

Effects of UVB radiation on a marine microphytobenthic community growing on a sand-substratum under different nutrient conditions

Angela Wulff¹

Marine Botany, Botanical Institute, Göteborg University, P.O. Box 461, SE 405 30 Göteborg, Sweden

Sten-Åke Wängberg

Plant Physiology, Botanical Institute, Göteborg University, P.O. Box 461, SE 405 30 Göteborg, Sweden

Kristina Sundbäck, and Claes Nilsson

Marine Botany, Botanical Institute, Göteborg University, P.O. Box 461, SE 405 30 Göteborg, Sweden

Graham J. C. Underwood

Department of Biological Sciences, University of Essex, Colchester, CO4 3SQ, U.K.

Abstract

The effect of ultraviolet-B radiation (UVBR) on a microphytobenthic community, dominated by diatoms, was studied under different nutrient conditions in a 9 day outdoor experiment on the Swedish west coast. The microalgal assemblage was isolated from the sediment and resettled onto acid-cleaned sand placed in petridishes. The experimental treatments were: “ambient” or “enhanced” UVBR with no nutrient addition, “ambient” or “enhanced” UVBR with nutrient addition (N, P, Si). Enhanced UVBR (+15%) was provided by a computer-controlled system. Primary productivity, carbon allocation, chlorophyll *a* (Chl *a*) concentrations, composition of algae and pigments and intra- and extracellular carbohydrate fractions were measured. Most UVBR effects were seen in treatments without nutrient addition. Exposure to enhanced UVBR resulted in statistically significant decreases in primary productivity, Chl *a* and in the biomass of *Haslea ostrearia* and *Nitzschia* spp. The relative amount of carbon allocated to proteins increased when exposed to enhanced UVBR. The effect of enhanced UVBR on microalgae subjected to nutrient addition was less pronounced, and observed for Chl *a*, algal intracellular storage of carbohydrates (glucans) and concentration of extracellular “colloidal” carbohydrates. Enhanced UVBR + nutrients resulted in a significantly greater ratio between glucan and total carbohydrates and a decreased concentration of colloidal carbohydrates compared to the ambient UVBR + nutrients treatment. These results indicate that availability of inorganic nutrients acts to mitigate the negative effects of UVBR on microphytobenthic communities and that UVBR acts as a selective force during early growth and succession.

Marine shallow-water sediments are widely distributed habitats in both tidal and nontidal areas. The microbenthic communities often serve a crucial ecological function by forming the basis of the food web in these habitats, which provide nursery grounds for fish and fish prey (Mallin et al. 1992). Furthermore, benthic microalgae have been shown to increase the sediment erosion threshold through their production of extracellular polymeric substances (EPS) (Smith and Underwood 1998). As the water column in these areas is often very low (<1 m), ultraviolet-B radiation (UVBR;

280–320 nm) may well reach detrimental levels at the sediment surface, and the potential for UVBR to penetrate into the sediment, and through scattering even exceed the incoming radiation, has been shown (Garcia-Pichel and Bebout 1996). Ambient UVBR has been shown to affect benthic marine microalgal communities through a reduction in primary productivity and altered carbon allocation (Odmark et al. 1998; Wulff et al. 1999), and by changing species composition (Santas et al. 1997). Moreover, elevated UVBR affects the vertical migration and positioning of epipelagic cells in subtidal diatom films, while reducing concentrations of EPS (Underwood et al. 1999).

Results from previous studies on planktonic cultures suggest the importance of available inorganic nutrients to reduce the negative effects of UVBR (Cullen and Lesser 1991). It has been shown that microbenthic communities can be limited by nutrients (Nilsson et al. 1991), particularly in sandy sediments where nutrient concentrations are generally lower than in fine grained sediments.

Aiming at an ecological understanding of the effect of UVBR on microbenthos, several previous experiments have been conducted on intact sediment communities. These experiments, on time scales varying from a few days to several

¹ Corresponding author (angela.wulff@marbot.gu.se).

Acknowledgments

We thank Kristineberg Marine Research Station for providing excellent working facilities. S. Odmark, R. Oja, and U. Stagell assisted during the fieldwork and U. Stagell did the carbon allocation analyses. Financial support was provided by the Swedish Environmental Protection Agency, the Swedish Natural Science Research Council, and the Swedish Council for Planning and Coordination of Research. Additional support was given by the Marine Research Centre at Göteborg University and the funds of Hierta-Retzius, Wilhelm and Martina Lundgren, Captain Carl Stenholm, and Helge Ax:son Johnson.

months, have revealed statistically significant effects of both ambient and enhanced UVBR (Sundbäck et al. 1997; Odmark et al. 1998; Wulff et al. 1999). The observed effects, however, have not been very clear-cut, particularly regarding structural components, such as biomass and species composition. There may be several explanations for the lack of unambiguous effects. One reason could be the use of intact sediment communities either in situ or in sediment cores. Thus, one explanation, supported by previous experiments (Odmark et al. 1998 and refs therein) could be that well-established microbenthic communities are characterized by an initial “lag phase” in their response to moderate environmental changes. As this “lag phase” often appears long (2–3 weeks), experimental effects, such as flaking of the sediment surface in cores, occur before any treatment effects in structural variables can be observed. Another suggestion is that established microbenthic communities are less sensitive to UVBR compared with communities in an earlier successional phase (Santas et al. 1997), during which UVBR may function as a selective force. Therefore, to simplify the sediment system for the purpose of investigating specifically the response of its algal component, in the present study we isolated the natural algal assemblage onto thin sand-substratum instead of using intact sediment cores. This technique also allowed us to study the response of a growing microalgal community, and the selective pressure of UVBR, on an initially nutrient-free substratum. This strategy mimics the frequent and important processes of both new colonisation of a “clean” sandy substratum, as well as recolonisation after disturbances.

For ecologically relevant studies of UVBR effects, it is crucial that the spectral composition of the light is realistic; for example, DNA repair mechanisms after UVBR damage are dependent on the ratios between PAR, UVAR, and UVBR (Karentz et al. 1991). A shortcoming in the majority of previous UVBR experiments, on both planktonic and benthic microalgal communities, has been the use of fixed levels of enhanced UVBR for a few hours a day. This implies that a realistic ratio between PAR, UVAR, and UVBR has not been retained. To overcome this problem in the present experiment, we let the UVBR-enhancement follow the natural variation in the ambient UVBR.

