UV-A mediated modulation of photosynthetic efficiency, xanthophyll cycle and fatty acid production of Nannochloropsis

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Title: UV-A mediated modulation of photosynthetic efficiency, xanthophyll cycle and fatty acid production of *Nannochloropsis*

Authors: Eduardo Forján[1], Inés Garbayo[1], Marta Henriques[2], Jorge Rocha[2], José M. Vega[3] and Carlos Vílchez[4]

Affiliations: Facultad de Ciencias Experimentales, Algal Biotechnology Group, Universidad de Huelva, 21071 Huelva, Spain[1]

Chemical Engineering Department, University of Coimbra, 3030-290 Coimbra, Portugal[2]

Facultad de Química, Departamento de Bioquímica Vegetal y Biología Molecular, Universidad de Sevilla, 41012 Sevilla[3]

International Centre for Environmental Research (CIECEM), Universidad de Huelva, Parque Dunar, Almonte, 21760 Huelva, Spain[4]

Running title: UV effect on growth and lipid production of *Nannochloropsis*

*Corresponding author: Carlos Vílchez

Address: Facultad de Ciencias Experimentales, Algal Biotechnology Group, Universidad de Huelva, Avda Tres de Marzo s/n, 21071 Huelva, Spain

email cvilchez@uhu.es Phone +34.959.219947 Fax +34.959.219942

Abstract

Nannochloropsis, a green microalga, is source for commercially valuable compounds as extensively described and, in particular, is recognized as a good potential source of EPA (20:5v3), an important polyunsaturated fatty acid for human consumption for prevention of several diseases. Climate change might include variation in the UV levels, as one of the consequences derived from the antropogenic activity. This paper shows the response of *Nannochloropsis* cultures exposed for 7 days to UV-A added to PAR. Growth rates and photosynthetic activity were assessed to determine the impact of UV-A increased levels on the cell growth and basic metabolism activity. Xanthophyll pigments (zeaxanthin and violaxanthin), carotenoids (cantaxanthin and β-carotene) and PUFAs (miristic, palmitic, palmitoleic, araquidonic and eicosapentanoic acids) were measured for assessing the antioxidant response of the microalgae to added UV-A radiation to PAR. The results show that the modulated use of UV-A radiations can led to increased growth rates which are sustained in time by an increased light transduction activity. The expected antioxidant response to the incident UV-A radiation consisted of increases in zeaxanthin and β-carotene contents –synthesis of antioxidant carotenoids- and increases in the SFAs (saturated fatty acids) to PUFAs (polyunsaturated fatty acids) ratio. The results suggest that modulated UV-A radiation can be used as a tool to stimulate value molecules accumulation in microalgae through an enhanced both light transduction process and antioxidant response, while sustaining cell growth.

Keywords: microalgae, *Nannochloropsis*, UV-A, xanthophyll cycle, fatty acids

Introduction

Algae are often presented as best solar bioconverters, since outdoor algal mass cultures have been shown to convert solar energy into chemical energy with much higher efficiency than most of the efficient terrestrial crops (Barbosa et al. 2003; Laws and Berning, 1991). Moreover, microalgae are currently being considered as suitable raw material for production of biofuel (Chisti, 2007) and unsaturated lipids of commercial value including carotenoids and PUFAs (Vilchez et al. 1997; Wen and Chen, 2003; Pulz and Gross, 2004). However, economically valuable production of such compounds still depends on enhancing algal biology and bioprocess engineering aspects crucial for its massive production. The following aspects can be cited among those which should be improved: increasing photosynthetic efficiency to enable increased biomass yield on light; enhancing biomass growth rate; eliminating the light saturation phenomenon and reducing photoinhibition, and reducing susceptibility to photooxidation that damages cells. Though molecular level engineering could potentially be used for that, genetic modification of microalgae have received little attention (León et al. 2004) and, moreover, modified strains could meet some growth difficulties when produced at large scale, specially in open systems (Pulz and Gross, 2004; Rodolfi et al. 2009). However, some other tools from classic biotechnology can be used for enhancing both growth and anabolic activity of microalgae in such a way that production of value compounds (e.g. carotenoids and PUFAs) can be specifically improved. Among such those tools, profile and concentration of nutrients, light quality and regime, growth factors and environmental conditions are included (Courchesne et al. 2009; Hsieh and Wu, 2009; Rodolfi et al. 2009). In this context, UV radiation is an oxidative factor that has been previously used at our laboratory for inducing antioxidant responses such as carotenoid

accumulation (Salguero et al. 2005), in cultures of *Dunaliella bardawil*. From those results, it was infered that culture illumination with suitable UV-A to PAR ratios could enhance microalgal growth and β-carotene accumulation of *Dunaliella bardawil*. Moreover, the enhanced growth seemed to respond to a more efficient action of the antenna chlorophyll molecules under light stress conditions (Polle et al. 2002). However, little is known about photosynthesis yield on absorbed light energy (e.g. photosynthetic efficiency and biomass yield) and related changes in value antioxidants content of UV-A exposed microalgal cultures (Heraud and Beardall, 2000; Liang et al. 2006).

Nannochloropsis, a microalgae that belongs to the class Eustigmatophycea, it is source for commercially valuable compounds including carotenoids as extensively described (Lorenz and Cysewski, 2000; Rodolfi et al. 2003), and is also recognized as a good potential source of EPA (20:5v3), an important polyunsaturated fatty acid for human consumption for prevention of several diseases (Wen and Chen, 2003). Main accesory pigments of that microalga, violaxanthin and vaucheriaxanthin esters, play a major role in light harvesting (Macías-Sánchez et al. 2005). Some minor xanthophylls (canthaxanthin, anteraxanthin, zeaxanthin) and carotenes (β-carotene) are also present in much lower amounts (Lubián et al. 2000). This work attemps to show that UV-A radiation can enhance photosynthetic light transduction into biomass and regulate both type and concentration of antioxidants in microalgae.

Materials and methods

Microorganism and culture conditions

Nannochloropsis gaditana was kindly provided by ICMAN-CSIC, Cádiz. Standard cultures were grown with F/2 medium (Guillard and Ryther, 1962) modified with double nitrate and phosphate concentrations to avoid nutrient limitation at 25ºC, bubbled with air containing 5% (v/v) CO_2 and continuously illuminated with fluorescent lamps (140 μE m^{-2} s^{-1}, at the surface of the flasks).

