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Effect of UV stress on the fatty acid and lipid class composition in two marine microalgae *Pavlova lutheri* (Pavlovophyceae) and *Odontella aurita* (Bacillariophyceae)

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Abstract Polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic and docosahexaenoic acids (EPA and DHA), are abundantly synthesized by some phytoplankton species and play a key role in the marine food chain. However, they are generally considered to be sensitive to oxidation by UV radiation (UV-R). In order to investigate the effect of UV-R on the lipid composition of two marine microalgae, *Pavlova lutheri* and *Odontella aurita*, they were exposed to a combination of UVA-R and UVB-R with a total UV-R daily dose of 110 kJ m^{-2}. Chlorophyll *a*, photochemical efficiency, and lipid composition were then determined on days 3, 5, and 8 of UV-R exposure. In *P. lutheri*, exposure to UV-R treatment led to a decrease in the proportions of PUFAs, such as EPA and DHA, especially into structural lipids (glycolipids and phospholipids). Our findings reveal a reduction of 20% in EPA levels and 16% in DHA levels, after 8 days of UV-R treatment. In *O. aurita*, exposure to UV-R did not change the fatty acid composition of the total lipids and lipid fractions of the cells. EPA levels remained high (27–28% of total lipids) during the 8 days of treatment. Consequently, the n-3 fatty acid content of *P. lutheri* was altered which highlights the sensitivity of this species to UV-R, whereas the results obtained for *O. aurita* suggest a more UV-R resistance. As a result, in latitude countries with medium UV-R level, outdoor "race-way" culture of *O. aurita* could yield a high-EPA algal biomass, whatever the seasonal variations in UV-R.

Keywords *Pavlova lutheri* · *Odontella aurita* · UV radiation · Lipid classes · n-3 fatty acids

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Introduction

The considerable depletion of the ozone layer in the stratosphere, which has been directly attributed to human activities, means that increased amounts of UV radiation (UV-R) reach the earth's surface (Karentz and Bosch 2001). This phenomenon is mainly observed in the polar regions, but ozone depletion has also been measured at lower latitudes of the northern hemisphere (Stolarski et al. 1992). UVA-R (315–400 nm) and UVB-R (280–315 nm) induce genetically and physiologically deleterious effects on many life forms of terrestrial and aquatic ecosystems (Hessen et al. 1997; Beardall and Raven 2004; Holzinger and Lütz 2006). Previous studies have shown that UVB-R reduces the growth rate and photosynthetic activity of phytoplankton (Beardall et al. 1997; Rech et al. 2005; Fouqueray et al. 2007). UVB-R can also damage the ultrastructure of nuclear and chloroplastic DNA, causing mutations or cell death (Holzinger and Lütz 2006; Buma et al. 2006). In microalgae, UV-R is also known to have deleterious effects on several enzymes and biochemical pathways, especially

those involved in the synthesis of fatty acids (Hessen et al. 1997; Skerratt et al. 1998; Liang et al. 2006a).

Numerous authors have recently studied the effects of UV-R on the fatty acid composition of microalgae (Goes et al. 1994; Wang and Chai 1994; Skerratt et al. 1998; Leu et al. 2006a, b; Liang et al. 2006a, b); however, the results reported are often contradictory. Some authors have observed an overall increase in levels of both saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), and a decrease in polyunsaturated fatty acids (PUFA) levels of total lipids, when microalgae were exposed to UVB-R (Goes et al. 1994; Wang and Chai 1994). In other studies, exposure to UV-R did not cause any significant change in their fatty acid compositions (Sundbäck et al. 1997; Skerratt et al. 1998). Moreover, some authors have reported increased levels of PUFAs after exposure to UV-R (Skerratt et al. 1998; Liang et al. 2006a). The changes in lipid compositions related to UV-R therefore seem to vary considerably in different microalgal species.

In a natural environment, such as the open ocean, changes in the position of microalgae in the water column provide protection against UV-R, which is strongly absorbed by seawater. In contrast, in artificial aquaculture ecosystems, such as oyster-ponds or microalgae culture systems in outdoor pools, the shallow water and low turbidity provide only limited protection against UV-R for the phytoplankton. Therefore, the UV-R doses received by the algae are higher in these shallow artificial environments than in the open ocean.

In this context, the aim of this ecophysiological approach reported here was to study the effects, in experimental conditions, of UVA-R/UVB-R ratio and daily dose equivalent to that observed in the natural environment on the lipid composition of two microalgae, *Pavlova lutheri* (Pavlovophyceae) and *Odontella aurita* (Bacillariophyceae), which are industrially cultured in outdoor pools and used in aquaculture and human nutrition, respectively, owing to high content in PUFAs. This was done by exposing cultures in exponential growth phase to UV-R stress for eight consecutive days. The chlorophyll *a* (Chl *a*) and lipid contents, the photochemical efficiency (F_v/F_m), the distribution of the major lipid classes, and the lipid class and total lipid fatty acid compositions were then determined for each species during UV-R exposure.

