



# The effect of light intensity on the production and accumulation of pigments and fatty acids in *Phaeodactylum tricorutum*

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## Abstract

The diatom *Phaeodactylum tricorutum* is known to accumulate polyunsaturated fatty acids (PUFAs), especially omega-3 fatty eicosapentaenoic acid (EPA) 20:5n3, which is health beneficial and are essential in human and animal diets. The main genes involved in the biosynthesis of EPA have been reported, but the effect of light and other environmental factors on transcription of such genes is poorly understood. This paper evaluates the transcription of six genes related to EPA production, fatty acid methyl esters (FAMES), and pigments as a result of growing *P. tricorutum* under 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  irradiance up to 72 h. Regarding transcription levels, all genes displayed a wide variation range in time. Under 24 h of exposure to 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , transcription of *PTD6*, *PTD15*, *ELO6\_b1*, and *ELO6\_b2* significantly increased, whereas 72 h exposure led *PTD6* transcript levels to decrease significantly, and *PTD5 $\alpha$*  and *ELO6\_b1* to increase. The 72-h evaluation showed that the 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  irradiance resulted in a 4-fold increase pigments (chlorophyll and carotenoids) production, as well as in PUFAs (16.59%) production, including EPA (5.72%). Conversely, the 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  irradiance resulted in increased saturated fatty acids (SFAs) and decreased PUFA concentrations in the FAME distribution. The irradiance variation had a major effect on microalgal metabolism as a result of membranes remodeling, PUFA synthesis rerouting, SFA, and pigments. Such information could be utilized for improving pigments, EPA, and other PUFA syntheses from *P. tricorutum*.

**Keywords** Diatom · Eicosapentaenoic acid · Fucoxanthin · Gene expression · Polyunsaturated fatty acids · Microalgae

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## Introduction

Global efforts to improve sustainability have led to many studies on the economic viability of the use of microalgae for different biotechnological purposes, e.g., biofuel production, bioremediation, food, and animal feed. Besides, the production of high-value pharmaceuticals and nutraceuticals derived from microalgae can also be pursued (Li et al. 2014; Neumann et al. 2018).

In this scenario, the marine diatom *Phaeodactylum tricorutum* stands out mainly because it can accumulate up to 30% of EPA of the total fatty acids (Qiao et al. 2016), which is one of the most valuable omega-3 fatty acids. PUFAs can be classified in families like omega-3 ( $\omega$ -3) or omega-6 ( $\omega$ -6) fatty acids, depending on where the first unsaturation is located in the long fatty acid chain. The  $\omega$ -3 fatty acids are associated with human health benefits because they act as precursors for anti-inflammatory eicosanoids, are cellular membrane components, and are an essential nutrient for newborns and infants (Luchtman and Song 2013; Ulmann et al. 2017). For

these reasons, they are considered nutraceuticals and are often added to food supplements.

In addition to the importance for humans,  $\omega$ -3 are considered essential nutrients for the larvae development of many aquatic organisms like fish, shrimp, and bivalves (Marquez et al. 2019). Therefore, this industry has a strong demand for  $\omega$ -3, which is largely obtained from marine fish (Shah et al. 2018). Considering that consumption of these supplements will increase, and their natural sources are becoming scarce, it is imperative to develop alternative sources of  $\omega$ -3 (Mühlroth et al. 2013; Li et al. 2014).

Fatty acid synthesis in *P. tricorutum* begins in the chloroplast, where long chains are produced and then transported to the endoplasmic reticulum for the PUFA synthesis. This pathway requires energy and is catalyzed by desaturases and elongases that catalyze the double bond formation and elongation in aliphatic carbon chains, thus producing EPA (Arao and Yamada 1994; Zulu et al. 2018).

Domergue et al. (2002) cloned and characterized the desaturases genes *PDT5* and *PDT6*, and demonstrated their front-end activity in *P. tricorutum*. Based on this information, subsequent work demonstrated that the activity of these enzymes, through overexpression in this organism, exhibited a significant increase in EPA production, indicating that gene expression and transcription can be limiting factors in PUFA pathway (Peng et al. 2014; Zhu et al. 2017). The influence of stress exposure on transcription of fatty acid desaturases and fatty acid composition has also been analyzed in some studies, including nitrate starvation of *Isochrysis galbana* (Huerlimann et al. 2014), increased salinity in *Tetraselmis* sp. (Adarme-Vega et al. 2014), and the nitrogen/phosphorus ratio in *P. tricorutum* (Lopes et al. 2019), but the effects of light changes on *P. tricorutum* gene expression as related to lipids composition are not well understood yet.

Evaluating the transcriptome of *P. tricorutum* exposed to high light with 500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , Nymark et al. (2009) described the molecular mechanisms of capacity to adapt the light harvesting complexes (LHC) in response to the new environmental condition. The LHC are a complex structures of proteins and pigment molecules in the thylakoid membrane, which in low light improves energy capturing, and in excess of light rapidly induces thermal dissipation of excess absorbed energy (Lepetit et al. 2012; Buck et al. 2019). Among pigments, the most abundant carotenoid is fucoxanthin that forms the protein complexes called fucoxanthin-chlorophyll *a/c*-binding antenna protein (FCP) which are peripheral antennae of the photosystems (Domingues et al. 2012).

