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INFLUENCE OF UV-B RADIATION ON NITROGEN UTILIZATION BY A NATURAL ASSEMBLAGE OF PHYTOPLANKTON[1]

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A 7-day mesocosm experiment was conducted in July 1996 to investigate the effects of ambient UV-B radiation (UVBR) exclusion and two UVBR enhancements above ambient levels on NO_3^-, NH_4^+ and urea utilization in a natural plankton community (<240 μm) from the Lower St. Lawrence Estuary. The phytoplankton community was dominated by diatoms during the first 3 days and, afterward, by flagellates and dinoflagellates. The results of 4-h incubations just below the water surface show that, compared with ambient UVBR conditions, UVBR exclusion generally increased NO_3^-, NH_4^+, and urea uptakes. During the last 4 days of the experiment, the percent increase in the specific uptake rate of urea under excluded UVBR conditions varied between 17% and 130% and was a linear function of the ambient UVBR dose removed. During the first 3 days, the phytoplankton community dominated by diatoms was able to withstand UVBR enhancements without any perceptible effect on nitrogen uptake. However, during the post-diatom bloom period, UVBR enhancements resulted in decreases in NO_3^-, NH_4^+, and urea uptake compared with ambient UVBR conditions. The reduction of urea uptake under UVBR enhancements during the last 3 days varied between 23% and 64% and was linearly related to the enhanced UVBR dose. However, the different UVBR treatments did not affect the internal organic nitrogen composition (internal urea, free amino acids, and proteins) of the phytoplankton community experiencing vertical mixing in the mesocosms. The discrepancy between short-term uptake measurements at the surface and long-term effects in the mesocosms emphasizes the importance of vertical mixing on UVBR effects in natural ecosystems. This suggests that an increase in ambient UVBR would have a minimal effect on nitrogen utilization by natural phytoplankton assemblages if these are vertically mixed.

Key index words: **ammonium uptake; mesocosm experiment; nitrate uptake; phytoplankton; urea uptake; UV-B radiation**

A decrease in stratospheric ozone levels has been observed over Antarctica, mostly because of anthropogenic emission of chlorofluorocarbons (Farman et al. 1985, Sikorski et al. 1994, Hofmann 1996). A stratospheric ozone depletion was also observed, albeit to a smaller extent, over the Arctic (Hofmann and Deshler 1991, Müller et al. 1997) and even over temperate latitudes (Blumthaler and Ambach 1990, Kerr and McElroy 1993, Wardle et al. 1997). Ozone depletion over the Arctic was also linked to global warming resulting from an increase of greenhouse gases (Wardle et al. 1997). Because stratospheric ozone is the main compound responsible for the absorption of ultraviolet-B radiation (UVBR, 280–320 nm), the ozone depletion results in an increase in the amount of UVBR reaching the Earth's surface (Kerr and McElroy 1993, Booth and Madronich 1994, Frederick and Lubin 1994, Roy et al. 1994, Sikorski et al. 1994, Wendler and Quakenbush 1994, Lubin and Jensen 1995). Even if UVBR is rapidly attenuated in the water column, it may reach a part of the euphotic zone where photosynthesis, the basis of the food web, takes place.

Many studies have investigated the impacts of UVBR on marine organisms, especially on phytoplankton. These studies, conducted either in the laboratory or in the field on natural phytoplankton populations, have shown that UVBR may affect the physiology (Lorenzen 1979, Cullen and Lesser 1991, Smith et al. 1992, Behrenfeld et al. 1993a, b, Lesser et al. 1994, Boucher and Prézelin 1996) and the biochemical composition of phytoplankton (Vosjan et al. 1990, Sebastian et al. 1994, Döhler and Lohmann 1995, Schofield et al. 1995, Buma et al. 1996, Goes et al. 1996). Different authors have also reported that UVBR exposure could influence the structure of phytoplankton communities (Karentz and Spero 1995, Villafañe et al. 1995, Davidson et al. 1996).

Although nitrogen is an essential nutrient for phytoplankton growth, few studies have looked at the impacts of UVBR on nitrogen utilization by natural phy-

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toplankton communities. Nitrogen is needed for the synthesis of nucleic acids, amino acids, proteins, and pigments (Collos and Slawyk 1980, Dortch 1982, Wheeler 1983, Dortch et al. 1984). Therefore, a modification in nitrogen metabolism could affect phytoplankton growth, as well as its biochemical composition which would have consequences on the entire food web (Goes et al. 1995).

It has been previously shown that UVBR affects different steps of the nitrogen metabolism in phytoplankton. Laboratory experiments conducted on marine diatoms grown in batch cultures showed that UVBR depresses NO_3^- uptake during 2-day exposures (5 h per day; Döhler and Biermann 1987). In these experiments, Döhler and Biermann (1987) observed that a dark period following the UVBR exposure can allow diatom cells to recover their natural NO_3^- uptake capability. Döhler (1992) also observed a decrease in NO_3^- uptake during short-term (6 h) laboratory experiments on natural phytoplankton communities dominated by the flagellate *Phaeocystis pouchetii* from the Wadden Sea. Short-term exposure to artificial UVBR (1–5 h) of diatom-dominated natural populations from the Weddell Sea also induced a reduction of NO_3^- uptake (Döhler 1997). However, Döhler (1992) showed no variation in NO_3^- uptake in a natural phytoplankton assemblage consisting mainly of the dinoflagellate *Ceratium* spp. after 3 h of UVBR exposure. In addition, during a transect from 37°N to 55°N in the Pacific Ocean, Behrenfeld et al. (1995) found no correlation between changes in absolute NO_3^- uptake rates and UVBR dose, during short-term (4–8 h) surface incubations of natural phytoplankton populations under excluded, natural, and enhanced UVBR. Previous laboratory studies on batch cultures of the tropical marine diatom *Bellerochea yucatanensis* and the haptophycean *Pavlova* reported an inhibitory effect of UVBR on NH_4^+ uptake during short-term (5 h) exposures (Döhler 1995, Döhler and Buchmann 1995). Natural phytoplankton communities dominated by flagellates (Döhler 1992) and by diatoms (Döhler 1997) also showed a decrease in NH_4^+ uptake under UVBR exposure. Behrenfeld et al. (1995) also found that natural and enhanced levels of UVBR depressed NH_4^+ absolute uptake rates of natural assemblages of phytoplankton, during short-term incubations. They showed that this inhibition of NH_4^+ absolute uptake rate was dependent upon UVBR dose.

UVBR may also affect the internal pools of amino acids and the protein contents of phytoplankton cells. Döhler (1995) showed that short-term UVBR exposures can reduce the synthesis of amino acids in batch cultures of *Bellerochea yucatanensis*. However, in another study, Döhler (1984) reported an increase of internal pools of amino acids during 2-day UVBR exposures (5 h per day) of batch cultures of marine diatoms from the North Sea. Short-term incubations (7–8 h) of natural communities dominated by diatoms from the North Pacific Ocean (Goes et al. 1995) corroborated the results of the latter study, since they showed that internal pools of amino acids increase under natural ultraviolet radiation (UVR, 280–400 nm), as compared with excluded UVR conditions. Döhler (1984 and 1985) reported that a 2-day exposure (5 h per day) decreases protein content in batch cultures of several marine diatoms. Goes et al. (1995) reached the same conclusion with diatoms incubated under natural or excluded UV conditions. However, Buma et al. (1996) exposed cultures of three temperate marine diatoms to different levels of UVBR (on short-time scale) and observed either an increase in cellular protein content under realistic levels of UVBR, or the same protein content as compared with cultures nonexposed under higher levels of UVBR.

Results from the studies presented above show that UVBR effects on phytoplankton nitrogen metabolism are very species dependent. It is thus still difficult to predict how the specific effects of UVBR will translate on a natural multispecies assemblage. There is also a lack of information about the effect of ambient UVBR enhancements on the nitrogen metabolism of natural populations. In addition, some aspects of nitrogen utilization by phytoplankton have not been investigated. For example, the influence of UVBR on urea uptake has never been studied so far despite the importance of this nitrogen source for phytoplankton in the field (McCarthy 1972, Kristiansen 1983, Harrison et al. 1985). Finally, long-term studies (exceeding 2 days) are needed to help the extrapolation of short-term study results to natural environments.

The objectives of this study were to determine the long-term (7 days) effects of the exclusion and two enhancements of ambient UVBR on (1) the utilization of three nitrogenous sources—NO_3^-, NH_4^+, and urea—by a natural phytoplankton community and (2) the internal pools of NO_3^-, NH_4^+, urea, free amino acids, and the protein contents of this community.