Materials and methods

Microalgal cultures and UV-R treatments

Pavlova lutheri and *Odontella aurita* cultures were obtained from the microalgal collection of the "Centre de Ressources Biologiques" (MMS, Université de Nantes, France). The microalgae were grown under batch conditions in 500 mL Erlenmeyer flasks, using a working volume of 300 mL. The culture medium was artificial seawater (Harrison et al. 1980) complemented following De Brouwer et al. (2002), modified according to Perkins et al. (2006). The medium was prepared with deionized water and was autoclaved at 121°C for 20 min. The flasks were inoculated with exponentially growing cells and with an initial density of 10^5 cell mL^{-1}. The cultures were maintained at 16±1°C in a thermo-regulated chamber under 100 µmol photons m^{-2} s^{-1} provided by high-intensity discharge lamp (Osram HQI-BT, 400 W, Munich, Germany), with a 14/10-h light/dark cycle. The irradiance level was measured in the middle of the Erlenmeyer, as the photosynthetic active radiation (PAR), using a 4π US-SQS/L Quantum Sensor (Walz Instruments, Germany), coupled to a LI-189 Data Logger (LI-COR Biosciences, France).

All the experiments were carried out in a custom-made frame described by Rech et al. (2005), in which cells placed in a 500-mL glass beaker received PAR from below and UV-R from above. The UV-R source consisted of a Vilber-Lourmat VL315 BLB (Torcy, France), equipped with three Sylvania F15W-BLB T8 (SLI, Germany) auto-filtrating fluorescent tubes to provide the UVA-R (peak emission at 365 nm), and a Vilber-Lourmat VL115M, equipped with two Vilber-Lourmat 15 M tubes, and an additional filter to provide UVB-R (peak emission at 315 nm). The UV-R irradiances were measured using an HD-R 9021 UV-Radiometer (Delta-Ohm Instruments, Italy) coupled with an LP 9021 UVA-R probe (spectrum range, 315–400 nm) for UVA-R and an LP 9021 UVB-R probe (spectrum range, 280–315 nm) for UVB-R. The UV-R irradiances were adjusted by changing distance or inserting neutral density filters between the UV-R sources and culture flasks. Before the UV-R exposure, 100 mL of cultures in exponential growth phase was replaced by 100 mL of fresh EMA medium (so that the nutrients were present in non-limiting quantities). Microalgae (300 mL culture) were transferred into 500 mL glass beakers covered by a thin polyethylene film (0.1 m), which prevent evaporation of the medium. It has previously been confirmed that this film did not significantly alter UV-R spectra (Rech et al. 2005). The light treatment consisted 100 µmol photons m^{-2} s^{-1} of PAR, 5.84 W m^{-2} of UVA-R, and 0.28 W m^{-2} of UVB-R (0.61 Wm^{-2} UVA-R and 0.23 Wm^{-2} weighted (Fouqueray et al. 2007) according to Cullen et al. (1992)). The UVA-R/UVB-R ratio (approximately 4.5%) and the total daily dose of UV-R (110 kJ m^{-2}) were equivalent to those measured in June on a sunny day in the "Pays de la Loire" region and previously used in the ecophysiological experiments of Rech et al. (2005) and Fouqueray et al. (2007). Microalgae were exposed to UV-R for 5 h in the middle of the

photoperiod, and the UV-R treatment was repeated on eight consecutive days. The controls were grown under the same conditions as those used before UV-R stress (100 µmol photons m^{-2} s^{-1}, 14/10 h light/dark cycle, 16± 1°C). Each experiment was repeated three times.

Chlorophyll *a* content, fluorescence measurement, and lipid analysis

On days 3, 5, and 8, the in vivo Chl *a* fluorescence was measured on 2 mL algal samples (maintained at 15°C) with an FMS 1 modulated fluorimeter (Hansatech Ltd, UK), modified for use at low Chl *a* concentrations (Rech et al. 2003). Fluorescence measurements were made every day before and after the UV-R treatment (5 h). Samples were dark-adapted for 5 min to limit their dark recovery (there was no significant difference after 5, 15, or 30 min of dark adaptation, data not shown) before measuring the fluorescence parameters. Following dark adaptation, the minimum fluorescence level (F_o) was measured, and the maximum fluorescence level (F_m) was obtained using a saturating flash (4,300 µmol photons m^{-2} s^{-1} for 0.7 s). These parameters made it possible to calculate the variable fluorescence $F_v=F_m-F_o$ and the photochemical efficiency of PSII (F_v/F_m).

Algal biomass was analyzed as the concentration of Chl *a* and total lipid content in 1 mL of culture. Pigments were extracted in dimethylformamide, and total chlorophyll *a* was determined by spectrophotometry (Speziale et al. 1984). Cells were gently harvested by centrifuging at low speed (1,200×*g*, 10 min). The pellets obtained were then frozen and stored at −70°C prior to analysis. Chl *a* content and lipid analysis were carried out on day 0 (control), 3, 5, and 8 of UV-R treatment.