The aim of the study was to investigate the effects of UVBR in addition to nutrient limitation on a microphytobenthic community in early succession. The hypotheses to be tested were: (1) A microphytobenthic community in early succession is affected by a realistic enhancement (ca. 15%) of UVBR. (2) The sensitivity of the microphytobenthic community to UVBR-exposure is dependent on nutrient availability. Natural microalgal communities isolated onto initially nutrient-free sand-substratum were incubated outdoors. Two rate variables (primary productivity and carbon allocation) and five state variables (biomass and composition of algae, Chl *a*, composition of algal pigments and carbohydrate fractions) were considered.

Material and methods

Study site and experimental setup—The study was carried out in June 97 at Kristineberg Marine Research Station on

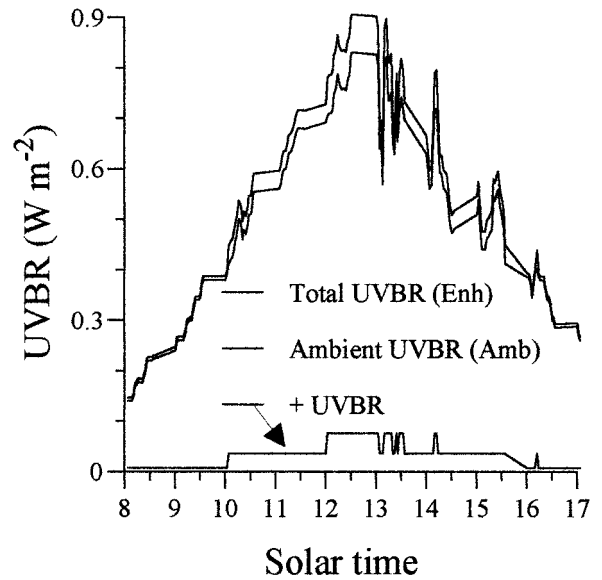


Fig. 1. One example from 13 June 97 showing how the computer-modulated UVBR enhancement worked. Radiation levels in the Amb and Enh treatments are shown together with the additions from the UVB fluorescent tubes.

the Swedish west coast (58°15'N, 11°27'E), an area with a maximum tidal amplitude of 20–30 cm. The natural microalgal assemblage of a sandy sediment from a shallow (ca. 0.5 m) bay was isolated (see Nilsson and Sundbäck 1991) and left to settle overnight onto a 5 mm layer of acid-cleaned quartz sand placed in 36 petridishes (Nunc 90 × 20 mm). On the next day, the petridishes were transferred to the experimental aquaria containing 0.2 μm filtered surface seawater. The aquaria (4.3 liters) were made of UVBR-transparent Plexiglas (GSO 2458 UV, 3 mm). The experiment was run in an outdoor system consisting of 24 aquaria (cf. Odmark et al. 1998) with one petridish in each. The aquaria were spread out in a basin and cooled by a surrounding flow of seawater. The water temperature in the aquaria varied between 18 and 21°C and differed less than 0.5°C between the aquaria. Every morning, ≈ 0800, 1 liter of the water in the aquaria was replaced by new 0.2 μm filtered surface seawater with either ambient or enriched levels of nutrients. Four treatments were established: ambient UVBR with no nutrient addition (Amb), enhanced UVBR with no nutrient addition (Enh), ambient UVBR with nutrient addition (Amb + Nutr), and enhanced UVBR with nutrient addition (Enh + Nutr). The water in the +Nutr treatments was enriched to a final concentration of 38 μM NO₃, 40 μM Si and 3.6 μM PO₄. Background levels of these nutrients were approximately 5 μM NO₃, 8 μM Si and 0.6 μM PO₄. The background N:P molar ratio suggests a N-limitation at the beginning of the experiment. The experiment ran for 9 days (5 June to 14 June 97). On day 0 (5 June), before treatments started, 12 petridishes were sampled for initial values.

The UVBR treatment—Enhanced UVBR was provided using a computer-controlled system (LabView) linked to a UVBR sensor (International Light 1400A photometer, detec-

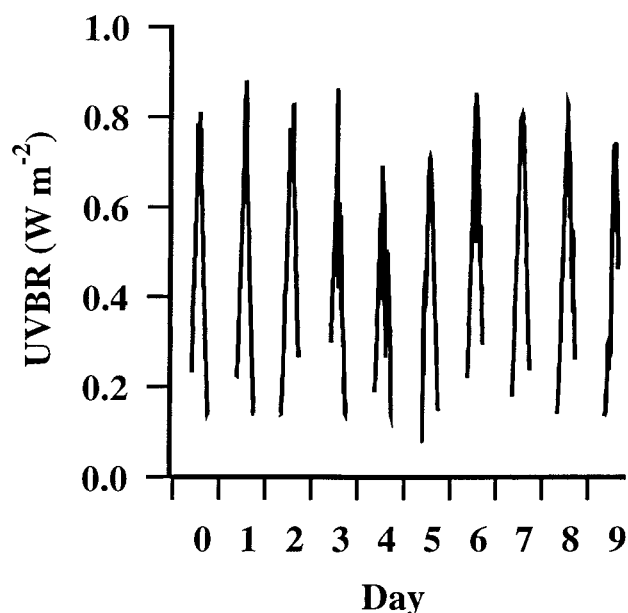


Fig. 2. Ambient UVBR measured between 0800 and 1700 h (solar time) during the experiment (5 June to 14 June 1997).

tor type SUL 240) that continuously measured the natural ambient UVBR. The enhanced level ($\approx 15\%$) given by the UVBR fluorescent tubes (Philips TL4W/12) varied throughout the daily exposure, mirroring the natural UVBR radiation curve. Modulation was not fully continuous, but had five trigger values (0.1, 0.4, 0.7, 1.0, and 1.3 W m^{-2}) and was run between 0800 and 1700 (solar time). Fig. 1. gives an example of the enhancement. The spectrum of the UVBR fluorescent tubes was measured with a spectroradiometer (Optronics Instrument OL 754) (Odmark et al. 1998). Wavelengths < 290 nm (UVCR) were screened off by daily replaced cellulose diacetate film (Erik S. Ekman AB, Stockholm). Empty lamp units ensured equal shading effects of the ambient treatments. When weighted with a Biological Weighting Function determined on the marine diatom *Phaeodactylum tricorutum* (Cullen et al. 1992), the enhancement level was $\approx 23\%$. This corresponds to an ozone reduction of 11–15%, using the assumed Radiation Amplification Factor defined as a 1.5–2% increase of the UVBR per % reduction in the ozone layer.

Light measurements—The weather was sunny during the experimental period, with maximum PAR intensities of ca. 1,800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Downwelling UVB irradiance was measured in air between 0800 and 1700 using an IL 1400A photometer equipped with a cosine corrected sensor (IL SUL 240) (Fig. 2). The stratospheric ozone layer during the experiment was on average 343 DU (± 4 S.D.) (TOMS-data).

