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Effects of changes in ambient PAR and UV radiation on the nutritional quality of an Arctic diatom (*Thalassiosira antarctica* var. *borealis*)

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Abstract

Polyunsaturated fatty acids (PUFAs) are essential macromolecules that are synthesized by phytoplankton during spring bloom, and they play a key role in the Arctic food web. They are, however, considered to be sensitive to oxidation by UV radiation (280–400 nm). Changes in the food quality of primary producers may affect the transport of biomass and energy in the whole ecosystem. Using a common Arctic diatom, we looked at the effect of ambient and increased UV radiation on its nutritional quality, specifically, the fatty acid composition and elemental ratios. In May 2004, in the archipelago of Svalbard (79° N), a unialgal culture of *Thalassiosira antarctica* var. *borealis* was subjected to a 17-day experiment in outdoor aquaria. The diatoms were kept in semi-continuous culture (40 1) and exposed to three treatments with different levels of UV radiation: none (UV-shielded), ambient, and enhanced. Fatty acid composition, C:N:P ratios, photosynthetic pigment composition, optimum quantum yield of PSII, and cell numbers were analysed over the experimental period. An initial increase in PAR (photosynthetically active radiation, 400–700 nm) intensities profoundly affected the fatty acid composition and substantially inhibited the synthesis of PUFAs, but the relative amounts of PUFAs were not reduced by UV radiation. Enhanced UV radiation did, however, cause a significant reduction in optimum quantum yield of PSII and affected some fatty acids, mainly 18:0 and 16:1 n-7, during the first week of the experiment. Both ambient and enhanced UV radiation caused significantly lower C:P and N:P ratios. At the same time, these treatments elicited a higher relative content of the photoprotective pigments diadinoxanthin and diatoxanthin. After acclimation to the new light levels these effects faded off. Thus, brief periods with high light exposure may cause significant changes in photosynthetic activity and food quality, but the capacity for photo-acclimation seems high. The impact of UV radiation seems to be less important for food quality than that of PAR during a sudden rise in total light intensity.

Keywords: Arctic; Fatty acid composition; PAR; Stoichiometry; *Thalassiosira antarctica* var. *borealis*; UV radiation

1. Introduction

In Arctic marine ecosystems the light climate is continuously changing—both in absolute intensity, due to variation in sea ice extent and thickness (Aas et al.,

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2002; Sakshaug, 2004) and in spectral composition, following dynamic and seasonal depletion events in the stratospheric ozone layer (Dahlback, 2002). In winter and spring 2005, ozone values above the Arctic Circle dropped to the lowest values ever recorded; at 18 km altitude more than 50% of the ozone had been destroyed (http://toms.gsfc.nasa.gov/ozone/ozone.html, Press release SCOUT-03 project, EU/WMO, 25 April 2005). This event was linked to extremely low stratospheric temperatures, which could be attributed, partly, to tropospheric warming. Further global warming could thus accentuate such periods of major ozone losses.

Heightened levels of UVB radiation (280–320 nm), expected from such large-scale depletion events, clearly pose a threat to the biota living at these latitudes. Food quality—especially fatty acids—at the base of the Arctic marine food web forms the foundation for the transport of biomass and energy to higher trophic levels. Most organisms use lipids as a primary energy storage for survival in times before and after the short productive season (Falk-Petersen et al., 1990; Klein Breteler et al., 2005). The transfer of lipids from diatoms to herbivorous zooplankton (mainly represented by three *Calanus* species) is of particular importance in high Arctic systems (Falk-Petersen et al., 2006). Polyunsaturated fatty acids (PUFAs) play a key role in food webs, because they are synthesized *de novo* only by photosynthetic organisms, yet they are essential for the many heterotrophs (Sargent and Whittle, 1981). Thus, any changes at the base of the food web may propagate to higher trophic levels. PUFAs are also of major importance in regulating membrane fluidity under low temperatures; using these, organisms ensure the functioning of vital physiological processes (Hall et al., 2002 and references therein).

Several studies have documented a negative impact of UV radiation on PUFAs in marine phytoplankton species; this impact has been attributed either to oxidation of previously synthesized fatty acids (Skerratt et al., 1998; Wang and Chai, 1994) or to disruption of their synthesis (Döhler and Biermann, 1994; Goes et al., 1994). Only one of these studies (Skerratt et al., 1998) dealt with polar phytoplankton species, utilizing similarly low temperatures. Temperature is a crucial parameter, since it may have a substantial impact on fatty acid composition itself (Thompson et al., 1992) as well as on the dynamics of repair mechanisms (Roos and Vincent, 1998). Most of the previous reports about UV-mediated effects on fatty acids were laboratory studies using artificial light and UV radiation sources; thus they differed with respect to both spectral composition and light intensity, compared to outdoor ambient conditions.

We sought to investigate the impact of ambient and enhanced UV radiation on the fatty acid composition of *Thalassiosira antarctica* var. *borealis* under realistic light and temperature conditions in the high Arctic. An outdoor experiment with three different treatments (UV-shielded, ambient, and enhanced UV radiation) was carried out in the high Arctic archipelago of Svalbard, Norway (79° N) in spring 2004. In addition to fatty acid composition, we analysed C:N:P ratios (i.e. cellular stoichiometry) as another proxy of food quality for consumers (Sterner and Elser, 2002). Other quantitative effects of UV radiation on algal biomass development, photosynthetic pigment composition, and photosynthetic efficiency (measured as optimum quantum yield of PSII) were included in order to evaluate the sensitivity of different physiological parameters to these light regimes.

2. Materials and methods

2.1. Location, weather conditions, and light measurements

The experiment was located in Ny-Ålesund, Spitsbergen (78° 55′ N, 11° 56′ E, Fig. 1), and occurred during typical Arctic spring-bloom conditions from 19.05.2004 to 04.06.2004. The weather was variable, with light cloud cover being the predominant type. The ambient PAR radiation at the experimental site, as well as the actual PAR radiation levels inside the aquaria (measured in the middle of an extra aquarium 17.5 cm above the bottom), were measured with 5-minute resolution using two LI-COR flat-head cosine quantum sensors connected to a LI-COR LI-1400 data logger. The sensors were calibrated to a reference PAR quantum sensor (type SKP215 from Skye Instruments), which was measuring continuously at the Sverdrup radiation station in Ny-Ålesund. The ambient spectral UV radiation was measured at the Sverdrup radiation station with a Bentham DM150 double monochromator UV spectrometer, fitted with J1002 cosine optimized-input optics from Schreder, scanning from 290 to 500 nm, with 1-nm spectral resolution, and taking 6 spectra per hour. The instrument is regularly calibrated at the Sverdrup Calibration lab with 1000 W FEL lamps traceable to NIST. Non-weighted hourly UVA (320–400 nm) and UVB doses (280–320 nm) were calculated from numerical integration of the spectral data. Biological weighting of the UV radiation was done using a function determined from measurements of inhibition of photosynthesis in phytoplankton communities from Antarctica (Cullen and Neale, 1997). Total ozone was measured with a five-channel GUV511 UV-filter radiometer from Biospherical Instruments.

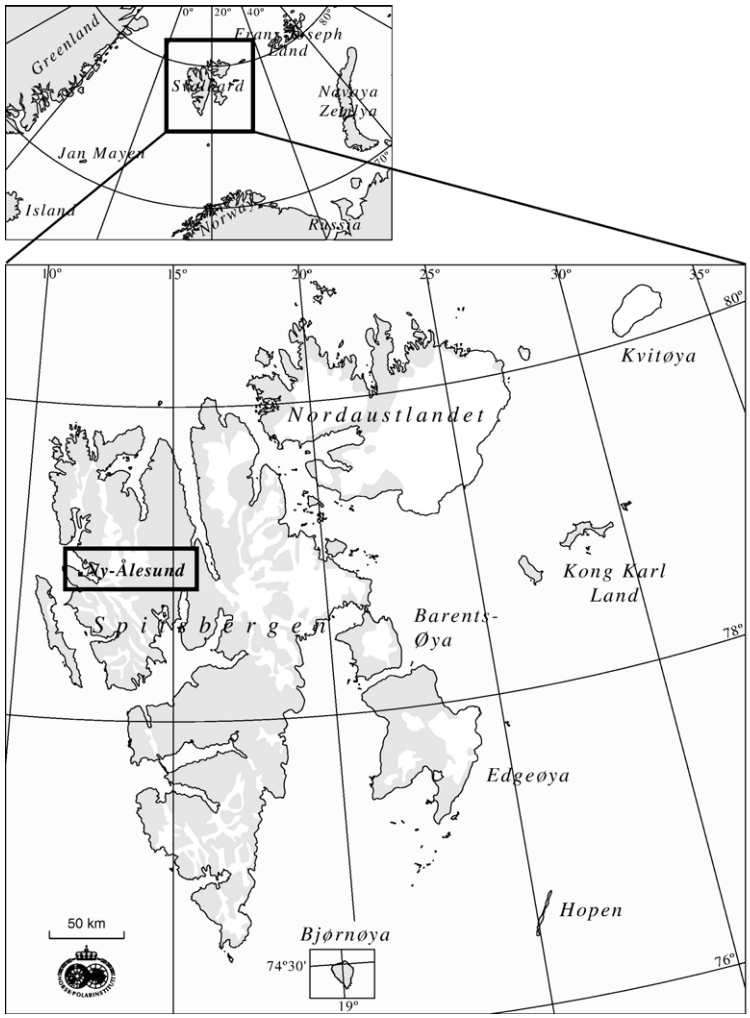

Fig. 1. Ny-Ålesund (Kongsfjorden) on Svalbard.