Growth rates determination

The growth rate was calculated from regressions of the linear portion of the growth curve expressed as the natural logarithm (ln) of number of cells versus time.

Oxygen evolution

The biological activity used to test cell viability was the photosynthetic activity. For photosynthetic activity determinations 1 ml cell culture of the microalgae was placed in a Clark-type electrode to measure O_2-evolution. Measurements were made at 25º C under saturating white light (1500 μE m^{-2} s^{-1}) or darkness (endogenous respiration).

Spectrophotometric determinations

Aliquots (5 ml) of the cultures were spinned down and the pellet obtained was freeze-dried and resuspended in 2.5 ml of absolute methanol to extract pigments. Chlorophyll and total carotenoids concentrations in the supernatant were determined either spectrophotometrically using the equations proposed by Wellburn (1994) or by HPLC

analysis for specific carotenoids (see below). Protein content was determined following the method described by Bradford (1976). Nitrate was determined spectrophotometrically as described by Cawse (1967).

HPLC analysis of carotenoids

Separation and chromatographic analysis of pigments was performed in a Merck Hitachi HPLC equipped with a UV-Vis detector as described by Young et al. (1997), using a RP-18 column and a flow rate of 1 ml min^{-1}. The mobile phase consisted on: solvent A, ethyl acetate; solvent B, acetonitrile/water (9:1, v/v) and the gradient programme applied was: 0-16 min, 0-60% A; 16-30 min, 60% A; 30-35 min, 100% B. Pigments detection was carried out at 450 nm, and their identification and quantification was achieved by injecting known amounts of pigment standards. Chlorophyll *a* and β-carotene were purchased from Sigma Chemical Co. (St. Louis, MO), all other standards were obtained from the Water Quality Institute VKI (Horsholm, Denmark).

Gas Chromatography

The followed method was described by Sato et al. (1988). After the fatty acids were sterified with a chloroform:methanol mixture (CHCl3:CH3OH) (1:2 v/v), the samples were injected in a gas chromatograph (TREMETRICS 9001) equipped with a PTV (Programmable Temperature Vaporiser) injector and a FID (Flame Ionisation Detector).The silica capillary column (DB225, J&W SCIENTIFIC) was 30 m long, with an outer layer of aluminium, intern and extern diameter of 0.15 and 0.25 mm respectively. The column had a 0.25 mm height apolar stationary phase of di-methyl siloxane. The carrier gas used was helio (N45, Air Liquide) at 2-3 ml/min. FID detector

was maintained at 300 ºC. The injector temperature, right after injection (1 mL of ether extract) rise to 250 ºC, in splitless mode. The oven's programme of temperatures started at 70 ºC for 1 min, followed by continuous increase of temperature (20 ºC/min) up to 180 ºC, a second increase (5 ºC/min) up to 220 ºC, kept at 220ºC for 5 min and, finally, a third increase (4 ºC/min) up to 240 ºC, kept at 240ºC for 2 min before the analysis ended. Chromatographic analyses of samples were performed by duplicate. The methyl esthers were identified by comparison of the retention times with those obtained using diverse references (Sigma-Aldrich). An internal reference was used (C19:0) for calibration.

Cell counting

The number of cells was determined by counting *N. gaditana* cells in a Neubauer chamber using an Olympus microscope model CX41.

Statistics

Unless otherwise indicated, figures show means and standard deviations of three independent experiments.

Results and Discussion

UV-A effect on growth parameters of *Nannochloropsis*

Added UV-A radiation to a given PAR irradiance influences growth parameters of *Nannochloropsis,* to different extent depending on the irradiance magnitude. The effect of UV-A radiation on *Nannochloropsis* was studied in growing cultures illuminated with a given PAR irradiance (140 µmol photons.m^{-2}.s^{-1}) that was added different UV-A irradiances (6, 15 and 24 µmol.m^{-2}.s^{-1}, respectively). Table 1 compiles light conditions of that experiment. The choice of these UV-A irradiances was based on results from preliminary experiments, in which no growth was observed in growing *Nannochloropsis* cultures illuminated with UV-A radiation above 25 µmol photons.m^{-2}.s^{-1} added to PAR (data not shown).

The experiment consisted of four growing *Nannochloropsis* cultures, three of them under continuous illumination of 140 µmol photons.m^{-2}.s^{-1} PAR and 6, 15 and 24 µmol photons.m^{-2}.s^{-1} UV-A, respectively, and a fourth one illuminated with 140 µmol photons.m^{-2}.s^{-1} PAR only. All cultures were prepared from a growing *Nannochloropsis* culture continuously illuminated with 140 µmol photon.m^{-2}.s^{-1} PAR only and all of them had the same initial cell density, about 10^8 cell.ml^{-1}. Figure 1 shows time-course cell density evolution of the cultures. From the beginning of the experiment, cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} UV-A showed the fastest growth. The growth of that cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} UV-A grew up much faster during the exponential phase, the growth end phase of that culture being reached earlier

than in the rest of cultures. On the contrary, growth of those cultures illuminated with higher UV-A irradiances, 15 and 24 μmol photons.m^{-2}.s^{-1} UV-A, was much slower than that of control cultures, in terms of cell density.

Growth rates of UV-A illuminated cultures were calculated from log phase of time-course cell density data, as explained in Materials and Methods. As shown in Figure 2, growth rate of those cultures illuminated with 6 μmol photons.m^{-2}.s^{-1} accounted for about 29% higher than growth rate of control cultures, whereas growth rate of those cultures illuminated with 15 and 24 μmol photons.m^{-2}.s^{-1} UV-A was much lower than that of control cultures. Therefore, our results show that *Nannochloropsis* growth might either be enhanced or be lowered by modulating the UV-A/PAR ratio selected for culture illumination. Thus, UV-A could act either through speeding microalgal growth or producing alguicide effect, always depending on the UV-A dose applied to the cultures.

In the field literature some examples of both possitive and negative UV-A effects on microalgal growth have been reported. Döhler et al. (1997) reported *Dunaliella* cell growth to become negatively affected by short-time exposure to UV radiation; on the contrary, Salguero et al. (2005) showed enhanced *Dunaliella* growth after long exposure to moderate UV-A radiation. In general, the toxic UV-A dose seems to depend on the specific microalgal sensitivity to the oxidative imposed conditions (Holm-Hansen et al. 1993) but, in particular, growth enhancement can be generally induced after short exposures (hours) at low UV-A irradiances.