Associated with human and animal health benefits, fucoxanthin is used in dietary supplement form acting as antioxidant, anti-obesity, anti-inflammatory, antidiabetic, and antihypertensive agents, and can be used as an additive in the feed industry (Peng et al. 2011; Wang et al. 2018). PUFAs and fucoxanthin have high commercial value, and their production

by microalgae can leverage environmental benefits, although challenges to the production of algal biomass must be overcome to attain large-scale production (Wang et al. 2018). A possibility is applying metabolic engineering, as shown in Dinamarca et al. (2017), where lipid biosynthesis was increased, especially triacylglycerols (TAGs) after overexpression of diacylglycerol acyltransferase in *P. tricorutum*. Hence, there is a need to understand the microalgae metabolism and identify new targets for genetic manipulation with new techniques like CRISPR (Nymark et al. 2016).

Based on the literature review, in order to improve the lipid metabolism gene response understanding, it is necessary to identify factors involved in gene expression modulation, especially those linked to environmental conditions, such as light intensity. In such context, this study focused on analyzing the differential expression of desaturases and elongases under two irradiance conditions. In this way, modulations in the gene expression ratio for fatty acid composition and the effect on the synthesis of pigments in *P. tricorutum* were investigated.

## Material and methods

### Culture and growth analyses

The microalga *Phaeodactylum tricorutum* strain CCAP1055/1 was cultivated in 4 L of *f/2* medium (Guillard and Ryther 1962) plus silica and was acclimated at 22 °C, 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at constant irradiance white fluorescent lamps, monitored using Li-250A light meter (Li-COR) and supplemented with 0.5%  $\text{CO}_2$  (*v/v*) for 7 days. The biomass was harvested by 5 min centrifugation at 2950 $\times g$  and washed 1 $\times$  in Guillard *f/2* medium-plus silica. The cells were resuspended at turbidity equivalent to 100 mg dry weight, according to Lopes et al. (2019), estimated indirectly through the absorbance read at 700 nm in UV-Vis spectrophotometer (Thermo Fisher Scientific), according to the equation: Biomass ( $\text{g L}^{-1}$ ) =  $\text{ABS}_{700} \times 0.0022$ ,  $R^2 = 0.994$  and fractionated into six borosilicate flasks with 2 L of medium. Three independent replicas used in each of the treatments at constant irradiances of 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , supplemented with 0.5%  $\text{CO}_2$  (*v/v*) at 22 °C. To avoid self-shading, cultures were partly replaced with fresh medium once every 24 h until turbidity equivalent to approximately 100 mg dry weight. At the sampling periods (0, 12, 24, and 72 h), 30 mL were taken to measure turbidity, pigments and gene expression.

### Pigments analyses

The pigments were extracted according to Strickland and Parsons (1972), for the photosynthetic pigments chlorophylls *a* (Chl *a*), *c* ( $c_1 + c_2$ ); content was calculated indirectly according to Jeffrey and Humphrey (1975), for carotenoids

(fucoxanthin, diadinoxanthin, and  $\beta$ -carotene) according to Carreto and Catoggio (1977), through absorbance readings at 480, 510, 664, and 630 nm.

### RNA extraction and qPCR analysis

For gene expression, the cells were obtained by centrifugation for 5 min at  $3500\times g$  and resuspended in 1 mL of RNA Later solution. Extraction was performed with the TRIzol reagent, and the concentration was estimated at 260 nm, with purity checked at ratios  $Abs_{260}/Abs_{280}$  and  $Abs_{260}/Abs_{230}$ . The transcription of the desaturase, *PDT5 $\alpha$* , *PDT5 $\beta$* , *PDT6*, *PTD15*; and elongase, *ELO6\_b1*, *ELO6\_b2*, genes were evaluated, with primers described by Lopes et al. (2019). For reference genes, *RPS* and *H4* were used as baseline expressions, synthesizing the 30S ribosomal subunit and Histone H4, respectively, according to Saut et al. (2007). Amplifications were performed with a StepOne thermal cycler with a Power SYBR Green RNA-to-Ct 1-Step, according to the manufacturer's instructions. In each run, RT-PCR efficiency was checked for each primer pair by constructing a standard curve from serial dilutions from a pool of all samples, and assays were carried out in triplicates and all reagents and equipment at Thermo Fisher Scientific. For statistical analysis, the equation of Livak and Schmittgen (2001) was used ( $2^{-\Delta\Delta CT}$  method).

### Lipid analysis

At the beginning and end of the cultivation, 1 L of cultured cells were withdrawn from each treatment at 0 and 72 h and was centrifuged at  $3500\times g$  for 10 min at 4 °C, and lyophilized to obtain biomass to analyze their fatty acid profile.