No direct measurements of UV radiation could be taken from inside the aquaria. Due to shading from the experimental setup, only diffuse radiation reached the middle of the aquaria, except for very short periods around noon on clear sunny days. Since the ratio between diffuse and global radiation depends on solar elevation and wavelength, and since it is higher for UV radiation compared to PAR, the calculation of realistic UV doses inside the aquaria used the following equation:

$$UV_{aq} = PAR_{aq} \times (UV_D/PAR_D), \quad (1)$$

where UV_{aq} is the calculated UV dose inside the aquaria, PAR_{aq} is the measured PAR radiation inside the aquaria, UV_D is the diffuse ambient UV radiation, and PAR_D is the diffuse ambient PAR radiation.

Since the only measurements available were ambient global radiation for both PAR and UV, Eq. (1) can be rewritten:

$$UV_{aq} = PAR_{aq} \times (UV_G/PAR_G) \times PAR_{G/D} \times UV_{D/G,} \quad (2)$$

where PAR_G is the ambient measured global PAR radiation, UV_G is the ambient measured global UV radiation, $PAR_{G/D}=PAR_G/PAR_D$, $UV_{D/G}=UV_D/UV_G$. The 2 last terms, $PAR_{G/D}$ and $UV_{D/G}$, which are both functions of solar elevation and wavelength, were empirically derived at the Sverdrup radiation station during 2 clear sunny days with standard sun/shade methods. Both of these last 2 terms can be simplified: they are a smooth function of solar elevation when there is direct solar radiation (sunshine); and they are equal to 1 when there is no sun ($PAR_G=PAR_D$, $UV_G=UV_D$). As a final adjustment in the

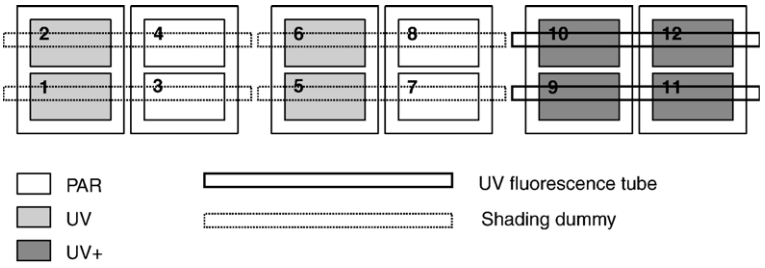

Fig. 2. Sketch over the experimental setup.

derivation of UV_{aq}, we weighted Eq. (2) with a number corresponding to the actual fraction of minutes with sunshine in each hour; the data for this came from the direct radiation measurements at the Sverdrup radiation station:

$$UV_{aq} = PAR_{aq} \times (UV_G/PAR_G) \times \sum_{1}^{60} (PAR_{G/D} \times UV_{D/G,})/60, \quad (3)$$

For the hourly sums, the minute values of $PAR_{G/D}$ and $UV_{D/G}$ are given different values depending on whether there is direct sun or not. In this way, the brief occurrences of direct radiation inside the aquaria around midday could also be corrected.

2.2. Experimental setup

The outdoor model ecosystem was modified from the setup described in Wängberg et al. (2001), see Fig. 2. It consisted of 12 aquaria with 40 l volume each (39.0×28.7×37.9 cm [length/width/height]), made of UV-transparent Plexiglas. The aquaria were placed in opaque plastic boxes and surrounded by a continuous flow of seawater for temperature control. The boxes were covered by UV-transparent Plexiglas lids, combined with different cut-off foils. Three different treatments were applied in four replicates each:

(1) UV-shielded (referred to as PAR), protected from UV radiation by an Ultraphan 400 foil (Digefra, Munich, Germany);
(2) ambient UV (referred to as UV), covered with cellulose acetate (Tamboer and Co Chemie B.V., Heemstede, Netherlands) to correct for the ca. 10% absorption of Ultraphan 400 in the PAR spectrum;
(3) enhanced UV (referred to as UV+), covered with cellulose acetate, and exposed to UV radiation provided by a fluorescence tube Q-Panel UVA-340 (Q-Panel Lab Products, Cleveland, USA) for 10 h daily (5:30 a.m. to 3:30 p.m., UTC). The UV spectrum of these light tubes (Fig. 3) closely resembles the solar spectrum between 280 and 350 nm. The relative increase in radiation intensity was largest between 295 and 330 nm (data not shown). The fluorescent tubes were pre-burned for at least 100 h and mounted 20 cm above the respective covers for the first 7 days. Since we observed no effect on biomass (measured fluorometrically as Chl-*a*) during the first week, it was decided to increase the UV dose; hence, the tubes were lowered and placed directly on the covers from day 7 onwards. The filter foils were replaced every 3rd day (Hessen and Faerovig, 2001). To ensure equal shading effects, dummies resembling lamp holders were mounted above the aquaria that lacked additional UV light. One additional aquarium in a box (without water) was used for measuring the diurnal change of the light intensities inside the aquaria. It was identical to the other aquaria, one UV-tube and a dummy provided the same shading conditions as in all other aquaria.

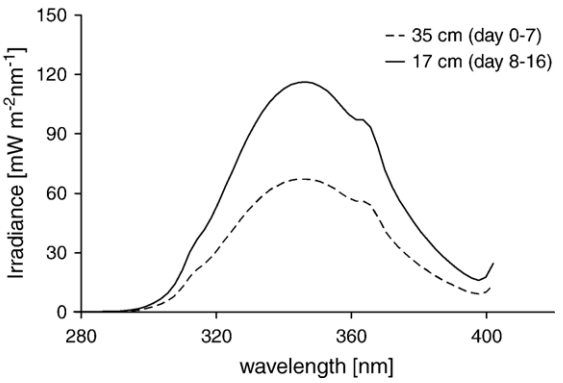

Fig. 3. Spectrum of the q-panel UVA 340 lamp, used in the UV+ treatment, measured from two different distances (corresponding to the different conditions encompassing days 0–7 or days 8–16).

Due to technical problems, the UV intensities in the UV+ treatment could not be recorded continuously. Since the output of the UV fluorescent tubes depends on air temperature and wind chill, it is possible to calculate only an estimate of the amount of extra radiation used in that treatment. For this, we measured the UV radiation at different distances from the UV tubes at 3 °C (mean air temperature during the experimental period: −0.5± 1.6 °C). Therefore, the values given here have to be considered as a theoretical maximum value that could have been achieved under stable temperature conditions and without the influence of wind, i.e. a considerable over-estimation. The maximum intensities of UV radiation in the ambient UV treatment around noon inside the aquaria corresponded to underwater values measured in Kongsfjorden at about 4 m depth on a sunny day for UVA, and at 1–2 m depth for UVB. PAR intensities inside the aquaria corresponded to 8–12 m depth in the fjord.

Seawater from the littoral zone of Kongsfjorden close to the experimental site was filtered through a SNAP-RING filter bag with 1 μm mesh size (Hayward Industrial Products Inc., New Jersey, USA). Each aquarium was filled with 32 l of it, then we added 3 l of a unialgal culture of *T. antarctica* var. *borealis*, and amended this with macronutrients in the following concentrations: 88 μM $NaNO_3$, 3.6 μM NaH_2PO_4, 10.6 μM Na_2SiO_3, and micronutrients, corresponding to a tenfold diluted f/2 medium (Guillard, 1975), which is referred to as f/20. The monocultures had been isolated at the Norwegian College of Fishery Sciences in Tromsø, Norway, from germinating resting spores contained in bottom (60 m) surface sediments collected in May 2001 in sub-Arctic Austnesfjorden (Lofoten). Culturing prior to the start of the experiment took place in a laboratory in Ny–Ålesund in f/20 medium at 3 °C and a light intensity of 100 μmol m^{-2} s^{-1}, provided by fluorescent tubes (OSRAM Lumilux de luxe 36W/950 daylight) for 14 h daily. The algae were exposed to weak ambient light during the night to adjust them to midnight-sun conditions. During the first 7 days of the experiment, 3 l (8.5%) of water from each aquarium were replaced daily by filtered seawater. From day 8 onwards, the volume in each aquarium was increased to 40 l, and 10 l (25%) were exchanged daily. The lower dilution rate during the first period was due to low biomass in the aquaria. The seawater added was taken directly from Kongsfjorden close to the experimental site. Sufficient nutrient supply was assured by regular addition of nutrients corresponding to f/20. To prevent the algae from sedimentation, all aquaria were stirred thoroughly three times daily. In four randomly selected aquaria, temperature loggers (Tinytag-12, Intab, Stenkullen, Sweden) were placed to measure the water temperature throughout the entire experimental period every 10th minute. Water sampled daily was not only used for a variety of analyses of the phytoplankton culture itself, but also as a food solution in a feeding experiment with the calanoid copepod, *Calanus glacialis* (Leu and Falk-Petersen in ms).