MATERIALS AND METHODS

Experimental set-up and irradiance measurements. The experiment was conducted from 17 to 23 July 1996 in four land-based stainless steel tanks of the Pointe-au-Père nearshore station (48°31′N, 68°28′W, Québec, Canada) divided into two polyethylene mesocosms (2.25 m deep, with a volume of 1500 L, for more details see Chatila et al. 1999). The eight mesocosms were filled simultaneously with water from the Lower St. Lawrence Estuary previously filtered on a 240 μm net. The entire experimental set-up and light regimes have been described in Belzile et al. (1998). To summarize, four UVBR treatments were experimented in duplicates: (1) natural UVBR (NUVB) as control, (2) without UVBR (WUVB), (3) UVBR enhanced at a low level above ambient (LUVB), and (4) UVBR enhanced at a high level above ambient (HUVB). For the WUVB treatment, the mesocosms were covered with a sheet of Mylar®D (0.13 mm thick; Dupont, Wilmington, DE). The UVBR enhancements were obtained using UV-B lamps (model XX15B from Spectronics Corporation, Westbury, NY; emission peak at 312 nm, preburned 100 h). To eliminate wavelengths <280 nm, each lamp was covered with a sheet of cellulose acetate (preburned and changed every day). Two lamps were placed 40 cm above the LUVB mesocosms and 3 cm above the HUVB mesocosms. Every day, the UV-B lamps were turned on at 09:00 and turned off at 17:30 (i.e. 4.5 h before and after solar midday). Similar shading conditions were created by installing three and one

dummies (wooden lamps replicate) over the NUVB and LUVB mesocosms, respectively. Since the Mylar sheet reduced ~10% of photosynthetically available radiation (PAR, 400–700 nm), no dummy was installed over the WUVB mesocosms to ensure similar shading conditions of PAR as in the NUVB mesocosms. Incident intensities of ambient, UVAR (ultraviolet-A, 320–400 nm) and UVBR were recorded every 10 min with an IL-1700 radiometer (International Light Company, Newburyport, MA) equipped with broadband photodetectors designed for PAR (SED033 detector/PAR filter/W diffuser), UVAR (SUD033 detector/UVA filter/W diffuser) and UVBR (SUD240 detector/SPS300 filter/W diffuser) providing a cosine-corrected irradiance. A measurement of incident intensity was also made using the on-deck cell of a PUV-511 radiometer (Biospherical Instruments, San Diego, CA). A PUV-500 radiometer (Biospherical Instruments) was used to make vertical profiles of irradiance in each mesocosm on four days. Both the PUV-500 and the PUV-511 radiometers provided a measurement of cosine-corrected downwelling irradiance at 305, 320, 340, and 380 nm and a measurement of downwelling, cosine-corrected PAR. A correction factor of 2.6 was applied to the irradiance at 305 nm measured with the PUV-500 and the PUV-511 radiometers to compensate for the underestimation caused by the lamp calibration method (Kirk et al. 1994). Solar spectra were determined, on July 17 and 18 around noon, with an Optronic Laboratories OL 752 spectroradiometer (courtesy of Dr. David Lean, Orlando, FL). The UV-B lamps emission spectra were measured using an Optronic Laboratories OL 754 spectroradiometer (courtesy of Dr. Howard Browman). In each mesocosm, water mass homogeneity was ensured by a Little Giant® pump (model 2-MD-HC). Constant water temperature (between 8.5° C and 11.3° C) was maintained in the mesocosms by circulating local estuarine water between the mesocosms. There was no significant difference in temperature between mesocosms (Belzile et al. 1998).

Sampling. During the experiment, samples were taken at 15 cm under the surface in each mesocosm. An initial sample was taken on the first day of the experiment, at 05:00, for the determination of external nutrients (NO_3^- + NO_2^-, NO_2^-, urea, PO_4^{3-} and $Si(OH)_4$), chlorophyll *a* (chl *a*) concentration and phytoplankton abundance. Then, external and internal nutrients (including NH_4^+), particulate organic carbon (POC) and nitrogen (PON), particulate proteins, free amino acids (FAA), and nitrogen uptake rates were measured twice a day, at 09:00 and 13:00, during the entire experiment. Samples for taxonomic identification of phytoplankton were collected on days 1, 2, 4, and 7, at 09:00. After the first sampling, samples for chl *a* concentrations and phytoplankton abundance were collected every 4 h during the first 4 days and, afterward, three times a day (at 09:00, 13:00, and 17:00) for chl *a* and twice a day (at 09:00 and 17:00) for phytoplankton abundance.

Laboratory analysis. For chl *a* determination, 50–100 ml subsamples were filtered through Whatman GF/F glass-fiber filters (Whatman, Clifton, NJ), which were subsequently frozen in liquid nitrogen and stored at −80° C. Chl *a* retained on the filters was then determined on a R010 Turner Designs fluorometer, after 24 h extraction in 90% acetone at 4° C (Parsons et al. 1984). Phytoplankton abundance (1–20 μm) was analyzed immediately after sampling using a FACSORT Analyzer flow cytometer (Becton-Dickinson, San Jose, CA) fitted with a 488 nm laser (Mostajir et al. 1999). Samples were fixed with acid Lugol for latter identification and enumeration of phytoplankton cells larger than 3 μm (Villegas 1999) using the Utermöhl technique (Lund et al. 1958). For FAA and proteins determination, 200–400 ml subsamples were filtered in duplicate through Whatman GF/F filters (precombusted at 500° C for 5 h). Filters were frozen in liquid nitrogen and then stored at −80° C. Separation and analysis of FAA and proteins were conducted following the method of Clayton et al. (1988). For PON and POC determination, 100–300 ml subsamples were collected on precombusted Whatman GF/F filters and stored at −20° C before being analyzed with a Perkin-Elmer CHN analyzer (model 2400) (Perkin Elmer, Norwalk, CT). For nutrient analysis, 200-400 ml subsamples were filtered through Whatman GF/F filters which had been previously combusted and rinsed with seawater. The vacuum differential for filtration was kept low (<13 kPa) and filtration stopped as soon as the liquid had passed through the filter in order to avoid losses of cell content (Thoresen et al. 1982). The filtrate was used for analyzing inorganic and organic nutrients. The concentration of external ammonium (Ext-NH_4^+) was immediately determined using the method of Solórzano (1969) described by Parsons et al. (1984). The remainder of the filtrate was frozen at −20° C in acid-cleaned polypropylene cryogenic vials and analyzed within 1 month for the determination of ambient NO_3^- + NO_2^-, NO_2^-, PO_4^{3-} and $Si(OH)_4$ (Ext-NO_3^-, i.e. NO_3^- + NO_2^- − NO_2^-, Ext-NO_2^-, Ext-PO_4^{3-} and Ext-$Si(OH)_4$) using a Perstorp FS III Autoanalyzer and ambient urea (Ext-urea) using a Technicon Autoanalyzer (diacethyl monoxime thiosemicarbizide method of Price and Harrison 1987). Internal concentrations of NO_3^- + NO_2^-, NO_2^-, NH_4^+ and urea (Int-NO_3^-, i.e. NO_3^- + NO_2^- − NO_2^-, Int-NO_2^-, Int-NH_4^+ and Int-urea) of cells retained on Whatman GF/F filters were determined after extraction with boiling deionized water (method C-2 in Thoresen et al. 1982) with the same methods as described above.

Nitrate, ammonium, and urea uptake rates were measured according to the ^{15}N tracer method of Dugdale and Wilkerson (1986). Separate trace additions of $K^{15}NO_3^-$ (99 atom%), $(^{15}NH_4^+)_2SO_4$ (99 atom%), and ^{15}N-urea (99 atom%) were made to 250 mL subsamples, for a final concentration of 0.1 μM for $^{15}NO_3^-$ and $^{15}NH_4^+$ and 0.05 μM for ^{15}N-urea. The inoculated subsamples were incubated in Whirlpak polyethylene bags (Nasco, Fort Atkinson, WI) which transmit UVR (Smith and Baker 1980, Worrest et al. 1980) and are not toxic (Prézelin and Smith 1993). The bags were submerged in the middle of the corresponding mesocosms, just below water surface. Two incubations were performed each day. Samples collected at 09:00 (i.e. before UV-B lamps were turned on) were incubated from 10:00 to 14:00, while samples collected at 13:00 were incubated from 14:00 to 18:00. After the incubation period, the subsamples were filtered onto combusted Whatman GF/F filters which were stored at −20° C. Prior to sample analysis, filters were oven dried at 60° C for 24 h and pelletized. Samples were then analyzed for ^{15}N isotope ratios, PON, and POC using an Europa Scientific ANCA mass spectrometer (Bedford Institute of Oceanography). Nitrogen uptake rates were calculated using the equation of Dugdale and Wilkerson (1986).

