-1}$, however at 2000 and 2500 µmol photons $m^{-2}s^{-1}$, it rapidly decreases to values below 0.2 (Farahin

et al. 2021a). On the other hand, *Nannochloropsis* sp cells experienced a decrease in F_v/F_m (0.5) when the light intensity increased up to 1200 µmol photons $m^{-2}s^{-1}$ (Sforza et al. 2012). The results observed in the present study suggest that, despite obtaining an F_v/F_m of 0.5, cells of *T. subcordiformis* may be undergoing positive acclimation to the established light conditions. They utilized photoprotection strategies against light stress, optimizing their cellular growth performance (Fig. 1a) and activating energy dissipation mechanisms that allowed them to regulate.

Montes-González et al. (2021) recorded higher values of chlorophyll *a* as light intensity increased (300 or 750 µmol photons $m^{-2}s^{-1}$), while the opposite was observed in our study (Fig. 5b). In our study, higher pigment concentrations were achieved under control conditions with low light intensity (20 µmol photons $m^{-2}s^{-1}$). High light intensity can cause cellular photodamage and trigger photooxidation mechanisms of pigments, leading to rapid degradation and breakdown of chloroplasts under prolonged exposure to high-intensity light (Kim et al. 2017; Farahin et al. 2021a). Our results are consistent with those of Farahin et al. (2021a), where chlorophyll content decreased as light intensity increased (from 300 to 2500 µmol photons $m^{-2}s^{-1}$), with complete absorption of the required light energy for cellular growth followed by low cellular chlorophyll content and decreased photosynthetic yield (F_v/F_m) under high irradiances.

On the other hand, in a 9-day culture of *T. suecica*, it was found that the highest chlorophyll *a* content was obtained under blue and white light (22 mg g^{-1}), while the lowest was under red and green light (16 mg g^{-1}) (Abiusi et al. 2014). In our case, the chlorophyll *a* content on day 14 was similar under all evaluated light wavelengths, but on day 26, white and green light were higher compared to red and blue light (Fig. 5d). All photosynthetic pigments have two light absorption bands: blue or blue-green (450–475 nm) and red (630–675 nm) (Masojıdek et al. 2013; Ra et al. 2018). These narrow bands of the photosynthetically active radiation (PAR) spectrum allow for the manipulation of specific and optimal light conditions for microalgae cultivation to control cellular and photosynthetic yields (Kim et al. 2017).

Lipid content and fatty acid profile

Microalgae store lipids with great commercial potential for various purposes. These synthesis of the lipids depends on growth parameters in the cultivation, affecting the fatty acid profile. These fatty acids hold biological significance and specific uses due to their diverse chemical properties (Lu et al. 2021). We found that a high photon flux density and different light wavelengths on the *T. subcordiformis* culture affected the total lipids obtained and a significant cellular response regarding the content of the fatty acids found (Fig. 6, Tables 3 and 4).

Dammak et al. (2016) reported 49 % lipids when *Tetraselmis* sp was grown under a light intensity of 182 µmol photons $m^{-2}s^{-1}$ in continuous light, also reporting a predominance of oleic and palmitic fatty acids, which represented 32.88 % and 30.89 % of total FAMEs, higher than that reported in our study. A similar case occurred for linolenic acid, where they reached 1.84 %, however, the EPA content was much lower than that found in our study (3.43 %), while under conditions of high photon flux density with continuous light evaluated in this study, we found an increase of 10.23 %. Other authors report lipid content under light intensities of 300, 1500, 2000, and 2500 µmol photons $m^{-2}s^{-1}$ in a semicontinuous culture of *T. tetrathele* for 36 days. They found that the highest lipid content was at 300 µmol photons $m^{-2}s^{-1}$ (11-15 %), while for higher intensities, production noticeably decreased (3-5 %). Although they obtained biomass productivities of 0.971 g L^{-1} day^{-1} and growth rates of 0.32 day^{-1} under 1500 µmol photons $m^{-2}s^{-1}$, these did not correlate with their lipid content, as lipid accumulation does not require energy under high growth rates (Farahin et al. 2021b). These data agree with those obtained in this study where high growth rates were achieved at high photon flux density, but low lipid productivities, which means that all the energy absorbed by *T. subcordiformis* was directed towards cellular growth rather than lipid accumulation. Other authors also indicate that lower lipid contents are accompanied by high growth rates and are mainly due to the reduction of the metabolic rate due to stress, where the absorbed carbon is directed towards storage molecules (Ra et al. 2018). In another study with *T. chui*, Lima et al. (2021) found between 8-17 % of total lipids when grown at 300 µmol photons $m^{-2}s^{-1}$ under different light intermittencies (continuous light, and 5, 50, and 500 Hz), demonstrating that frequencies did not generate significant differences in total lipid production and that a lower frequency resulted in a higher content of PUFAs.