2.3. Parameters analysed

2.3.1. Fatty acid composition

For lipid analysis, daily, 1 l from each aquarium was filtered onto glass fibre filters (GF/C, which were pre-washed with chloroform : methanol (2:1, v/v)). Samples were stored at −80 °C in 8 ml chloroform : methanol (2:1, v/v) until analysis. Total lipid extraction was carried out according to the procedure described in Folch et al. (1957). Afterwards, a known amount of 21:0, as internal standard, was added, and an acid-catalysed transesterification was carried out using 1% sulphuric acid in methanol (Christie, 1982). The extract was then cleaned using a silica column (Christie, 1982). The percentage composition of fatty acid methyl esters (FAME) was determined in an Agilent 6890 N gas chromatograph, equipped with a fused-silica, wall-coated, capillary column (50 m × 0.25 mm i.d., Varian Select FAME), with an Agilent 7683 injector, and with flame ionization detection. Hydrogen was the carrier gas; the oven thermal gradient rose from an initial 60 °C, to 150 °C at 30 °C min^{-1}, and then to a final temperature of 230 °C, increasing 1.5 °C min^{-1}. Individual components were identified by comparison with two known standards and were quantified using HPChemStation software (Hewlett-Packard).

2.3.2. Nutrients and cellular stoichiometry

To control the amount of nutrients available in the aquaria, samples for total nitrogen and phosphorus were taken on days 0, 7, and 16. They were fixed with 1% (v/v) 4 M sulphuric acid and stored in the dark at 4 °C until analysis. Samples for analysis of particulate C, N, and P were taken on days 7, 10, 13, and 16 by filtering 250 ml on a pre-combusted (3 h, 500 °C) GF/C filter. For particulate phosphate analysis, the filters were, in addition, pre-washed with HCl (0.1 M). All samples were stored frozen (−20 °C) until analysis. Particulate C and N were analysed on a Carlo-Erba CHN 1106 elemental analyser. Particulate P was measured on corresponding filters placed in 15 ml distilled water that was acidified (150 μl 4.0 M H_2SO_4), peroxodisulfate was added (0.15 g $K_2S_2O_8$), and the filters were autoclaved (121 °C, 1 h). The particulate fraction of P was then analysed spectrophotometrically by the standard ammonium–molybdate method (Hessen et al., 2002). Total P and N were analysed on unfiltered

water samples. Total N (P) were measured as NO_3 (PO_4) after wet oxidation with alkaline (acid) with peroxodisulfate. NO_3 and SiO_2 were analyzed colorimetrically in a segmented flow autoanalyzer, while PO_4 was analyzed by manual spectrophotometry.

2.3.3. Chlorophyll a and photosynthetic pigments

Chl-*a* concentration was analysed daily in order to calculate the appropriate dilution rate and to calculate the concentration of the feeding solution. At noon, 100-ml samples were taken from all aquaria, filtered on a GF/C filter, and extracted in 10 ml 90% acetone (v/v) for 2 h at room temperature in darkness. Chl-*a* concentrations were then determined using a Turner field fluorometer (TURNER 10-AU-005-CE, Turner Designs Inc., Sunnyvale, CA, USA). Unless stated otherwise, Chl-*a* values given in the Results section are obtained by this method.

On 6 days, additional samples were taken for a complete analysis of all photosynthetic pigments by high-performance liquid chromatography (HPLC). Thus, 250 ml were filtered onto a GF/C filter, put in liquid nitrogen (−196 °C), and transferred to −80 °C. The pigments were extracted in 1.5 ml 100% MeOH. The extract was sonicated for 30 s using a Vibra-cell sonicator, equipped with a 3 mm diameter probe (cf. Wright and Jeffrey, 1997). The extraction and HPLC analysis continued according to Wright and Jeffrey (1997), using an absorbance diode-array detector (Spectraphysics UV6000LP). The column used was a C18 Phenomenex Ultracarb 3 μm ODS (150×3.20 mm), with a guard column, SecurityGuard Phenomenex C18 (4×3.0 mm). Chlorophyll breakdown products were integrated and quantified at 668 nm. Apart from the absorbance peaks from Chl-c_1+c_2 and Chl-*a*, the remaining absorbance peaks at 668 nm were considered chlorophyll breakdown products, the absorbance units (area under peaks) were pooled and calculated as ratios to Chl-*a* (absorbance units of pooled peaks at 668 nm subtracted by absorbance units from Chl-c_1+c_2 and Chl-*a* and then divided by absorbance units of Chl-*a* at 668 nm). The HPLC system was calibrated with pigment standards from DHI, Water and Environment, Denmark. Peak identities were further confirmed by online recording of absorbance spectra (400–700 nm) as described in Wright and Jeffrey (1997). Pigments are expressed as ratios to Chl-*a* (weight/weight).

2.3.4. Cell numbers

For counting of cells, 20-ml samples were taken from all aquaria and fixed with 2% formaldehyde buffered with borax. The counting was done according to the method described by Utermöhl (1958). From each sample, about 1000 cells were counted to obtain a sufficient accuracy.

2.3.5. Optimum quantum yield of PSII

At 11 a.m. UTC, in the middle of the daily irradiation period, the algal cultures were stirred and a small sample (about 50 ml) was taken for measurements of *in vivo* chlorophyll fluorescence using a pulse-amplitude modulation fluorometer (Water-PAM, Walz, Effeltrich, Germany), coupled to a PC with WinControl software (Walz, Effeltrich, Germany). Measurements were made using round quartz cuvettes at ambient (outdoor) temperatures according to a procedure modified after Hanelt (1998). Optimum quantum yield of PSII (cf. Maxwell and Johnson, 2000) was calculated as the ratio of variable to maximum fluorescence (F_v/F_m) of dark-adapted algae. After application of a 5-s far-red pulse, the samples were kept in darkness inside the cuvette for 3 min. The initial fluorescence F_0 was measured by applying weak, red, measuring-light pulses; and F_m was determined by means of a saturating pulse of high light intensity (0.6 s). F_v was then calculated by subtracting the initial fluorescence from the maximum fluorescence: $F_v = F_m - F_0$.

2.4. Statistical analysis

Effects of UV radiation on optimum quantum yield of PSII, chlorophyll concentration, photosynthetic pigments, and cell number were analysed by one-way repeated measures analysis of variance (RM-ANOVA) for the whole experimental period, and with one-way ANOVA for single days. All data were tested for homogeneity of variance using Cochran's test. There were three treatment levels (PAR, UV, UV+) and different levels of time (depending on the parameter measured). Due to the unbalanced dataset (three replicates for the PAR treatment, but four replicates in UV and UV+ treatments), Unequal N HSD was used as a post hoc test. All univariate analyses were performed with non-transformed data, using STATISTICA 7 (StatSoft Inc.). A multivariate indirect gradient analysis (principal component analysis, PCA) of changes in fatty acid composition was performed using CANOCO for Windows 4.5 (ter Braak and Smilauer, 2002). All fatty acids accounting for less than 1% of total fatty acids in all samples were removed from the dataset before analysis. All analyses were run with non-transformed percentage values of fatty acid composition.

3. Results

3.1. Radiation conditions and temperature

Noon values of photosynthetically active radiation (PAR), measured in air, varied between 700 and

1200 μmol m^{-2} s^{-1}. PAR intensities (logged in the middle of one empty aquarium) exerted a pronounced diurnal cycle, with noon values ranging from 200 to 540 μmol m^{-2} s^{-1} (mean: 360 μmol m^{-2} s^{-1}). Corresponding values measured near midnight, depending on cloud conditions, were between 20 and 70 μmol m^{-2} s^{-1}. In the middle of the aquarium, under cloudless conditions, the PAR intensity was 20% of the above-surface PAR, while the corresponding percentage during overcast weather was between 50% and 60% (Fig. 4A).