UVBR dose calculations. To estimate UVBR dose-response relationships for nitrogen uptake rates, two kinds of UVBR cumulative doses were calculated: the UVBR cumulative dose removed in the WUVB treatment and the enhanced UVBR cumulative dose in LUVB and HUVB treatments. Phytoplankton cells underwent UVBR treatments not only during surface incubations, but also before incubations, while circulating in the mesocosms. Considering that the objective of this study was to determine the long-term effects of UVBR, it is important to evaluate the cumulative UVBR dose that cells received (or did not receive in the case of the WUVB treatment) from the beginning of the experiment until the end of each surface incubation. To obtain this cumulative dose, two UVBR doses were added together for each incubation and each treatment: the UVBR cumulative dose received in the mesocosm before sampling and the UVBR cumulative dose received just below the water surface during the incubation. The UVBR cumulative dose in the mesocosm before sampling was computed for each treatment as the 305 nm irradiance averaged over the water column of the mesocosm, according to the equation of Riley (1957), and integrated from the beginning of the experiment (day 1, 05:00) until the time of sampling for each incubation. The UVBR cumulative dose during the incubation was calculated as the 305 nm irradiance recorded a few cm below the water surface integrated over the 4 h of incubation. We considered that the Mylar®D screen completely eliminated UVBR since it transmits 50% of irradiance at 325 nm (Belzile et al. 1998). Therefore, for each incubation, the UVBR cumulative dose removed in the WUVB treatment corresponds to the UVBR cumulative dose received at the same time in the NUVB

treatment from the beginning of the experiment until the end of the incubation. The enhanced UVBR cumulative doses in LUVB and HUVB treatments were computed for each incubation as the difference between the UVBR cumulative dose in the LUVB or HUVB treatment and the UVBR cumulative dose in the NUVB treatment from the beginning of the experiment until the end of the incubation. Ambient UVBR cumulative doses removed and enhanced UVBR cumulative doses calculated here are absolute doses at 305 nm.

Statistical analysis. One-way analyses of variance (ANOVA) were used for the comparison of averages of the different variables studied, between UVBR treatments, for each sampling time (Zar 1984). The null hypothesis (H_0) stipulates that averages of the measured variable are equal between UVBR treatments. The ANOVA was completed by an *a posteriori* contrast test, the test of Fisher's LSD (Least Significant Difference; Zar 1984). This test allows the identification of averages that are significantly different between the four UVBR treatments. In the text, differences between UVBR treatments are considered significant when $P < 0.05$. Simple linear regressions (Zar 1984) were used to estimate the relationships between the percent changes in the uptake rates of NO_3^-, NH_4^+, and urea and the UVBR dose received during the incubations. Analyses of covariance (ANCOVA) were also used to compare the slopes between samples collected in the morning and those collected in the afternoon (Zar 1984). The null hypothesis (H_0) stipulates that the regression lines of the morning samples and the afternoon samples have the same slope.

RESULTS

Variations in irradiance regime. Temporal variations of incident PAR, UVAR, and UVBR are presented in Fig. 1. During the first 2 days, maximal incident PAR, UVAR, and UVBR intensities were, respectively, 1923 $\mu E \cdot m^{-2} \cdot s^{-1}$, 33.63 and 1.48 $W \cdot m^{-2}$. Days 3–5 were cloudy and incident irradiance was much lower. Maximum irradiances were 596 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for PAR, 14.22 $W \cdot m^{-2}$ for UVAR and 0.50 $W \cdot m^{-2}$ for UVBR. During the last 2 days, incident PAR, UVAR, and UVBR intensities increased up to 1861 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 33.89 $W \cdot m^{-2}$, and 1.43 $W \cdot m^{-2}$ respectively. Daily incident irradiances are presented in Table 1. During the sunny days (days 1, 2, 6, and 7), daily incident PAR varied between 28 $E \cdot m^{-2}$ and 46 $mol \cdot m^{-2}$, while daily incident UVAR and UVBR ranged from 621 $kJ \cdot m^{-2}$ to 928 $kJ \cdot m^{-2}$ and from 13 $kJ \cdot m^{-2}$ to 34 $kJ \cdot m^{-2}$, respectively. During the three cloudy days (days 3–5), incident irradiances decreased drastically with values ranging from 7 $mol \cdot m^{-2} \cdot d^{-1}$ to 11 $mol \cdot m^{-2} \cdot d^{-1}$ for PAR, from 168 $kJ \cdot m^{-2} \cdot d^{-1}$ to 280 $kJ \cdot m^{-2} \cdot d^{-1}$ for UVAR and from 6 $kJ \cdot m^{-2} \cdot d^{-1}$ to 9 $kJ \cdot m^{-2} \cdot d^{-1}$ for UVBR. Due to shading effects from the sides of the tanks and UV lamps (or dummies) in early morning and late afternoon, only 37% of incident irradiance reached the water surface of the mesocosms (Belzile et al. 1998).

Light attenuation in all mesocosms was the same during the whole experiment. The mean depths of the 1% of surface UVBR and UVAR were 0.9 m and 1.6 m, respectively. The bottom of each mesocosm received, on average, 4% of the surface PAR (Belzile et al. 1998).

Weighted spectral irradiances measured at the surface of the water on day 1 at 11:00 for the four UVBR treatments are presented in Fig. 2. The spectral irradiances between 290 nm and 347 nm were weighted by the biological weighting function for ρNH_4^+ of Behrenfeld et al. (1995) There was an evident increase in irradiance in the UVBR range in both LUVB and HUVB treatments compared with NUVB conditions. The relative increases in irradiance between 290 nm and 320 nm, as compared with NUVB conditions, were 173% and 253% in the LUVB and HUVB treatments, respectively. Between 320 nm and 347 nm, the relative increases were 19% and 28%, respectively. It is acknowledged that the lamps provided an increase in the daily UVBR dose much higher than would be expected under any projected decrease in stratospheric ozone. Since the UV-B lamps provided a constant UVBR intensity between 9:00 and 17:30, while ambient UVBR was highly variable, the UVBR enhancement also produced unnatural UVBR:UVAR:PAR ratios, especially in the early morning and late afternoon. Furthermore, the spectrum of the WUVB treatment diverged from that of the NUVB treatment in the UVAR wavelengths (Fig. 2). In the WUVB treatment, the relative decreases in weighted irradiance, compared with NUVB conditions, were 91% between 290 and 320 nm and 35% between 320 and 347 nm, respectively.

Variations in external nutrients. External nutrient (Ext-NO_3^-, Ext-NH_4^+, Ext-urea and Ext-$(Si(OH)_4)$ concentrations showed large temporal variability (Fig. 3). Since there was no significant difference in the concentrations of nitrogenous nutrients between the four UVBR treatments, the mean and standard deviation of the concentrations in all the different mesocosms are presented in Figs. 3a, 3b, and 3c. During the first 3 days, Ext-NO_3^- concentrations decreased from ~9 μM to <1 μM (Fig. 3a). They remained <0.5 μM during the last 4 days. From day 1 to day 3, Ext-NH_4^+ concentrations decreased from 0.32 μM to ~0.10 μM (Fig. 3b). During the next 4 days, Ext-NH_4^+ concentrations exhibited an increase on days 4 and 6, when they reached 0.28 μM and 0.17 μM, respectively. Ext-Urea concentrations were around 1 μM on day 1 and remained around 0.5–0.8 μM for the rest of the experiment (Fig. 3c). Ext-PO_4^{3-} concentrations decreased from ca. 0.4 μM to <0.3 μM during the first 2 days of the experiment (data not shown). Afterward, they exhibited a great variability until the end of the experiment. As with nitrogenous nutrients, no significant effect of UVBR treatments was detected on Ext-PO_4^{3-} concentrations. On the first day, Ext-$Si(OH)_4$ concentrations ranged from 7.4 μM to 10.5 μM (Fig. 3d). During the following 3 days, they decreased to values <3 μM on day 4. During the last 4 days, Ext-$Si(OH)_4$ concentrations continued to decrease down to <2 μM in the NUVB, WUVB, and LUVB treatments, while they remained relatively constant in the HUVB treatment. No significant difference was found between UVBR treatments at the beginning of the experiment, while HUVB treatments exhibited significantly higher Ext-$Si(OH)_4$ concentrations than the NUVB treatment during the last three days, except on day 6 at 09:00.