Lipid and fatty acid analyses

All chemicals used in the experiments were of analytical grade and were purchased from Carlo Erba (Val de Reuil, France). Total lipids were extracted with methanol/chloroform (2/1, *v*/*v*) after adding 200 µL of 2.8 g L^{-1} NaCl, a modified version of Bligh and Dyer's (1959) method, using manual crushing (Dounce cells grinders) coupled with sonication (twice, for 15 and 30 min, respectively). Chloroform (1 mL) was added between the two sonication steps in order to allow phase separation. The chloroform layer, containing the lipids, was collected, and a second extraction was carried out by adding 2 mL of chloroform to the remaining methanol/water phase. The solvents were removed by evaporating under vacuum, and all samples were dissolved in a known volume of chloroform. Until they were analyzed, the lipid extracts were stored at −20°C under nitrogen gas (N_2) to limit oxidization.

Total lipid extracts were fractionated on reversed phase silica gel columns (Sep-Pak Plus silica cartridges, Waters, France) after an activation step with 20 mL of methanol followed by 20 mL of chloroform. Neutral lipids were eluted using 20 mL of chloroform, polar lipids such as glycolipids were eluted with 40 mL of chloroform/methanol (5/1, *v*/*v*), and the phospholipids were recovered in 30 mL of methanol (Sukenik et al. 1989). The different fractions obtained were dried by evaporating under a stream of N_2. Thin-layer chromatography of the total lipid extract was carried out in order to check the purity of each fraction (Henderson and Tocher 1992).

Lipids were quantified by the sulfuric acid charring method of Marsh and Weinstein (1966), using palmitic acid as standard. The sulfuric acid charring method gave reproducible results and made it possible to carry out quantitative determinations of lipid contents in small culture volumes.

The solvent was then evaporated under N_2, and the fatty acids were extracted after saponifying with CH_3OH-NaOH 0.5 M at 80°C for 20 min, according to the method of Slover and Lanza (1979). The fatty acid methyl esters (FAMEs) were formed directly by treating the different extracts with boron trifluoride-methanol (BF_3-MeOH, Sigma-Aldrich, France) at 80°C for 20 min. The FAMEs were then extracted with iso-octane after adding 35% NaCl. All samples were analyzed with a FOCUS gas chromatography apparatus (Thermo Electron Corporation, France) equipped with a flame ionization detector and a fused-silica capillary column (CP Sil-88 25 m×0.25 mm id capillary column, Varian, France). Samples were injected using an autoinjector AI 3000 (Thermo Electron Corporation, France). The injector and detector temperatures were 250°C and 280°C, respectively; and the oven temperature was increased from 120°C to 220°C at a rate of 6°C min^{-1}. N_2 was used as the carrier gas. Pure standards (Sigma-Aldrich) were used to identify the fatty acids by comparing the peak retention times of the samples and the standards. Pentadecanoic acid was used as an internal standard to quantify the fatty acid content.

Data analysis

All the results were analyzed by one-way analysis of variance (ANOVA). Post hoc analyses were then made by Student–Newman–Keuls (SNK) test to estimate the differences between treatment groups. Differences were considered significant at $p < 0.05$. All statistics were performed with SigmaStat (version 3.1) software (SPSS).

Results

In this section, for each of the parameters estimated, the control corresponds to the sum of results obtained

on days 3, 5, and 8, when the cells grown at 100 μmol photons m^{-2} s^{-1} without UV-R. No significant variations in parameters, excepted photochemical efficiency, were observed in the controls over the time of UV-R treatment (data not shown).

Biomass production, chlorophyll *a* content, and photochemical efficiency

Algal biomass production was estimated as the Chl *a* and total lipid contents expressed in μg mL^{-1} of culture. Relative to control (Table 1), these two parameters decreased in both species only after 8 days of UV-R exposure (Chl *a*, $p=0.115$, $p=0.298$; lipid content, $p=0.246$, $p=0.808$; one-way ANOVA; *P. lutheri* and *O. aurita*, respectively). The growth slowed down and stopped under UV-R treatment. Chl *a* content was also expressed in μg × 10^6 $cell^{-1}$ of culture (Table 1). The results shown that the cellular Chl *a* concentration of *P. lutheri* and *O. aurita* did not change significantly ($p=0.52$ and $p=0.638$, respectively, one-way ANOVA), no matter how many consecutive days of UV-R stress they had been exposed. Nonetheless, the cellular Chl *a* concentration of *P. lutheri* tended to decline after 8 days of treatment, whereas that of *O. aurita* tended to increase during UV-R exposure.

In the two species, UV-R affected the value of the fluorescence parameter F_v/F_m (photochemical efficiency) from day 3 of treatment (Fig. 1). The F_v/F_m of the control was similar for the two species (0.63±0.01 for *O. aurita* and 0.65±0.02 for *P. lutheri*) and did not show any significant change over time ($p=0.176$ and $p=0.243$, respectively, one-way ANOVA). Photochemical efficiency measured in the middle of the photoperiod showed a decrease of 12% to 25% in *O. aurita* and of 22% to 40% in *P. lutheri* after 5 h of UV-R exposure. However, the algal culture recovered from the exposure to UV-R as shown by the F_v/F_m measurements taken each day before the start of the UV-R treatment; so, *O. aurita* exhibited F_v/F_m values corresponding to 99% of the control on day 3, 96% on day 5, and only 90% on day 8; *P. lutheri* was similarly affected at first, with 95% on day 3, 99% on day 5, but was more affected, 74% less than in the control on day 8. Compared to the control and in all cases, 5 h of UV exposure caused a decrease in the photochemical efficiency, which reached 32% and 55% on day 8 for *O. aurita* and *P. lutheri*, respectively.