Biochemical rationale behind such UV-A effects is still to be investigated. In general, it has been clearly stated that high UV-A doses cause oxidative stress in growing microalgae which face it by antioxidative response mechanisms induction, including carotenoid accumulation (Jahnke 1999; Salguero et al. 2005). Continuous exposure of growing microalgae to high UV-A doses ends in growth cesation due to directly affects photosynthesis, observed as a loss of PS II electron transport efficiency and increased radical formation (White and Jahnke, 2002).

The rationale behind possitive effects for microalgal growth after exposure to low UV-A doses still remains unclear. It has been published that high photosynthetic efficiencies lead to high microalgal per absorbed photon productivities (Melis et al. 1999). Usually, high photosynthetic efficiencies are obtained in diluted cultures with low chlorophyll per cell content. The last -low chlorophyll content- is one of the consequences of cell acclimation to excess light that can not be absorbed by the cells (Melis et al. 1999), like happens to UV-A exposed cultures. To assess whether UV-A exposed cultures could be synthetising more efficient antenna chlorophyll molecules, light-dependent chlorophyll activity of UV-A exposed cultures was determined. First, maximum cell chlorophyll content was determined both in UV-A exposed and control cultures of *Nannochloropsis*. Results are shown in Figure 3.

Surprisingly, even though the highest cell density of UV-A exposed cultures was found in those cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} (up to about 20% higher than control cultures, Figure 1), the maximum cell chlorophyll content of cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} was about 7% lower than that of control cultures (Figure 3).

Production of more biomass with less chlorophyll should be the result of a more efficient chlorophyll action. To probe that, light-dependent oxygen production (photosynthetic activity) was measured in log-phase UV-A exposed and control cultures. Light-dependent chlorophyll activity of UV-A exposed cultures was calculated from dividing produced oxygen (per mg chlorophyll) by amount of photons incoming the cultures (considering photon flux density at the flask surface). Results show that in terms of efficiency -produced oxygen per chlorophyll unit and incoming photon-, the photosynthetic yield of chlorophyll molecules in those cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} was higher than that of control cultures. That conclusion arises from results in Figure 4. Therefore, it can be concluded that modulated use of UV-A radiation for microalgal growth can result in production of more efficient chlorophyll which in the end leads to increased culture productivity.

UV-A effect on primary anabolism

Light transduction process mediated by a more efficient chlorophyll should result in a higher photosynthetic energy production rate (ATP and NADPH), thus speeding primary nutrient assimilation including nitrate transport and reduction into ammonium and its subsequent incorporation into aminoacids. That way, also protein synthesis should become speeded. This should be consistent with higher nitrate consumption rates and, theoretically, with higher protein production rates by the cells. In order to determine whether optimal UV-A irradiances speeded both those rates, nitrate uptake and protein synthesis per cell were determined in growing cultures of *Nannochloropsis gaditana*. Results are shown in Figure 5.

From time-course nitrate consumption kinetics of *Nannochloropsis* full nutrient cultures growing under different UV-A irradiances, nitrate consumption rates were calculated for each one of the cultures. As shown in Figure 5, nitrate consumption rate obtained for *Nannochloropsis* cultures illuminated with 6 μmol.m^{-2}.s^{-1} UV-A was 15% higher than that for control cultures, decreasing dramatically for cultures illuminated with higher UV-A irradiances. Besides, protein production rates were obtained from time-course protein accumulation kinetics in *Nannochloropsis* full nutrient cultures illuminated with UV-A irradiances. Similar differences were found in terms of protein production rate, with the exception of those cultures incubated under the highest UV-A irradiance (24 μmol.m^{-2}.s^{-1}), which is a sign of the alguicide effect of that UV-A dose for which no protein synthesis was observed.

Nitrate transport to the cells is active and exerted by an ATP-dependent permease (Florencio y Vega, 1983). The subsequent assimilatory reduction into nitrite and ammonium is also an energy-dependent enzyme-mediated redox process for which NADH is required (Kessler, 1964). The higher nitrate uptake activity in cultures illuminated with 6 μmol photons.m^{-2}.s^{-1} UV-A is consistent with a higher photosynthetically produced energy demand and, therefore, with a more efficient chlorophyll and higher oxygen production as shown in the results. The higher nitrate uptake in UV-A exposed cultures also resulted in an increased protein accumulation (Figure 5). Moreover and as already shown in Figure 4, the quantum yield –calculated as described in Materials and Methods- of growing cells in cultures illuminated with 6 μmol photons.m^{-2}.s^{-1} UV-A was clearly higher –up to 27%- than that of control cultures, which means that light incoming UV-A exposed cultures was more efficiently

used than in control cultures. This is coherent with the presence of a more efficient chlorophyll in the cells of those UV-A cultures, as expected according to results in Figure 2. In the end and in good agreement with that, biomass yield in cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} UV-A was also higher –up to 28%- than that of control cultures (Figure 6). Concluding, the results show that a more efficient light transduction is behind the enhanced growth of those cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} UV-A.

As commented, nitrate consumption rate at the highest UV-A irradiance did interestingly not result in net protein biosynthesis, indicating that the photosynthetically produced energy (ATP and NADPH) did not yield biomass production even though nitrate consumption and therefore, in theory, its subsequent reduction into ammonium, were taking place. In those cultures incubated under the highest UV-A irradiance, growth was almost inhibited. This unexpected phenomena could partly be explained in terms of maintenance energy requirements of inhibited cultures. Maintenance energy is defined as energy required for functions other than production of biomass (Pirt, 1965) and may include turnover of cell components, osmotic work, cell motility and active transport process (Pirt, 1975). In inhibited cultures, the presence of inhibitory substances which may affects transport processes, membrane structure stability and ionic strenght negatively, among other critical factors for cell growth (Maiorella et al. 1983), may result in higher maintenance energy requirements by the cells to maintain their cells structure intact. For that reason, for growing cells under optimal conditions maintenance energy requirements is almost negligible (Yan and Okos, 1987). Our observations of significant nitrate uptake (active transport) and even photosynthetic activity (metabolic energy production) in almost inhibited cultures of *Nannochloropsis*

illuminated with 24 µmol photons.m^{-2}.s^{-1} UV-A are therefore fully consistent with a higher demand of maintenance energy by the cells.