Stratospheric ozone values were high throughout the experimental period, but showed a decreasing trend—from 400 to 360 DU—during the experiment (R^2 = 0.74). Measured in the middle of one aquarium in the ambient UV treatment, daily doses of unweighted UVA and UVB ranged from 334 to 685 kJ m^{-2}, and 9 to 28 kJ m^{-2}, respectively (Fig. 4B). Inside the aquaria of the ambient UV treatment, there were no clear time trends with regard to daily doses. In the UV+ treatment, after lowering the fluorescent tubes on day 7, the daily

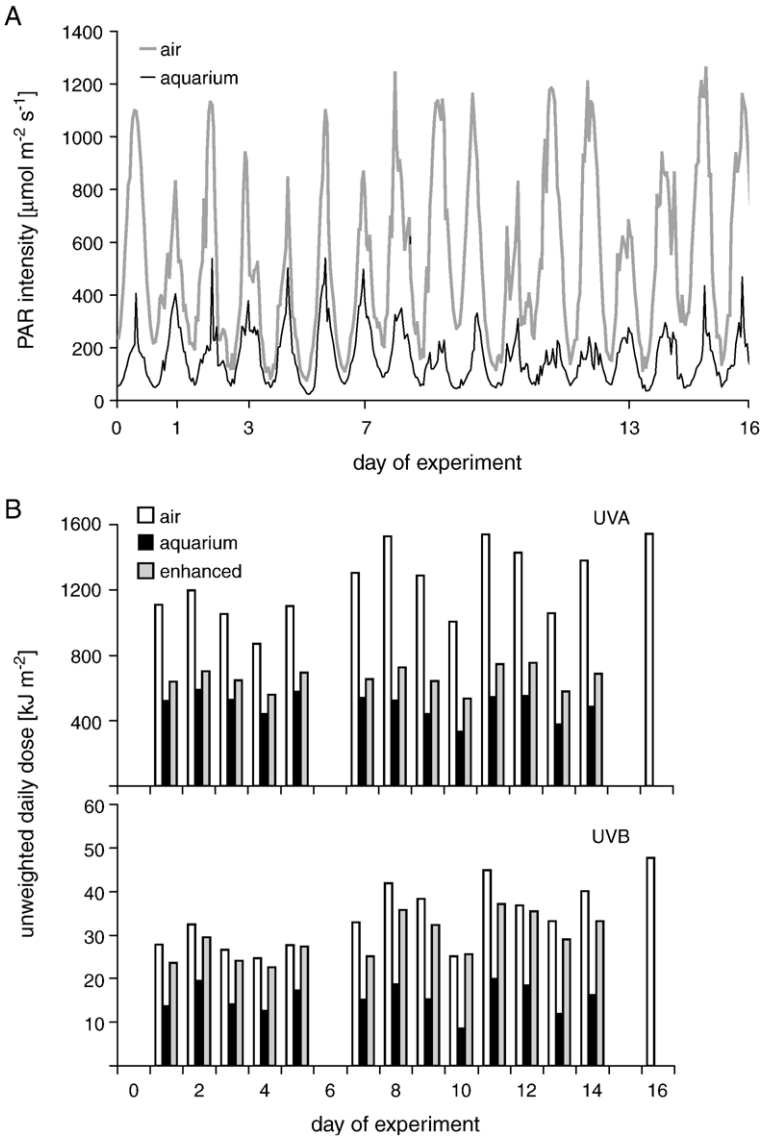

Fig. 4. A. Temporal variations of incident PAR (μmol m^{-2} s^{-1}) 19.05.–04.06.2004, measured inside the aquarium and outside. Shown are average values per hour, calculated from measurements every 5th minute. B. Unweighted daily doses for UVA and UVB (kJ m^{-2}), measured in the air and calculated for the aquarium (ambient) and the UV+treatment (enhanced). Missing values are due to incomplete measuring series for the respective days.

doses of UVB were significantly higher (*t*-Test, $p<0.05$); but for UVA, the difference was not significant. During the first week of the experiment, with the UV+ treatment, daily doses of UVA and UVB were 18% and 48% higher, respectively, than the average ambient doses. Upon weighting of the UV radiation data (Cullen and Neale, 1997), the increases were seen to be 22% and 69% higher than average ambient doses. By day 7, the unweighted increases were 37% and 86%, respectively, for UVA and UVB. But on all days the daily dose in the UV+ treatment remained at lower values than those measured at the surface.

Compared to underwater measurements in the fjord around noon on a sunny day, the noon UVA intensities in the UV ambient treatment (mean 15 W m^{-2}) corresponded to values measured at 4 m depth (min 6.4 W m^{-2}=9 m; max 24.5 W m^{-2}=2 m). For UVB, the corresponding values were: mean=0.62 W m^{-2}; min=0.21 W m^{-2}; max=1.0 W m^{-2}, which were equal to field measurements at 2 m, 4 m, and 0 m depth. The maximum intensities in the UV+ treatment (for day 0–7, and day 8–16, respectively) were 2.6 and 4.6 W m^{-2} higher than UVA in the ambient treatment; for UVB, the added intensities were 0.2 W m^{-2} during the first week and 0.34 W m^{-2} during the second. The water temperature during the entire experiment displayed diurnal cycles, fluctuating mainly between 0 and +2 °C. This was close to ambient surface conditions in the fjord, where temperatures ranged from −1 to +1.5 °C during the time of the experiment.

3.2. General phytoplankton development

All aquaria underwent the same pronounced changes in phytoplankton responses over the experimental period. Thus for clarity, we have chosen to first present the general time course development of the major parameters before proceeding to the specific responses on the irradiation treatments.

3.2.1. Fluorometrically determined Chl-a concentrations and cell numbers

In all aquaria, Chl-*a* concentrations were between 1 and 2 μg l^{-1} at the start of the experiment, and increased slowly towards 10 μg l^{-1} on day 7. During the second part of the experimental period (day 8–16), Chl-*a* varied between 20 and 30 μg l^{-1}. The cumulative biomass increase was calculated from the fluorometrically determined Chl-*a* values (Fig. 5). Note that these estimated concentrations did by far exceed the real Chl-*a* concentrations (given above), since this estimate also includes the biomass removed daily by dilution. Hence, Fig. 5 shows an exponential increase of Chl-*a* over the duration of the experiment that would have occurred ideally without diluting the cultures. One aquarium from the PAR treatment differed markedly from this development: Here, concentrations remained at 2 μg l^{-1} for the duration of the experiment; data from this replicate were therefore excluded from further analysis. Cell numbers increased from $0.7–1\times10^6$ cells per litre on day 3 to values between 3 and 6×10^6 cells per litre on days 13 and 16. There were no significant differences between the different treatments on any day. Contamination of the aquaria with species other than *T. antarctica* was found to be negligible.

3.2.2. Fatty acid composition

The fatty acid composition showed distinct changes during the course of the experiment, irrespective of the

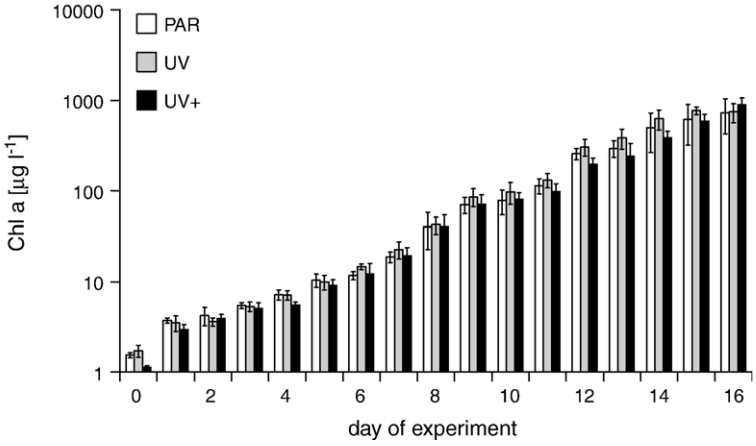

Fig. 5. Cumulative biomass increase of *T. antarctica* during the experiment; shown are theoretical Chl-*a* concentrations that would have been measured without dilution. Calculations are based upon fluorometrically measured values (mean values and standard deviation, $n=3$ in PAR and $n=4$ in UV and UV+).