Lipid and fatty acid analyses

Changes in cell lipid concentration, in the two species studied, is presented in Table 1. In *P. lutheri*, the cell lipid concentration increased significantly after 8 days of UV-R exposure ($p<0.001$, one-way ANOVA). However, the lipid content of *O. aurita* increased until the fifth day of UV-R stress and then decreased after the eighth day of treatment ($p=0.001$, one-way ANOVA) but was not different of the control ($p=0.397$, one-way ANOVA).

The influence of UV-R on the levels of each of the lipid fractions of *P. lutheri* and *O. aurita* is reported in Table 2. In both the species, the percentages of neutral lipids (NL), glycolipids (GL), and phospholipids (PL) did not significantly change when the cells were exposed to UV-R (*P. lutheri*, $p=0.529$, $p=0.735$, and $p=0.652$, respectively; *O. aurita*, $p=0.864$, $p=0.828$, and $p=0.641$, respectively;

Table 1 Chlorophyll *a* and lipid concentrations of *Pavlova lutheri* and *Odontella aurita* cultured under photosynthetic active radiation irradiance of 100 μmol photons m^{-2} s^{-1} (control) and after UV radiations exposure on each experimental day (means ± SE, $n=3$)

	Control	Day 3	Day 5	Day 8
Chl *a* content (μg mL^{-1})				
Pavlova lutheri	0.88±0.09 b	0.84±0.04 b	ND	0.54±0.06 a
Odontella aurita	0.80±0.11 b	0.59±0.10 ab	0.62±0.09 ab	0.54±0.06 a
Total lipid content (μg mL^{-1})				
Pavlova lutheri	16.86±1.88	15.37±0.12	ND	14.21±1.61
Odontella aurita	12.20±3.38	12.73±3.16	14.03±2.81	10.51±1.57
Chl *a* concentration (μg × 10^6 $cell^{-1}$)				
Pavlova lutheri	0.30±0.04 a	0.31±0.01 a	ND	0.27±0.01 a
Odontella aurita	3.84±0.51 a	3.94±0.81 a	4.45±1.17 a	4.51±1.15 a
Lipid concentration (μg × 10^6 $cell^{-1}$)				
Pavlova lutheri	5.80±0.11 a	5.68±0.15 a	ND	7.15±0.13 b
Odontella aurita	62.67±9.91 a	81.07±3.37 b	96.31±6.16 c	67.35±7.16 a

After one-way analysis of variance, Student–Newman–Keuls (SNK) multiple comparison test results are arranged in increasing order from left to right: a<b<c ($p<0.05$). No significant variations in all parameters were observed in the controls over the time of UV radiation treatment

ND not determined

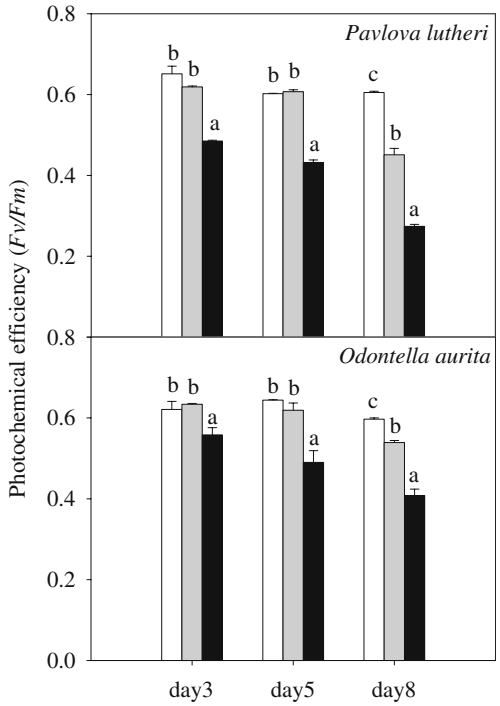

Fig. 1 Photochemical efficiency (F_v/F_m) measured in *Pavlova lutheri* and *Odontella aurita* control (*white bars*) before (*gray bars*) and after (*black bars*) 5 h of UV radiations treatment on 3, 5, and 8 days of culture under an irradiance of 100 µmol photons m^{-2} s^{-1} (means ± SE, n=3). After one-way analysis of variance, Student–Newman–Keuls (SNK) multiple comparison test results are arranged in increasing order from left to right: a<b<c (p<0.05)

one-way ANOVA). Under the experimental conditions the NL and GL represented the major lipid fractions in both species. In *P. lutheri*, the levels of NL, GL, and PL were between 40–43%, 44–45%, and 12–15% of total lipids, respectively. Similarly, in *O. aurita*, the percentages of NL and GL accounted for 41–46% and 50–55%, respectively, while the PL did not exceed 5% of total lipids.