Sampling—Initial samples were taken on day 0 and thereafter on two more occasions, day 6, and day 9. On each sampling occasion, three petridishes (= aquaria) were sampled from each treatment and then discarded. From each pe-

tridish, subsamples of the upper 2 mm sediment layer were taken using cut-off plastic syringes.

Microalgae—Two samples (i.d. 4.7 mm) from each petridish were pooled, diluted, and counted in an epifluorescence microscope. As the algal community was dominated by unattached (epipellic) life forms, no ultrasonication was applied to detach cells from sand grains. The sample was vigorously shaken by hand for one minute and after ca. 5 s (to allow sand grains to settle), several individual subsamples (40 μl) of the algal suspension were pipetted onto a microscope slide and fluorescing cells were counted. When possible, cells were identified to species level, and otherwise allocated to size and shape groups. Cell volumes were measured, calculated and converted to carbon using a factor of $0.089 \times 10^{-12} \text{ g C } \mu\text{m}^3$.

The dimensions of ca 30 cells of the commonest species were measured from each of the initial and the final samples in order to test whether cell dimensions were significantly affected by the treatments. The species measured were *Entomoneis* cf. *pseudoduplex* Osada and Kobayasi, *Haslea ostrearia* (Gaillon) Simonsen, and three size classes of *Cylindrotheca* cf. *closterium* (Ehrenberg) Reimann and Lewin.

Algal pigments—Two samples (i.d. 4.7 mm) were taken from each core, pooled, and stored in liquid nitrogen (-196°C). For extraction and sample preparation see Sundbäck et al. (1997). Pigments were analysed by high performance liquid chromatography according to Wright and Jeffrey (1997). Chl *a* was quantified as mg L^{-1} according to Wright and Jeffrey (1997) and converted to mg m^{-2} . The composition of other pigments were expressed as ratios to Chl *a*.

Carbohydrate fractions—Samples (i.d. 8.7 mm) were frozen, lyophilised, and stored at -20°C (Underwood et al. 1995). Total sediment carbohydrate concentrations were measured by adding 2 ml of distilled water to 40 g dry sediment, followed by 1 ml of 5% aqueous phenol (wt/v) and 5 ml of concentrated H_2SO_4 (see Underwood et al. 1995). Extracellular “colloidal” carbohydrates were separated from the microbenthic community by extraction with saline water (salinity 21) followed by centrifugation. This colloidal carbohydrate fraction was further separated into polysaccharides (EPS) and low molecular weight compounds by precipitation in cold 70% (final volume) ethanol (Underwood et al. 1995). Intracellular storage carbohydrates (glucans) were extracted from the sediment pellet with 1 h incubation in 0.1 N H_2SO_4 (Smith and Underwood 1998). The phenol-sulphuric acid assay (Dubois et al. 1956) was used to determine carbohydrate concentrations, with D-glucose dissolved in water or 0.1 N H_2SO_4 used as standards for colloidal/EPS and glucan fractions, respectively.

Primary productivity—Four samples (i.d. 4.7 mm) from each petridish were pooled by two into High-Performance glass scintillation vials (Packard; for UV transmittance see fig. 1 in Wulff et al. 1999). To each vial, 2 $\mu\text{Ci } ^{14}\text{C}$ -bicarbonate was added together with 10 ml GF/F filtered seawater. The samples were incubated for ≈ 1 h under their

Table 1. Summary of variables showing significant UVBR effects. (+) show a significant positive effect and (–) a significant negative effect of enhanced UVBR (ANOVA, $P < 0.05$).

	Amb vs Enh	Amb+Nutr vs. Enh+Nutr
Species composition	(–)Day 9	No effect
Chlorophyll <i>a</i>	(–)Day 9	(–)Day 9
Glucan % of total carbohydrates	No effect	(+)Day 9
Colloidal carbohydrates	No effect	(–)Day 9
Primary productivity	(–)Day 6	No effect
Carbon allocation, % to proteins	(+)Day 6, 9	No effect

respective treatment (Amb or Enh). On day 0, the incubations started at 1500 and on days 6 and 9 at 1300. After incubation, the samples were filtered onto 0.4 μm polycarbonate membrane-filters (Poretics, 25 mm), and transferred to scintillation vials. For further processing and calculations see Nilsson et al. (1991). Calculations were corrected for dark fixation.

Carbon allocation—Four samples (i.d. 4.7 mm) were taken from each petridish and pooled by two into High-Performance glass scintillation vials (Packard) together with 10 ml filtered seawater (Whatman GF/F). To each sample, 5 μCi ^{14}C -bicarbonate were added and the samples were incubated under ambient radiation for 2 h, starting at 1500 on day 0, and at 1300 on days 6 and 9. After incubation, the samples were filtered onto glassfibre filters (Whatman GF/F). The filters were washed with 2 ml unlabelled seawater and placed in vials with 0.8 ml distilled water, and stored in -20°C . Fractionation of the organic carbon into polysaccharides, proteins, low molecular weight compounds (LMW) and lipids was done (Li et al. 1980) with a further separation into polar and neutral lipids using a silica acid column (see Sundbäck et al. 1997).

Statistical analyses—The data were analysed by 1-factor ANOVA for each sampling date. Cochran's test was used to check for heterogeneous variances, and when heterogeneous, transformed according to Underwood (1997). Post-hoc analyses were made by the Student-Newman-Keul test. P -values < 0.05 were accepted for significant differences.

Results

Statistically significant UVBR treatment effects are summarized in Table 1. The majority of effects were observed in treatments with no nutrient addition.