Table 1
Changes in fatty acid composition of *T. antarctica* during the experiment

	Day 0	Day 1			Day 3			Day 7			Day 13			Day 16		
		PAR	UV	UV+	PAR	UV	UV+	PAR	UV	UV+	PAR	UV	UV+	PAR	UV	UV+
14:0	9.0±0.0	5.4±0.2	5.5±1.0	5.0±0.5	6.7±0.4	6.7±0.4	6.3±0.3	5.5±0.2	6.2±0.7	5.8±0.5	11.0±2.5	10.3±1.6	10.0±0.8	7.1±1.5	8.4±1.1	9.1±0.4
16:0	22.4±0.2	28.2±0.2	29.5±3.2	28.9±0.7	31.9±1.2	30.1±2.9	33.5±1.9	25.2±1.1	24.3±1.7	25.6±1.0	29.7±5.7	28.8±5.4	24.7±1.2	27.5±1.8	30.0±1.1	25.9±1.6
16:1 n-9	0.6±0.0	1.0±0.2	0.8±0.1	1.2±0.1	1.9±0.4	1.2±0.1	1.9±0.2	1.4±0.3	1.2±0.3	1.3±0.1	1.1±0.6	1.5±0.8	0.7±0.1	1.4±1.5	1.0±0.4	0.4±0.1
16:1 n-7	38.8±0.2	20.2±2.0	17.8±5.3	14.3±5.7	20.8±1.0	20.7±3.8	13.7±2.1	17.6±1.1	18.3±3.3	14.8±2.8	23.0±12.1	23.9±6.5	26.3±3.6	27.8±13.4	25.6±5.8	30.1±3.0
16:2 n-7	1.1±0.0	0.4±0.0	0.5±0.1	0.3±0.1	0.5±0.0	0.7±0.1	0.4±0.1	0.6±0.1	0.8±0.2	0.7±0.2	1.1±0.4	1.0±0.5	1.3±0.1	0.6±0.3	0.7±0.2	1.0±0.1
16:3 n-4	3.0±0.1	1.0±0.1	0.9±0.3	0.7±0.3	1.2±0.0	1.2±0.2	0.7±0.2	0.7±0.6	1.0±0.4	0.7±0.2	2.4±0.9	1.2±0.4	1.7±0.2	1.3±0.7	1.0±0.3	1.7±0.3
16:4 n-1	2.5±0.1	1.0±0.1	1.2±0.4	0.9±0.3	1.5±0.1	2.3±0.4	1.6±0.2	3.8±0.5	5.1±1.1	4.5±1.2	5.7±1.4	4.8±1.4	6.9±1.2	2.5±0.8	2.9±0.4	4.3±0.5
17:0	0.9±0.0	0.8±0.1	0.8±0.1	0.8±0.1	0.9±0.1	1.0±0.0	0.9±0.0	1.1±0.1	1.2±0.1	1.2±0.2	1.3±0.4	1.6±0.4	1.7±0.4	0.9±0.1	1.0±0.1	1.2±0.2
18:0	4.2±0.1	22.7±1.5	21.1±3.2	25.5±3.0	16.8±1.2	18.6±2.6	20.8±1.5	22.0±2.0	19.7±5.1	24.1±4.0	6.3±2.5	9.1±5.2	8.1±1.4	12.6±13.1	12.0±7.1	6.4±1.9
18:1 n-9	1.1±0.0	6.7±1.2	5.8±0.6	11.0±4.7	6.7±0.6	5.4±0.6	10.9±2.4	5.6±0.4	4.7±1.3	6.7±1.5	2.4±1.5	4.2±2.9	2.3±0.4	4.1±4.6	3.0±1.8	1.7±0.6
18:1 n-7	0.3±0.0	0.6±0.0	0.5±0.1	0.8±0.1	0.5±0.0	0.5±0.1	0.7±0.0	0.5±0.0	0.4±0.0	0.5±0.0	0.4±0.1	0.3±0.1	0.3±0.0	0.5±0.1	0.4±0.0	0.3±0.0
18:2 n-6	0.4±0.0	2.8±0.3	2.1±0.1	3.3±0.6	1.9±0.4	1.6±0.3	2.2±0.8	1.7±0.1	1.2±0.3	1.4±0.4	0.8±0.0	1.4±0.7	0.9±0.1	1.0±0.5	1.7±0.6	0.9±0.1
18:4 n-3	5.5±0.1	3.0±0.3	2.7±0.9	2.1±0.8	2.8±0.4	3.1±0.6	2.0±0.3	3.8±0.4	3.9±1.0	3.1±0.5	6.3±2.2	3.7±0.7	5.1±0.7	4.7±1.9	3.7±0.6	5.7±1.0
20:5 n-3	7.2±0.2	3.2±0.3	3.2±1.0	2.5±1.0	1.8±0.1	3.3±1.3	1.4±0.9	6.7±1.0	8.2±1.9	6.5±1.7	4.9±2.6	4.4±3.4	5.9±1.9	5.2±2.5	5.2±1.2	7.9±0.8
22:6 n-3	0.8±0.0	0.6±0.1	0.5±0.1	0.4±0.2	0.3±0.2	0.3±0.1	0.2±0.1	1.2±0.2	1.3±0.3	1.1±0.3	0.8±0.3	1.0±0.5	1.0±0.4	0.8±0.3	0.9±0.2	1.3±0.2

Fatty acids are given as percentage values of total lipid; shown are means±SD, $n=3$ for PAR, $n=4$ for UV and UV+.

different radiation treatments (Table 1, Fig. 6). The most conspicuous alteration of the profile was observed at the beginning (between day 0 and day 1), and samples taken on the last day of the experiment (day 16) were most similar to the day 0 samples (see Table 1). The percentage fatty acid composition of the total lipids of *T. antarctica* before the onset of the experiment was dominated by 16:0 (22%) and 16:1 n-7 (39%). Among the saturated fatty acids, 14:0 accounted for 9% and 18:0 for 4%; while the predominant polyunsaturated fatty acids (PUFAs) were 16:3 n-4, 16:4 n-1, 18:4 n-3, and 20:5 n-3. Of these, the last two n-3 fatty acids were most abundant, at 5.5% and 7.2%, respectively. The PUFA 22:6 n-3 accounted for less than 1% of the total fatty acid composition. Over the first day, the proportion of 16:1 n-7 decreased to between 14% and 20%. Also over this first day, percentages of total PUFAs were halved compared to their initial levels (Fig. 6). At the same time, 18:0 increased from 4% to values between 21% and 26% in all treatments, and 16:0 increased from 22% to 29%. A monounsaturated fatty acid, 18:1 n-9, accounted for only 1% on day 0, but increased to 7–11% on day 1. Results from day 3 differed from day 1 only slightly: some PUFAs were present in even lower

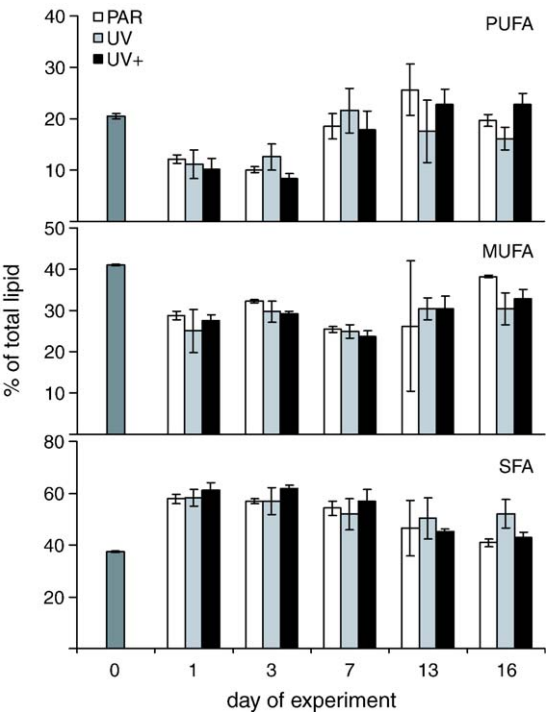

Fig. 6. Changes in polyunsaturated (PUFA), monounsaturated (MUFA), and saturated fatty acids (SFA) during the experiment and in the three different treatments (as % of total lipid).

Table 2
Molar ratios and concentrations [μg l^{-1}] of C, N, and P in *T. antarctica*; *: UV treatments which differed from the PAR control in a statistically significant way (One-way ANOVA, Unequal N HSD, $p<0.05$); shown are means±SD, $n=3$ for PAR, $n=4$ for UV, UV+

	Treatment	Day 7	Day 13	Day 16
C:N	PAR	12.8±1.2	10.7±2.8	9.0±2.2
	UV	13.0±2.3	10.1±1.9	7.1±0.6
	UV+	13.8±3.0	10.1±1.3	8.5±2.2
C:P	PAR	117.8±3.4	120.5±30.3	175.5±88.8
	UV	75.8±11.3*	136.9±51.1	132.6±46.8
	UV+	81.6±4.1*	136.3±21.0	151.9±30.1
N:P	PAR	9.3±1.2	11.3±0.7	18.7±4.6
	UV	5.9±0.4*	13.4±4.1	18.4±4.9
	UV	6.1±1.5*	13.7±2.6	19.0±7.4
C	PAR	2035±230	2120±152	2408±1181
	UV	2032±110	2745±497	2526±503
	UV+	2149±137	1950±333	2197±501
N	PAR	188±41	219±24	300±70
	UV	185±26	338±63	413±48
	UV+	189±50	236±79	317±115
P	PAR	45±3.8	39±5.7	36±0.7
	UV	70±7.6*	60±14.8	52±14.2
	UV+	68±4.6*	39±5.1	38±9.3

proportions, and there was significantly less 18:0 (17–22%) and higher average percentages of 16:0. After 1 week, 16:0 decreased slightly in all aquaria, 18:0 increased again to the levels that had been observed on day 1, while 18:1 n-9 declined. All PUFAs increased substantially: 20:5 n-3 reached values similar to day 0 (7–8%); 16:4 n-1 and 22:6 n-3 were even higher than in the beginning (4–5% and 1.1–1.3%, respectively). On day 13, 14:0 had doubled compared to day 7 and accounted for more than 10%. Both 16:0 and 16:1 n-7 increased as well. 18:0 dropped to values below 10% in all treatments, and 18:1 n-9 showed lower values than before. 20:5 n-3 and 22:6 n-3 decreased slightly, while 18:4 n-3 and 16:4 n-1 increased. Results from the last sampling of the experiment showed general resemblance to the starting fatty acid composition.