The results concerning the effects of UV-R on the total lipid fatty acid composition of *P. lutheri* and *O. aurita* are presented in Tables 3 and 4, and those of each lipid fractions (NL, GL, and PL) in Figs. 2 and 3. In *P. lutheri*, the fatty acid composition of total lipids (Table 3), GL and PL (Fig. 2), followed similar patterns during exposure to UV-R stress. MUFA levels had increased, whereas PUFA levels had decreased after 8 days of treatment. With regard to the GL and PL fatty acid composition, SFA levels were also higher after 8 days of UV-R treatment. In the case, the low proportions of PUFAs and n-3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) in total lipids were correlated to the lower EPA level in GL, as EPA and DHA levels in PL. With regard to the NL fatty acid composition, exposure to UV-R treatment caused no significant modifications of the fatty acid composition of *P. lutheri*. In *O. aurita*, the fatty acid compositions of total lipids (Table 4) and each lipid fraction (Fig. 3) did not change significantly during UV-R treatment.

Discussion

Recently, the deleterious effects of UV-R on cellular components and several metabolic pathways of marine phytoplankton have been thoroughly examined in experiments conducted under controlled conditions in the laboratory using monoclonal cultures (Rech et al. 2005; Liang et al. 2006a, b; Fouqueray et al. 2007). Similarly, this work is a first approach to investigating the effects of UV-R stress (PAR+UVA-R+UVB-R) on lipid metabolism in *P. lutheri* and *O. aurita*. Several studies have already shown that exposure to light, including UV-R, considerably alters the fatty acid composition and therefore the nutritional value (Hessen et al. 1997; Leu et al. 2006b) in numerous microalgae (Goes et al. 1994; Wang and Chai 1994; Skerratt et al. 1998; Liang et al. 2006a, b). However, as far as we are aware, no data exists about the influence of UV-R on the lipid composition of the diatom *O. aurita* and, more generally, on the fatty acid composition of the different lipid classes of microalgae.

Table 2 Relative levels (percentage of total lipids) of the major lipid fractions in *Pavlova lutheri* and *Odontella aurita* cultured under an irradiance of 100 µmol photons m^{-2} s^{-1} (control) and after UV radiation exposure on each experimental day (means ± SE, n=3)

	Control	Day 3	Day 5	Day 8
Neutral lipids				
Pavlova lutheri	41.33±2.51	40.52±0.66	ND	43.12±0.74
Odontella aurita	43.03±5.53	41.12±5.55	43.50±6.68	45.64±1.64
Glycolipids				
Pavlova lutheri	44.93±0.68	44.96±0.66	ND	44.52±0.74
Odontella aurita	52.45±4.90	55.21±8.24	53.53±6.87	50.19±1.21
Phospholipids				
Pavlova lutheri	13.75±2.76	14.52±1.76	ND	12.36±0.27
Odontella aurita	4.53±1.64	3.67±2.69	2.97±0.20	4.27±1.64

See footnote to Table 1

Table 3 Total lipid fatty acid composition (percent molar) of *Pavlova lutheri* cultured under an irradiance of 100 µmol photons m^{-2} s^{-1} (control) and after UV radiation exposure on each experimental day (means ± SE, n=3)

	Control	Day 3	Day 8
Saturated fatty acids			
14:0	13.8±1.5	13.8±0.2	12.5±0.9
16:0	16.9±0.7 a	16.3±0.2 a	19.1±0.6 b
18:0	3.1±0.2 a	3.0±0.5 ab	4.3±0.7 b
Sum SFAs	33.8±2.3	33.1±0.8	35.9±2.1
Monounsaturated fatty acids			
16:1 n-7	14.6±0.6 b	14.4±0.1 b	11.6±1.2 a
18:1 n-7+18:1 n-9	4.0±0.3 a	3.9±0.2 a	12.0±1.4 b
Sum MUFAs	18.7±0.9 a	18.2±0.2 a	23.4±2.6 b
Polyunsaturated fatty acids			
18:2 n-6	1.2±0.4	1.1±0.1	1.8±0.8
18:3 n-3	0.7±0.2	0.5±0.1	0.9±0.3
18:4 n-3	7.2±0.9	8.3±0.3	7.6±0.3
20:4 n-6	0.1±0.1	Tr	0.3±0.2
20:5 n-3 (EPA)	22.6±0.5 b	23.4±0.4 b	18.2±0.8 a
22:5 n-6	0.9±0.1	0.8±0.1	0.7±0.1
22:6 n-3 (DHA)	13.4±0.5 b	13.5±0.3 b	11.2±0.6 a
Sum PUFAs	46.1±1.8 b	47.6±0.9 b	40.5±0.9 a
Unidentified	1.5±1.1	1.1±0.1	0.1±0.0
n-3	43.9±1.5 b	45.7±0.9 b	37.9±1.5 a
n-6	2.2±0.6	1.9±0.1	2.6±0.9

See footnote to Table 1

DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *Tr* traces

Effect of UV-R on chlorophyll *a* content and photosynthetic capacity

It is generally recognized that UV-R degrades photosynthetic pigment and proteins of chloroplasts, especially the D1 protein of the photosystem II (PSII), which is involved in the mechanisms of photosynthesis (Renger et al. 1989; Sinha and Häder 2002). UV-R has been shown to induce photoinhibition of the biosynthesis pigments, particularly of Chl *a* in different species of microalgae (Döhler and Lohmann 1995).