Microalgae—During the experiment, the microalgal biomass varied between 60 and 3,500 mg C m^{-2} (ca. $30\text{--}340 \times 10^4$ cells cm^{-2}), being dominated by diatoms. The communities underwent a general succession where the proportion of diatoms $< 20 \mu\text{m}$ decreased, while larger epipellic species increased. The biomass was significantly stimulated by nutrient addition, with the highest values being found on day 9 in the two enriched treatments (Fig. 3). Most of this increase was due to the growth of two diatom species, *En-*

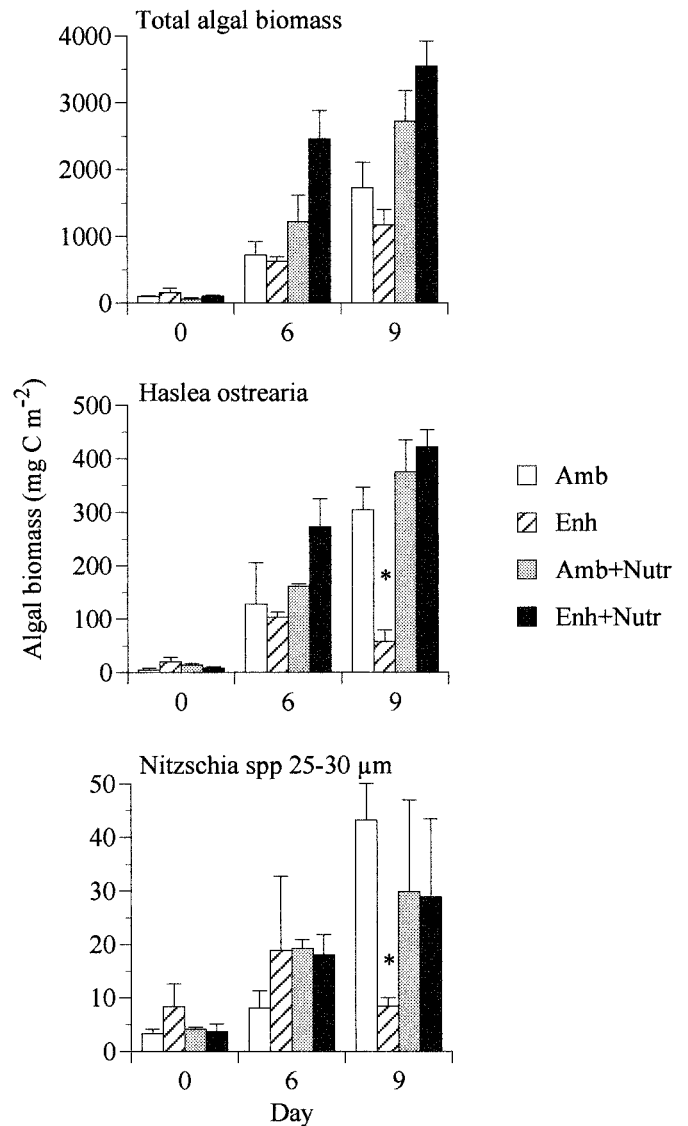


Fig. 3. Total microalgal biomass and biomass of the diatom species *H. ostrearia* and *Nitzschia* spp. in sediment from the four experimental treatments. Bars show mean values for three replicate cores + SE. (*) indicate significant UVBR effects.

tomoneis cf. *Pseudoduplex*, that constituted 60–80% of the biomass on the final day, and *Haslea ostrearia*. To some extent also *Cylindrotheca* spp. and *Nitzschia* spp. within the size range 15–30 μm contributed to the biomass increase.

The UVBR treatment had no significant effect on either the total biomass or total cell numbers, although on day 9, the biomass appeared lowest in the Enh treatment (Fig. 3). However, when individual algal species or groups were considered, significant differences between Amb and Enh treatments were found, but not between the two nutrient-enriched treatments. Among the commoner species, both the biomass and relative abundance of *Haslea ostrearia* and *Nitzschia* sp. 25–30 μm were significantly lower on day 9 in the Enh treatment (Fig. 3). The cell size of the two commonest species (*Entomoneis* cf. *pseudoduplex* and *H. ostrearia*) was not significantly affected by treatments.

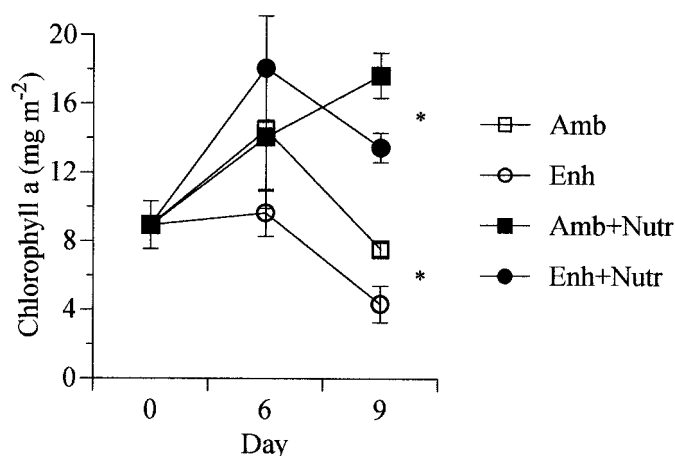


Fig. 4. Chlorophyll *a* concentration in sediment from the four experimental treatments. Symbols show mean values for *n* replicate cores + SE; *n* = 12 for day 0, and *n* = 3 for days 6 and 9. (*) indicate significant UVBR effects.

Photosynthetic pigments—For Chl *a*, there was a significant UVBR effect in both nonenriched and enriched treatments on day 9 (Fig. 4). The Chl *a* values varied between 2.5–22.4 mg m⁻², the lowest value being found for the Enh treatment on day 9 and the highest for the Amb + Nutr treatment on day 6. The Chl *a* values were initially very low, increased in 5 days but decreased to day 9 in all treatments except Amb + Nutr. For pigment ratios to Chl *a*, no significant UVBR effects were found. For the degradation/intermediate pigment chlorophyllide *a*, the enhanced treatments, both enriched and non-enriched, showed higher values throughout the experiment (Table 2). No pheophytin *a* was present. The ratios of the major diatom pigments (Chl *c*₁*c*₂, fucoxanthin, diadinoxanthin together with diatoxanthin and betacarotene) to Chl *a*, were significantly stimulated by the nutrient addition on days 6 and 9 (Table 2). The dinoflagellate pigment peridinin, as well as zeaxanthin (marker pigment for cyanobacteria in the absence of green algae), both showed a very patchy distribution throughout the experiment and no significant treatment effects were found.

Carbohydrate fractions—In all four treatments, the concentrations of total carbohydrate, colloidal carbohydrates (Table 2), EPS and glucan (not shown) increased over the experimental period, particularly between day 0 and day 6. There were significantly higher concentrations of total carbohydrates in the nutrient enriched treatments and a significant UVBR effect was observed as a lower concentration of colloidal carbohydrates in the Enh + Nutr compared to Amb + Nutr on day 9 (Fig. 5). However, a tendency of lower total (Enh + Nutr, day 9) and colloidal (Enh, day 6) carbohydrate concentrations was observed. Moreover, there was a significant UVBR effect on the ratio of glucan to total carbohydrate content, with higher glucan: total carbohydrate ratios in the Enh + Nutr compared with Amb + Nutr treatment (day 9) (Fig. 5).

