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Ultraviolet-B radiation resistance of benthic diatoms isolated from tidal flats in the Dutch Wadden Sea

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ABSTRACT: Seven species representative of the benthic diatom community of the tidal flats in the Dutch Wadden Sea hardly differed in their sensitivity to ultraviolet-B radiation (UVBR). Some isolates had been cultured in the laboratory for up to 20 yr. Cell numbers of all species increased at a rate similar to unexposed cultures up to a DNA-weighted daily UVBR dose of 3.5 kJ m^{-2} d^{-1} (biologically effective dose, normalized at 300 nm); only at higher UVB irradiance levels did the growth rate become reduced. No clear relationship between mean cell size and UVBR sensitivity was observed. The benthic diatoms that were tested are apparently adapted to the natural, high UVB irradiance incident at tidal flats during spring and summer. Thus, even a sharp UVBR increase resulting from severe stratospheric ozone reduction would hardly affect tidal flat diatom communities by influencing cell division rate. In contrast, growth of representatives of the phytoplankton community was already seriously affected by doses that were 10 times lower. This is in agreement with their natural, low UVBR exposure.

KEY WORDS: Benthic diatoms · UVB sensitivity · Growth

INTRODUCTION

Benthic diatoms form a thin brown layer covering the sediment of tidal flat areas. These algal mats are of great ecological importance since they contribute considerably to primary productivity available to both the benthic and the pelagic community above the flats (Admiraal 1984). Another key function of benthic diatoms is that they stabilize the sediments in shallow seas by excretion of polymeric substances that stick particles together (Grant et al. 1986, Vos et al. 1988, Paterson 1989, Hoagland et al. 1993).

The benthic microalgal community usually consists of a large number of species. Tide and wave action, properties of the sediment or the flood water, and chemical and optical characteristics of the overlaying water column determine growth, production and species composition of the various communities (Admiraal 1984). The effect of ultraviolet-B radiation (UVBR) on benthic diatoms of tidal flat areas has never been investigated properly (Kramer 1990), in spite of the fact that UVBR should be considered as a factor of great ecological significance. Benthic diatoms may be exposed to high levels of UVBR throughout the vegetative season, especially when growing on elevated tidal flats, where they are directly exposed to ultraviolet radiation during most of the tidal cycle.

In many macro- and microalgae, photosynthesis and growth are reduced upon exposure to UVBR (Worrest 1982, Cullen & Lesser 1991, Ekelund 1991, Häder 1993, Larkum & Wood 1993). When the level of UVB irradiation was increased, the species composition of phytoplankton in artificial microecosystems was seen to be affected (Worrest 1982, Bothwell et al. 1993). On the other hand, increased UVBR has had little effect on species diversity of Antarctic diatom communities over the past 20 yr (McMinn et al. 1994). UVBR may also affect the balance between primary producers and consumers of benthic communities (Bothwell et al. 1994). In the present study a selection of benthic diatom species

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representative of a tidal flat microalgal community was exposed to different levels of UVBR to test the effect of UVBR on growth rate reduction. A comparison in UVBR sensitivity with pelagic microalgae of the Dutch Wadden Sea and of the open waters of the bordering North Sea was set up to test the hypothesis that sediment-inhabiting diatoms are better adapted to UVBR than pelagic algae. In contrast to pelagic algae, that usually circulate vertically in the surface layer of open water and are therewith exposed to a variable light field, benthic diatoms in the intertidal mudflats of the Dutch Wadden Sea are exposed much more continuously to incident UVBR, even directly when emersed during low tide. Growth rate reduction as an indicator of UVBR stress was chosen since the most-studied effect on microalgae, photosynthetic rate reduction, which is usually measured during short incubations, may not reflect UVB stress suffered over more realistic longer time scales (i.e. in excess of several generations) that apply in field situations. Notice that it has been pointed out by Cullen & Neale (1994) that short-term measurements of the rate of photosynthesis can easily be out of balance with growth; there is too little information on which to compare sensitivities of photosynthesis versus cell division (Cullen & Neale 1994).