In our study, no significant difference in cellular Chl *a* concentration was obtained during UV-R treatment in both the species studied. Nevertheless, in *P. lutheri*, a slight decrease in the Chl *a* level was observed after exposure to UV-R for 8 days. Our results also showed that the photochemical efficiency of *P. lutheri* decreased considerably during UV-R treatment, which could justify the conclusion that this species was highly sensitive to UV-R, as Döhler and Lohmann (1995) also suggested. In *O. aurita*, cellular Chl *a* concentration tended to increase slightly during UV-R treatment. Fouqueray et al. (2007) obtained similar results in *O. aurita* and in two other species of diatom under similar UV-R conditions. According to these authors, *O. aurita* seems to be less sensitive to UV-R and quickly develop mechanisms that protect it against the damage caused by UV-R stress (Fouqueray et al. 2007).

In this work, from day 3, exposure to UV-R for 5 h per day significantly affected the photosynthetic capacities (photochemical efficiency) of both microalgae. A similar but greater effect has previously been reported in arctic diatoms by Leu et al. (2006b). This photoinhibition most probably contributes to the decrease in biomass production. Both species display a significant recovery each day after UV-R treatment; although the fact this was incomplete on day 8 suggested that the photodamage produced could not be fully repaired by the next day. Recovery after UV-R exposure was higher in *O. aurita* than in *P. lutheri*.

Effect of UV-R on lipid content and major lipid fractions

In microalgae, UV-R is known to reduce the capacity to absorb inorganic nutrients (Döhler and Biermann 1994; Hessen et al. 1997). A deficiency in any of these nutrients (nitrogen, phosphate, and dissolved inorganic carbon) is often considered to be the cause of many changes in the biochemical composition of microalgae (Reitan et al. 1994; Parrish and Wangersky 1997; De Castro Araújo and Tavano Garcia 2005; Khozin-Goldberg and Cohen 2006). In our study, the cellular lipid content of *P. lutheri* had increased after 8 days of UV-R treatment, while that of *O. aurita* increased until day 5, and then decreased on day 8. These results could be explained by the fact that most microalgae accumulate lipids when exposed to nutrient-limited conditions (Illman et al. 2000; Scragg et al. 2002). Moreover, several studies have also shown that algal cell sensibility to UV-R depends on nutrient availabilities which are involved in repair processes like protein re-synthesis from nitrogen (Beardall et al. 2009).

The reduction of nutrient availability is often associated with increased proportions of triacylglycerols (reserve lipids) and a reduction of GL (structural lipids) (Alonso et al. 2000; Khozin-Goldberg and Cohen 2006). In this study, no change in relative proportions of the lipid fractions was observed when the cells of *P. lutheri* and *O. aurita* were exposed to UV-R. In both species, NL and GL constituted the majority lipid classes (*P. lutheri*, 40–43% and 44–45%; *O. aurita*, 41–46% and 50–55%, respectively). The sum of structural lipids (GL+PL) accounted for 57–59% of total lipids in *P. lutheri* and for 54–59% of total lipids in *O. aurita*. These high levels of structural lipids, particularly

Table 4 Total lipid fatty acid composition (percent molar) of *Odontella aurita* cultured under an irradiance of 100 µmol photons m^{-2} s^{-1} (control) and after UV radiation exposure on each experimental day (means ± SE, n=3)

	Control	Day 3	Day 5	Day 8
Saturated fatty acids				
14:0	11.4±2.9	12.0±1.5	11.1±3.0	11.6±4.1
16:0	13.7±3.4	13.7±3.1	13.9±1.5	14.3±3.8
18:0	2.2±1.1	2.6±2.2	2.1±0.9	2.0±0.1
Sum SFAs	30.1±3.2	30.7±4.4	29.9±1.9	30.3±0.5
Monounsaturated fatty acids				
16:1 n-7	22.5±1.8	23.4±2.6	23.9±2.6	23.7±3.7
18:1 n-7 + 18:1 n-9	10.1±0.9	9.5±0.9	10.4±0.7	10.7±0.4
Sum MUFAs	32.6±1.3	32.9±2.8	34.2±2.5	34.3±3.3
Polyunsaturated fatty acids				
18:2 n-6	0.7±0.5	0.7±0.7	0.9±0.4	1.0±0.3
18:3 n-3	0.8±0.6	0.4±0.4	0.5±0.5	0.2±0.1
18:4 n-3	1.3±0.9	0.9±0.6	0.7±0.4	0.6±0.3
20:4 n-6	0.3±0.4	0.3±0.2	0.2±0.2	0.2±0.2
20:5 n-3 (EPA)	25.6±3.6	25.7±4.9	26.1±3.3	26.3±5.4
22:5 n-6	0.4±0.1	0.4±0.3	0.4±0.3	0.3±0.2
22:6 n-3 (DHA)	3.8±0.4	3.8±0.3	3.8±0.3	3.5±0.5
Sum PUFAs	32.8±3.9	32.2±4.8	32.6±3.7	32.0±6.2
Unidentified	5.5±1.9	4.2±2.8	3.3±1.6	3.5±2.4
n-3	31.5±3.6	30.7±4.5	31.1±3.4	30.6±5.7
n-6	1.4±0.4	1.5±0.5	1.5±0.7	1.4±0.4

See footnote to Table 1

DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

GLs, are characteristic of microalgae in the exponential growth phase (Fidalgo et al. 1998; Alonso et al. 2000; Mansour et al. 2003). UV-R therefore did not modify the proportions of the different lipid classes (NL, GL, and PL) in either species during this growth phase.