3.2.3. Stoichiometry and nutrients

Molar ratios of particulate C:N were between 12 and 14 in all treatments on day 7; they then decreased to less than 10 during the latter part of the experiment (Table 2). In contrast, the particulate C:P ratios increased during the experiment, from around 100 on day 7 to 150 on day 16. The ratio between N and P increased more markedly, from between 6 and 10 on day 7 to nearly 20 on day 16. Total nitrogen and phosphorus concentrations were measured on days 0, 7, and 16, and were >1500 and 150 μg l^{-1}, respectively.

3.2.4. Photosynthetic pigments

The ratios of fucoxanthin to Chl-*a* (weight/weight) and of diadinoxanthin+diatoxanthin to Chl-*a* (Dd+Dt/Chl-*a*) decreased during the experiment (Table 3); but betacarotene to Chl-*a* ratios were stable, around 0.02 on all days. The total sum of chlorophyll breakdown products to Chl-*a* increased over time, from 0.4 to 0.5–0.6.

3.2.5. Optimum quantum yield of PSII

Optimum quantum yield measurements on 9 days (day 6 to day 15) showed values between 0.35 and 0.56 for F_v/F_m (Fig. 7). In all aquaria, the optimum quantum yield of PSII decreased toward the end of the experiment. A measurement of the culture before the start of the experiment yielded $F_v/F_m=0.55$.

3.3. Effects of UV radiation

An overview of the changes that UV had on the different parameters is given in Table 4. Effects of UV radiation on fatty acid composition, photosynthetic

Table 3
Photosynthetic pigments as ratios to Chl-*a*; *: UV treatments which differed from the PAR control in a statistically significant way (One-way ANOVA, Unequal N HSD, $p<0.05$), shown are means±SD, $n=3$ for PAR, $n=4$ for UV and UV+

	Treatment	Day 1	Day 7	Day 13
Dd+Dt/Chl-*a*	PAR	0.049±0.003	0.043±0.002	0.031±0.004
	UV	0.058±0.008	0.057±0.004	0.036±0.003
	UV+	0.084±0.011	0.071±0.006*	0.029±0.001
Fucoxanthin/Chl-*a*	PAR	0.393±0.094	0.348±0.045	0.232±0.023
	UV	0.496±0.044	0.437±0.054	0.206±0.017
	UV+	0.610±0.055	0.452±0.021	0.221±0.005
Betacarotene/Chl-*a*	PAR	0.019±0.008	0.018±0.001	0.024±0.001
	UV	0.023±0.003	0.017±0.001	0.019±0.001
	UV+	0.026±0.002	0.018±0.001	0.017±0.001*
Chl-*a* breakdown/Chl-*a*	PAR	0.278±0.031	0.353±0.045	0.398±0.033
	UV	0.298±0.020	0.285±0.017	0.301±0.008*
	UV+	0.314±0.024	0.317±0.036	0.316±0.020*

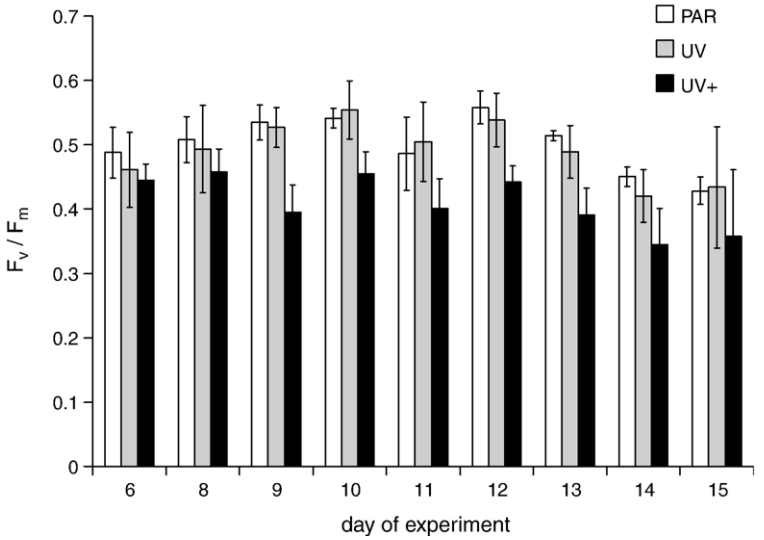

Fig. 7. Optimum quantum yield of PSII days 6–15; shown are mean values and SD ($n=3$ for PAR, $n=4$ for UV and UV+). F_v/F_m in the UV+ treatment is significantly lower than in the other two treatments (RM ANOVA, $p<0.05$).

pigments, and particulate C:N:P were found only on days 1–7; whereas optimum quantum yield of PSII was also affected later in the experiment, as measured on days 8 to 15.

3.3.1. Fatty acid composition

In general, only the algae exposed to artificially enhanced UV radiation showed treatment-dependent changes in their fatty acid composition. An overview over the significance of UV effects on the fatty acid composition for the analysed days is given in Table 5. Although it is mainly on day 3 that significant changes in the fatty acid composition were detectable, the same trends (though not statistically significant) were seen on days 1 and 7 as well. Thus, for clarity, we have chosen to describe the effects of UV radiation on fatty acid composition as divided into two periods: day 1–7 and day 13–16. We believe this represents well the transition from "initial" to "acclimated" conditions.

Results from the first week of the experiment showed treatment-dependent effects, with the UV+ treatment differing from the other two. But on day 13 and 16, no significant treatment effects were detectable. A principle component analysis (PCA) of the fatty acid composition percentage data (Fig. 8) shows also the effect of both time and treatment for the period day 1–7. While the amount of PUFAs changed significantly with time, the

Table 4
Summary of effects of UV radiation on all variables studied

	Day 1	Day 3	Day 7	Day 13	Day 16
Chl-*a* fluorometrically	−	−	−	(+)[a]	−
Cell number	n.d.	−	−	−	−
Fatty acid composition	+	+	−	−	−
Photosynthetic pigments (HPLC)	−	n.d.	+	−	n.d.
Particulate C:N:P	n.d.	n.d.	+	−	−
Optimum quantum yield of PSII	n.d.	n.d.	n.d.[b]	+	n.d.[b]

−: no effect; +: effect; n.d.: not determined.
[a] UV+ had a significantly lower content of Chl-*a* compared to UV, but not to PAR (unequal N HSD: $p=0.03$).
[b] F_v/F_m was measured on days 6, and 8–15; UV+ showed significantly lower values over the whole period (RM-ANOVA, $p<0.05$), while no difference was detectable between PAR and UV.

Table 5
Effects of UV radiation on fatty acid composition: one-way ANOVA for single days, homogeneity of variances tested by Cochran-test, post-hoc: unequal N HSD, Level of significance: $p<0.05$

	Day 1	Day 3	Day 7	Day 13	Day 16
PUFA	n.s.	UV+<UV	n.s.	n.s.	n.s.
20:5 n-3	n.s.	–	n.s.	n.s.	n.s.
18:4 n-3	n.s.	UV+<UV	n.s.	n.s.	n.s.
16:1 n-7	n.s.	UV+<PAR UV+<UV	n.s.	n.s.	n.s.
18:0	UV<UV+	n.s.	n.s.	n.s.	–
18:1 n-9	–	UV+>PAR UV+>UV	n.s.	–	–
16:1 / 16:0	n.s.	UV+<UV	n.s.	n.s.	n.s.

n.s.: no significant differences between the treatments; significant differences are indicated by an inequality that states which treatment had larger values for a given parameter.
–: homogeneity of variances not given; no ANOVA possible.