MATERIALS AND METHODS

All the benthic diatoms were isolated from tidal flats in the Ems-Dollard estuary, Wadden Sea, The Netherlands. Some of the species had been kept in the laboratory for several years without having been exposed to UVBR (*Navicula salinarum, Nitzschia closterium, Amphiprora* cf. *paludosa, Stauroneis constricta, and Nitzschia thermalis*), while others were isolated from the field just prior to the experiments (*Nitzschia apiculata, N. salinarum, N. closterium, Amphiprora* sp., *Navicula flanatica*). All the species tested are common in tidal microalgal mats of this region; they may reach monospecific occurrence at times (namely *N. salinarum*). Cultures were grown in artificial seawater medium supplied with nutrients, trace elements and vitamins as described by Admiraal & Werner (1983). The 'old' cultures were maintained at a salinity of 33‰, while the freshly isolated species were kept at a salinity of 15‰ (local salinity at isolation). Cultures were grown at a photosynthetically active irradiance (PAR) level of 200 µE m^{-2} s^{-1} in an 8 h light 16 h dark cycle at a temperature of 12°C. Experiments were carried out in polystyrene culture vessels or trays. Polystyrene is UVB transparent but eliminates the UVC that is emitted by the UVB lamp (Philips TL 12) used for the experiments (Fig 1) During the UVB treatments, cultures were illuminated from above by fluorescent tubes (Philips TTD, 18 W) to supply PAR; UVB was supplied from below. UVBR exposures were performed for 3 h a day in the middle of the PAR light period. Controls were placed on perspex plates that were non-transparant to UVBR (see also Steeneken et al. 1995).

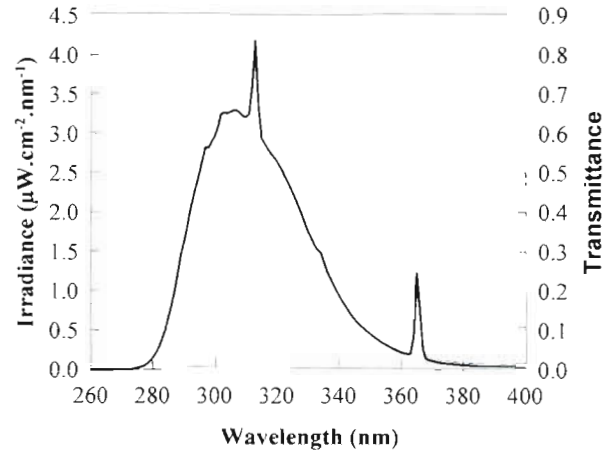

Fig. 1. Emission spectrum of the UVB lamp used (Philips TL 12, bold line), measured with an Optronic OL752 spectroradiometer, and transmission spectrum (dotted line) of the UVB transparent perspex + polystyrene used for the experiments

The light spectrum (Fig. 1) received by the algae was thus determined by characteristics of both perspex and the polystyrene from the bottles or trays; transmission characteristics of both materials were measured with a Cary spectrophotometer (Cary, UV/Vis, Varian). Spectral emission (Fig. 1) of the UVB lamp was measured with an Optronics OL 752 spectroradiometer. Weighted daily UVBR doses were calculated from the spectrum received by the algae and the DNA action spectrum of Setlow, normalized at 300 nm (Setlow 1974). The daily weighted UVB doses (Biologically Effective, BED, normalized at 300 nm) used in these experiments were 3.4 and 6.8 kJ m^{-2} d^{-1} ($BED_{DNA300nm}$).

Since benthic diatoms attach to solid substrates, growth rate measurements were carried out in 2 ways. First, an algal suspension was divided over 2 trays, each containing 6 wells with a working volume of 7 ml $well^{-1}$. During these experiments 1 well was emptied completely by scraping the cells from the bottom of the well every other day. The whole volume of this well was counted.

The other method was to grow the cultures in 200 ml polystyrene culture vessels. Twice a day the vessels were shaken vigorously to avoid strong attachment of the cells to the walls of the vessels. Every other day samples were taken from these vessels and counted. Experiments were carried out in triplicate. Cells were fixed with formaldehyde and counted in Neubauer counting chambers. Long-term growth rates were calculated from semi-log linear regression analysis of

plots spanning several days of cell number increase. Reductions in growth were plotted against UVBR doses weighted by the DNA action spectrum and not with the absolute weighting functions of Cullen et al. (1992) because we wished to compare the benthic diatom response with that of phytoplankton exposed to natural UVBR sources (the data of Behrenfeld et al. 1993). UV effects on pelagic algae are described elsewhere (Buma et al. 1996b).

The cell size of the various species was determined by measuring the dimensions of the cells microscopically and by calculating the volume of simplified cell shapes such as discs or cylinders. Average cell volumes of the cultures were used throughout. Notice that these benthic diatoms are usually seen in valve view and hardly in girdle view. It is therefore not possible to distinguish any cell volume differences that might result from additional girdle band formation during growth.