UV-A effect on carotenoid accumulation

As previously described in Introduction, UV-A is an oxidative radiation that stimulates antioxidant responses in microalgae including carotenoid accumulation. In our work, we have investigated both quantity and type of accumulated carotenoids in growing cells of *Nannochloropsis* cultures incubated under UV-A irradiances. Figure 7 shows time-course carotenoid accumulation in *Nannochloropsis* cultures illuminated with those different UV-A irradiances in Table 1. Although maximum cell density achieved in those cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} UV-A was just about 15% higher than that in control cultures (Figure 1), maximum volume carotenoid productivity (Figure 7A) in those cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} UV-A accounted for about 60% higher than in control cultures. That means UV-A –properly combined in suitable to PAR ratios- greatly stimulated carotenoid accumulation in *Nannochloropsis*. Indeed, maximum carotenoid content per cell of *Nannochloropsis* cells in UV-A illuminated cultures (6 µmol photons.m^{-2}.s^{-1} UV-A) accounted for about 30% higher than that content of control cells (Figure 7B).

Abundance of the main carotenoids in *Nannochloropsis* cell extracts from cultures incubated under UV-A irradiances was investigated by chromatographic separation by HPLC. Figure 8 shows maximum cell content of each one of the main carotenoids under the different light conditions assayed. Maximum β-carotene and cantaxanthin contents were found in those cultures illuminated with 6 µmol photons.m^{-2}.s^{-1} UV-A, the content of control cells being the second in quantity. However, a different pattern for

carotenoid abundance was found for violaxanthin and zeaxanthin. As observed in Figure 8, all those cultures irradiated with UV-A showed similar or higher zeaxanthin content than that of control cells and, interestingly, similar or lower violaxanthin content than that of control cells.

Based on fluorescence quenching measurements, zeaxanthin and xantophyll cycle were assigned a photoprotection role (Demmig et al., 1987; Demmig-Adams, 1990; Baroli and Niyogi, 2000). In excessive light zeaxanthin is formed from violaxanthin through an enzyme-mediated (violaxanthin de-epoxidase) reduction reaction (Gilmore, 1997; Eskling et al. 1997). In presence of oxidative factors including UV radiations, xantophyll cycle is expected to form zeaxanthin in order to neutralize increases in free radical species which affect cell metabolism negatively (Demmig-Adams, 1990; Gilmore, 1997; Niyogi et al. 1998; Baroli and Niyogi, 2000). Zeaxanthin to violaxanthin ratio in UV-A irradiated cultures of *Nannochloropsis* increases if UV-A irradiance is increased. Therefore, the xantophyll cycle turns into its photoprotection role to express the required antioxidant response to face the impossed oxidative stress.

In order to assess whether the enhanced carotenoid production in UV-A exposed cultures of *Nannochloropsis* is part of a more general effect consisting of an enhanced lipid production in UV-A exposed algal cultures, the time-course content of some saturated and unsaturated fatty acids was also determined. A typical chromatogram of fatty acids from *Nannochloropsis* standard cultures, obtained as described in Materials and Methods, is shown in Figure 9. As observed in Figure 10, the content of the main saturated fatty acids in *Nannochloropsis* (miristic and palmitic fatty acids, Figure 10A) increased both with increased UV-A irradiance and with exposure time of algal cultures

to UV-A radiation, with the exception of those cultures exposed to 24 μmol.m^{-2}.s^{-1}. On the contrary, the content of the main unsaturated fatty acid of *Nannochloropsis* (eicosapentanoic acid, EPA, Figure 10B) decreased both with increased UV-A irradiance and with exposure time of algal cultures to UV-A radiation. Consequently, the need of a stronger antioxidant response of *Nannochloropsis* when exposed to UV-A radiations seems to rather come from an enhanced carotenoid biosynthesis than other unsaturated lipids. That suggests induced-UV regulation of lipid-based antioxidant response in *Nannochloropsis*.

Our results show that in spite of expecting the antioxidant response to predominantly occur only under culture conditions at which growth becomes negatively affected (e.g. lack of nutrients, excess light, UV radiations, herbicides), an optimal UV-A irradiance can be found such that both enhanced carotenoid accumulation and enhanced growth may take place simultaneously. Moreover, both quantity and profile of specific fatty acids can be modified by UV-A, therefore being possible to modify cell composition in biomolecules depending on the wanted end use of the algal biomass. In our opinion, that can be used as a tool for carotenoid and, in a wider view, lipid production processes with viable cultures of microalgae.

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Table 1. Irradiance and light quality during *Nannochloropsis* growth. PAR: Photosynthetically Active Radiation. PFD: Photon Flux Density.

Irradiation and light quality (µmol photons $m^{-2} \cdot s^{-1}$)	**Total PFD (µmol photons $m^{-2} \cdot s^{-1}$)**	**UV-A/PAR ratios**
6 UV-A + 140 PAR	146	0.043
15 UV-A + 140 PAR	155	0.107
24 UV-A + 140 PAR	164	0.171

Captions to Figures

Figure 1. Time-course cell density of UV-A irradiated *Nannochloropsis* cultures. Cells grown under standard culture conditions were illuminated with PAR (140 µmol photons m^{-2} s^{-1}) plus either 0 (control cells, -□-), 6 (-■-), 15 (-●-) or 24 (-▲-) µmol photons m^{-2} s^{-1} UV-A. Cell density was measured as described in Materials and methods.

Figure 2. Growth rates of UV-A irradiated *Nannochloropsis* cultures. Growth rates were calculated from time-course cell density data, as described in Materials and Methods.

Figure 3. Maximum cell chlorophyll content of UV-A irradiated *Nannochloropsis* cultures. Cells grown under standard culture conditions were illuminated with PAR (140 µmol photons m^{-2} s^{-1}) plus either 0 (control cells), 6, 15 or 24 µmol photons m^{-2} s^{-1} UV-A. Chlorophyll was extracted and determined spectrophotometrically as described in Materials and Methods.