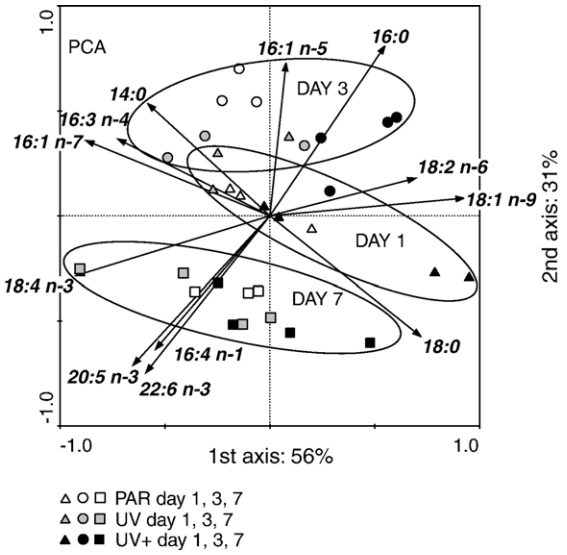

Fig. 8. Principal component analysis (PCA) of the fatty acid composition from all samples on days 1, 3, and 7.

differences between the treatments were mainly detectable in the percentages of 16:1 n-7, 18:0, 18:1 n-9, and to a lesser extent 18:4 n-3. The relative amount of 16:1 n-7 was significantly lower in the UV+ treatment compared to the other two treatments (day 3, Unequal N HSD after ANOVA, $p<0.05$). At the same time, 18:1 n-9 was found to be significantly higher in the algae exposed to enhanced UV radiation compared to the other two treatments (day 3, Unequal N HSD after ANOVA, $p<0.05$). In addition, 18:0 was consistently highest in the UV+ treatment, the difference being statistically significant on day 1 only. Both the sum of PUFAs and 18:4 n-3 had lower abundances in the UV+ treatment (statistically significant on day 3 between UV+ and UV), while 20:5 n-3 was not affected by UV radiation.

Although not statistically significant, the tendencies for treatment-dependent differences on the last sampling day of the experiment were contrary to the situation during week 1 (see Table 1, Fig. 6): The UV+ treatment was characterized by the highest amounts of PUFAs (16:4 n-1, 18:4 n-3, 20:5 n-3, and 22:6 n-3, together accounting for nearly 20%) and 16:1 n-7 (30%). At the same time, in this treatment the lowest values of 18:0, 18:1 n-9, and 16:0 were found.

3.3.2. Cellular stoichiometry

UV radiation had no detectable effect on the particulate C:N ratios (Table 2), but on day 7, C:P and N:P ratios in all UV-irradiated treatments were significantly lower than in the PAR treatment ($p<0.05$ Unequal N HSD after ANOVA). No differences were found between ambient and UV+ treatments and no significant effects were detected during the last part of the experiment.

3.3.3. Chl-a and photosynthetic pigments

The dominant trend is that fluorometrically determined Chl-*a* values did not differ significantly between the different treatments on any day. There was, however, one exception: on day 13, aquaria exposed to UV+ had a significantly lower Chl-*a* content (Unequal N HSD after ANOVA, $p<0.05$) compared to aquaria exposed to ambient UV radiation only (one-way ANOVAs for day 1, 3, 7, 13 and 16, $p>0.05$). The uniformity of the Chl-*a* trend is confirmed by a statistical analysis of Chl-*a* data obtained by HPLC analysis, which did not show any significant difference between the treatments.

None of the samples showed more than trace amounts of Chl-c_1+c_2. Compared with the PAR treatment, higher ratios of fucoxanthin:Chl-*a* and (Dd+Dt):Chl-*a* were found in the UV+ treatment on days 1 and 7 (Table 3). However, only the difference in the (Dd+Dt):Chl-*a* on day 7 was statistically significant. For betacarotene:Chl-*a*, a higher ratio was found in the PAR treatment compared with the UV+ treatment on day 13. The amount of chlorophyll breakdown products as a ratio to Chl-*a* did not differ between the treatments, apart from day 13, where the PAR treatment showed a significantly higher ratio compared to UV+.

3.3.4. Optimum quantum yield of PSII

From day 6 to 15 optimum quantum yield of PSII varied between 0.35 and 0.45 for the UV+ treatment, while algae in PAR and UV treatments showed higher values (0.43 to 0.56 in PAR, 0.42 to 0.53 in UV). Compared to PAR, there was no detectable inhibition of optimum quantum yield of PSII due to ambient UV radiation, but the enhanced UV treatment resulted in a statistically significant lower optimum quantum yield of PSII (Fig. 7) ($p<0.05$, RM-ANOVA).

4. Discussion

4.1. Relevance of exposure levels and experimental conditions

The radiation levels that the cells experienced were well inside the range of naturally occurring intensities at these latitudes. Compared to parallel measurements in Kongsfjorden during the same period, the PAR levels corresponded to intensities at 8–10 m depth at noon, and maximum UV intensities in the ambient UV treatment were equal to intensities (of UVA and UVB, respectively)

measured at 2–4 and 1–2 m depth. In all cases, even in the UV+ treatment, the daily doses in the experiment were lower than the corresponding daily doses measured in air. The transition in light intensity between the pre-experimental phase and the experimental conditions was made as smooth as possible, with the transfer taking place at 8:00 p.m. local time. However, especially at these high latitudes, algae are experiencing rapid changes in their natural habitat as well (see discussion later). A relatively short exposure time resulted in the major effects that we note here, but upon extended exposure to high PAR and UV radiation, the phytoplankton seemed to be able to adapt to it. Similar acclimation to UV radiation (after 14 days) was found in a chlorophyte from Antarctica (Lesser et al., 2002), whereas acclimation took place much faster in a study of the subtropical diatom *Chaetoceros gracilis* (Hazzard et al., 1997). Nutrient levels during the experiment were even higher than those usually reported during winter–early-spring in Kongsfjorden (Leu et al., submitted for publication-b; Hop et al., 2002) and certainly well above limiting conditions. Temperatures were close to ambient values measured in the fjord at the same time.

4.2. Treatment effects: UV radiation vs. PAR

The UV radiation, which was similar to natural conditions found in the high Arctic, affected the fatty acid composition and the cellular stoichiometry of *T. antarctica* var. *borealis.* The algae also responded by reducing the optimum quantum yield of PSII and increasing the ratio (compared to Chl-*a*) of the photoprotective pigments diadinoxanthin and diatoxanthin. However, these effects occurred during only the first part of the experiment. During the later part of the experiments, no effects of the applied UV radiation were found in any of the investigated parameters, apart from optimum quantum yield of PSII. This held true even though the cells that were given enhanced UV radiation received increased doses from day 7 onwards.

The largest changes in the fatty acid composition of *T. antarctica* occurred immediately after the start of the experiment, and were independent of the different treatments. Compared to the conditions during pre-cultivation in the laboratory, light intensity was the parameter that changed most profoundly. At the outset, temperatures decreased from + 2.6 °C (average for last 24 h prior to experiment start) to − 0.2 °C (average for the first 24 h of experiment). According to the homeoviscous adaptation theory (Sinensky, 1974), we might have expected that with a decrease in temperature, the fatty acid composition would have shown an increase in PUFAs, but we observed the opposite. Thus, we assume that the differences in fatty acid composition were caused primarily by the change in irradiation intensity. Supporting this view, the pattern of changes in the fatty acid composition due to enhanced PAR resembled closely the pattern we observed after UV exposure. In addition, PUFAs were substantially reduced by increased PAR. According to Thompson et al. (1990), for different species of marine phytoplankton the content of PUFAs and the relative amounts of 16:0 and 16:1 n-7 are dependent on light intensity. This is in accordance with our results for *T. antarctica*, in that at the start of the experiment, the decrease in the relative fatty acid content was strongest in 16:1 n-7 and in the PUFAs. At the same time, 18:0, 18:1 n-9, and 18:2 n-6 increased considerably. An increase in 18:0, 18:1 n-9, and 18:2 n-6, in combination with a drop in the content of PUFAs, points to an inhibition in the synthesis of PUFAs. Arao and Yamada (1994) describe different possible pathways for the synthesis of 20:5 n-3 in a marine diatom; in these pathways, the first products were the three C18 fatty acids mentioned above. In our experiment, PUFA synthesis resumed between day 3 and day 7; by that time PUFA levels were essentially back to start conditions. On the other hand, it was only on day 16 that 16:1 n-7 and 18:0 reached values similar to those found on day 0.

The close coupling between PAR-and UV-induced effects on algal physiology and biochemistry in these results underlines the need for outdoor experiments under natural light intensities; this work also highlights the importance of a realistic UV:PAR ratio during exposure. Not only can these major spectral components cause similar changes in the investigated organisms, but exposure to high PAR intensities has also been shown to decrease phytoplankton sensitivity towards UV stress in general (see Vernet, 2000 and references therein; Roos and Vincent, 1998). In *Isochrysis galbana*, an increase in PAR intensity, both during pre-cultivation and UV exposure, led to a reduced effect of UV radiation on n-3 fatty acids (Wang and Chai, 1994).