Lipids were extracted from the dry biomass using a solvent-based extraction method, according to Borges et al. (2016). Dry biomass (0.5 g) was placed into test tubes (in three replicates) with 1.5 mL of a chloroform-methanol (2:1 v/v) mixture. The solution was exposed to ultrasound (Quimis USC-1400/40 kHz) for 20 min. The mixture was then centrifuged for 2 min at  $2000\times g$ . The lipid extraction process was repeated three times for each tube. The liquid phase was transferred to previously weighed flasks. Afterward, the solvent was evaporated under vacuum in a rotary evaporator, and the flasks were then reweighed. The total lipid fraction was determined based on the differences in flask weights, and the lipid content was calculated based on the percentage of the initial dry weight.

The derivatization of the lipid fraction of microalgae was carried out according to Lemões et al. (2016), in which the sample containing the lipid fraction (300 mg) was placed in a test tube, and a mixture of 3 mL of boron trifluoride/methanol was added. The mixture was heated in a water bath at 70 °C for 20 min. For recovery of the FAMES, the mixture derived was washed in a separatory funnel with 15 mL of hexane and 20 mL of distilled water. The organic and aqueous phases

were then separated, the organic phase containing the fatty esters was dried, and the solvent was evaporated at 50 °C.

Analyses were performed using a Shimadzu GCMS-QP2010 Plus chromatographic system equipped with a split/split less injector coupled with a mass detector. The operating temperatures of the detector were 280 °C for the interface and 230 °C for the source. Detection was performed using a full scan from  $m/z$  30 to  $m/z$  500 with a scan time of 0.20 s. The ionization mode electron impact was at 70 eV. The operating conditions of the chromatograph were as follows: injector 250 °C, column 80 °C (initial temperature, 0 min), followed by a gradient of 10 °C  $\text{min}^{-1}$  to 180 °C and then 7 °C  $\text{min}^{-1}$  to the final temperature of 330 °C, gas flow 1.3 mL  $\text{min}^{-1}$ , pressure 88.5 kPa, average linear velocity 42 cm  $\text{s}^{-1}$ , and 1 mL injection volume with the split ratio 1:100. A crossbond 5% dimethyl polysiloxane diphenyl 95% column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ; Restek) was used. The fatty acid methyl esters were identified by comparison with known standards (Supelco 37 Component FAME Mix) and were quantified using the standardized areas method.

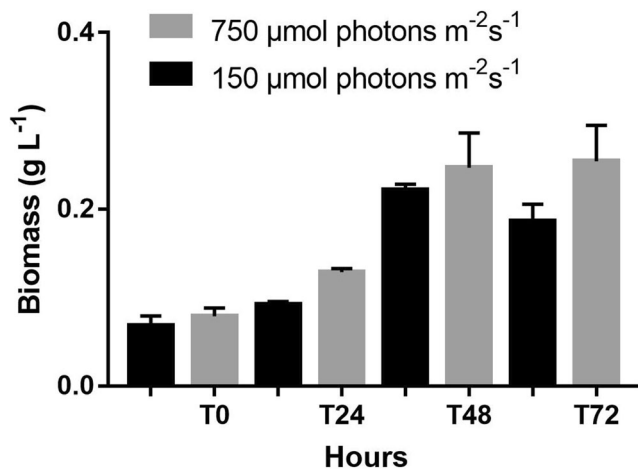
### Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.00 for Windows. The data were assessed for normality with the Kolmogorov-Smirnov test. The data were compared by one-way analysis of variance (ANOVA) with replicate post hoc comparisons made using the Tukey's test for lipid content and growth. For pigments and gene expression, the Mann-Whitney test one-tailed was applied. A significance of  $P < 0.05$  was applied to all statistical tests performed. Results are presented as mean  $\pm$  standard deviation ( $n = 3$ ).