Figure 4. Photosynthetic efficiency measured as light-dependent chlorophyll activity of UV-A exposed cultures. Quantum yield was calculated from dividing produced oxygen (per mg chlorophyll) by amount of photons incoming the UV-A exposed cultures and control cultures (considering photon flux density at the flask surface). Oxygen evolution was followed by Clark electrode measurements on microalgal samples.

Figure 5. Nitrate consumption rate and maximum protein content of UV-A irradiated *Nannochloropsis* cultures. Cells grown under standard culture conditions were illuminated with PAR (140 µmol photons m^{-2} s^{-1}) plus either 0 (control cells), 6, 15 or 24 µmol photons m^{-2} s^{-1} UV-A. Nitrate in the culture medium and protein content inside the cells were measured as described in Materials and methods. Nitrate consumption rates were calculated from slopes of time-course nitrate concentration in the culture medium.

Figure 6. Biomass yield of UV-A exposed cultures. Biomass yield of each culture under the radiation conditions assayed is the averaged produced biomass to incoming photon flux density ratio calculated from the early exponential growth phase data of the UV-A exposed cultures and control cultures. Photon flux density was measured at the flask surface.

Figure 7. Carotenoid productivity of UV-A irradiated *Nannochloropsis* cultures. Cells grown under standard culture conditions were illuminated with PAR (140 µmol photons m^{-2} s^{-1}) plus either 0 (control cells), 6, 15 or 24 µmol photons m^{-2} s^{-1} UV-A. At the indicated times, algal samples were taken to determine total carotenoids per culture volume (A) and total carotenoid content per cell (B). Carotenoids were determined as described in Materials and Methods.

Figure 8. Maximum content of specific carotenoids in cells of UV-A irradiated *Nannochloropsis* cultures. Cells grown under standard culture conditions were illuminated with PAR (140 µmol photons m^{-2} s^{-1}) plus either 0 (control cells), 6, 15 or 24 µmol photons m^{-2} s^{-1} UV-A. Carotenoids were determined by HPLC as described in Materials and Methods.

Figure 9. Typical chromatogram of fatty acids from *Nannochloropsis* obtained as described in Materials and Methods.

Figure 10. Content of main fatty acids in cells of UV-A irradiated *Nannochloropsis* cultures. Cells grown under standard culture conditions were illuminated with PAR (140 µmol photons m^{-2} s^{-1}) plus either 0 (control cells), 6, 15 or 24 µmol photons m^{-2} s^{-1} UV-A. At the indicated times (48h and 96h), content of palmitic acid (C14:0) and miristic acid (C16:0) (main saturated fatty acids of Nannochloropsis) and eicosapentanoic acid (C20:5n3) (main unsaturated fatty acid of Nannochloropsis) were determined by GC as described in Materials and Methods. Total sum of both saturated fatty acids is shown in (A), and EPA content is shown in (B).