4.3. The effect of UV radiation

4.3.1. Fatty acid composition

The development of the fatty acid composition of *T. antarctica* var. *borealis* that we observed did not support earlier reports about UV radiation causing specific damage or reduced synthesis of PUFAs. The only n-3 fatty acid responding negatively to UV radiation was 18:4 n-3. However, this fatty acid is of minor importance in diatoms (Dalsgaard et al., 2003), and accounted

for only 2–6% of the total fatty acids in *T. antarctica*. But 20:5 n-3, the most abundant PUFA in diatoms, was not reduced by UV radiation. Nor was 16:4 n-1, which, due to its high number of double bonds, a priori, should be most sensitive to oxidative stress caused by UV radiation (Halliwell and Gutteridge, 1989; Cosgrove et al., 1987). Rather, these two fatty acids were even higher in the algae exposed to enhanced UV radiation (compared to the other two treatments at the end of the experiment, although this was statistically significant only for 16:4 n-1). Our results for 16:4 n-1 contradict the findings of Goes et al. (1994), who suggested that the ratio between 16:0 and 16:4 could be used as a marker for UVB stress, after observing a selective reduction of the latter in the marine prasinophyte *Tetraselmis* sp. In support of our findings, Döhler and Biermann (1994) also described an increase of 20:5 n-3 in response to irradiation with UVB. There have been reports of species-specific variation in the response of 20:5 n-3 to UV radiation (Skerratt et al., 1998; Wang and Chai, 1994) with a decrease of 20:5 n-3 in some species.

As an alternative to the idea that radiation could have a direct effect on a specific type of fatty acid, we favour the concept that the changes are downstream effects resulting from a generally weakened state. For this, we have other indications: First, if oxidative stress were the mechanism responsible for the observed changes, fatty acids with a higher number of double bonds should be more severely affected than monounsaturated fatty acids. And second, 16:1 n-7 has been shown to be generally correlated with growth rate (e.g. Kattner and Brockmann, 1990; Thompson et al., 1990). From this point of view, a decrease in this fatty acid could be seen as merely a general indicator of stress due to shifting conditions, rather than a specific target of high radiation levels.

4.3.2. Cellular stoichiometry

A significant decrease in the molar ratio of particulate C:P and N:P under both ambient and UV+ radiation was found on day 7 (we have no data for day 1), while the C:N ratio was not affected. This supports findings regarding freshwater algae (Tank et al., 2003; Watkins et al., 2001; Xenopoulos et al., 2002) and is verified by several tests (laboratory experiments) with various marine and freshwater species (Leu et al., submitted for publication-a, Leu et al., in press). The reduction of C:P and N:P ratios was caused by higher P concentrations in the UV exposed treatments, whereas C concentrations were not affected by UV radiation (Table 2, Unequal N HSD). We suggest therefore that these changes in C:P ratios can be attributed to an increase in cellular P, rather than to a decrease in C, which agrees with data from Wängberg et al. (1998). The most likely explanation for the UV-dependent decrease of C:P and N:P is therefore an increased need for nutrients for cellular repair, a view suggested by Hessen et al. (1995).

The C:N ratios on day 7 were higher than most (but not all) values reported in the literature (e.g. Shifrin and Chisholm, 1981; Berges et al., 2002; Viso and Marty, 1993). This high ratio could indicate nitrogen limitation, but this is highly unlikely, due to the addition of N in excess. We therefore suggest that high, or rapidly increasing PAR intensities probably caused an inhibition of nitrogen assimilation, which disappeared after some period of adaptation to the new radiation regime (this could also explain the decrease in C:N and increase in N:P and C:P on days 13 and 16). It has previously been shown that nitrogen metabolism can be affected in this manner by UV radiation (Döhler et al., 1995), and it is not unlikely that high intensities of PAR could have a similar effect. Interestingly, Mock and Kroon (2002) found that for Antarctic phytoplankton, the physiological response to nitrogen limitation is very similar to a reaction towards light stress. Here, we see that high irradiation levels or a rapid light increase probably caused effects similar to nutrient stress.

4.3.3. Photosynthetic pigments

UV radiation has been found to stimulate the diadinoxanthin cycle (i.e. the de-epoxidation of Dd to Dt under strong light) usually as an increased conversion of Dd to Dt (Goss et al., 1999). But there are different opinions regarding time scales for the conversion of Dd to Dt—from seconds, to minutes (Olaizola and Yamamoto, 1994; Willemoes and Monas, 1991), and hours (Demers et al., 1991). Our sampling design did not allow for estimating the conversion between Dd to Dt, particularly not if the conversion takes place within minutes. Therefore we used the ratio of (Dd + Dt) / Chl-*a* to indicate a photoadaptive response to high light intensities (Brunet et al., 1993). The treatment effect on day 7—a higher ratio of these photoprotective pigments in both UV treatments—disappeared on day 13 and paralleled the results for stoichiometry and fatty acid composition (partly), indicating that over the experimental period, the microalgae accomplished at least partially an acclimation to UV radiation.

High concentrations of Chl-*a* degradation products indicate senescence in microalgae and might be a consequence of light stress. But the ratios found here were so high that we assume a contamination of our samples by dead algal cells; these probably had sedimented to the bottom of the aquaria, and were resuspended when the water was stirred before sampling.

4.3.4. Optimum quantum yield of PSII

For optimum quantum yield of PSII, we found no differences between the PAR treatment and the ambient UV treatment. This confirms the results by Lesser et al. (1996), who undertook an outdoor experiment with a diatom-dominated phytoplankton community at a similarly high latitude (78° S near McMurdo Station in Antarctica). In addition, the values they found for optimum quantum yield of PSII were very similar to ours, and optimum quantum yield of PSII declined as a response to high PAR intensities (increased radiation from 17% to 41% of ambient). In a field study north of Svalbard in May 2003, for near-surface phytoplankton, optimum quantum yield of PSII values of approximately 0.5 were also found (McMinn and Hegseth, 2004). In our UV+ treatment, however, we observed a decrease in optimum quantum yield of PSII over the measurement period (day 6–15), indicating that the applied level of extra UV posed a stress factor to the algae. This is particularly interesting because none of the other parameters were negatively affected by UV radiation beyond day 7. Optimum quantum yield of PSII, therefore, seems to be a rather sensitive parameter for documenting UV stress.

4.4. Changes in food quality vs. food quantity

Neither Chl-*a* concentrations nor cell numbers were significantly affected by UV radiation. In general, the nutritional quality was more sensitive to light manipulations than biomass. However, since the food quality was not affected negatively (no decrease in PUFAs, and no increase in C:P ratios) and the quantity remained unchanged, it seems to be unlikely that ambient UV radiation has severe food-web effects. In a feeding experiment, in which the herbivorous copepod *C. glacialis* was fed the algal cultures from the study described here, no treatment-dependent changes of zooplankton fatty acid composition, growth, survival, or moulting rate were found (Leu and Falk-Petersen in ms). The effect of increased PAR on the nutritional quality, by contrast, was considerable.

4.5. Ecological considerations

The sudden change and exposure to ambient radiation conditions at the start of this experiment resembles the situation in the field during ice breakup in Arctic spring (compared to pre-experimental conditions, light intensity increased several-fold). The onset of the spring bloom in the Marginal Ice Zone is tightly linked to the retreat of the ice edge (Sakshaug and Slagstad, 1991; Sakshaug, 2004). The formation of a halocline between the surface melt water and deeper, more saline water masses stabilizes the water column through stratification. Consequently, phytoplankton biomass during the bloom phase is concentrated in the uppermost water layer (Engelsen et al., 2004), where it is exposed to high intensities of both PAR and UV radiation. Satellite data revealed a strong phytoplankton bloom in the Marginal Ice Zone of the northern Barents Sea about 2 weeks after the ice edge had receded (Engelsen et al., 2002). This matches the time required for adaptation that we observed in our study. Furthermore, *T. antarctica* var. *borealis* is a species often dominating the early phase of a bloom and seems to be associated with sea ice (Falk-Petersen et al., 2000; Wiktor, 1999). It is likely that it belongs to the group of pioneers during a spring bloom, starting the spring production from ice-associated seed material (Michel et al., 2002; Syvertsen, 1991). We suggest, therefore, that the observed reaction patterns in the biochemistry and physiology of this diatom are important for our understanding of the timing and the development of an ice-edge bloom. Turbulent mixing or sudden variation in cloud cover or in stratospheric ozone concentrations are further possible causes for rapid changes in radiation exposure, changes that phytoplankton in the Arctic are frequently subjected to. Thus, it is important to consider the brief and dynamic nature of the high Arctic spring bloom and to take into account the meteorological conditions in order to fully understand the role of PAR and UV in the ecology of the autotrophs. While the capacity for adaptation seems high, sudden light exposure after a long period of low light appears to affect quality more than quantity, with PAR having a stronger impact on these changes than UV.

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