### Results

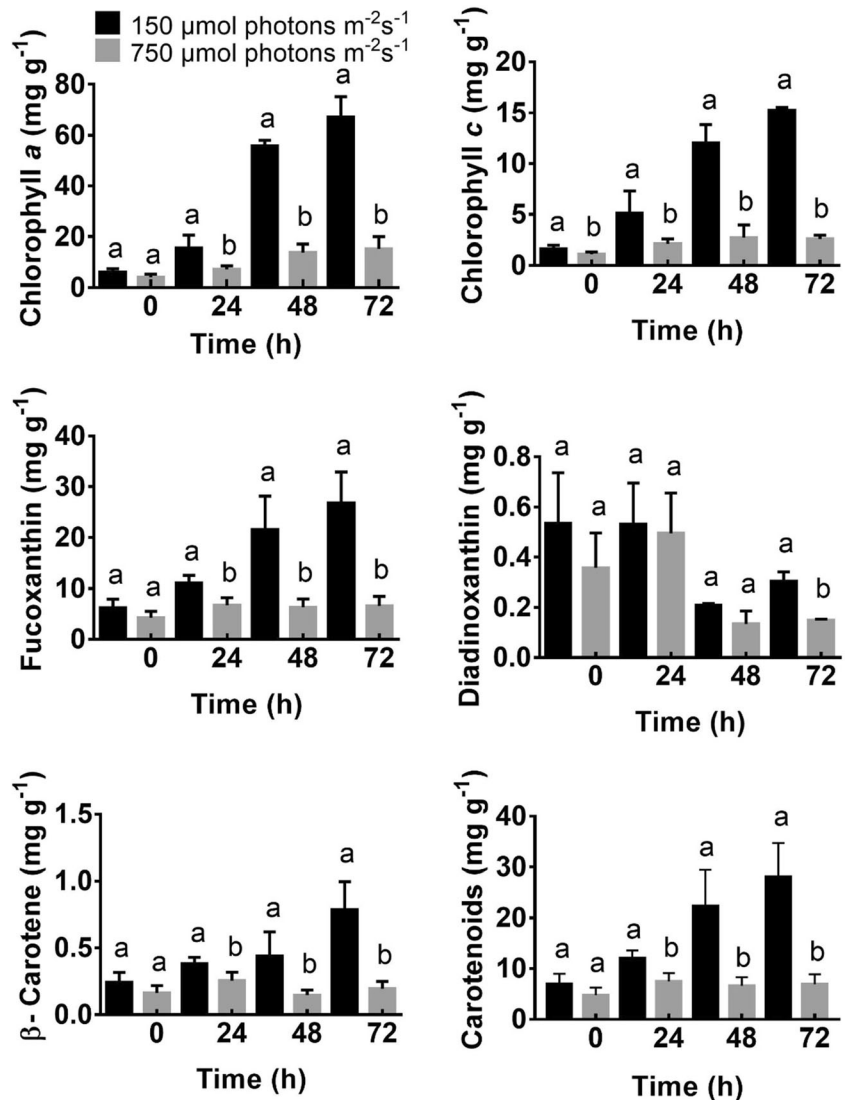
This study analyzed the influence of light on physiological responses of *P. tricornutum* exposed to up to 72 h to irradiation conditions of 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Concerning biomass production, no statistically significant differences was observed between the light irradiances up to 72 h of cultivation, as shown in Fig. 1. Regarding the photosynthetic pigments, there was a significant difference among treatments. At the lower irradiance, an increase was observed in the synthesis of Chl *a*, *c* ( $c_1 + c_2$ ) and carotenoids, especially after 72 h, and the highest increase was observed in Chl *a*  $15.37 \pm 3.54$  to  $67.19 \pm 6.18 \text{ mg g}^{-1}$ , as well as fucoxanthin  $6.58 \pm 1.25$  to  $26.79 \pm 4.36 \text{ mg g}^{-1}$  (Fig. 2).

The EPA biosynthesis pathway in *P. tricornutum* involves the transcription of six main gene products (four desaturation steps and one elongation step), which were quantified in this work, as presented in Fig. 3. Among the four desaturase genes analyzed (*PTD5 $\alpha$* , *PTD5 $\beta$* , *PTD6*, and *PTD15*), in the first



**Fig. 1** Growth of *Phaeodactylum tricornutum* during exposure to 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  up to 72 h of cultivation. All data points are presented as the mean ( $n = 3$ )  $\pm$  SD

