

Experimental test of the effect of ultraviolet-B radiation in a planktonic community

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Abstract

Ultraviolet-B (UVB, 280–320 nm) radiation is a natural component of sunlight that harms organisms and disturbs natural communities in surface waters. A natural planktonic assemblage of organisms (<240 μm) was studied in a mesocosm experiment for 7 d under varying conditions of UVB radiation: UVB excluded, natural radiation, and UVB enhanced at two different levels. The dynamics of several populations at different trophic levels comprising heterotrophic bacteria (<1 μm), heterotrophic flagellates (2–10 μm), small phytoplankton (<5 μm), large phytoplankton (5–20 μm), and ciliates (15–35 μm) were monitored during the experiment. Enhanced UVB provoked a significant decrease in the number of ciliates (66%) and large phytoplankton (63%) relative to natural UVB conditions. The severe effects of UVB radiation on ciliates and large phytoplankton communities shown here would strongly limit upward transfer of mass and energy. The decline of predator abundance (ciliates) under UVB stress relative to natural conditions resulted in a positive feedback between enhanced UVB radiation and prey abundances, shown by increased abundances of bacteria (49%), heterotrophic flagellates (up to 300%), and small phytoplankton (41%). Similarly, with respect to carbon partitioning, the decrease in ciliate and diatom carbon biomass (64 and 56%, respectively) under enhanced UVB exposure was balanced by an increase in the carbon biomass of heterotrophic bacteria (48%), heterotrophic flagellates (126%), and autotrophic flagellates (162%). As a manifestation of enhanced UVB at the community level, the ecosystem develops toward a microbial food web in preference to an herbivorous food web. Thus, enhanced UVB radiation can change the structure and dynamics of the pelagic food web.

The pelagic planktonic community functions through a web of energy and nutrient exchanges mediated by a diverse array of producers and consumers, which ultimately depend on the energy supplied by sunlight. Following the discovery of stratospheric ozone depletion (Farman et al. 1985) and the resulting increase in intensity of biologically harmful UVB radiation (280–320 nm) reaching Antarctic waters, the majority of UVB studies have focused on phytoplankton be-

cause of their importance as primary producers. Several investigations have shown that phytoplankton are sensitive to current UVB levels, which can reduce the rate of primary production (Smith et al. 1980; Cullen and Lesser 1991; Smith et al. 1992; Vincent and Roy 1993); inhibit growth, photosynthesis, and cell division (Calkins and Thordardottir 1980; Jokiel and York 1984; Karentz et al. 1991); and change the species composition (Neale et al. 1994; Davidson et al. 1996). UVB exposure also adversely affects both the survival and the reproduction of the major multicellular consumers, such as copepods (Karanas et al. 1979; Naganuma et al. 1997; Zagarese et al. 1997). However, viruses (Suttle and Chen 1992) and bacteria (Herndl et al. 1993; Müller-Niklas et al. 1995; Jeffrey et al. 1996) are likely to be even more susceptible to UVB stress because of their smaller size and shorter generation time (Karentz et al. 1994).

Stratospheric ozone depletion has been recorded also throughout temperate and polar regions (Blumthaler and Ambach 1990; Lubin and Jensen 1995; Müller et al. 1997). Moreover, in the Arctic region, ozone loss rates have reached

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values comparable to those recorded over the Antarctic in recent years (Rex et al. 1997 and references therein) and are predicted to continue to decline for one or more decades (Shindell et al. 1998). In the Arctic and into the north temperate zone, the ozone layer diminished 15–20% during the 1991–1992 winter and 30% in 1996. During spring 1997, it decreased as much as 45% below normal in the high Arctic and about 7% below normal over the midlatitude regions of Canada (Tarasick and Fioletov 1997). It is therefore clear that the effects of enhanced UVB radiation on midlatitude aquatic ecosystems, and not just those in polar regions, need to be better understood.

The nature of pelagic ecosystem responses to increased UVB radiation, via its effect on trophic interactions, is to date the most elusive ecological question related to UVB impact studies. Recent investigations have variously either confirmed or rejected the influence of UVB radiation on the relationship between predator and prey. Bothwell et al. (1994) showed that ultraviolet radiation reduced not only the photosynthesis and growth of benthic diatoms but also inhibited algal consumers (Diptera: Chironomidae). In an oligotrophic lake, Sommaruga et al. (1996) observed the high sensitivity of a heterotrophic nanoflagellate (*Bodo saltans*) to solar ultraviolet radiation, which reduced the rate of bacterivory by up to 70%. Similarly, Ochs (1997) demonstrated the harmful effects of ultraviolet radiation on the grazing activity of heterotrophic flagellates on autotrophic picoplankton. Although Keller et al. (1997a), using a mesocosm approach, concluded that the enhancement of UVB radiation during a winter/spring bloom did not affect the marine food web, trends of diminishing abundances of organisms were consistently observed at different trophic levels in the enhanced UVB treatment. For example, relative to natural conditions, phytoplankton abundance and biomass tended to be reduced under UVB stress. This was also the case for total copepod abundances (adults, copepodites, and nauplii). Furthermore, there was a nonsignificant tendency for the ratio of nanoplankton to microplankton biomass (measured as *in vivo* fluorescence) to be lower in enhanced UVB conditions. The interpretation of these results is rather difficult because of the high temporal and replicate variability among the controls (e.g., mesocosms) for natural conditions (Keller et al. 1997a). Keller et al. (1997b), using the same mesocosm experimental design, reported the significant reduction of phytoplankton abundance and biomass and of copepod nauplii numbers under UVB stress in a stratified coastal system. However, they did not observe a significant effect of UVB radiation on microzooplankton. They concluded that despite the 10-fold increase in biologically damaging UVB applied in their experiment, effects were not seen at the higher trophic levels. They suggested that it was most likely because of the rapid extinction of UVB in the highly colored coastal water.