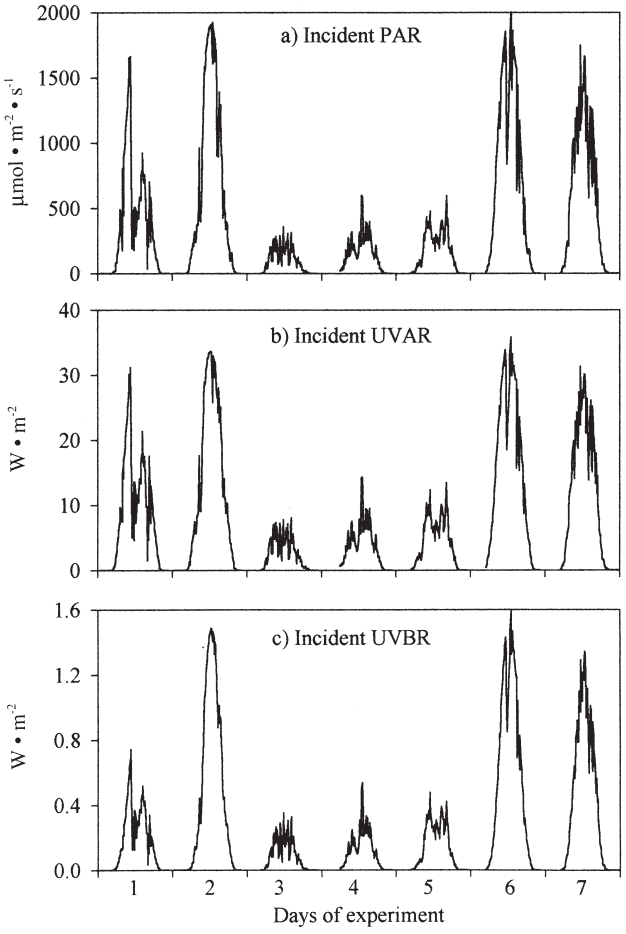

FIG. 1. Temporal variations of incident (a) PAR (400 to 700 nm), (b) UVAR (320 to 400 nm) and (c) UVBR (280 to 320 nm) from 17 (day 1) to 23 July (day 7) 1996 measured at the study site.

Variations in phytoplankton abundance, biomass, and community structure. Temporal variations in phytoplankton abundance (1–20 µm), as determined by flow cytometry, and in chl *a* concentration are presented in Fig. 4. During the first 2 days, phytoplankton abundance was around 10–20 × 10^6 cells·L^{-1} (Fig. 4a). Then the abundance exhibited a rapid increase in all treatments to reach 30–40 × 10^6 cells·L^{-1} on day 3. During days 4 and 5, phytoplankton abundance continued to increase slowly to reach 48 × 10^6 cells·L^{-1}. During the last 2 days, cell concentration declined down to 30–40 × 10^6 cells·L^{-1}. Phytoplankton abundances were not significantly affected by the UVBR treatments, except on day 5 when the abundance was higher in the WUVB and LUVB treatments than in NUVB treatment at 09:00. Chl *a* concentration increased from ~5 to 20 µg chl *a*·L^{-1} in all treatments, during the first 3 days, and remained at this level during days 4 and 5 (Fig. 4b). Then chl *a* concentration decreased to ~10 µg chl *a*·L^{-1} for NUVB, LUVB, and HUVB treatments, but down to <5 µg·L^{-1} in the WUVB treatment. During days 6 and 7, the exclusion of solar UVBR resulted in a significant decrease in chl *a* concentration compared with NUVB conditions.

TABLE 1. Daily incident irradiance from 17 (day 1) to 23 July (day 7) 1996.

	PAR (mol·m^{-2}·d^{-1})	UVAR (kJ·m^{-2}·d^{-1})	UVBR (kJ·m^{-2}·d^{-1})
Day 1	28	621	13
Day 2	45	886	33
Day 3	7	168	6
Day 4	9	230	7
Day 5	11	280	9
Day 6	46	928	34
Day 7	37	752	28

The absolute and relative abundance of diatoms, dinoflagellates, and flagellates changed during the experiment (Table 2). From day 1 to day 7, in the NUVB treatment, the abundance of diatoms, dominated by the centric diatoms *Chaetoceros* spp. and *Thalassiosira* spp., increased from 0.5 × 10^6 cells·L^{-1} to 5 × 10^6 cells·L^{-1}. Meanwhile, dinoflagellates, dominated by *Katodinium* spp., *Heterocapsa* spp., and *Prorocentrum* spp., increased from 0.1 × 10^6 cells·L^{-1} to 0.5 × 10^6 cells·L^{-1} and flagellates increased from 0.5 × 10^6

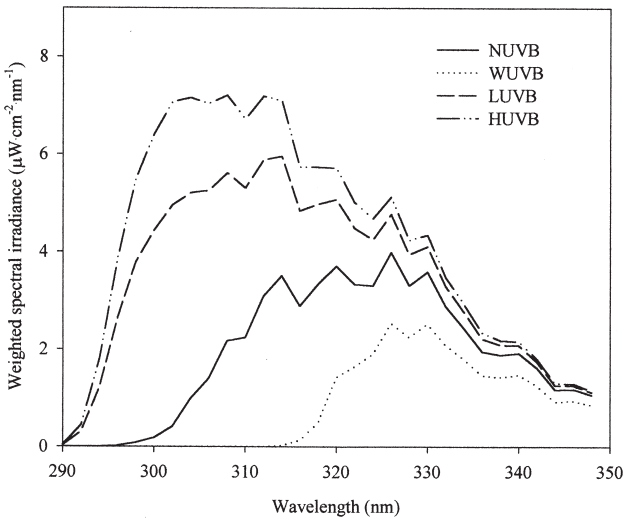

FIG. 2. Weighted spectral irradiances at the water surface on 17 July 1996 (day 1) at 11:00 in the mesocosms under ambient UVBR (NUVB), excluded UVBR (WUVB), low UVBR enhancement (LUVB) and high UVBR enhancement (HUVB). The spectral irradiances are weighted by the biological weighting function for ρNH_4^+ inhibition normalized to 1 at 300 nm (Behrenfeld et al. 1995). It should be noted that the irradiance in the WUVB treatment was calculated by multiplying the irradiance in the NUVB treatment by the transmission spectra of the Mylar sheet as measured by a spectrophotometer. Contrary to the NUVB treatment, no dummy lamp was installed over the WUVB mesocosms to compensate for the ~10% reduction of PAR by the Mylar sheet. Consequently, 11% was added to the Mylar transmission spectra for wavelengths between 320 nm and 700 nm in order to obtain a transmission of 1 at 700 nm.

cells·L^{-1} to 5.0×10^6 cells·L^{-1}. During the first 4 days, the phytoplankton community was dominated by diatoms (>40% of total phytoplankton abundance) in all treatments. At the end of the experiment, the phytoplankton community structure changed. Diatoms were significantly less abundant under excluded and enhanced UVBR than under NUVB conditions; at this time, diatoms represented only 32% and 23% of total phytoplankton abundance in LUVB and HUVB treatments, respectively.

Temporal variations in biochemical indices. Temporal variations of the ratios of POC:PON, FAA:PON, Int-NO_3^-:PON, and Int-NH_4^+:PON are illustrated in Fig. 5. The POC:PON molar ratio was between 6 and 8 during the first 4 days and increased up to ~12 during the rest of the experiment (Fig. 5a). It was not significantly affected by UVBR treatments. FAA represented 10%–20% of PON during the first 5 days of the experiment (Fig. 5b). The FAA:PON ratio increased to 20%–40% on day 6 and remained at this level during the last 2 days. Again, UVBR treatments had no significant effect on the FAA:PON ratio. Proteins represented around 80% of PON at the beginning of the experiment (data not shown). The protein:PON ratio decreased slightly during the first 3 days and remained around 60%–70% for the rest of the experiment, in all UVBR treatments. Although Int-urea was measured during the whole experiment, it never accumulated and, therefore, data are not reported here. During the first 3 days, Int-NO_3^- increased from 1% to ~4% of PON (Fig. 5c). Then it decreased to ~1% on day 5. A slight increase was observed on day 6. Int-NH_4^+ decreased from 2%–4% to <1% of PON during the first day and remained relatively stable during the rest of the experiment (Fig. 5d). Some statistically significant differences were detected between UVBR treatments for Int-NO_3^- (on day 6 a.m. [morning]) and for Int-NH_4^+ (on days 1 p.m. [afternoon], 4 p.m., and 6 a.m.).