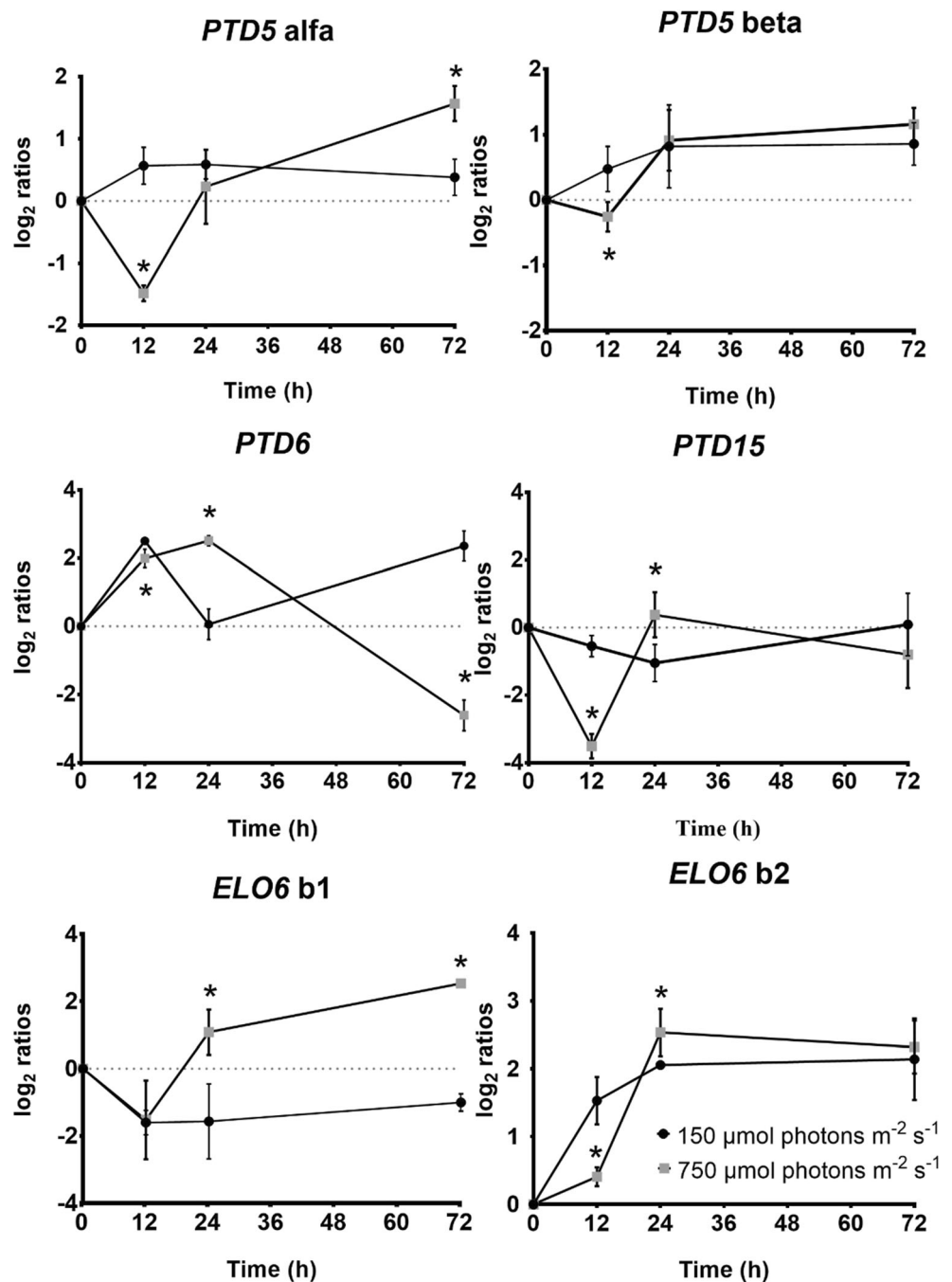
**Fig. 2** Chlorophyll *a*, *c* (*c1* + *c2*) and carotenoids (fucoxanthin, diadinoxanthin, and  $\beta$ -carotene) concentrations during 72 h of exposure at 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . All data points are presented as the mean ( $n = 3$ )  $\pm$  SD; lowercase letters *a* and *b* indicate significant differences among irradiations,  $P < 0.05$



12 h of exposure, the genes most negatively affected by high irradiance were *PTD5 $\alpha$* , *PTD5 $\beta$* , and *PTD15*. The opposite was found at 24 h because the genes *PTD6* and *PTD15* presented a significant increase in expression. After 72 h of exposure at 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , *PTD5 $\alpha$*  was more than one-fold higher, and there was a significant reduction in transcription of *PTD6* in the treatments. With regard to the elongase gene expression, there was no significant variation in the first 12 h of the highest irradiance; however, after 24 h of exposure at 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  irradiance, a significant increase was found in *ELO6\_b1*, *ELO6\_b2*, and at 72 h, only the transcript level of *ELO6\_b1* was increased.

Figure 4 presents comparative data about the fatty acid profiles, the most significant change was observed for saturated fatty acids (SFAs), which increased 16.57% in the treatment with higher light intensity (750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), and the principal fatty acid was 16:0 (palmitic acid) with 36.72% of the FAMES. The monounsaturated fatty acid

**Fig. 3** Relative transcript levels of four desaturase genes (*PDT5 $\alpha$* , *PDT5 $\beta$* , *PDT6*, and *PDT15*) and two elongases (*ELO6\_b1* and *ELO6\_b2*) for PUFAs synthesis in *Phaeodactylum tricornutum* in culture during 72 h of exposure at 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  irradiance. Each data point indicates the mean ( $n = 3$ )  $\pm$  SD. \*Significant differences between irradiances,  $P < 0.05$



(MUFAs) concentrations were similar above 30% of total fatty acids, but significant difference was observed for synthesis of 16:1 (palmitoleic acid).

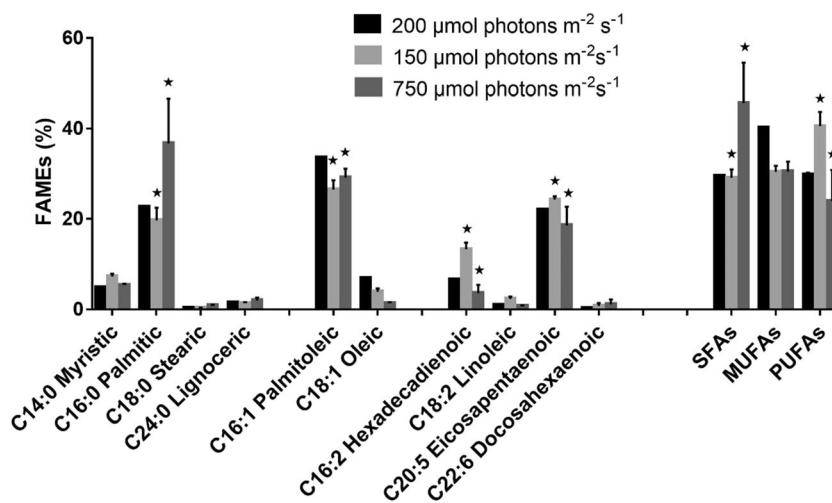
Concerning PUFA biosynthesis, it was possible to verify the presence of C22:6 docosahexaenoic acid (DHA) in both treatments, and its content was not significantly altered by the light intensity. Regarding EPA, the variations in concentrations were 24.26% in the lower, and 18.54% (*w/w*) in the higher irradiance, a variation of 5.72% was detected, which suggests that its synthesis was negatively affected by excess light conditions. In contrast, an increase of 16.59% in PUFAs was observed at the lower