FIGURE 1

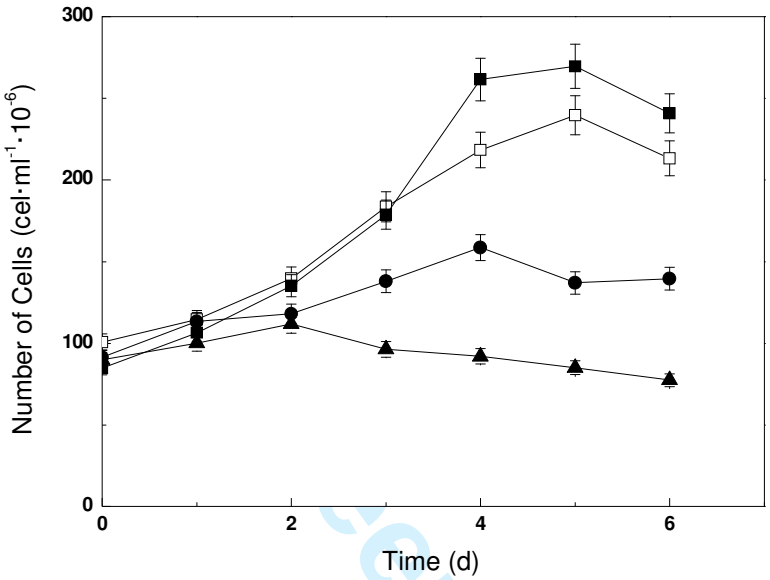


FIGURE 2

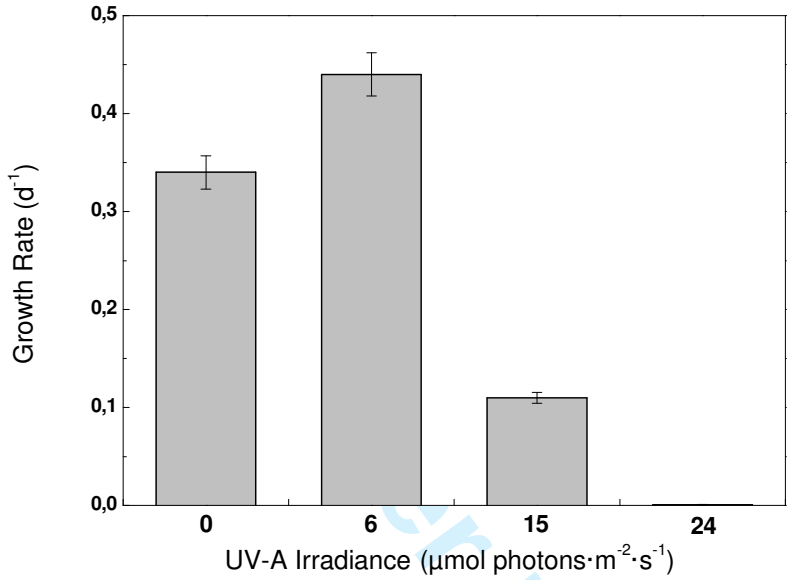


FIGURE 3

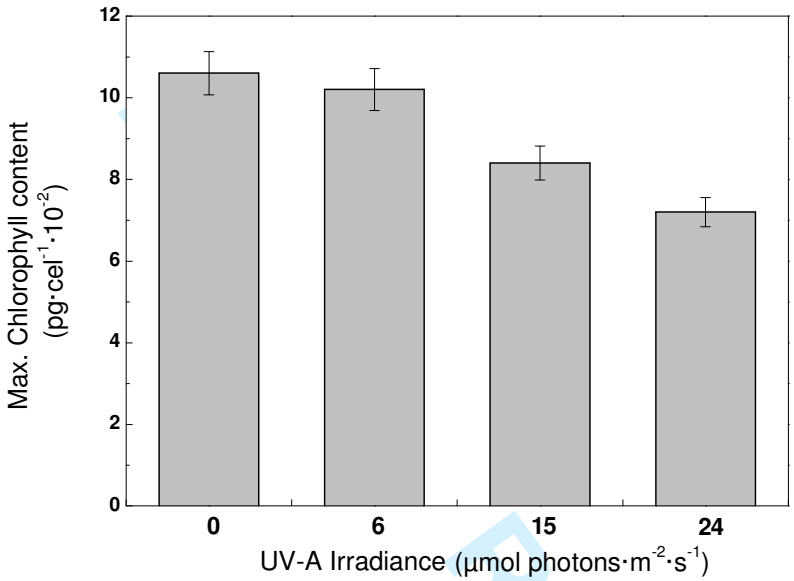


FIGURE 4

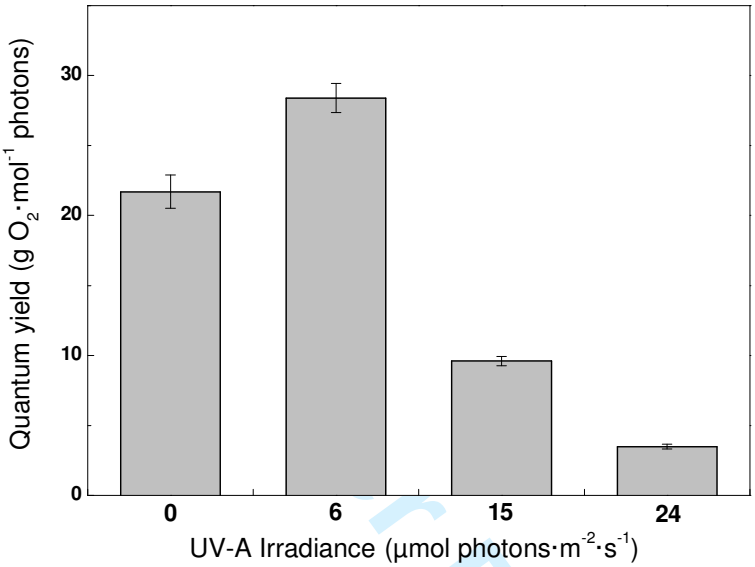


FIGURE 5

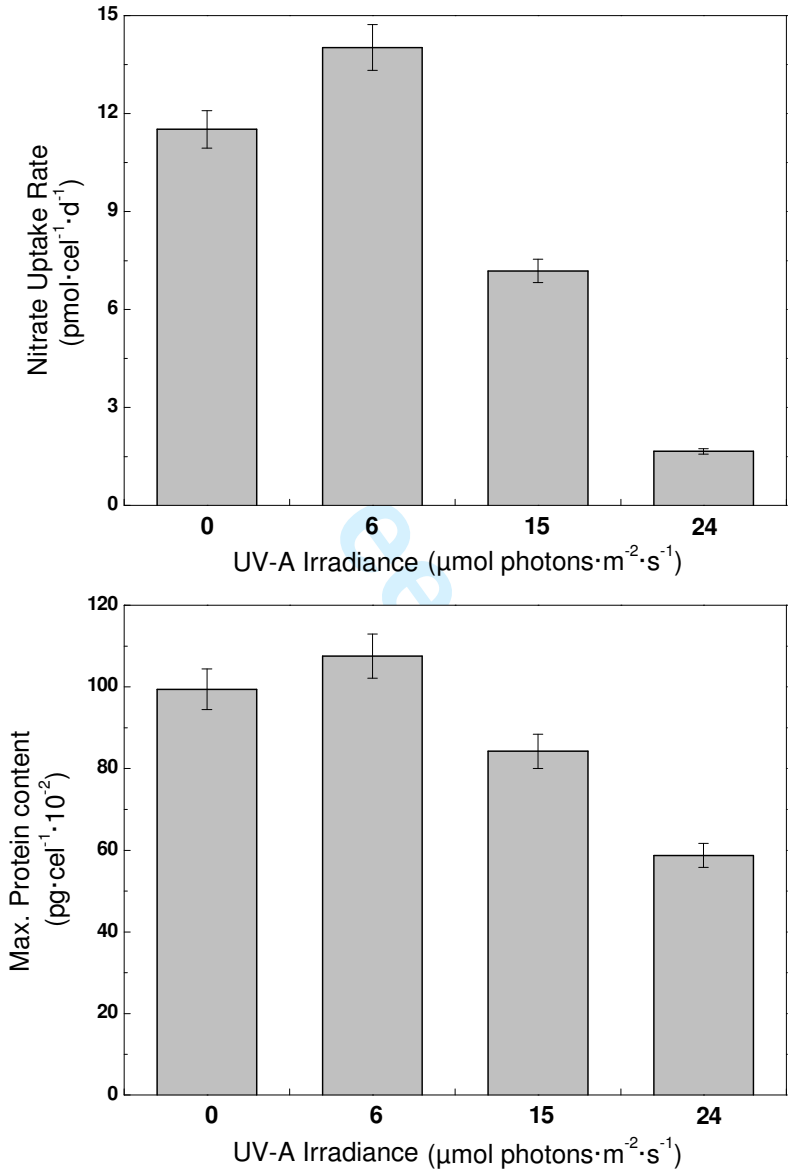

Nitrate Uptake Rate
(pmol·cel^{-1}·d^{-1})
15
12
9
6
3
0
0
6
15
24
UV-A Irradiance (µmol photons·m^{-2}·s^{-1})
Max. Protein content
(pg·cel^{-1}·10^{-2})
120
100
80
60
40
20
0
0
6
15
24
UV-A Irradiance (µmol photons·m^{-2}·s^{-1})