Primary productivity—Primary productivity varied between 17 and 221 mg C m⁻² h⁻¹, with highest values found

in the nutrient-enriched treatments (Fig. 6). On Day 6, primary productivity was significantly lower in Enh compared with Amb. On day 9, no significant UVBR effects were seen, although Enh appeared lower than Amb. On both days 6 and 9, the primary productivity was stimulated by the nutrient addition (Fig. 6).

Carbon allocation—Significant treatment effects were found for the percentages of total carbon allocated to proteins on day 6 and day 9 (Fig. 7). On both days, the percentage carbon allocated to proteins was higher in the Enh treatment than in any of the other treatments. If the actual amounts of carbon allocated to each fraction are compared instead of percentage values, less carbon was allocated to all fractions in Enh than the other treatments on day 6. On day 9, significantly more carbon was allocated to all fractions (ratios to total carbon), except neutral lipids, in the two nutrient-enriched treatments than in the two nonenriched treatments (Table 2).

Discussion

Our results support the hypothesis that a moderate increase in UVBR can have an impact on microphytobenthic communities in early succession. The effects on primary productivity, carbon allocation, and to some extent carbohydrate fractions corroborate results from previous work on intact microbenthic communities (Wulff et al. 1999 and refs therein), while significant impacts on structural variables, e.g., species composition and Chl *a*, were not generally observed in these previous experiments on undisturbed sediment. The fact that most effects were observed for the UVBR-treatment with no nutrient addition supports the hypothesis that nutrient limitation increases the sensitivity of the microphytobenthic community to UVB-exposure.

Methodology—The use of a ‘colonisation’ method, i.e., starting the experiment with a newly-settled natural microphytobenthic assemblage, allowed us to follow the development of a community while still in a phase of growth and succession. We also managed to avoid unwanted experimental effects, such as a ‘lag phase’ and flaking of the sediment surface. Furthermore, our results show that our ambition to create a nutrient-limited habitat was successful, since the microalgal community clearly responded to nutrient additions, reflected as increased algal biomass, primary productivity and concentrations of all carbohydrate fractions.

Although isolated from sandy sediment, the developing microalgal assemblage was not a pure epipsammic community, but was dominated by epipellic (motile) taxa. This can be attributed to the isolation method and the experimental set up which favored nonattached motile life forms (cf. Nilsson and Sundbäck 1991). However, all microalgal species encountered are found on various types of sediment surfaces in the area. The species-specific effects observed during the succession of the diatom community, agree with previous observations on periphyton communities colonising artificial substratum (Bothwell et al. 1993; Santas et al. 1997).

Table 2. Different fractions of carbon allocation (ratios to total carbon), pigment ratios to Chl *a*, total carbohydrate concentrations (μg glucose equivalents cm^{-2}), and % EPS to colloidal carbohydrates. All values shown \pm SE.

Variable	Day	Amb	Enh	Amb+Nutr	Enh+Nutr	<i>n</i>
LMW	0	0.32 \pm 0.01				12
	6	0.30 \pm 0.02	0.26 \pm 0.00	0.29 \pm 0.01	0.29 \pm 0.01	3
	9	0.29 \pm 0.02	0.25 \pm 0.02	0.33 \pm 0.02	0.33 \pm 0.02	3
Polysaccharides	0	0.22 \pm 0.01				12
	6	0.19 \pm 0.01	0.20 \pm 0.01	0.24 \pm 0.03	0.23 \pm 0.01	3
	9	0.23 \pm 0.00	0.21 \pm 0.01	0.25 \pm 0.01	0.25 \pm 0.03	3
Polar lipids	0	0.11 \pm 0.00				12
	6	0.11 \pm 0.01	0.10 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.01	3
	9	0.09 \pm 0.00	0.09 \pm 0.00	0.09 \pm 0.00	0.09 \pm 0.01	3
Neutral lipids	0	0.03 \pm 0.00				12
	6	0.13 \pm 0.00	0.12 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.01	3
	9	0.10 \pm 0.01	0.09 \pm 0.02	0.07 \pm 0.00	0.09 \pm 0.02	3
Polar lipids:Neutral lipids	0	4.90 \pm 0.91				12
	6	0.83 \pm 0.12	0.90 \pm 0.18	1.02 \pm 0.20	1.01 \pm 0.12	3
	9	1.02 \pm 0.12	1.16 \pm 0.29	1.29 \pm 0.09	0.99 \pm 0.17	3
Chlorophyllide <i>a</i>	0	0.01 \pm 0.00				12
	6	0.02 \pm 0.02	0.08 \pm 0.04	0.31 \pm 0.07	0.38 \pm 0.07	3
	9	0.08 \pm 0.02	0.14 \pm 0.08	0.18 \pm 0.00	0.36 \pm 0.11	3
Chl <i>c</i> ₁ + <i>c</i> ₂	0	0.33 \pm 0.02				12
	6	0.35 \pm 0.02	0.35 \pm 0.02	0.46 \pm 0.03	0.50 \pm 0.04	3
	9	0.30 \pm 0.00	0.28 \pm 0.05	0.36 \pm 0.01	0.41 \pm 0.04	3
Fucoxanthin	0	0.69 \pm 0.01				12
	6	0.78 \pm 0.02	0.78 \pm 0.05	1.14 \pm 0.12	1.14 \pm 0.07	3
	9	0.84 \pm 0.06	0.80 \pm 0.08	1.05 \pm 0.02	1.24 \pm 0.11	3
Diadinoxanthin+diatox.	0	0.19 \pm 0.01				12
	6	0.59 \pm 0.03	0.63 \pm 0.01	0.84 \pm 0.09	0.86 \pm 0.05	3
	9	0.69 \pm 0.00	0.77 \pm 0.05	0.89 \pm 0.00	0.98 \pm 0.08	3
Betacarotene	0	0.00 \pm 0.00				12
	6	0.08 \pm 0.01	0.09 \pm 0.01	0.12 \pm 0.03	0.13 \pm 0.01	3
	9	0.11 \pm 0.00	0.08 \pm 0.04	0.12 \pm 0.00	0.14 \pm 0.01	3
Total carbohydrates	0	41.81 \pm 107.2	47.58 \pm 37.7	66.18 \pm 60.8	38.2 \pm 25.3	3
	6	272.7 \pm 35.0	187.4 \pm 50.7	490.2 \pm 65.0	389.7 \pm 120	3
	9	145.1 \pm 23.5	196.8 \pm 19.1	442.0 \pm 83.0	276.5 \pm 30.8	3
% EPS of coll. carbohydr.	6	60.1 \pm 28.1	52.5 \pm 17.2	37.1 \pm 13.2	58.7 \pm 14.0	3
	9	71.2 \pm 27.1	53.3 \pm 47.9	67.4 \pm 26.9	69.4 \pm 11.3	3