Although our knowledge about the effects of UVB radiation on organisms in the laboratory has improved over the last decade, it is still not possible to predict the changes that might occur in the natural environment. To extend the results from laboratory and mesocosm studies to whole ecosystems, some fundamental experimental design criteria must be met. First, the organisms used in the UVB experiment must be

representative of natural conditions, not only in their abundance but also in their sensitivity to UVB radiation (Ochs 1997). Organisms cultured in the laboratory in the absence of UVB radiation might have reduced concentrations of molecular sunscreens, both colored and colorless (Goodwin 1980; Garcia-Pichel and Castenholz 1993) or reduced repair capabilities and thus, be more susceptible to UVB damage than natural organisms (Paerl et al. 1985). Secondly, it is the relative sensitivity of different members of the community that determines the net impact on the ecosystem. Although physiological experiments and UVB dose-response curves for a particular group of organisms from a single trophic level may elucidate UVB damage and repair processes, the sensitivity levels to UVB radiation differ between organisms (e.g., predator and prey: Bothwell et al. 1994), and the results of these experiments cannot be applied directly to the natural environment, which is governed by a complex web of trophic interactions. Thirdly, experimentally enhanced UVB doses must be representative of plausible natural conditions, and natural UVB controls are also necessary for comparison. Fourthly, in addition to the measurement of the dynamics of several trophic levels, changes in the dynamics of environmental variables such as nutrients and temperature should also be investigated in enhanced UVB studies. Finally, in the pelagic system, the fate of planktonic organisms is closely linked to mixing dynamics (Neale et al. 1998). The euphotic zone of the pelagic system is heterogeneous with respect to UVB response, permitting periodic damage at the surface as well as periodic repair in deeper waters. Good experimental design will ensure that mixing is uniform, reproducible, and representative of the natural conditions.

The objective of this study was to examine the influence of UVB radiation on trophic interactions and thereby, on the community structure of a temperate pelagic food web. A mesocosm approach was adopted, taking into account the five important criteria listed previously. The experimental design used organisms from a natural system, comprising a planktonic assemblage from the St. Lawrence Estuary. Several trophic levels, including heterotrophic bacteria, heterotrophic flagellates, small and large phytoplankton, and ciliates, were studied concurrently under varying UVB regimes. The suite of measurements undertaken incorporated a wide range of physical and chemical variables. The water column was homogeneously mixed, and the light regime mimicked the photic zone as already described by Belzile et al. (1998). The present paper shows that, in the absence of large predators (e.g., copepods), the acute UVB sensitivity of the ciliate community and large phytoplankton determined the structure of the final community under different conditions of ultraviolet radiation.

Materials and methods

Experimental procedure—Mesocosm experiments were performed on the south shore of the lower St. Lawrence Estuary (Québec, Canada: 48.6°N, 68.2°W) during 17–23 July 1996. A time-series experiment was carried out for 7 d using eight land-based mesocosms (2.25-m depth), each con-

taining 1,500 liters of St. Lawrence Estuary surface water previously passed through a 240- μm Nitex screen.

Experimental design and light conditions—The experimental setup, including natural and experimental light conditions, has been described in detail elsewhere (Belzile et al. 1998). Briefly, pairs of mesocosms were submitted to four UVB treatments: (1) natural UVB (NUVB) as control, (2) low UVB enhancement (LUVB), (3) high UVB enhancement (HUVB), and (4) without UVB (WUVB). The UVB intensities were increased using two LUVB and three HUVB lamps (model XX15B, Spectronics) with an emission peak at 312 nm, which were turned on from 0900 to 1730 h. The shorter wavelengths not encountered in nature but emitted by the lamps were eliminated by using aged 0.13-mm cellulose acetate sheets that were changed daily. In the WUVB treatment, natural UVB radiation was removed by a 0.13-mm Mylar® D sheet. Incident irradiance was recorded every 10 min during the experiment using an IL 1700 radiometer (International Light). UV irradiance through the water column was measured using a PUV 500 profiling radiometer (Biospherical Instruments) and an Optronic Laboratories OL 752 spectroradiometer. The PUV 500 radiometer provided a measure of cosine-corrected downwelling irradiance at 305, 320, 340, and 380 nm and downwelling, cosine-corrected photosynthetically available radiation (PAR, 400–700 nm). A correction factor of 2.6 was applied to the absolute irradiances at 305 nm measured with the PUV 500 to compensate for the underestimation caused by the lamp calibration method (Kirk et al. 1994).

The irradiance attenuation coefficient (K_d) in the mesocosms was calculated based on measurements using the PUV 500 radiometer. Temperature regulation was achieved by circulating local estuarine water around the mesocosms. The water temperature was measured every hour between the two mesocosms in each tank with thermocouples (type “J”) connected to a datalogger (21X, Campbell Scientific). In each mesocosm, vertical profiles were obtained using the temperature sensor of the PUV 500 radiometer. To ensure a homogeneous water mass, the water in each mesocosm was continuously mixed using a small pump (Little Giant®, model 2-MD-HC) achieving a turnover time of 1 h.

Nutrients—Samples for nutrient measurements were collected from each mesocosm at 0900 and 1300 h every day. Aliquots of 40–50 ml of sample were filtered (<10 cm Hg) through Whatman GF/F glass-fiber filters. Inorganic nutrients were analyzed in the filtrates. The concentration of ammonium was immediately determined using the method of Solórzano (1969) as described by Parsons et al. (1984). The remainder of the filtrate was frozen at -20°C for later analysis of nitrate plus nitrite, nitrite and silicate using a Perstorp FS III Auto analyzer (Parsons et al. 1984).