FIGURE 6

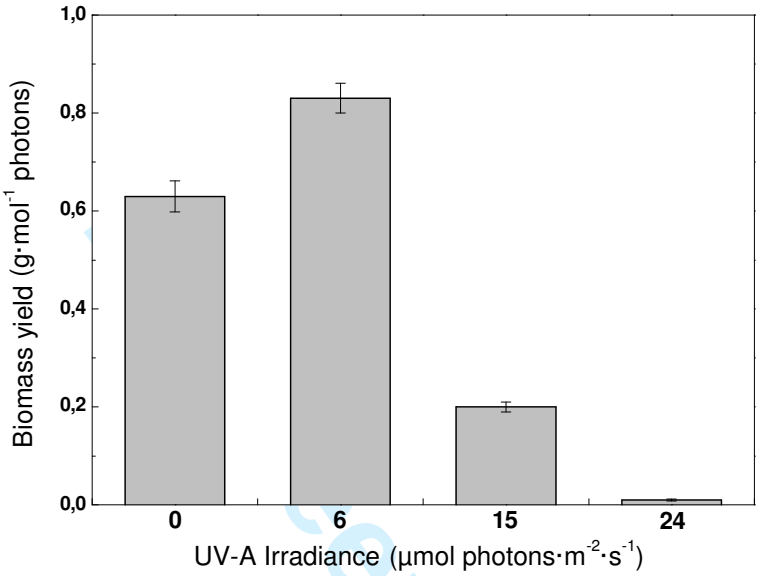


FIGURE 7

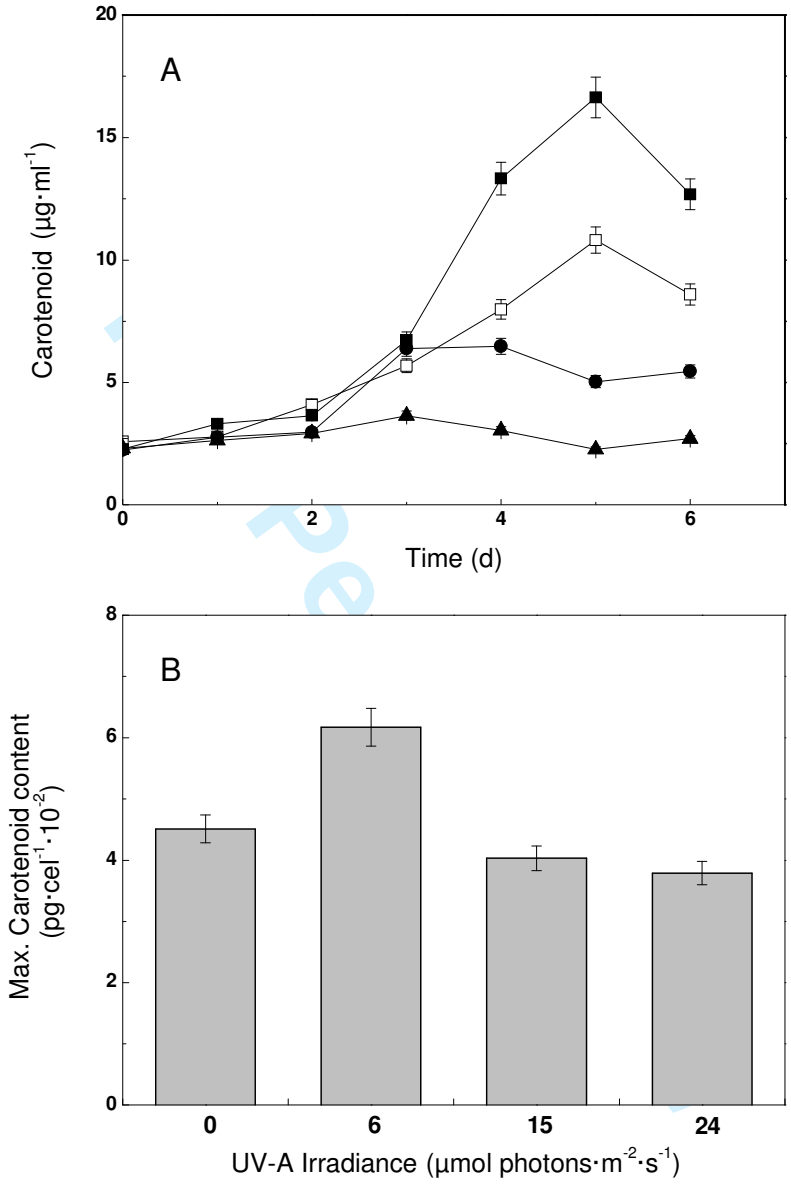

A
Carotenoid (µg·ml-1)
Time (d)
B
Max. Carotenoid content (pg·cel-1·10-2)
UV-A Irradiance (µmol photons·m-2·s-1)
0
6
15
24