light intensity (150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Many fatty acid compounds of 18 carbon atoms were observed in low concentrations in both treatments, and a few were observed only in traces such as 15:0 pentadecanoic, 20:0 arachidonic, and 22:0 arachidic acid, as shown by Table S1.

### Discussion

It is well known that changes in environmental conditions, like nitrogen restriction, can affect the modulation of lipid

**Fig. 4** Total fatty acid methyl esters (FAMES) profile in batch culture of *Phaeodactylum tricornutum* in initial biomass grown at 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  for 7 days and after 72 h under irradiances of 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Results are expressed as the mean  $\pm$  SD (standard error) of three biological replicates. \*Significant differences between irradiances,  $P < 0.05$ . SFAs saturated fatty acids, MUFAs monounsaturated fatty acids, PUFAs polyunsaturated fatty acids



biosynthesis (Huerlimann et al. 2014; Remmers et al. 2017). Light plays a central role in photosynthetic organisms; therefore, in this work, the microalgae *P. tricornutum* was evaluated for its physiological response to different light intensities, 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , in gene expression ratio, fatty acids biosynthesis, biomass production, and synthesis of photosynthetic pigments which were analyzed because of their high potential as nutraceuticals and therefore economic importance.

*Phaeodactylum tricornutum* is a marine organism well adapted to changes in light available in environment that can rapidly acclimate to the established laboratory conditions, being resilient to sudden changes of irradiance due to photoacclimation mechanisms, variable light environments characteristic of turbulent waters (Nymark et al. 2009; Domingues et al. (2012)). It was possible to observe in Fig. 1. the minor differences in growth could be related to dilution made at 24 h of exposure, to avoid self-shading, which may have caused unbalanced growth. An increase in growth rate in high irradiance was also observed in previous reports such as Chrismadha and Borowitzka (1994) using 56 to 1712  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and Nogueira et al. (2015) using 50 a 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

Regarding the photosynthetic pigments such as chlorophyll *a*, *c* and fucoxanthin, it was observed a significant increase at lowest irradiance. The photosynthetic capacity depends on a specific range of light intensities on diatoms, which may provide different mechanisms and strategies for optimizing the photosynthetic rate for optimum exploitation of available photons (Heydarizadeh et al. 2017). Thus, it responds to irradiance conditions that include adjustments in the amount and proportion of chlorophyll *a*, *c*, and fucoxanthin, as well as the size of the photosynthetic unit, which alters the carbon assimilation capacity in photoadaptive response, also reported by Heydarizadeh et al. (2019) and Gundermann et al. (2019).

The high irradiance treatment was observed to inhibit the synthesis of photosynthetic pigments when photocatalytic

stress light conditions occur, specialized chloroplast structures lose the ability to absorb excess excitation and a photoprotection mechanism, non-photochemical quenching (NPQ), responsible for the dissipation of light in the form of heat comes to action, which was reported by Nymark et al. (2009), Domingues (2012) and Remmers et al. (2017). However, the diadinoxanthin content presented the lowest variation between the irradiances used in this study, since this pigment is more related to the xanthophyll cycle (conversion of diadinoxanthin into diatoxanthin) and associated with protection of photo-oxidative damage (Domingues 2012; Lepetit et al. 2012). Buck et al. (2019) obtained genetic modification in *P. tricornutum* with range of NPQ and verified, based on physiological effect of high light, the diatoxanthin and proteins LHC1/2/3 act mediated inducible thermal energy dissipation.