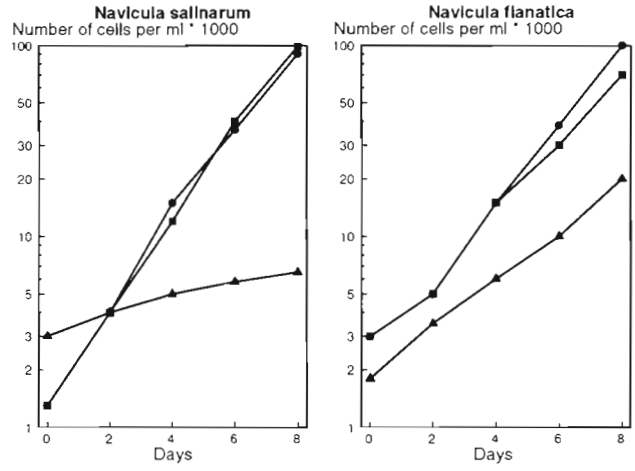

Fig. 2. *Navicula salinarum* and *Navicula flanatica*. Examples of growth curves during UVBR exposure for benthic diatoms grown in bottles. (■) 3.5 kJ m^{-2} d^{-1}; (▲) 6.8 kJ m^{-2} d^{-1}; (●) reference

RESULTS

Both methods for determining growth rates of the diatoms showed identical results. Therefore only results of the culture bottle experiments are presented (Fig. 2). The cultures had to be exposed to a daily weighted UVBR dose of at least 3.4 kJ m^{-2} d^{-1} to establish UVBR induced growth rate reduction, with the exception of the freshly isolated *Nitzschia closterium* (which is also found in the plankton; see Table 1): only above this UVBR level was significant reduction observed.

Nitzschia apiculata and the freshly isolated strain of *Navicula salinarum* showed growth rate reduction only at 6.8 kJ m^{-2} d^{-1} (Fig. 3). *Stauroneis constricta* showed no significant growth rate reduction at all. No significant differences were found between cultures maintained in the lab for several years and the freshly isolated strains. Surprisingly, the 'old' strain of *Nitzschia closterium* (see 'Materials and methods') seemed less vulnerable than the freshly isolated strain (Fig. 3).

It has been suggested (Karentz et al. 1991, Bothwell et al. 1993) that UVBR sensitivity is related to cell size which influences protection of the cell nucleus through enhanced UVBR absorption by the cytoplasm. Since there is a large variation in size within the group of microalgae tested, UVBR sensitivity was also plotted against mean cellular volume as