Temporal variations in nitrogen uptake rates. The patterns of utilization of NO_3^-, NH_4^+, and urea varied during the experiment (Fig. 6). At the beginning of the experiment, phytoplankton were mainly using NO_3^-, with maximum absolute uptake rates on day 2 in all four treatments. Then NO_3^- uptake decreased and, after 3 days, urea was the main nitrogenous nutrient used by the microalgae (Figs. 6a and 6c). During the experiment, NH_4^+ uptake rates did not exhibit any definite pattern (Figs. 6b and 6e). However, during the last 2 days, the proportion of NH_4^+ taken up relative to other nitrogenous sources was significantly higher under enhanced UVBR than under ambient UVBR conditions (data not shown). The specific uptake rates of the different nitrogen sources showed about the same patterns as the absolute uptake rates (Figs. 6d–6f).

One-way ANOVAs showed that the UVBR treatments had significant effects on the uptake rates of NO_3^- on some occasions during the experiment. Compared with ambient UVBR conditions, exclusion

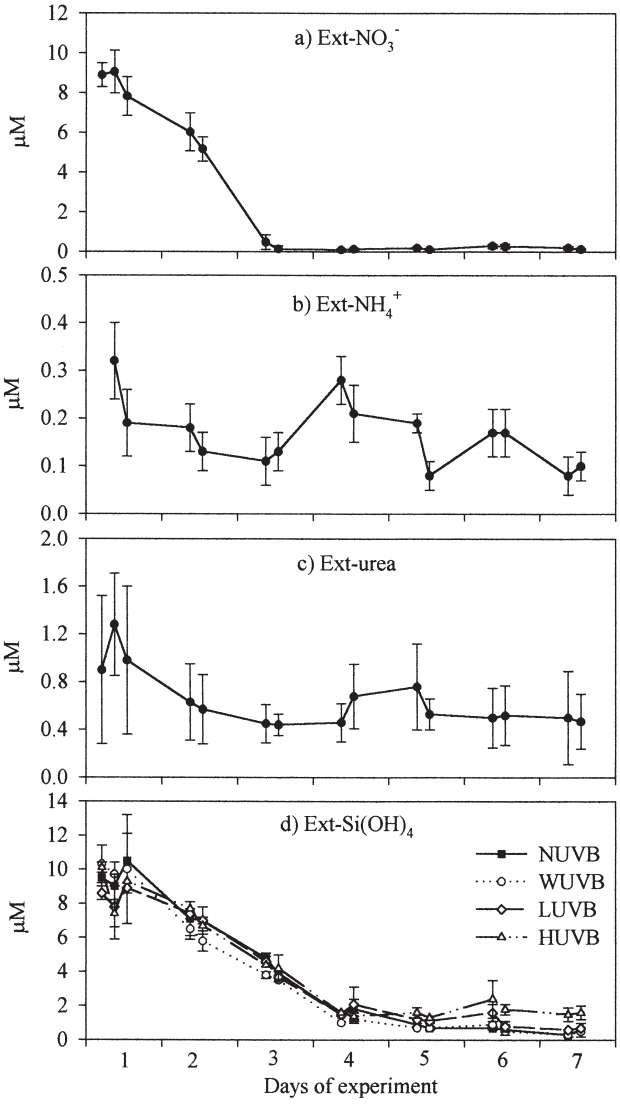

FIG. 3. Temporal variations of the external concentrations of (a) nitrate, (b) ammonium, (c) urea and (d) silicic acid in the mesocosms under ambient UVBR (NUVB), excluded UVBR (WUVB), low UVBR enhancement (LUVB) and high UVBR enhancement (HUVB) (average ± SD). In (a), (b), and (c), the averages and standard deviations are calculated from all the values of all mesocosms. In (d), they are calculated separately for each UVBR treatment.

of solar UVBR resulted in a significant increase by 166% of the absolute uptake rate of NO_3^- (ρNO_3^-) on day 2 (a.m.) and a decrease by 26% on day 5 (p.m.) (Fig. 6a). High UVBR enhancement caused significant decreases of ρNO_3^- on days 1 (64% and 100%), 5 (39% and 55%), and in the samples collected in the afternoon of day 7 (40%). Under low UVBR enhancement, decreases by 23% of the uptake rates were observed during days 5 and 7 (p.m.). The same effects of UVBR treatments were observed on the specific uptake rate of NO_3^- (VNO_3^-), except that the exclusion of UVBR even increased the uptake

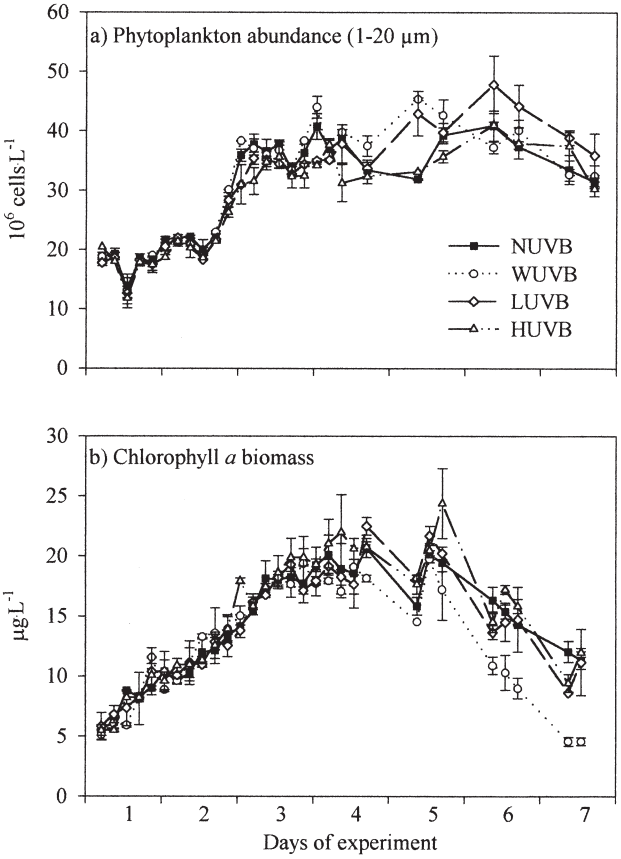

FIG. 4. Temporal variations of (a) phytoplankton abundance and (b) chlorophyll *a* concentration in the mesocosms under ambient UVBR (NUVB), excluded UVBR (WUVB), low UVBR enhancement (LUVB) and high UVBR enhancement (HUVB) (average ± SD).

rates by 44%–97% on days 6 and 7 (a.m.) (Fig. 6d). During these days, PON concentrations in the WUVB treatment were significantly lower than in the other mesocosms (data not shown).

The uptake rates of NH_4^+ were also affected by UVBR treatments on some occasions. In morning samples, the absolute uptake rate of NH_4^+ (ρNH_4^+) under WUVB conditions was enhanced by 25% on days 1 and 7 and depressed by 27% on day 6 as compared with NUVB conditions (Fig. 6b). The HUVB treatment resulted in a decrease of ρNH_4^+ by 16% on day 6 (p.m.), while low UVBR enhancement did not affect the absolute uptake rates at any time during the experiment. The exclusion of UVBR increased the specific uptake rates of NH_4^+ (VNH_4^+) by 23%–88% during days 1 (a.m.), 5 (a.m.), 6 (p.m.), and 7 (Fig. 6e). The HUVB treatment depressed VNH_4^+ by 23% and 20% in the afternoon samples of days 4 and 7, while LUVB conditions decreased the specific uptake rate by 12% in the afternoon samples of day 4.

There was no effect of the UVBR treatments on the absolute uptake rate of urea (ρurea) during the first day of the experiment (Fig. 6c). However, when compared with NUVB conditions, exclusion of ambient UVBR resulted in enhanced ρurea in the samples collected in the morning of days 2 (83%), 3 (77%), 5 (17%), and 7 (62%) and in the afternoon samples of day 6 (17%). The HUVB treatment depressed ρurea by 25%–64% on days 3 (p.m.), 5, 6 (p.m.), and 7. Under LUVB conditions, decreases of the absolute uptake rate by 19%–39% were observed on days 5, 6 (p.m.), and 7 (a.m.). UVBR treatments had the same effects on the specific uptake rate of urea (*V*urea) than on ρurea, except that more sampling periods were affected (Fig. 6f). For example, as compared with the NUVB treatment, *V*urea also increased by 17% and 31% in the afternoon samples of days 5 and 7 under WUVB conditions, decreased by 37% in the afternoon samples of day 7 under LUVB, and was reduced by 27% and 48% in the morning samples of days 3 and 4 under HUVB conditions.

To test if there was a relationship between the increase in urea uptake rates and the UVBR cumulative dose removed in the WUVB treatment, the percent increase in specific uptake rate of urea under WUVB

Table 2. Average (SD) abundance of the various phytoplankton groups in the mesocosms under ambient UVBR (NUVB), excluded UVBR (WUVB), low UVBR enhancement (LUVB) and high UVBR enhancement (HUVB). The relative abundance (% total) is also indicated. (* = significantly different from the NUVB treatment at 0.05).