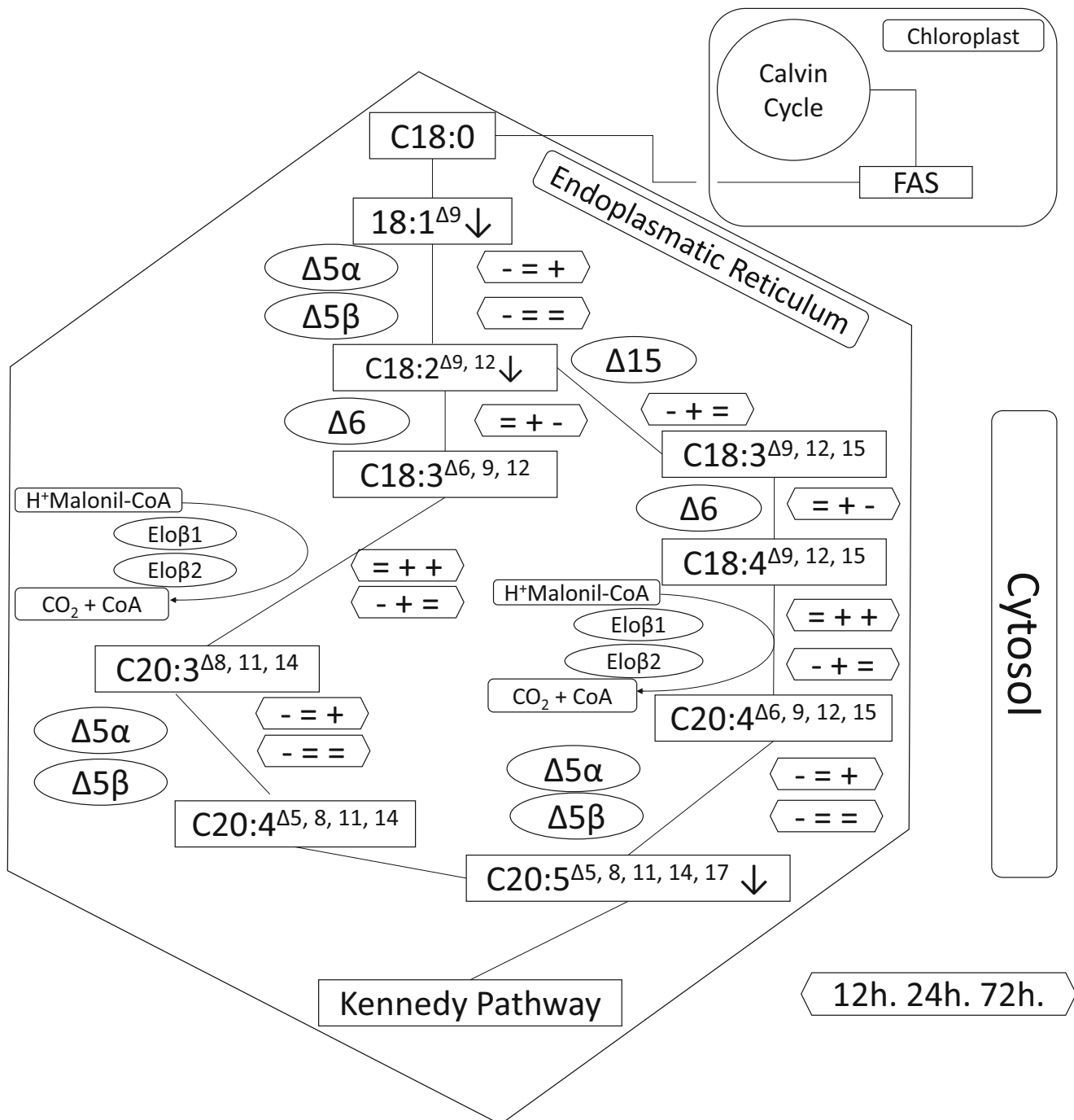
The analysis conducted in this study allowed observing changes in the profile of fatty acids: SFA, MUFA, and PUFA. Qiao et al. (2016) studied lipid synthesis in *P. tricornutum* and did not find significant differences in growth and lipid content when altering several variables such as salinity (15, 20, 28, and 35 ppt) and light intensity (50, 100, and 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and only found significant differences under nitrogen starvation stress (1.24  $\text{mg L}^{-1}$ ). Remmers et al. (2017) evaluated luminosities of 60 to 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and obtained the variation of 25.5 to 18%, respectively, of EPA in membrane lipid fraction in *P. tricornutum* cultivated in systems 16:8 h light/dark cycles and nitrogen starvation.

During photosynthesis light directly influences lipid synthesis, especially PUFAs that are essential for membrane synthesis and maintenance, as well as for cell division (Li et al. 2014). According to these authors, at low growth rates, an SFA (16:0) and MUFA (16:1) accumulation occurs, which is used to store the excess carbon accumulated during photosynthesis. Here, we showed that even with similar growth, the increase in light intensity increased SFA, while it decreased PUFAs. The importance of EPA in the structure of thylakoid

membranes and photosynthesis dynamics can be confirmed by the high proportion of this fatty acid found in glycerolipids strongly associated with photosynthesis and plastid membranes. In *P. tricornutum*, according to Abida et al. (2015), EPA is the major fatty acid in monogalactosyl diacylglycerides (MGDG) and phosphatidylcholine (PC)—approximately 40% and 35% of the total associated fatty acids, respectively—two of

the most abundant glycerolipids together with sulfoquinovosyl diacylglyceride (SQDG), which are responsible for maintaining membrane fluidity across all chloroplast membranes during the low light at photosynthesis.