Effect of UV-R on the fatty acid composition of total lipid content and major lipid fractions

The fatty acid composition of the total lipids and major lipid fractions depended mainly on the species and the

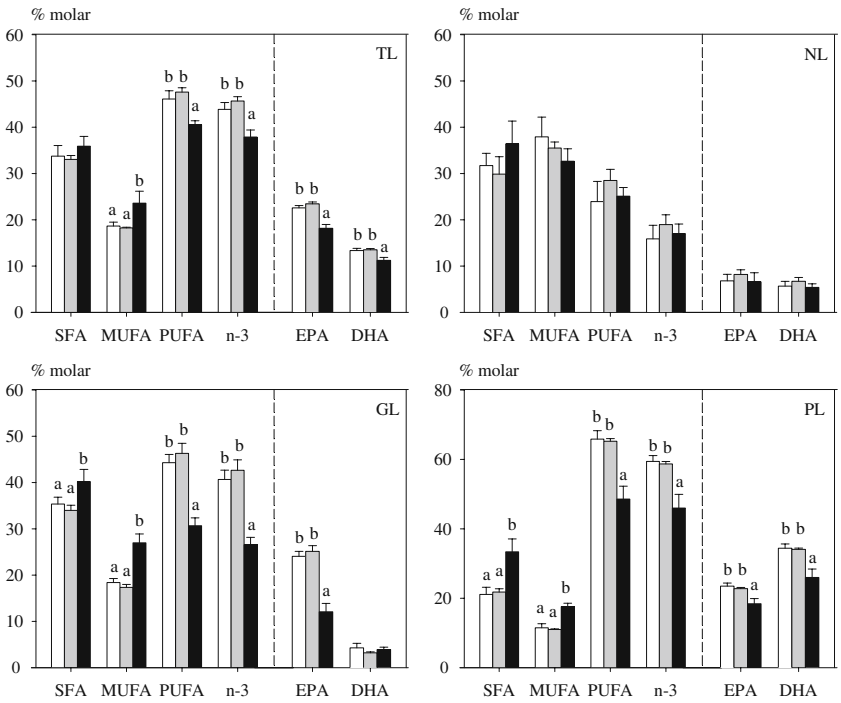

Fig. 2 Fatty acid composition (percent molar) of total lipid content and major lipid fractions in *Pavlova lutheri* cultured under an irradiance of 100 µmol photons m^{-2} s^{-1} (control, *white bars*) and after 3 (*gray bars*) and 8 days (*black bars*) UV radiations (UV-R) exposure (means ± SE, n=3). No significant variations in all parameters were observed in the controls over the time of UV-R treatment. See heading to Fig. 1. *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n-3* n-3 fatty acids, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *TL* total lipids, *NL* neutral lipids, *GL* glycolipids, *PL* phospholipids