UVBR treatment effects—Interestingly, the clearest UVBR effect on species level in our experiment was the decreased abundance of one of the commonest species, *H. ostrearia*, a taxon known to adapt well to high PAR levels (Mouget et al. 1999). *H. ostrearia* is known for its blue water-soluble pigment marennine, which functions as a light-shield in the long-wavelength region (Schubert et al. 1995), but apparently has no shielding function for UVBR. Other affected taxa belonged to the genus *Nitzschia*. Santas et al. (1997) and Karentz et al. (1991) also found negative UVBR effects on some *Nitzschia* species which might be explained by differences in morphology (cell shape, chloroplasts), as well as habitat (Karentz et al. 1991). Moreover, the genus *Nitzschia* has also been shown to be adversely affected by UVBR through a reduced motility (Moroz et al. 1999).

Increasing cell size has been pointed out as a typical effect

of UVBR exposure, both on individual species level, as cell division is hampered (Behrenfeld et al. 1992; Wångberg et al. 1997), and on community level, as species with larger cell-size could be favored (Bothwell et al. 1993). We found no evidence for increasing cell size because of UVBR exposure, neither for the individual commonest species nor for the entire community.

Significantly lower Chl *a* values were found for the Enh and Enh + Nutr and UVBR effects on biomass in terms of Chl *a* have been found in similar experiments where microalgae have been under primary succession (Bothwell et al. 1993). In the present study, we found a tendency of an elevated ratio of betacarotene to Chl *a* in the UVBR treatments, and such a UVBR response of diatoms was also found in a previous study (Underwood et al. 1999). We did not measure the amount of UV-absorbing compounds, since

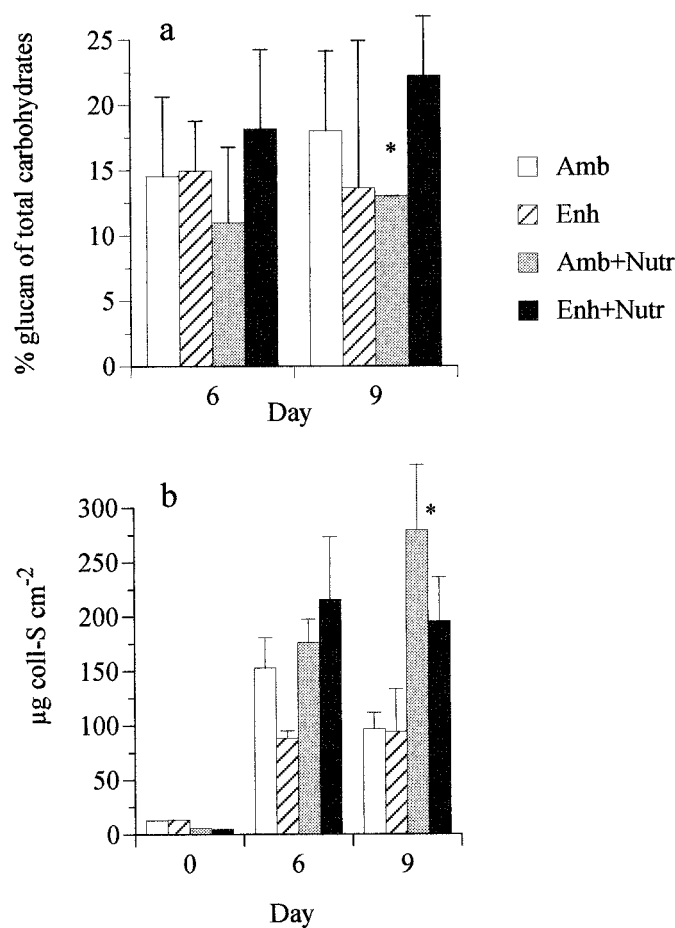


Fig. 5. (a) Percentage of glucan of total carbohydrates and (b) concentration of colloidal carbohydrates in sediment from the four experimental treatments. Bars show mean values for three replicate cores + SE. (*) indicate significant UVBR effects.

previous studies have suggested that benthic diatoms do not rely on these compounds for protection against UV/excessive light (Peletier et al. 1996; Wulff et al. 1999).

The present study confirms previously found UVBR effects on microphytobenthic primary productivity (Sundbäck et al. 1997; Odmark et al. 1998; Wulff et al. 1999), and will not be discussed further. The UVBR treatment effects found for the percentage carbon allocated to proteins and LMW were in this study more pronounced than in our previous experiments on microphytobenthos (Odmark et al. 1998; Wulff et al. 1999). The larger percentage of C allocated to proteins in the Enh treatment indicates that a larger proportion of the carbon was used for growth when carbon dioxide fixation decreased. Microalgal cells tend to maintain protein synthesis rather than storage products synthesis under adverse environmental conditions (Marañón et al. 1995). The difference in total carbon dioxide fixation between treatments during the allocation incubations agreed with the effects found for primary productivity, despite the longer incubation time and the fact that all allocation incubations were done under ambient radiation. The observed UVBR effects on primary productivity and carbon allocation are thus consequences of differences in the long-term succes-

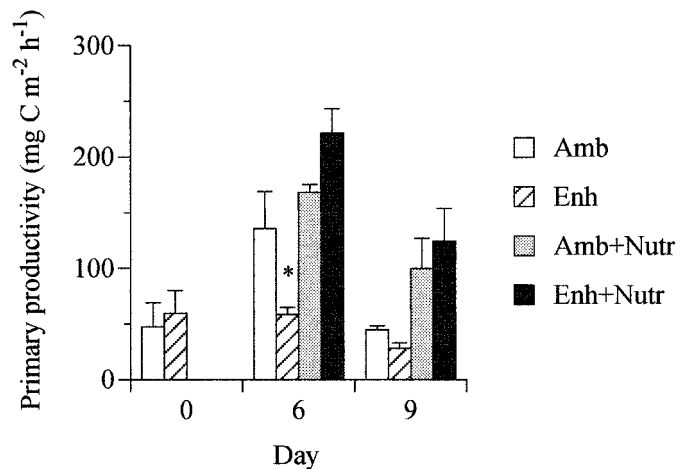


Fig. 6. Primary productivity in sediment from the four experimental treatments incubated under their different treatments, respectively. Bars show mean values for n replicate cores + SE; $n = 6$ for day 0, and $n = 3$ for days 6 and 9. (*) indicate significant UVBR effects.

sion/adaptation between treatments. Also the treatment effects on intracellular carbohydrates agree with our previous findings, i.e., increased proportions of glucan under elevated UVBR (Underwood et al. 1999). Furthermore, colloidal carbohydrate concentrations have been shown to be positively related to primary productivity (Smith and Underwood 1998), an effect at least partly corroborated in the present experiment where both primary productivity and concentrations of colloidal carbohydrates were lower in the Enh treatment.