		UVBR treatments							
		NUVB abundance		WUVB abundance		LUVB abundance		HUVB abundance	
Taxonomic groups		10^6 cells·L^{-1}	% total	10^6 cells·L^{-1}	% total	10^6 cells·L^{-1}	% total	10^6 cells·L^{-1}	% total
Diatoms	day 1	0.46 (0.17)	44	0.51 (0.13)	59	0.58 (0.15)	57	0.63 (0.11)	56
	day 2	0.54 (0.20)	43	0.61 (0.16)	59	0.68 (0.17)	57	0.77 (0.13)	55
	day 4	2.92 (0.34)	49	3.87 (0.32)	60	2.94 (0.24)	56	2.49 (0.18)	56
	day 7	4.99 (0.30)	50	3.21 (0.99)*	44	3.06 (0.49)*	32	2.33 (0.07)*	23
Dinoflagellates	day 1	0.10 (0.04)	9	0.06 (0.01)	7	0.09 (0.02)	10	0.09 (0.01)	8
	day 2	0.12 (0.05)	9	0.07 (0.01)	7	0.12 (0.02)	10	0.11 (0.02)	8
	day 4	0.99 (0.04)	17	0.94 (0.09)	15	0.72 (0.01)	14	0.70 (0.14)	15
	day 7	0.54 (0.13)	5	0.69 (0.32)	9	1.21 (1.00)	12	2.07 (1.47)	18
Flagellates	day 1	0.48 (0.03)	47	0.29 (0.05)	34	0.32 (0.06)	33	0.41 (0.08)	36
	day 2	0.57 (0.03)	47	0.35 (0.05)	34	0.39 (0.08)	33	0.51 (0.07)	37
	day 4	2.02 (0.07)	34	1.62 (0.29)	25	1.63 (0.25)	31	1.34 (0.64)	29
	day 7	5.02 (3.39)	45	3.37 (0.79)	46	5.32 (0.55)	56	6.11 (1.30)	58

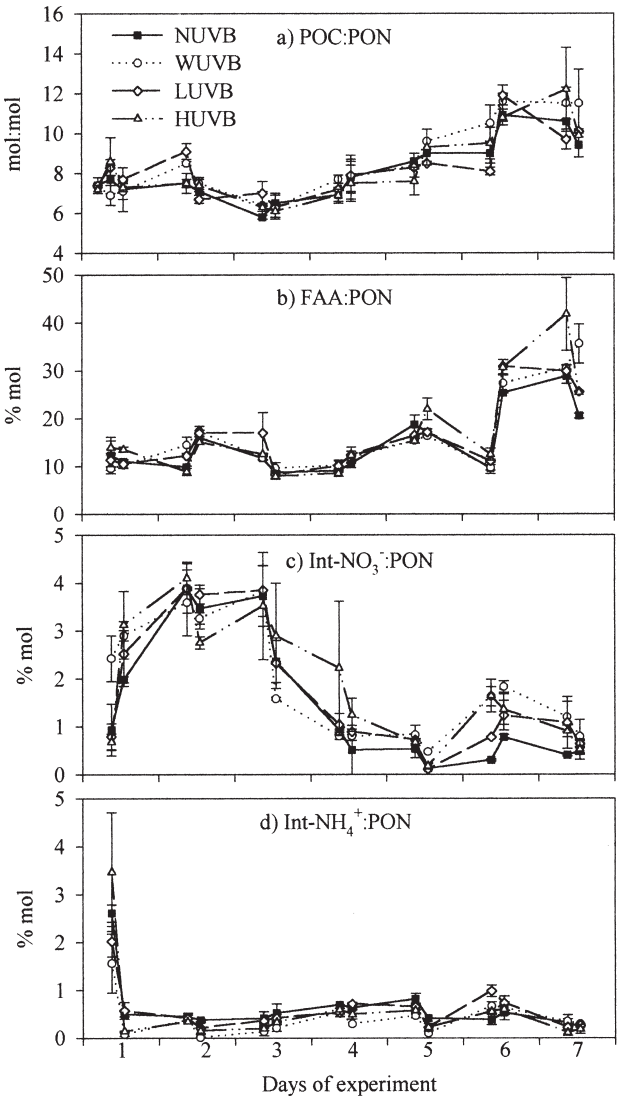

FIG. 5. Temporal variations in the ratios of (a) particulate organic carbon (POC) to particulate organic nitrogen (PON), (b) free amino acids (FAA) to PON, (c) intracellular nitrate (Int-NO_3^-) to PON, and (d) intracellular ammonium (Int-NH_4^+) to PON in the mesocosms under ambient UVBR (NUVB), excluded UVBR (WUVB), low UVBR enhancement (LUVB), and high UVBR enhancement (HUVB) (average ± SD).

conditions was calculated relative to the NUVB treatment. The percent increase in *V*urea was computed as $[(V\text{WUVB} - V\text{NUVB})/V\text{NUVB}] \times 100$, where *V*NUVB and *V*WUVB are *V*urea under ambient and excluded UVBR conditions, respectively. The relationship for days 2–7 is presented in Fig. 7. During the post-diatom bloom period, the increase in *V*urea was a linear function of the UVBR dose removed during the incubations ($r^2 = 0.81$, $P < 0.001$, regression analysis). However, during the diatom bloom, the percent increase in *V*urea was not dependent upon the UVBR dose removed (Fig. 7). To know if the variations in *V*urea were correlated with the UVBR enhancements, the percent decrease in *V*urea in enhanced UVBR treatments was calculated relative to the NUVB treatment (Fig. 8a). It was computed as $[(V\text{NUVB} - V\text{LUVB})/V\text{NUVB}] \times 100$ for the LUVB treatment and as $[(V\text{NUVB} - V\text{HUVB})/V\text{NUVB}] \times 100$ for the HUVB treatment. *V*NUVB, *V*LUVB, and *V*HUVB are *V*urea in NUVB, LUVB, and HUVB treatments, respectively. In both morning and afternoon samples, the percent decrease in *V*urea was linearly correlated with the enhanced UVBR dose ($r^2 = 0.88$, $P < 0.01$ for morning samples and $r^2 = 0.97$, $P < 0.001$ for afternoon samples, regression analyses). The slopes of the linear regressions for the percent decrease in *V*urea in samples collected in the morning and in the afternoon were statistically different (ANCOVA, $P < 0.05$). The decrease in *V*urea under enhanced UVBR conditions was generally less important in morning samples. A comparison of the different behavior of urea uptake under enhanced and excluded UVBR is shown in Fig. 8, only for the last 3 days. The difference in slopes between the dose-response curves presented in Figs. 8a and 8b shows that the WUVB treatment had a stronger effect on urea uptake than the HUVB and LUVB treatments.

DISCUSSION

External nutrients and phytoplankton community. During the experiment, the phytoplankton dynamics were characterized, from day 1 to day 3, by an early diatom bloom, largely dominated by *Chaetoceros* spp. and *Thalassiosira* spp. After 3 days, the diatom bloom resulted in an almost complete exhaustion of Ext-NO_3^- and, to a lesser extent, Ext-$Si(OH)_4$. During the post-diatom bloom period (days 4 to 7), total phytoplankton abundance and chl *a* concentration declined, while the abundance of autotrophic flagellates and dinoflagellates progressively increased in all treatments. During the whole experiment, the total cell numbers (1–20 μm) remained similar in all treatments.

During the post-diatom bloom period, the increase of the POC:PON ratio probably reflects the nutrient-limited condition of the diatom assemblage which, in our experiment, was maintained in suspension by the water circulation system. The increase in flagellates and dinoflagellates during that period suggests that they were not nutrient limited. They were, therefore, probably responsible for most of the nitrogen uptake during the last 3 days of the experiment.