Planktonic communities—Dynamics of ciliates (15–35 μm), heterotrophic flagellates (2–10 μm), heterotrophic bacteria (<1 μm), small phytoplankton (<5 μm), and large phytoplankton (5–20 μm) were monitored during the weeklong experiment. To estimate ciliate microzooplankton and heterotrophic flagellate concentrations, samples were taken

once a day (0900 h). Estimates of naked ciliates and full loricae tintinnids were made with a Zeiss inverted microscope using 100-ml water samples that had been preserved with acid Lugol (0.4% final concentration) and sedimented for 24 h. Although heterotrophic flagellates were enumerated in two size classes of 2–5 and 5–10 μm according to the method of Booth (1993), data are presented in a single size class of 2–10 μm . Briefly, 25-ml water samples were fixed with glutaraldehyde (1.0% final concentration, 10 min in the dark) and then drawn down onto a 25-mm black Nuclepore polycarbonate membrane (0.2- μm pore size) using low vacuum (<10 cm Hg). The filter was placed on a slide and examined using an epifluorescence microscope with a $\times 100$ objective. Samples for bacterial and phytoplankton counting were taken every 4 h during the first 4 d (0100, 0500, 0900, 1300, 1700, and 2100 h) and twice daily (0900 and 1700 h) during the following 3 d. For counting bacteria, 10-ml water samples were fixed with formalin (3.7% final concentration) and kept at 4°C . The bacterial cells were then stained with DAPI (4', 6-diamidino-2 phenylindole, Sigma Chemical, 2.5 $\mu\text{g ml}^{-1}$ final concentration) for 1 h at 4°C (Porter and Feig 1980). Bacteria were then enumerated using an ACR 1400SP flow cytometer (Bruker, Wissembourg) according to Monfort and Baleux (1992). Both small and large phytoplankton abundances were analyzed immediately after sampling using a FACSORT Analyzer flow cytometer (Becton-Dickinson) fitted with a 488-nm laser. Prior to analysis, phytoplankton were kept in the dark at 4°C for a maximum of 2 h, and samples from different treatments were analyzed in random order. The flow rate was set to 12 $\mu\text{l min}^{-1}$, and the acquisition time was at least 5 min. Two separate samples from each mesocosm were analyzed after the addition of 10- μm beads (Immunocheck, Coulter) as an internal standard. The data were logged using Cell Quest software and analyzed with Attractors software, both from Becton-Dickinson. In addition to counting phytoplankton using the flow cytometer, cell sizes of the abundant large phytoplankton (diatoms) were measured once a day (0900 h) for all treatments using optical microscopy for nonfixed samples. Furthermore, the abundance and mean cell size of the different algal species (>3 μm) were determined using the samples fixed by acid Lugol with an inverted microscope according to the methods of Lund et al. (1958) and Smayda (1978), respectively. These results are not shown in the present paper, but they have been used to estimate the carbon biomass of different algal groups.

The genus and species of ciliates and autotrophic and heterotrophic flagellates, as well as those of large phytoplankton, were identified by consulting several taxonomic guides and references (Kofoid and Campbell 1939; Grassé 1952; Bick 1972; Cardinal et al. 1977; Kudo 1977; Corliss 1979; Maeda and Carey 1985; Sournia 1986; Ricard 1987; Chrétiennot-Dinet 1990; Sime-Ngando et al. 1995; Tomas 1997).

Estimation of carbon biomass of planktonic organisms—Ciliate carbon biomass was estimated based on a mean ciliate diameter of 19.2 μm ($n = 801$), using volume to carbon ratios of 0.19 $\text{pg C } \mu\text{m}^{-3}$ (Putt and Stoecker 1989). It should be noted that the most abundant ciliate species presented in this study ranged in size from 17 to 22 μm . The cell di-

ameters of heterotrophic flagellates were assumed to be 3.5 and 7.5 μm for the size classes 2–5 and 5–10 μm , respectively. The biovolume of heterotrophic flagellates was converted to carbon using a factor of 0.22 $\text{pg C } \mu\text{m}^{-3}$ (Booth 1993). The bacterial carbon biomass was assumed to be 20 $\text{fg C bacterium}^{-1}$. This value had previously been obtained for bacteria from the St. Lawrence Estuary by Sime-Ngando et al. (1995) based on the equation of Simon and Azam (1989). This conversion factor for the bacterial carbon biomass agrees with that proposed by Zweifel et al. (1993). Phytoplankton was divided into two groups: (1) diatoms, and (2) all autotrophic flagellates including dinoflagellates. The biovolume of diatoms and autotrophic flagellates was converted to carbon biomass using the conversion factor of 0.22 $\text{pg C } \mu\text{m}^{-3}$ (Booth 1993).

Statistical treatments—A one-way analysis of variance with UVB treatment as the grouping factor was performed on each data set for each sampling time. When significant differences occurred, Bonferroni-adjusted pairwise comparisons were used, as well as Dunnett's test, with NUVB treatment acting as the control group. The probability values (P) given hereafter are indicative of significant results for these statistical tests.

Results

Temperature, light, and UVB irradiance—The cooling system provided good regulation of water temperature in the tanks. During the 7 d of the experiment, the water temperature varied between 8.5 and 11.34°C with no significant differences between the tanks (Belzile et al. 1998). Three periods during the experiment were distinguished according to the incident irradiance: 2 sunny days, followed by 3 cloudy days, and finally 2 more sunny days (Fig. 1A–C). The unweighted daily ambient UVB dose for a sunny day was 33 kJ m^{-2} (18 July), of which only about 40% reached the surface water of the mesocosms because of shading effects. At the water surface, the increases in daily unweighted UVB doses using lamps were 40.5 kJ m^{-2} (LUVB) and 59.2 kJ m^{-2} (HUVB). The attenuation coefficient (K_d) of PAR, 400–700 nm, in the mesocosms was 1.46 m^{-1} (3.7% surface PAR at the bottom of the mesocosms). In the St. Lawrence Estuary, this value varied in the range 0.29–0.53 m^{-1} (1% surface PAR at 8.6–16.0 m) in July (Levasseur et al. 1984). The water mass in the mesocosms thus simulated the whole euphotic zone. Biological weighting functions (BWFs) were applied because the lamps and the sun have different spectral shapes with respect to UVB. The results from applying some well-known BWFs (Cullen et al. 1992; Behrenfeld et al. 1993; Boucher and Prézélin 1996) to the spectral irradiance of Fig. 1D are illustrated in Table 1. The increase in UV irradiance relative to NUVB for a sunny day at noon was 1.10- and 1.15-fold greater in the LUVB and HUVB, respectively (Table 1). When weighted with three BWFs for inhibition of photosynthesis, the increased UV irradiance was greater by 1.75–3.61-fold for the LUVB treatment and 2.10–4.81-fold for HUVB (Table 1).