Light and nutrient effects not related to UVBR—The initial decrease (day 0 to day 6) in Chl *a* could be explained by the cells originating from a 1 cm thick sediment layer were suddenly exposed to light conditions at the sediment

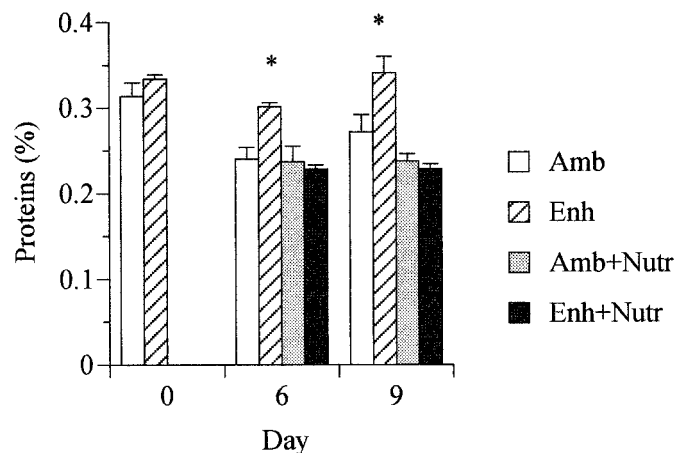


Fig. 7. Carbon allocation to proteins (percentages of total incorporated carbon) of sediment from the four experimental treatments incubated under ambient radiation. Bars show mean values for n replicate cores + SE; $n = 6$ for day 0, and $n = 3$ for days 6 and 9. (*) indicate significant UVBR effects.

surface. The observation that the Chl *a* per cell decreased by a factor of 2 in six days and by a factor of 4 in nine days may reflect this change in the light environment. Chl *a* per cell has been reported to decrease by a factor of 5 when nutrient-replete cultures have been shifted from an irradiance of 10 to 1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Goericke and Montoya 1998). Furthermore, the new substratum, consisting of white sand with high light reflectance, would have offered little protection from high irradiance, even if the cells migrated down into the sediment (Kühl et al. 1994). Between day 6 and day 9, one can assume that the cells had adapted to the high light conditions and further decreases in Chl *a* per cell is more likely to be due to nutrient-deplete conditions. The fact that the addition of inorganic nutrients did not match the increase in algal carbon (assuming a Redfield C/N/P ratio) suggests that the nutrient-enriched treatments could in fact have also become nutrient limited towards the end of the experiment. This is further supported by the differences in UVBR effects observed in Chl *a* and glucan:total carbohydrate ratios between the nutrient-enriched treatments on day 9, and by the algal carbon/Chl *a* ratios (*see below*). A more pronounced nutrient limitation in the nonenriched treatments is supported by the significant differences on day 9 in primary productivity, carbon allocation and increased percentage of polymeric EPS present between nutrient-enriched and nonenriched treatments, but not between the UVBR treatments.

The algal carbon to Chl *a* ratios increased from about 10–250, with the highest values found on day 9. Although our carbon values were determined by converting biovolumes to carbon using fixed factors, changed ratios still give valuable information. An increasing C/Chl *a* ratio could indicate a decreasing growth rate due to nutrient limitation (de Jonge 1980). As we found increasing ratios in the nutrient-enriched, as well as in the nonenriched treatments, decreasing Chl *a* values through light adaptation, in combination with nutrient limitation in all treatments could have changed the ratios. When algal cells are nitrogen limited, the available nitrogen is used for building structural components other than photosynthetic or metabolic apparatus, implying an increased biomass at the expense of decreased Chl *a*.

The pigments diadinoxanthin and diatoxanthin have been suggested to protect against excessive light through a xanthophyll cycle similar to the violaxanthin–antheraxanthin–zeaxanthin cycle found in higher plants (Demmig-Adams and Adams 1996). The increase of the diadinoxanthin plus diatoxanthin ratios to Chl *a* in all treatments supports the idea that the diatoms as an assemblage were stressed by the strong light in general, and not only specifically by UVBR. The amount of diadinoxanthin in the diatom *H. ostrearia* has indeed previously been reported to increase in high irradiance ($\approx 750 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Mouget et al. 1999). On the other hand, an increase in the relative amount of diadinoxanthin plus diatoxanthin as a response to an increased UVBR has been found in the diatom *Cyclotella* sp., but this response seems to be species-specific since no such response was detected in the diatoms *Nitzschia* sp. and *Thalassiosira nordenskioldii* (Buma et al. 1996). In general, the ratios of accessory pigments to Chl *a* increased with nutrient

addition and might indicate a nutrient limitation for the non-enriched treatments.

Conclusions

The microphytobenthic community was clearly affected by the enhanced UVBR (1, 2, 3, 4) and in addition some general light effects were observed (5):

(1) Sediment-living microalgal communities during early growth and succession appear to be more sensitive to enhanced UVBR than already established communities; (2) Both functional and structural variables of the community are affected by UVBR; and thus, (3) UVBR acts as a selective force during early growth and succession; (4) Nutrient limitation increases the sensitivity of the sediment-living microalgal communities to UVBR exposure; (5) Chl *a* concentrations decrease and carbon to Chl *a* ratios can increase substantially due to high irradiance and, furthermore, this effect is more pronounced in a substratum with high light reflectance.