Lipid content in *Tetraselmis* also has been evaluated by assessing different wavelengths. Abiusi et al. (2014) reported a content of 19 %, 17.3 %, 21.8 %, and 17.3 % for a culture of *T. suecica* in white, red, blue, and green light, respectively, very similar to those achieved in our study. For the predominant fatty acids in that study, they found higher amounts of palmitic acid under blue light (21.88 %) and green light (21.19 %), while the EPA content was much lower than that recorded in this study. Under white light, they obtained 4.24 %, 2.60 % under blue light, and 4.28 % under green light. In another study (Ra et al 2018) on four strains of microalgae, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannaochloropsis salina*, and *N. oceanica*, a lipid content greater than 50 % was found when grown under green light, but this was was not the case for our *T. subcordiformis*, where the lipid content only reached 18 %. Ra et al (2018) indicated that under green wavelength stress, the lipid

yield for these microalgae could be improved and it could be used as a lipid accumulation method. On the other hand, the EPA content of the microalgae in that study was much lower than that achieved in *T. subcordiformis*, representing 6.43 %, while for *P. tricornutum*, only 1.01 % was achieved (Ra et al. 2018). Kim et al. (2017) also recorded the content of palmitic and oleic acid in *Tetraselmis* sp under red and blue light conditions, finding a content of 32.3 % and 30.4 % for palmitic acid and 30.6 % and 29.5 % for oleic acid, higher than those found in our study. They considered that to obtain greater fatty acid productivity and biomass using light filters, light conditions such as the number of transmitted photons, the culture volume, and cell response to light intensity should be considered since the light filter reduces the number of photons supplied to the cultures by 72 ~ 80 %.

Conclusions

This study established a high photon flux density culture to evaluate cell growth and biomass productivity of a *T. subcordiformis*. culture. High photon flux density gave growths rates 37% higher than the control and had lower lipids and Chl_a concentrations. The growth rates obtained for the light and dark cycles tested differed by less than 20%. On the other hand, in the wavelength treatments compared to the control, similar growth rates were observed. For light:dark cycle treatments and wavelength treatments, fatty acid content was >65% for the sum of four fatty acids, namely, palmitic (C16:0), palmitoleic (C16:4), oleic (C18:1n9c), and stearidonic (C18:4) acids. Thus, the dominant fatty acids profile was same for all treatments, although treatments did account for variations within each of these four fatty acids. These results demonstrate the ability of *T. subcordiformis* to grow under different light regimes and the potential to increase cell growth and fatty acid content. We provide an analysis of photosynthetic yields and pigment production, as well as nutrient consumption in the culture. These data suggest the growth potential of the microalgae to be explored in future investigations in the scaling-up of cultivation.

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Author contribution *Laura Montoya*: Conception and design, Methodology, Drafting of the article, Analysis and interpretation of the data. *Andrés Arbeláez*: Conception and design, Final approval of the article, Writing-Correction and Editing, Critical revision of the article for important intellectual content. *Néstor Giraldo*: Conception and design, Final approval of the article, Writing-Correction and Editing, Critical revision of the article for important intellectual content. *Lucía Atehortúa*: Final approval of the article, supervision, technical, or logistic support, writing-correction and editing.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no conflict of interest.

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