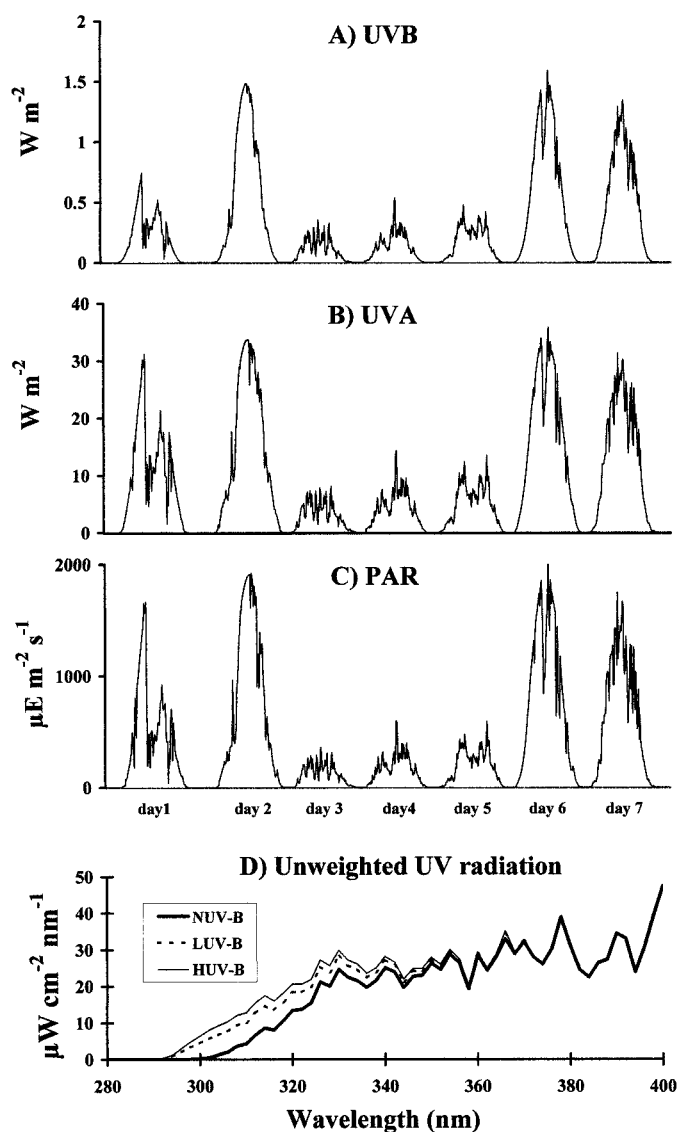


Fig. 1. Incident irradiance recorded every 10 min during the experiment. Ambient (A) UVB (280–320 nm), (B) UVA (320–400 nm), (C) PAR (400–700 nm), and (D) unweighted UV radiation intensities just below the water surface calculated for a sunny day at noon.

Nutrients—Nitrate concentration decreased from day 1 to day 3 in all treatments, from 10 to $<0.9 \mu\text{mol liter}^{-1}$ (Fig. 2A). Nitrate was depleted after day 3. Ammonium concentrations increased in all treatments from $0.10 \mu\text{mol liter}^{-1}$ on day 3 to $0.28 \mu\text{mol liter}^{-1}$ on day 4 (0900 h) (Fig. 2B) but did not show any consistent pattern with treatment.

Dynamics of planktonic populations—The ciliate community was composed of the following species: *Strobilidium spiralis*, *Strobilidium epacrum*, *Strombidium acutum*, *Lohmaniella oviformis*, *Askenasia* sp., *Uronema marinum*, and *Laboea* sp. The abundances were minimal until day 3, then increased after day 4 (Fig. 3A). They were significantly different between the four treatments ($P < 0.001$) on the last day of the experiment. On day 7, the highest ciliate concen-

Table 1. Relative irradiance increases provided by the lamps, for LUVB and HUVB enhancements, just below water surface on a sunny day around noon, compared to relative increases of incident irradiance associated with ozone depletion over Antarctica. These increases have been calculated from data presented in Fig. 1D. Spectral irradiances were weighted with the BWF for inhibition of photosynthesis in the temperate latitude diatom *Phaeodactylum* (Cullen et al. 1992), the best fit action spectrum for photoinhibition (Behrenfeld et al. 1993), and the daily averaged BWF for the in situ inhibition of primary production in natural communities of Antarctic diatoms (Boucher and Prézelin 1996). The data for McMurdo Station are from Cullen and Neale (1997) and correspond to a decrease of ozone thickness from 350 to 175 DU.

	Incident solar irradiance at McMurdo	LUVB	HUVB
Unweighted UV	1.06	1.10	1.15
BWF for inhibition of photosynthesis (Cullen et al. 1992)	1.24	1.75	2.10
BWF for photoinhibition (Behrenfeld et al. 1993)	—	3.26	4.31
BWF for in situ inhibition of photosynthesis (Boucher and Prézelin 1996)	—	3.61	4.81

tration was observed in WUVB, and progressively fewer ciliates were seen in treatments NUVB, LUVB, and HUVB, corresponding to increasing exposure to UVB. Ciliate concentrations were reduced 33% on day 7 in LUVB and 66% in HUVB relative to those in NUVB. Tintinnids, including *Helicostomella kiliensis*, *Helicostomella subulata*, *Tintinnopsis* sp., *Ptychocyclus obtusa*, *Favella* sp., *Parafavella* sp., and some other unidentified species, were also present in this study. However, they were excluded from further analysis because their concentrations remained very low and relatively constant at about 700 cells liter⁻¹ during the experiment, and no significant difference was detected between treatments (data not shown).

Heterotrophic flagellate (2–10 μm) communities consisted mainly of *Monosiga marina*, *Bodo* sp., with assorted unidentified flagellates and choanoflagellates. On day 2, the abundance of heterotrophic flagellates was higher in the

WUVB treatment ($P < 0.05$) than in the other treatments (Fig. 3B), indicating the negative effect of UVB on heterotrophic flagellates. Thereafter, the number remained stable until day 4 in all treatments. The population of heterotrophic flagellates then increased significantly ($P < 0.01$) on day 7 by up to 300% in LUVB and by up to 250% in HUVB relative to those in NUVB.