References

- BEHRENFELD, M. J., J. T. HARDY, AND H. LEE II. 1992. Chronic effects of ultraviolet-B radiation on growth and cell volume of *Phaeodactylum tricorutum* (Bacillariophyceae). *J. Phycol.* **28**: 757–760.
- BOTHWELL M. L., D. SHERBOT, A. C. ROBERGE, AND R. J. DALEY. 1993. Influence of natural ultraviolet radiation on lotic periphytic diatom community growth, biomass accrual, and species composition: Short-term versus long-term effects. *J. Phycol.* **29**: 24–35.
- BUMA, A. G. J., H. J. ZEMMELINK, K. SJOLLEMA, AND W. W. C. GIESKES. 1996. UVB radiation modifies protein and photosynthetic pigment content, volume and ultrastructure of marine diatoms. *Mar. Ecol. Prog. Ser.* **142**: 47–54.
- CULLEN, J. J., AND M. P. LESSER. 1991. Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: Results for a marine diatom. *Mar. Biol.* **111**: 183–190.
- , P. J. NEALE, AND M. P. LESSER. 1992. Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. *Science* **258**: 646–650.
- DE JONGE, V. N. 1980. Fluctuations in the organic carbon to chlorophyll *a* ratios for estuarine benthic diatom populations. *Mar. Ecol. Prog. Ser.* **2**: 345–353.
- DEMMIG-ADAMS, B., AND W. W. ADAMS. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trend. Plant Sci.* **1**: 21–26.
- DUBOIS, M., K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350–356.
- GARCIA-PICHEL, F., AND B. M. BEBOUT. 1996. Penetration of ultraviolet radiation into shallow water sediments: High exposure for photosynthetic communities. *Mar. Ecol. Prog. Ser.* **131**: 257–262.
- GOERICKE, R., AND J. P. MONTOYA. 1998. Estimating the contribution of microalgal taxa to chlorophyll *a* in the field-variations of pigment ratios under nutrient- and light-limited growths. *Mar. Ecol. Prog. Ser.* **169**: 97–112.
- KARENTZ, D. K., J. E. CLEAVER, AND D. L. MITCHELL. 1991. Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *J. Phycol.* **27**: 326–341.

- KÜHL, K., C. LASSEN, AND B. B. JØRGENSEN. 1994. Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes. *Mar. Ecol. Prog. Ser.* **105**: 139–148.
- LI, W. K. W., H. E. GLOVER, AND I. MORRIS. 1980. Physiology of carbon photoassimilation by *Oscillatoria thiebautii* in the Caribbean sea. *Limnol. Oceanogr.* **25**: 447–456.
- MALLIN, M. A., J. M. BURKHOLDER, AND M. J. SULLIVAN. 1992. Contributions of benthic microalgae to coastal fishery yield. *Trans. Am. Fish. Soc.* **121**: 691–693.
- MARAÑÓN, E., E. FERNÁNDEZ, AND R. ANADÓN. 1995. Patterns of macromolecular synthesis by natural phytoplankton assemblages under changing upwelling regimes: In situ observations and microcosm experiments. *J. Exp. Mar. Biol. Ecol.* **188**: 1–28.
- MOROZ, A. L., J. M. EHRMAN, T. A. CLAIR, R. J. GORDON, AND I. KACZMARSKA. 1999. The impact of ultraviolet-B radiation on the motility of the freshwater epipellic diatom *Nitzschia linearis*. *Global Cha. Biol.* **5**: 191–199.
- MOUGET, J.-L., G. TREMBLIN, A. MOTANT-MANCEAU, M. MORANÇAIS, AND J.-M. ROBERT. 1999. Long-term photoacclimation of *Haslea ostrearia* (Bacillariophyta): Effect of irradiance on growth rates, pigment content and photosynthesis. *Eur. J. Phycol.* **34**: 109–115.
- NILSSON, C., AND K. SUNDBÄCK. 1991. Growth and nutrient uptake studied in sand-agar microphytobenthic communities. *J. Exp. Mar. Biol. Ecol.* **153**: 207–226.
- NILSSON, P., B. JÖNSSON, I. LINDSTRÖM SWANBERG, AND K. SUNDBÄCK. 1991. Response of a marine shallow-water sediment system to an increased load of inorganic nutrients. *Mar. Ecol. Prog. Ser.* **71**: 275–290.
- ODMARK, S., A. WULFF, S.-Å. WÄNGBERG, C. NILSSON, AND K. SUNDBÄCK. 1998. Effects of UVB radiation in a microbenthic community of a marine shallow-water sandy sediment. *Mar. Biol.* **132**: 335–345.
- PELETIER, H., W. W. C. GIESKES, AND A. G. J. BUMA. 1996. Ultraviolet-B radiation resistance of benthic diatoms isolated from tidal flats in the Dutch Wadden Sea. *Mar. Ecol. Prog. Ser.* **135**: 163–168.
- SANTAS, R., C. LIANOU, AND D. DANIELIDIS. 1997. UVB radiation and depth interaction during primary succession of marine diatom assemblages of Greece. *Limnol. Oceanogr.* **42**: 986–991.
- SCHUBERT, H., G. TREMBLIN, J.-M. ROBERT, S. SAGERT, AND Y. RINCÉ. 1995. *In-vivo* fluorescence measurement of photosynthesis of *Haslea ostrearia* Simonsen in relation to marennine content. *Diat. Res.* **10**: 341–349.
- SMITH, D. J., AND G. J. C. UNDERWOOD. 1998. Exopolymer production by intertidal epipellic diatoms. *Limnol. Oceanogr.* **43**: 1578–1591.
- SUNDBÄCK, K., S. ODMARK, A. WULFF, C. NILSSON, AND S.-Å. WÄNGBERG. 1997. Effects of enhanced UVB radiation on a marine benthic diatom mat. *Mar. Biol.* **128**: 171–179.
- UNDERWOOD, A. J. 1997. Experiments in ecology. Cambridge.
- UNDERWOOD, G. J. C., C. NILSSON, K. SUNDBÄCK, AND A. WULFF. 1999. Short-term effects of UVB radiation on chlorophyll fluorescence, biomass, pigments and carbohydrate fractions in a benthic diatom mat. *J. Phycol.* **35**: 656–666.
- , D. M. PATERSON, AND R. J. PARKES. 1995. The measurement of microbial carbohydrate exopolymers from intertidal sediments. *Limnol. Oceanogr.* **40**: 1243–1253.
- WÄNGBERG, S.-Å., A. PERSSON, AND B. KARLSON. 1997. Effects of UV-B radiation on synthesis of mycosporine-like amino acid and growth in *Heterocapsa triquetra* (Dinophyceae). *J. Photochem. Photobiol.* **37**: 141–146.
- WRIGHT, S. W., AND S. W. JEFFREY. 1997. High-resolution HPLC system for chlorophylls and carotenoids of marine phytoplankton. In Jeffrey, Mantoura, and Wright [eds.], *Phytoplankton pigments in oceanography*. UNESCO.
- WULFF, A., C. NILSSON, K. SUNDBÄCK, S.-Å. WÄNGBERG, AND S. ODMARK. 1999. UV radiation effects on microbenthos—a four month field experiment. *Aquat. Microb. Ecol.* **19**: 269–278.

Received: 6 October 1999

Amended: 13 March 2000

Accepted: 21 March 2000