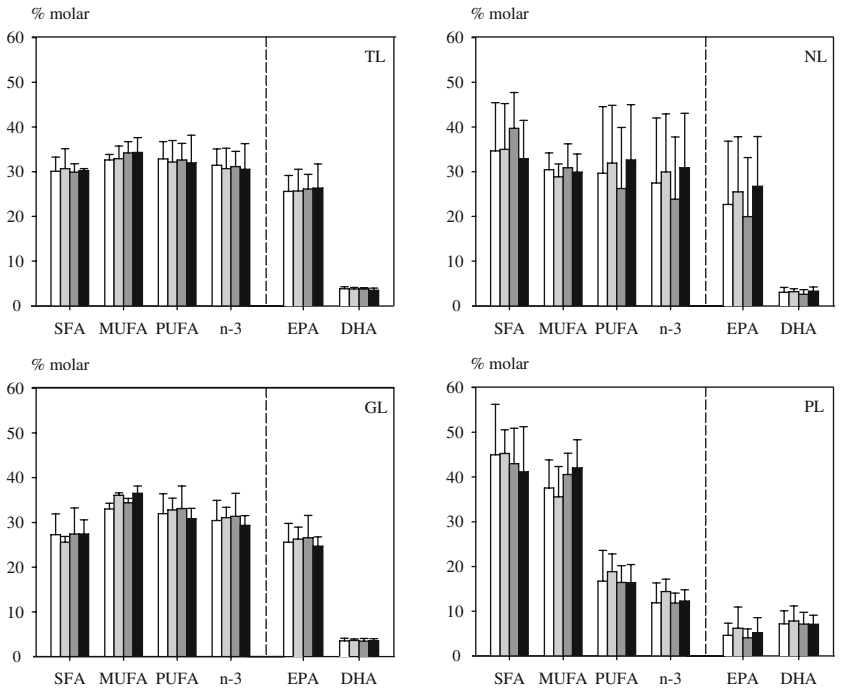

Fig. 3 Fatty acid composition (percent molar) of total lipid content and major lipid fractions in *Odontella aurita* cultured under an irradiance of 100 µmol photons m^{-2} s^{-1} (control, *white bars*) and after, 3 (*light gray bars*), 5 (*dark gray bars*), and 8 days (*black bars*) UV radiations (UV-R) exposure (means ± SE, n=3). No significant variations in all parameters were observed in the controls over the time of UV-R treatment. See heading to Fig. 1. *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n-3* n-3 fatty acids, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *TL* total lipids, *NL* neutral lipids, *GL* glycolipids, *PL* phospholipids

number of days of exposure to UV-R. In *P. lutheri*, UV-R treatment led to an increase in MUFA levels and a decrease in the proportions of PUFAs and n-3 fatty acids, such as EPA and DHA. These modifications in the total lipids are mainly due to changes in fatty acid compositions of polar lipids (GL and PL). These findings are consistent with those of Wang and Chai (1994) showing that UVB-R induces a reduction in the n-3 fatty acids levels (EPA and DHA) in eight species of microalgae. These authors viewed *P. lutheri* as a species with intermediate UVB-R tolerance, showing a decrease of approximately 25% of the n-3 fatty acids level (EPA+DHA) after exposure to UVB-R during 6 days (5 h per day; daily dose, 12 kJ m^{-2}). Our results show a decrease of 20% in EPA level and of 16% in DHA level after 8 days of UV-R treatment (5 h per day; ratio UVB/UVA-R, 4.5%; daily dose, 110 kJ m^{-2}). Similar results were also reported by Goes et al. (1994) in *Tetraselmis* sp., after PAR+UVB-R treatment, and by Liang et al. (2006b) in *Chaetoceros muelleri* grown under PAR+UVA-R+UVB-R treatment. In *O. aurita*, the exposure of cells to UV-R did not change the fatty acid composition of the total lipids, and EPA levels remained high during the 8 days of UV-R treatment. Similar results were obtained by Skerratt et al. (1998) for the diatom *Odontella weissflogii* exposed to PAR+UVA-R+UVB-R treatment for 24 h, with two quantities of UVB-R tested. Our data also show that the fatty acid compositions of the different lipid fractions of *O. aurita* were not significantly modified during UV-R treatment.

In aquatic environments, particularly in seawater surface, high O_2 concentrations and UV-R can expose microalgae to severe stress. Indeed, the high oxidizing power of UV-R led to the formation of powerful oxidants, such as intracellular peroxides and oxiradicals (Hessen et al. 1997). These elements induce fatty acids peroxidation that constitutes one of the main types of damage caused to cells by oxygen and UV-R. Moreover, Cosgrove et al. (1987) have shown that the sensitivity to oxidation of PUFAs is directly correlated to the number of double bonds. This oxidation could be one of the reasons for the reduction of PUFAs levels (EPA and DHA) induced when microalgae were exposed to UV-R stress. In microalgae sensitive to UV-R, peroxidation seems mainly to affect structural lipids (GL and PL) as plasma membranes and cellular organelles (such as chloroplasts) are predominantly composed of polar lipids rich in PUFAs (EPA and DHA) (Vothknecht and Westhoff 2001). As a result, lipid membrane oxidation and degradation could induce the formation of free fatty acids (Skerratt et al. 1998).

The decrease of PUFAs levels may also be explained by changes in the cellular mechanisms related to lipid metabolism (Hessen et al. 1997). The acetyl-CoA carboxylase, a key enzyme involved in de novo fatty acids synthesis, is dependent on the availability of adenosine triphosphate (ATP; Harwood 1988). During PUFAs biosynthesis, the desaturation and elongation also require much larger amounts of ATP than SFA and MUFA production (Thompson et al. 1990). Although plant cells

have different mechanisms to protect them against intracellular oxidative stress caused by UV-R, the simultaneous reduction of ATP production and CO_2 fixation appear to be properties commonly shared by cells exposed to UV stress (Hessen et al. 1997; Goes et al. 1994). It therefore seems possible that the low availability of ATP could account for the decline in the proportions of PUFAs in microalgae exposed to UV-R stress. Inhibition of the activity of some of the enzymes involved in the biosynthesis of unsaturated fatty acids (desaturases and elongases) could be one explanation for the decrease in PUFAs induced by UV-R. Indeed, Leu et al. (2006a) suggested that lower levels of 18:1 n-9 fatty acids observed in *Selenastrum capricornutum* could result from the inhibition of Δ9-desaturase induced by UV-R stress.

The results obtained in this study for *P. lutheri* can be explained as above and highlight the sensitivity of this species to UV-R. Moreover, the results obtained for the diatom *O. aurita* allow us to suggest that this species is more resistant and seems to be able to partially acclimate to UV-R. So, unlike *P. lutheri*, in latitude countries with medium UV-R level, outdoor "race-way" culture of *O. aurita* could yield a high-EPA alga biomass, whatever the seasonal variations in UV-R.

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