Bacteria exhibited two different responses during the weeklong experiment. Before day 4 (0900 h, Fig. 3C), bacterial abundances increased less in UVB-enhanced treatments relative to NUVB, probably due to the negative effect of UVB exposure. From day 5 onward (0900 h), their concentration in HUVB was significantly higher than those for all other treatments ($P < 0.01$). Bacterial concentrations under enhanced UVB radiation on day 7 (1700 h) were 26% higher in LUVB and 45% higher in HUVB relative to those under NUVB.

The small (<5 μm) phytoplankton community encompassed Prymnesiophyceae and Chrysophyceae (*Ochromonas* sp.), for which the Prymnesiophyceae (<4 μm) represented >94% of the cell numbers (Mostajir unpubl. data). It should be noted that some species of Prymnesiophyceae, Chrysophyceae, and Dinophyceae (large phytoplankton) could be mixotroph, but they could not be distinguished by the method used here (flow cytometer). Consequently, they have been categorized as autotroph. The abundance of small phytoplankton initially increased, but it then stabilized during days 3–6 before decreasing slightly toward the end of the experiment (Fig. 3D). Their concentrations became significantly higher in HUVB and LUVB on day 7 (0900 h) compared to the NUVB and WUVB treatments ($P < 0.05$). The number of small phytoplankton during the last 2 d of the experiment increased between 34 and 41% in LUVB and between 15 and 36% in HUVB, both with respect to those in NUVB.

The large (5–20 μm) phytoplankton community was made up of members of the Prymnesiophyceae (*Prymnesium* sp.), Prasinophyceae (*Tetraselmis* sp., *Pyramimonas* sp., and *Mantonella squamata*), Cryptophyceae (*Plagioselmis* sp., *Hiella marina* and *Teleaulax acuta*), Dinophyceae (*Katodinium rotundatum*, *Heterocapsa* sp., *Glenodinium* sp., and *Prorocentrum minimum*), and centric Bacillariophyceae (*Thalassiosira conferta*, *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira nordenskiöldii*). The overall abundance of

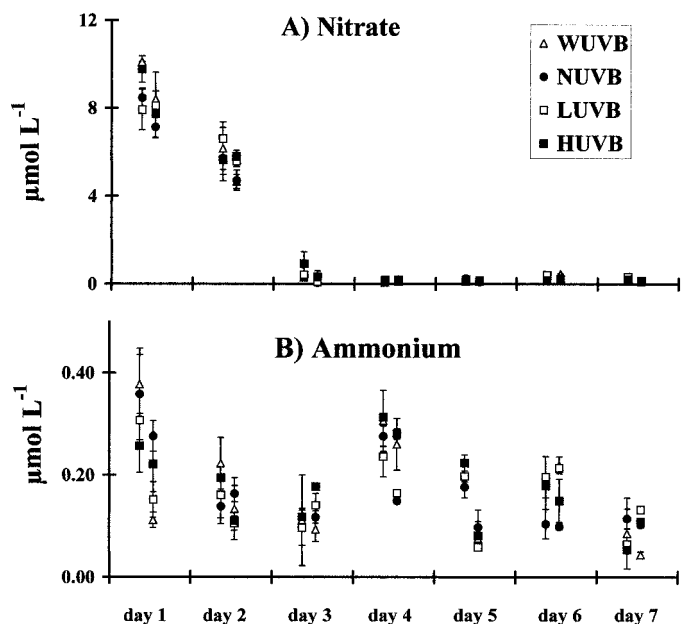


Fig. 2. Variations in the concentrations of (A) nitrate, and (B) ammonium at 0900 and 1300 h during the experiment under varying conditions of UVB radiation. Bars represent the range of the observations.

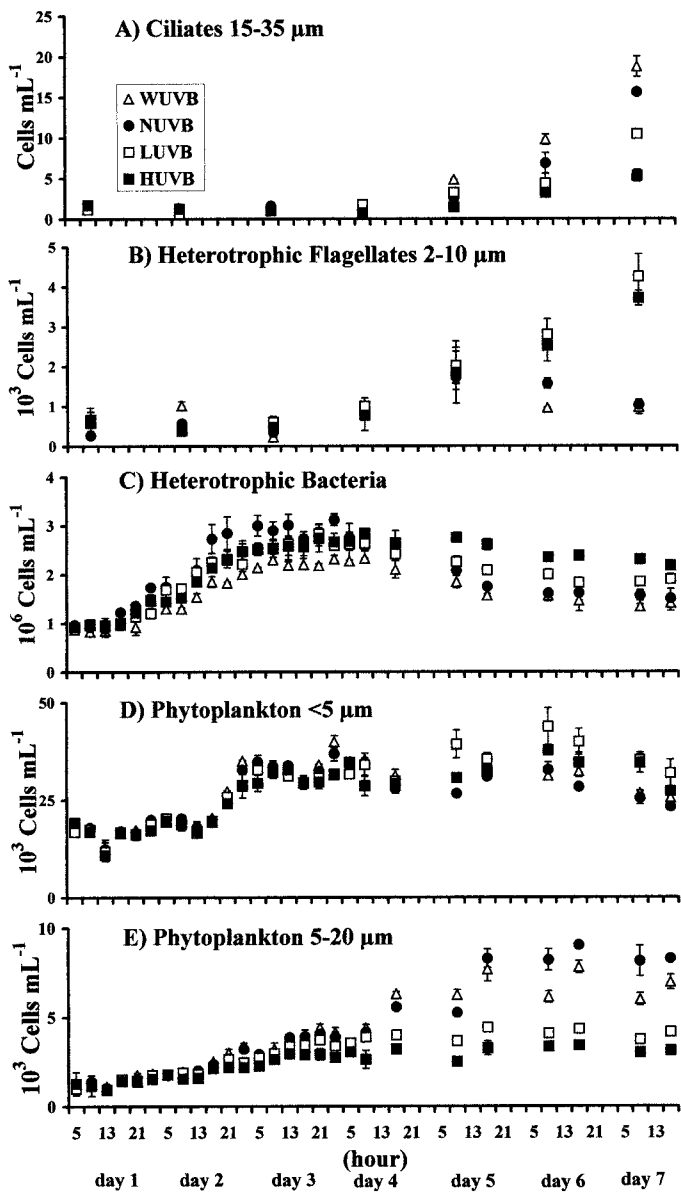


Fig. 3. Temporal variations of abundances (cells per milliliter) for major groups of the microbial food web: (A) ciliates, (B) heterotrophic flagellates, (C) heterotrophic bacteria, (D) small phytoplankton, and (E) large phytoplankton. Bars represent the range of the observations.