Internal nutrients, FAA, and proteins. Intracellular NO_3^-, NH_4^+, FAA, and proteins were similar under all UVBR conditions. These results are in disagreement with previous studies showing a strong effect of UVBR on internal pools of FAA and protein contents of diatoms in culture (Döhler 1984, 1985, Buma et al. 1996) or in the field (Goes et al. 1995). This absence of UVBR effects on internal nitrogen can possibly be attributed to the fact that vertical mixing in the mesocosms allowed phytoplankton cells to escape periodically from

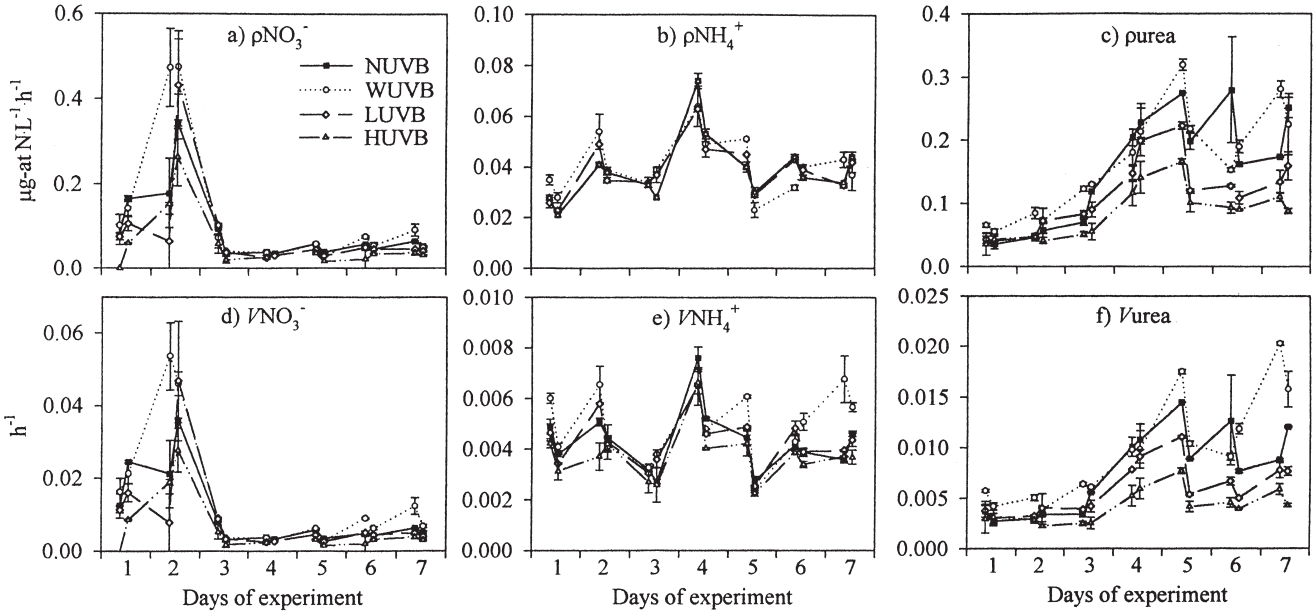

FIG. 6. Temporal variations of (a, b, and c) absolute uptake rates (ρ) and (d, e, and f) specific uptake rates (V) of nitrate, ammonium, and urea in the mesocosms under ambient UVBR (NUVB), excluded UVBR (WUVB), low UVBR enhancement (LUVB), and high UVBR enhancement (HUVB) (average ± SD).

the high UVBR levels at the surface. Time-averaged UVBR exposures experienced by phytoplankton under these conditions were therefore much lower than either the measured near-surface intensities or the UVBR doses experienced by the cells suspended just below water surface during the nitrogen uptake measurements. These results clearly show the importance of vertical mixing on UVBR effects in a natural phytoplankton community and caution against the extrapolation of near-surface incubation data for assessing water column effects.

NO_3^- uptake. During the first three days, NO_3^- uptake rates were highly variable, reflecting the rapid increase in diatom abundance and the associated exhaustion of Ext-NO_3^-. In addition, the low specific and cellular uptake rates of NO_3^- measured during day 1, when NO_3^- was abundant, suggest that the phytoplankton community was not fully acclimated to the new environmental conditions.

From day 3 on, NO_3^- uptake rates were reduced in response to the depletion of external NO_3^-. The exclusion of ambient UVBR tended to increase the specific and absolute uptake rates of NO_3^-, mainly in the samples collected in the morning of sunny days. However, during days 6 and 7, due to a lower PON concentration under excluded UVBR conditions, absolute uptake rates of NO_3^- in this treatment were not significantly different from those under ambient conditions. Several studies have shown that ambient UVBR inhibits photosynthesis in phytoplankton cells (Lorenzen 1979, Cullen and Lesser 1991, Cullen et al. 1992, Smith et al. 1992, Behrenfeld et al. 1993b, Villafañe et al. 1995) which may result in a shortage in ATP (Vosjan et al. 1990). Since NO_3^- enters phytoplankton cells by active transport requiring energy (Collos and Slawyk 1980, Wheeler 1983), a lack of ATP due to UVBR inhibition of photosynthesis may

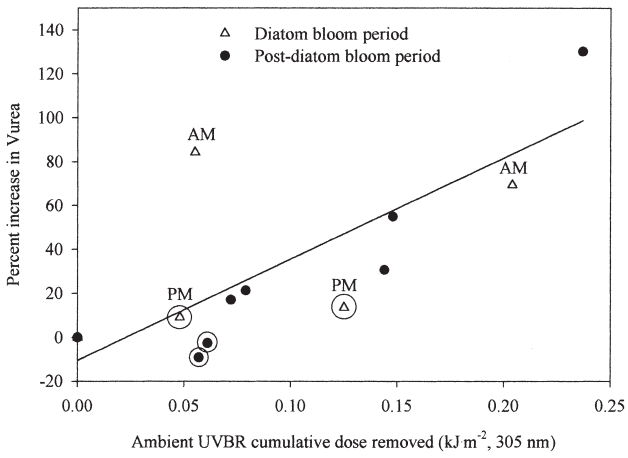

FIG. 7. Percent increase in specific uptake rate of urea (Vurea) as a function of the ambient unweighted UVBR cumulative dose removed in the WUVB treatment during the incubation periods of days 2−7. The increase in Vurea was calculated relative to the NUVB treatment (see text for details). The circled data represent the samples where the differences in Vurea between ambient and excluded UVBR conditions were not significant. The solid line represents the linear regression between both variables during the post-diatom bloom period ($r^2 = 0.81$, $P < 0.001$). The value of day 6 (a.m.) was excluded from the graph because of the unusual variability between replicates under ambient UVBR. The morning (a.m.) and afternoon (p.m.) samples are indicated for the diatom-bloom period.

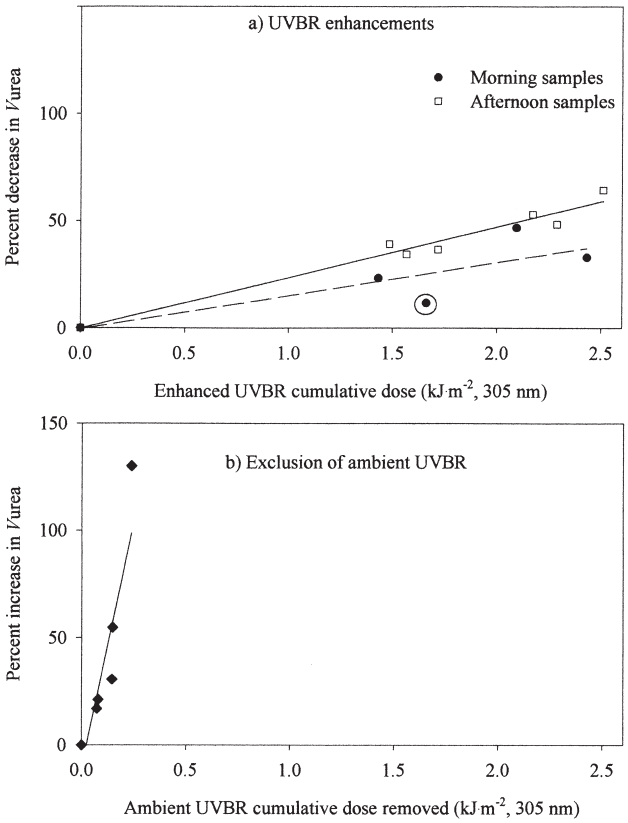

FIG. 8. UVBR dose-responses for specific uptake rate of urea (V_{urea}) during the last 3 days of the experiment. (a) Percent decrease in V_{urea} as a function of the unweighted enhanced UVBR cumulative dose in enhanced UVBR treatments during the incubation periods. The decrease in V_{urea} was calculated relative to the NUVB treatment (see text for details). The solid and dashed lines represent the linear regressions between both variables for afternoon ($r^2 = 0.97$, $P < 0.001$) and morning ($r^2 = 0.88$, $P < 0.01$) samples, respectively. The circled datum represents a sample where the difference in V_{urea} between ambient and enhanced UVBR was not significant. (b) Percent increase in V_{urea} as a function of the unweighted ambient UVBR cumulative dose removed in the WUVB treatment during the incubation periods. The increase in V_{urea} was calculated relative to the NUVB treatment as for Fig. 7. The solid line represents the linear regression between the percent increase in V_{urea} and the UVBR dose removed ($r^2 = 0.81$, $P < 0.001$). The value of day 6 (a.m.) was excluded from the graph because of the unusual variability between replicates under ambient UVBR.

cause a decrease in NO_3^- uptake rates. The negative impact of ambient UVBR on specific NO_3^- uptake rate observed during this study could, therefore, be a secondary response to a primary UVBR damage to photosynthesis, as suggested by Behrenfeld et al. (1995). It is noteworthy that the effect of the WUVB treatment on NO_3^- uptake was more important than that of UVBR enhancements. Since the Mylar screen does not only eliminate UVBR but also reduces UVAR, the difference in NO_3^- uptake between NUVB and WUVB treatments could result from the effect of both UVBR and UVAR. The increase in NO_3^- uptake rates under WUVB, relative to NUVB conditions, was mainly observed during midday incubations when ambient UVR was maximum. In addition, there was no increase in NO_3^- uptake rates under WUVB conditions during cloudy days. These results suggest that ambient UVR levels experienced during the afternoon of sunny days or during cloudy days were not high enough to affect NO_3^- uptake rates.