We verified the gene expression for FA synthesis, and a summary of the results is presented in Fig. 5. EPA biosynthesis begins with 18:1 (oleic acid) and can occur in the  $\omega$ -3 pathway



**Fig. 5** Gene expression changes on PUFAs pathways in *P. tricornutum* under 750 compared to 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ : Squares: pathway steps. Arrows inside the squares: significant change of products in lipids profile  $p < 0.05$ . Arrow up: increase, arrow down: decrease. Ellipses:

genes. The change in gene expression with light increase respectively after 12, 24, and 72 h is represented on the hexagons by “+,” “=,” and “-,” which means respectively, “increase,” “no change,” and “decrease” in the gene expression after the respective periods

with 18:3 ( $\alpha$ -linolenic acid) or in the  $\omega$ -6 pathway with 18:2 (linoleic acid), forming 18:4 (stearidonic acid) or 18:3 ( $\gamma$ -linolenic acid), respectively. Although these lipid fractions were not detectable in FAME analyses, the findings demonstrate a possible limitation in the substrate availability in both pathways. We observed low expression of *PTD6* at high irradiance for this enzyme, especially after 72 h of exposure, which could be associated with a decrease of EPA, indicating that the synthesis can be controlled at the beginning of the route. In a study with a similar approach with a variation of the N/P ratio, Lopes et al. (2019) verified that desaturases *PTD6* and *PTD5* alpha were modulated by changes in N/P ratio, but the EPA levels were unaffected by these conditions.

No difference between treatments was found on the *PTD5* $\beta$  gene, with its product catalyzing the first step of unsaturation of 18:1 (oleic acid) and the last desaturation step of 20:4 (ETA) to 20:5 (EPA), while its ortholog *PTD5* $\alpha$  showed an increase in the transcript. The same was observed for gene *ELO6\_b1*, which is responsible for elongation of C18:4 (stearidonic acid) into C20:4 and C18:3 into C20:3 in both alternative pathways. This enzyme was significantly affected by light, being more expressed at 72 h of exposure to high irradiance, while the same was not observed for *ELO6\_b2*, which remained at the same transcription level, reinforcing the understanding of different mechanisms of regulation that contribute to the activity of this limiting step under different environmental conditions. These data show that the regulation of unsaturation and elongation is strongly related to adjustments to environmental stresses.

The methyl-end desaturase Delta 15 is responsible for interconversion between the two pathways,  $\omega$ -6 and  $\omega$ -3, and has been identified in many organisms, though not in mammals (Lee et al. 2016). For this reason, mammals must acquire essential fatty acids such as LA and ALA ( $\omega$ -3) from foods or nutritional supplements, in order to synthesize longer chain  $\omega$ -3 fatty acids such as EPA and DHA (Plourde and Cunnane 2007). In contrast, a significant difference was observed, which could be related to more efficient interconversion, in response to a change in the natural environment. This hypothesis is based on the fluidics of membranes at lower temperatures that are signaled by light for marine algae since EPA synthesis can be produced from lipid precursors from both the  $\omega$ -3 and  $\omega$ -6 pathways.

The genes responsible for the synthesis pathway were analyzed, and important differences were observed: Light significantly affected the lipids profile, while lower light intensity was observed to be more favorable to PUFA synthesis, especially EPA. For future research, it would be interesting to verify other genes, especially *Phatr2\_9316* and *PtFAD6*, and enzymes  $\Delta 9$  and  $\Delta 12$  (respectively), which are responsible for the formation of 18:1 and 18:2 and represent the starting point of EPA synthesis. It would be interesting to create conditions for overexpression of these genes and determine if it is possible to obtain more precursors of PUFA synthesis.

## Conclusions

The physiological effect in *P. tricornutum* exposed for up to 72 h at two irradiances, 150 and 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  was investigated. The highest production in carotenoids and pigments syntheses occurred at the lowest irradiance, with 67.19 and 26.79  $\text{mg g}^{-1}$  of chlorophyll *a* and fucoxanthin, respectively.

Analysis of the gene expression responsible for the synthesis of PUFAs indicated a considerable variation in the transcript level of such genes, which hindered the association of their modulation. Furthermore, analysis of the formed products indicated that the lipid profile was strongly altered by this important environmental factor. The MUFA content was similar for both treatments. Under 750  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  exposure, the SFA content was 45.6% and PUFAs 23.92%, in contrast to 29.04 and 40.51% under lower irradiance. A significant increase in PUFA synthesis was achieved in the 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  light treatment, resulting in 5.73% increase in the most important fatty acid for this study, EPA.

Although microalgae have been shown to be suitable for many biotechnological applications, such potential could be substantially enhanced by maximizing the MUFA, SFA, and PUFA yield. In this way, the economic viability of industrial cultivation is expected to be clearly demonstrated.

The key conclusion is that a clear step to reach that objective has been taken in this study by showing that cultivation in 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  constant luminosity is the most viable path to obtain fucoxanthin and EPA. Additionally, *PTD6* expression could be correlated to EPA increase, so that it was possible to reroute *P. tricornutum* metabolism in order to increase the molecules of interest by altering environmental conditions.

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