large phytoplankton (5–20 μm) increased during the first 4 d of the experiment. Thereafter, their abundances became lower in the two enhanced UVB treatments, relative to those in NUVB and WUVB treatments, and were significantly different ($P < 0.01$) from day 5 until the end of the experiment for all treatments (Fig. 3E). Large phytoplankton abundances under enhanced UVB treatments were reduced during days 5 to 7 from 30 to 50% in LUVB and from 52 to 63% in HUVB relative to those observed in NUVB.

Dose-response curves at 305 nm—To evaluate the influence of UVB on each trophic level and to explain the community structure changes following 1 week of UVB expo-

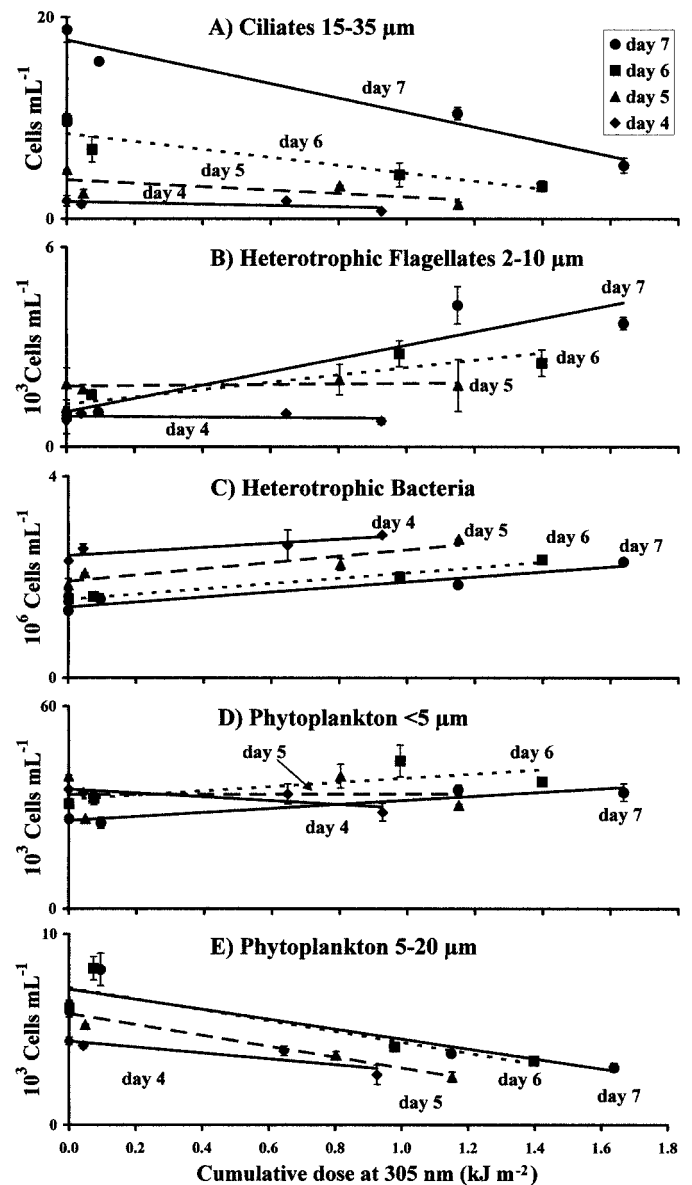


Fig. 4. Dose-response curves at 305 nm at 0900 h for five planktonic compartments: (A) ciliates, (B) heterotrophic flagellates, (C) heterotrophic bacteria, (D) small phytoplankton, and (E) large phytoplankton. Each point represents the cell concentration of each compartment at 0900 h (Fig. 3) in relation to the cumulative UVB dose from days 1 to 4, to 5, to 6, and to 7. The UVB dose was calculated as the daily 305-nm irradiance averaged over the water column (Belzile et al. 1998) according to Riley (1957). Bars represent the range of the observations.

sure, the dose-response curves were obtained for each component of the microbial food web. A linear fit was used to compare the various dose-response curves. These curves for ciliate abundances from days 4 to 7 of the experiment are shown in Fig. 4A. Negative slopes of the dose-response curves were significantly different from zero for day 6 (ANCOVA, $P < 0.01$) and were more pronounced for day 7 ($P < 0.001$). It should be stressed that this detrimental UVB impact was also observed for NUVB relative to the treatment

Table 2. Carbon biomass ($\mu\text{g C liter}^{-1} \pm \text{SE}$) of ciliates, heterotrophic flagellates, heterotrophic bacteria, diatoms, and autotrophic flagellates on days 1 and 7 under NUVB and HUVB enhancements.