Results of NO_3^- uptake rates during the diatom bloom period show that, when the phytoplankton assemblage was acclimated to its new environment (after 1 day), UVBR enhancements did not result in any effect on absolute and specific uptake rates of NO_3^-. During the post-diatom bloom period, UVBR enhancements decreased the absolute and specific uptake rates of NO_3^- on some occasions. However, no correlation was found between the percent decrease in NO_3^- uptake rates and the enhanced UVBR cumulative dose, at 305 nm, received by the cells (regression analysis, data not shown). These results agree with the conclusions reached by Behrenfeld et al. (1995) during a similar experiment done with a natural phytoplankton community from the North Pacific Ocean. In contrast, other studies showed systematic UVBR inhibitions of NO_3^- uptake by marine diatoms (*Lauderia annulata* and *Synedra planctonica*) grown in batch cultures (Döhler and Bierman 1987) or by a natural phytoplankton population dominated by the flagellate *Phaeocystis pouchetii* from the Wadden Sea (Döhler 1992). This discrepancy could result from differences in the UVBR dose or in the species composition of the studied communities (Döhler and Biermann 1987, Döhler 1992).

NH_4^+ uptake. During the experiment, the variations in absolute and specific uptake rates of NH_4^+ followed the changes in Ext-NH_4^+. The different UVBR treatments did not affect the uptake of NH_4^+ during the diatom bloom period. However, during the post-diatom bloom period, the uptake rates of NH_4^+ were enhanced under UVBR exclusion. During this period, low and high UVBR enhancements resulted in reductions of the absolute and specific uptake rates of NH_4^+ on some occasions. The results from the diatom bloom period are in disagreement with the study of Behrenfeld et al. (1995) who found that the exclusion of ambient UVBR increased ρNH_4^+ during short-term incubations (4–8 h) of natural phytoplankton assemblages from the North Pacific Ocean. However, we obtained similar effects as Behrenfeld et al. (1995) during the post-diatom bloom period. These results suggest that, with respect to NH_4^+ uptake, the post-diatom bloom community dominated by flagellates and dinoflagellates was more sensitive to ambient UVBR than the bloom community mainly composed of centric diatoms. Behrenfeld et al. (1995) also reported that inhibitions of the absolute uptake rate of NH_4^+, under enhanced UVBR conditions, were dependent upon the UVBR dose. However, in the present study, the variations in ρNH_4^+ and VNH_4^+ between ambient and enhanced UVBR conditions were not correlated

with the enhanced UVBR cumulative dose received by the cells. For both exclusion and enhancements of UVBR, the discrepancies between the results of this study and those of Behrenfeld et al. (1995) could be explained, as discussed earlier, by differences in the UVBR dose or in the composition of algal communities (Döhler and Biermann 1987, Döhler 1992). Another explanation is that Behrenfeld et al. (1995) used only short-term incubations (4–8 h), while we tested long-term UVBR exposure where acclimation processes could occur. At the end of the post-diatom bloom, the percentage of NH_4^+ taken up under UVBR enhancements was significantly higher than under ambient UVBR. During this period, the proportion of small phytoplankton in the algal community increased under enhanced UVBR compared with ambient conditions (Mostajir et al. 1999). Small cells with large surface:volume ratios exhibit high potentials for nutrient absorption by diffusion and active transport (Raven 1986). A difference in the relative abundance of small and large cells in the phytoplankton population could explain the higher percentage of NH_4^+ taken up under enhanced UVBR as compared to ambient UVBR conditions.

Urea uptake. During the diatom bloom period, absolute and specific uptake rates of urea were low, then they increased and urea became the main nitrogenous source for phytoplankton growth during the post-diatom bloom period.

After the first day of acclimation, the exclusion of ambient UVBR generally increased the uptake rates of urea. In addition, urea uptake rates were negatively affected by UVBR enhancements during the post-diatom bloom period. This is the first evidence that UVBR can be deleterious to urea uptake by a natural assemblage of phytoplankton. The increase in *V*urea under UVBR exclusion was a linear function of the UVBR dose removed during the post-diatom bloom but not during the diatom bloom. This apparent discrepancy of urea uptake characteristics between the diatom bloom and the post-diatom bloom periods suggests that diatoms and flagellates respond differently to UVBR exclusion. The fact that, during the diatom bloom, afternoon samples were less sensitive to ambient UVBR could indicate that, during this period, the phytoplankton was able to develop photoprotective mechanisms against UVR during the day. This would corroborate the findings of Figueroa et al. (1997) who tested the effects of UVR on the carbon fixation of a natural Antarctic community dominated by nanoflagellates sampled in the morning and at noon. They found that the inhibition of carbon fixation by UVR was higher in morning samples than in noon samples and concluded that photoprotection mechanisms might operate during the day protecting the cells from UVR. The effect of UVBR exclusion on *V*urea was drastic since the augmentation of *V*urea could be as high as 130% for the removal of a cumulative dose of <0.25 kJ·m^{-2}.

UVBR enhancements also affected urea uptake, especially during the post-diatom bloom period. The negative effect of UVBR enhancements on *V*urea was more important in afternoon samples than in morning samples. This difference in *V*urea during the daytime can not be explained by a modification in the specific composition of the community during such a short period of time (Harris 1980). These results indicate that urea uptake by the phytoplankton community was less efficient during the afternoon than during the morning. This could be due to morning UVBR damage to phytoplankton cells in the mesocosms along with an ability of these cells for night repair. However, the absence of UVBR effect on the nitrogen composition of the vertically mixed phytoplankton community does not support this hypothesis. Therefore, the differences in *V*urea between morning and afternoon samples can not be explained with our present data.

As suggested for NO_3^- uptake, the physiologic impacts of UVBR on urea uptake by phytoplankton could be the consequence of some UVBR damage to photosynthesis. Indeed, the inhibition of phytoplankton photosynthesis by UVBR may reduce the production of electron donors required for the assimilation of urea. A lack of these compounds might, therefore, affect urea assimilation, which would in turn depress its uptake rate since assimilation is often the limiting step in nitrogen metabolism (Dortch 1982, Wheeler 1983).

As mentioned earlier, the Mylar screen used to eliminate UVBR also reduces UVAR. Therefore, the large effect of the WUVB treatment on urea uptake could result in part from the reduction of UVAR. This strong impact of ambient UVAR compared with the smaller effect of UVBR enhancements suggests that UVAR has a more significant impact on nitrogen uptake than UVBR.

CONCLUSIONS

The results of this study show that, during short-term surface incubations, the enhancement of UVBR affects phytoplankton nitrogen uptake. In particular, this is the first evidence that UVBR enhancement can inhibit urea uptake and that this inhibition is a linear function of the UVBR dose. Our results also show that the effect of UVBR on the uptake of NO_3^-, NH_4^+, and urea may change rapidly, from day to day and even within a day. Consequently, one should expect a great deal of variability in the response of phytoplankton assemblages to UVBR regarding nitrogen utilization. This study also focused on the long-term effects of UVBR on nitrogen utilization by a natural assemblage of phytoplankton. The results reveal a discrepancy between the impact of UVBR during short-term uptake measurements at the surface and the reduced long-term UVBR effects on the nitrogen composition and biomass of the phytoplankton community experiencing vertical mixing. This discrepancy emphasizes the role of vertical mixing on UVBR effects in natural ecosystems. The results of the present paper suggest that an increase in UVBR resulting from stratospheric

ozone depletion would have a minimal effect on nitrogen utilization by natural phytoplankton assemblages if these are vertically mixed.

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