FIGURE 8

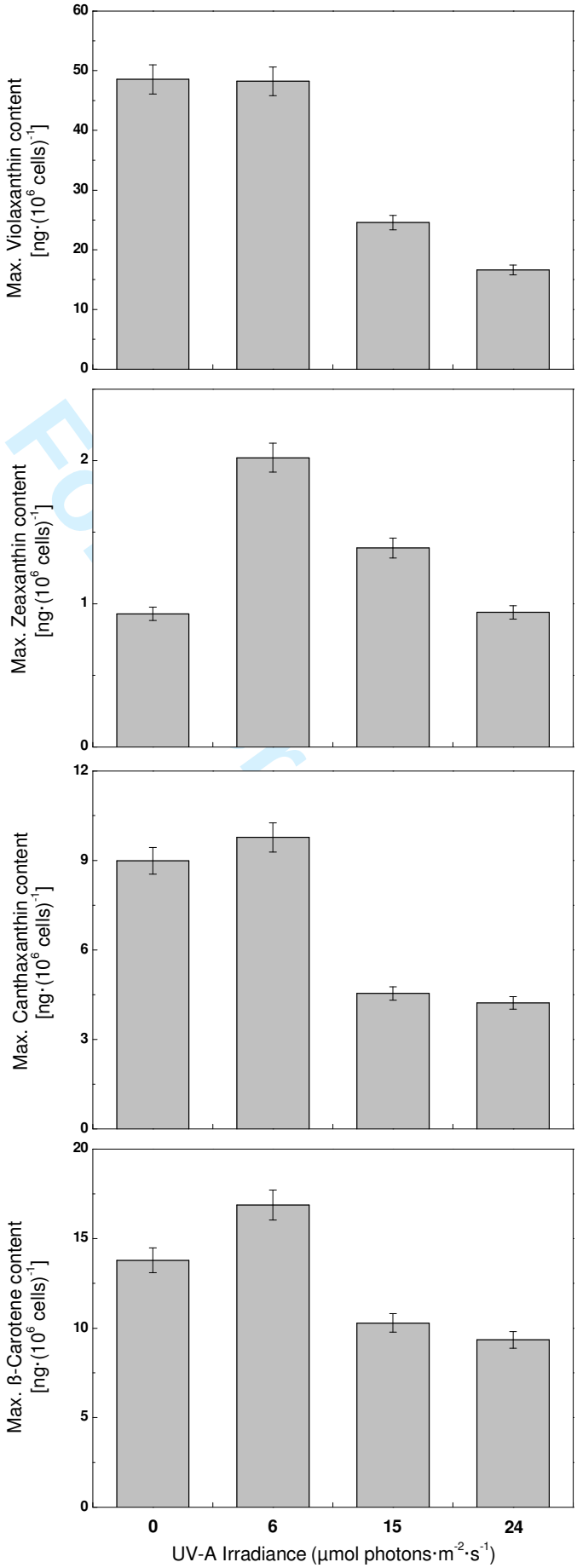

Max. Violaxanthin content [ng·(10^6 cells)^-1]
Max. Zeaxanthin content [ng·(10^6 cells)^-1]
Max. Canthaxanthin content [ng·(10^6 cells)^-1]
Max. ß-Carotene content [ng·(10^6 cells)^-1]
0
6
15
24
UV-A Irradiance (µmol photons·m^-2·s^-1)

FIGURE 9

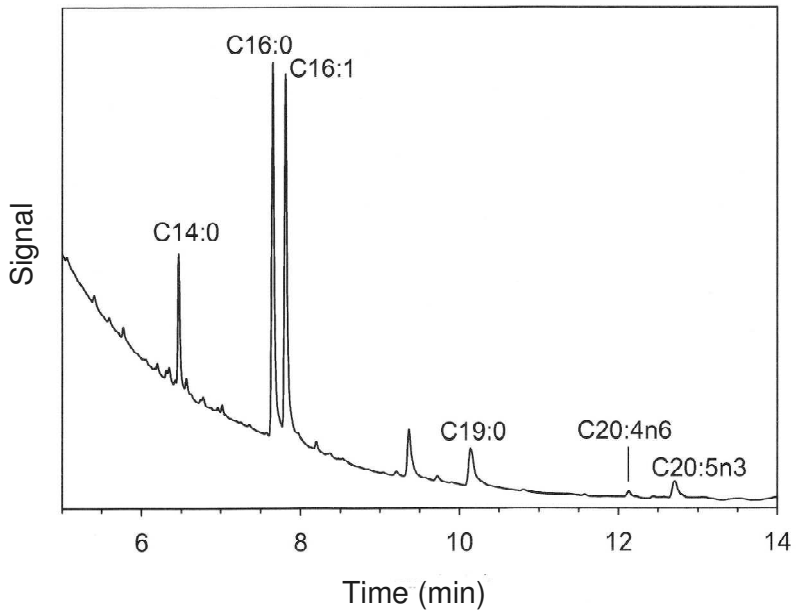
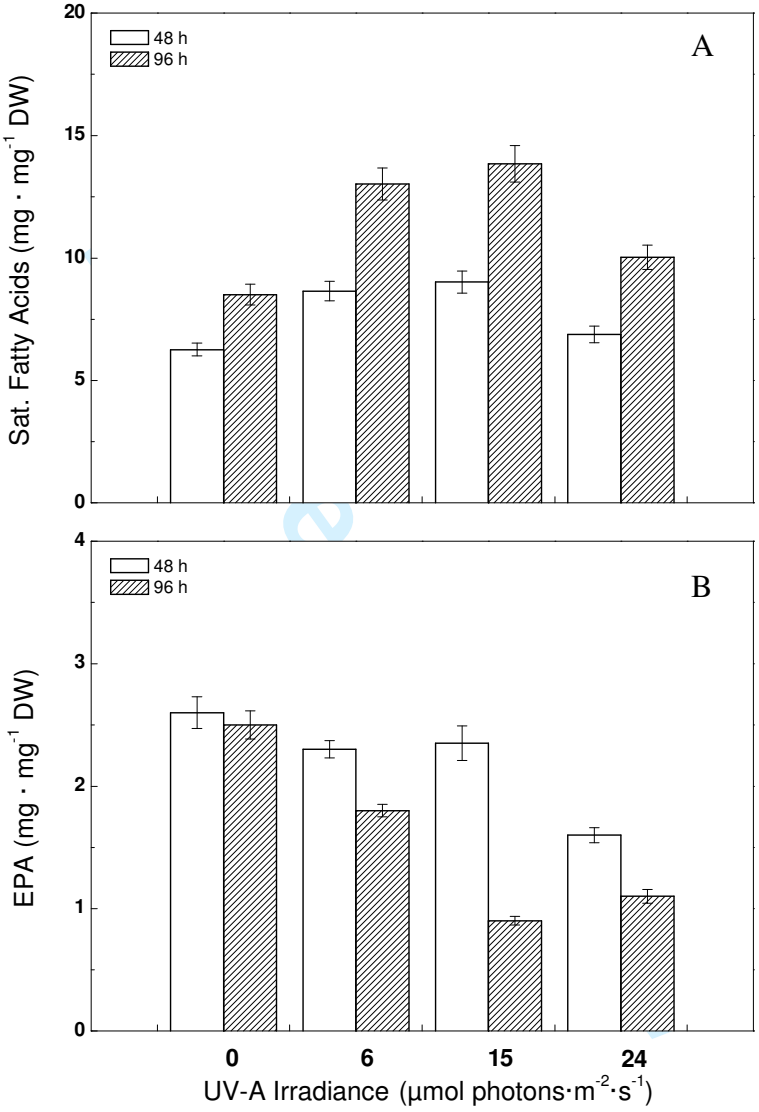


FIGURE 10


A
48 h
96 h
Sat. Fatty Acids (mg · mg-1 DW)
20
15
10
5
0
B
48 h
96 h
EPA (mg · mg-1 DW)
4
3
2
1
0
0
6
15
24
UV-A Irradiance (µmol photons·m-2·s-1)