Planktonic compartments ($\mu\text{g C liter}^{-1}$)	Day 1 (all treatments)	Day 7 (NUVB)	Day 7 (HUVB)	Change between NUVB and HUVB on day 7
Ciliates	1 \pm 0.2	11 \pm 0.1	4 \pm 0.5	-64%
Heterotrophic flagellates	11 \pm 3	35 \pm 1	79 \pm 2	+126%
Heterotrophic bacteria	19 \pm 1	31 \pm 2	46 \pm 1	+48%
Diatoms	203 \pm 38	1,330 \pm 7	590 \pm 85	-55%
Autotrophic flagellates	50 \pm 7	420 \pm 45	1,100 \pm 283	+162%

where UVB was removed. Heterotrophic flagellate dose-response curves (Fig. 4B) exhibited positive slopes, which were significantly different from zero for days 6 and 7 ($P < 0.01$). The trend for bacterial concentrations at the end of the experiment was the inverse of that apparent for ciliate abundances (Fig. 3C vs. Fig. 3A). Positive slopes of bacterial dose-responses curves (Fig. 4C) were significantly different from zero for days 5 ($P < 0.01$), 6, and 7 ($P < 0.001$). Small phytoplankton showed the progressive inversion of negative slope at day 4 toward a significant positive slope at day 7 ($P < 0.01$; Fig. 4D). In contrast, the dose-response curves for large phytoplankton (Fig. 4E) exhibited negative slopes from day 4 ($P < 0.05$) to day 7 ($P < 0.001$ for the last 3 d).

Partitioning of carbon in the food web—Table 2 summarizes the carbon biomass of the different planktonic groups on days 1 and 7 under NUVB and HUVB treatments. The estimated ciliate carbon increased from 1 $\mu\text{g C liter}^{-1}$ at the beginning of the experiment to 4 $\mu\text{g C liter}^{-1}$ in HUVB and 7 $\mu\text{g C liter}^{-1}$ in LUVB, compared to 11 $\mu\text{g C liter}^{-1}$ in NUVB at the end of the experiment. Ciliates benefited even more in the WUVB treatments, where the carbon biomass increased to 13 $\mu\text{g C liter}^{-1}$ (17% more than NUVB). The estimated carbon contribution of ciliates to the total estimated carbon of organisms was lower by 66 and 29%, respectively, for HUVB and LUVB and was greater by 180% for WUVB, all relative to NUVB.

Apart from ciliates, carbon contributions of all other heterotroph organisms increased under enhanced UVB treatments relative to NUVB conditions by the end of the experiment. At this time, biomass of heterotrophic flagellates was 102 and 79 $\mu\text{g C liter}^{-1}$ under LUVB and HUVB treatments, respectively, compared to 35 $\mu\text{g C liter}^{-1}$ under NUVB. The estimates of heterotrophic bacterial carbon at day 7 were 27, 31, 37, and 46 $\mu\text{g C liter}^{-1}$ under WUVB, NUVB, LUVB, and HUVB treatments, respectively.

The carbon biomass of the two groups of phytoplankton showed opposite trends with respect to UVB exposure. The estimated carbon biomass of the diatoms for NUVB conditions was 1,330 $\mu\text{g C liter}^{-1}$, which was reduced 56% under HUVB treatments by day 7. In contrast, the carbon biomass of autotrophic flagellates was 420 $\mu\text{g C liter}^{-1}$ for NUVB conditions and increased up to 1,100 $\mu\text{g C liter}^{-1}$ under HUVB treatments by the end of the experiment (Table 2).

Discussion

This investigation provides the first evidence of the susceptibility to UVB radiation of a crucial component of the pelagic food web: the ciliates. Ciliates play a key role in marine food webs. They regulate autotrophic picoplankton ($\leq 2 \mu\text{m}$) (Rassoulzadegan et al. 1988; Bernard and Rassoulzadegan 1990) and bacterioplankton (Fenchel 1980; Rivier et al. 1985; Sherr and Sherr 1987) through predation and nutrient regeneration, notably nitrogen (Paasche and Kristiansen 1982; F errier-Pag es and Rassoulzadegan 1994) and phosphorus (Allali et al. 1994). Ciliates can transfer the bulk of both matter and energy fluxes from lower trophic levels, i.e., pico- and nanoplankton (0.2–20 μm), to higher ones, e.g., metazoan zooplankton (Stoecker and Egloff 1987; Wiadnyana and Rassoulzadegan 1989; Jonsson and Tiselius 1990; Dolan 1991; Hartmann et al. 1993; Sime- Ngando et al. 1995) and fish larvae (De Mendiola 1974). A slight perturbation in such transfers could have ramifications throughout the marine food web. The high sensitivity of ciliates to UVB radiation was demonstrated in this study as a reduction of 66% of ciliate abundances under HUVB treatments relative to NUVB (Figs. 3A, 4A). The reduction of ciliate abundances might be due to the direct effect of UVB radiation on ciliates, to the physiological changes of prey that could produce qualitative food limitations, or to a combination of such direct and indirect effects. The diminution of ciliate abundances resulted in a positive feedback between UVB and prey abundances. Dose-response curves at 305 nm for heterotrophic flagellates, bacteria, and small phytoplankton at the end of the experiment (Fig. 4B–D; see results presented in Table 2) are interpreted to reflect trophic interactions between these prey and ciliates. Reduced ciliate numbers, under both enhanced UVB treatments, allowed the development of heterotrophic flagellates at a higher rate than under NUVB and WUVB conditions (Figs. 3B, 4B). In these latter cases, ciliate abundances and consequent grazing pressures were much stronger. Similarly, bacterial and small phytoplankton abundances increased under enhanced UVB conditions relative to NUVB and WUVB by the end of the experiment, when the ciliate number was reduced (Figs. 3C–D, 4C–D).

The predator–prey interactions illustrated in Fig. 3A–D show that the higher heterotrophic flagellate concentrations in enhanced UVB treatments relative to NUVB and WUVB did not induce a significant shift in their potential prey populations (i.e., bacteria and small phytoplankton). This seems to indicate a higher impact for ciliates than for heterotrophic flagellates on

bacteria and small phytoplankton. Some investigations have shown a reduction of grazing impact of heterotrophic flagellates under enhanced UVB (Sommaruga et al. 1996; Ochs 1997), which probably resulted from the inactivation of the flagella (Hessen et al. 1995). Sommaruga et al. (1996) have shown that UVB radiation decreased the swimming speed of the heterotrophic flagellate *B. saltans* by 3.5 times and changed the characteristic elongated cell morphology of this flagellate into a spherical one that was nonmotile. Disruption of flagellar movement could impair motility of the organism (Häder and Häder 1989), disrupt flagella-induced feeding currents (Fenchel 1986), or both (Ochs 1997).

The community of large phytoplankton exhibited a net reduction under enhanced UVB treatments relative to WUVB and NUVB (Figs. 3E, 4E). The carbon biomass of diatoms was reduced to 56% under HUVB treatment relative to NUVB at the end of the experiment (Table 2). Because large predators (copepods) were removed in this study and the tintinnid abundance remained very low and similar between treatments, differences in large phytoplankton numbers, especially diatoms, between treatments can be directly attributed to UVB stress. This influence, while also likely present for small phytoplankton, may have been masked because of the increased grazing pressure exerted by ciliates in NUVB and WUVB conditions. These results further illustrate the impact of UVB stress in predator-prey relationships as described previously.

The dynamics of nutrients should also be considered for the interpretation of the community structural changes. During the first 3 d of the experiments, the gradual increase in algal abundance was accompanied by a decrease in nitrate (Fig. 2A) and silicate concentrations (data not shown). Ammonium concentrations increased in all treatments on day 4 (0900 h) and remained higher than $0.44 \mu\text{mol liter}^{-1}$ for the rest of the experiment (Fig. 2B). Urea concentrations were also high in the mesocosms (Fauchot 1998). In response to these changes in ambient nitrogen levels, Fauchot (1998) has shown that the nitrogen nutrition of the phytoplankton community shifted from nitrate to ammonium and urea during the experiment. As expected, this shift in nutrient availability was also accompanied by a gradual decrease in diatoms and by an increase in the abundance of the autotrophic flagellates toward the end of the experiment, as demonstrated in terms of carbon biomass in Table 2. Fauchot (1998) has also shown that neither excluded UVB treatment nor enhanced UVB treatments had a significant effect on the level of nitrate and ammonium in the mesocosms. Therefore, the UVB-induced changes in plankton community reported in this study seem to result from a direct effect of UVB radiation on the organisms rather than an indirect effect via UVB-induced change in nutrients.

The experimental design used in this investigation allowed the study of the interactive responses of different trophic levels within a natural planktonic assemblage under varying conditions of UVB radiation. The relative UVB enhancements in this study were somewhat greater than the relative enhancement associated with the ozone hole over Antarctica. When weighted with three BWFs for inhibition of photosynthesis, the increased UVB radiation was 1.75–3.61-fold greater for the LUVB treatment and 2.10–4.81-fold greater for HUVB relative to NUVB

(Table 1). However, it should be kept in mind that application of BWFs is optimal when limited to the organisms or cellular components from which they are derived (Behrenfeld et al. 1993). Moreover, for each BWF, inhibition by UV radiation can be accurately assessed only for the set of environmental conditions (e.g., mixing or cloudiness) on the day of sampling (Neale et al. 1998). In addition, due to the rapid attenuation of the shorter wavelengths with depth in this study, the UVB doses diminished 50% at 14 cm below the water surface in the mesocosms. At 1-m depth, planktonic cells received 23% of surface PAR but only 0.6% of surface UVB at 305 nm. Because the water mass in this investigation was continuously mixed and because of the rapid attenuation of the shorter wavelengths with depth, the water column of the mesocosm simulated the whole euphotic zone. Therefore, in addition to nighttime repair processes, planktonic organisms would have been able to photorepair UVB damage in the deeper waters of the mesocosm.

Our results thus show that the decline of the ciliate population under enhanced UVB allows the development of their potential prey, namely heterotrophic flagellates, bacteria, and small phytoplankton, which are subject to lower grazing pressure relative to those under NUVB and UVB-excluded conditions. It is well known that microzooplankton, particularly ciliates, comprise the most important means for transferring production from the lower trophic levels to the higher ones. The small phytoplankton account for up to 70% of the total chlorophyll biomass in oceanic environments (Claustre and Marty 1995) and remain unavailable to direct predation by large zooplankton. Moreover, large zooplankton (copepods) eat planktonic ciliates readily (Wiadnyana and Rassoulzadegan 1989). Due to the severe decline of the ciliate abundances under enhanced UVB (66%), predators that depend on ciliates, such as metazoan zooplankton and fish larvae, would suffer a deficit of 66% of substrate and energy fixed by the bacteria and small phytoplankton. By contrast, the decrease in large phytoplankton numbers, mainly diatoms, suggests that their predators would experience a deficiency of 63% of prey, which can also perturb the transfer of matter and energy from primary producers to herbivorous consumers.

The partitioning of carbon in the food web presented in Table 2 denotes the net reduction in the carbon biomass of ciliates (64%) and diatoms (56%, large phytoplankton) under HUVB treatments relative to NUVB. The reduction in the carbon biomass of ciliates and diatoms was balanced by an increase in the carbon biomass of heterotrophic bacteria (48%), heterotrophic flagellates (126%), and autotrophic flagellates (162%) under HUVB treatments relative to NUVB. This experiment thus demonstrates a considerable shift in the food web structure induced by enhanced UVB in the absence of large zooplankton (e.g., copepods).

Conclusions

Two trophic pathways can be distinguished in the pelagic marine environment, namely herbivorous and microbial food webs (Legendre and Rassoulzadegan 1995). The first one, which links large phytoplankton and zooplankton to fish, is

characteristic of productive oceanic regions. In contrast, the less productive microbial food web comprises small eukaryotic algae, cyanobacteria, heterotrophic bacteria, and protozoa. The results from this experiment suggest that the adverse effect of enhanced UVB on ciliates interrupts the trophic transfer of matter and energy, thereby channeling more carbon into the microbial food web within the pelagic ecosystem. Moreover, although the large zooplankton were removed in this study, the reduced number of large phytoplankton (i.e., diatoms) under enhanced UVB could hinder the development of the herbivorous food web. The changes in the community structure (from large to small organisms) and probably in the dynamics of the pelagic food web under enhanced UVB have broad implications for the pelagic environment and could induce the trapping of carbon in small organisms. To elucidate responses of the whole pelagic food web to enhanced UVB, more investigations that include higher trophic levels, notably large zooplankton, and carbon flux measurements are necessary.

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