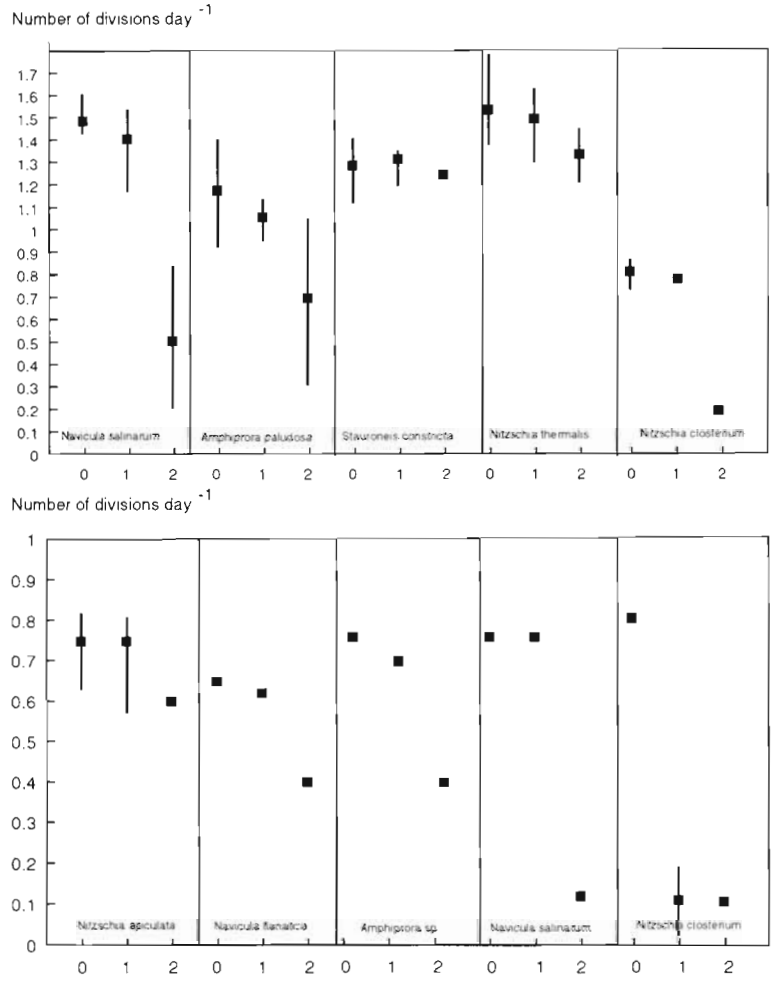

Fig. 3. Effect of 2 UVBR treatments on the growth of 8 benthic diatom species. 0: reference; 1: 3.5 kJ m^{-2} d^{-1}; 2: 6.8 kJ m^{-2} d^{-1}. Vertical bars indicated upper and lower extreme of number of doublings d^{-1} between triplicate culture. 'Old' strains: top row; freshly isolated strains: bottom row

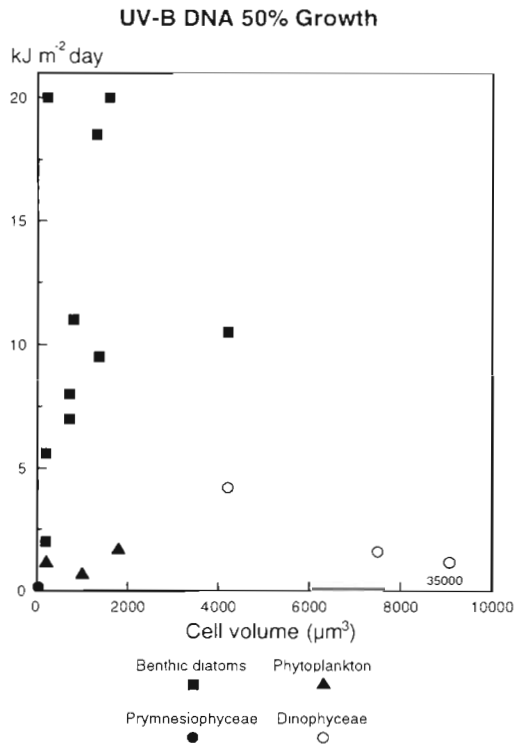

Fig. 4. Relation between UVBR sensitivity, expressed as the estimated daily UVB dose where 50% growth rate reduction occurs, and mean cellular volume in benthic and pelagic microalgae

measured during the experiments (Fig. 4). However, no clear relationship between mean cell size and UVBR sensitivity was observed (see Fig. 4).

DISCUSSION

The daily UVBR dose at which we still didn't record any growth reduction (3.4 kJ m^{-2} d^{-1}) is well above natural UVBR levels in the Dutch Wadden Sea, even in summer. Behrenfeld et al. (1993) reported maximal daily UVBR doses (DNA weighted with Setlows action spectrum, normalized at 300 nm) between 1 and 2 kJ m^{-2} d^{-1}, calculated from incident irradiance at the Pacific Ocean's water surface in summer at mid-latitudes; this is roughly the level measured near the ground in our sampling area (data of RIVM, The Netherlands). In fact, the UVBR effect on growth that we measured was probably overestimated. At natural UVAR and PAR conditions resistance of algal species is probably even higher than at the light conditions offered in our experiments, where PAR was only 200 µmol m^{-2} s^{-1}. Wavelengths in the UVAR and PAR range of the spectrum induce repair of damage due to UVBR stress (Karentz et al. 1994, Buma et al. 1995, 1996a); the formation of thymine dimers in nuclear DNA after UVBR exposure can be reversed readily by simultaneous or subsequent exposure to UVAR and PAR (Buma et al. 1995, 1996a). These wavelengths were underrepresented in our experiments compared to the field situation: PAR intensity was definitely lower than incident natural PAR irradiances on Dutch tidal flats during summer, which are up to 2500 µmol m^{-2} s^{-1}. Our UVBR lamp did emit some UVAR, but (see Fig. 1) at a much lower level compared with natural UVAR. The effect of UVAR on growth rate of a mat forming cyanobacterium was also reported by Quesada et al. (1995). Data presented by Jokiel & York (1984) showed that rapid adaptation of phytoplankton to extremely high levels of solar UVAR and PAR is also possible.

The benthic diatoms tested did differ in their UVBR sensitivity but they all had a very high UVBR resistance. *Nitzschia closterium* was the exception but this species is also found abundantly in the water column. No differences were found between cultures kept in the laboratory for many years (a stock culture of *Stauroneis constricta* was even kept in the laboratory for 20 yr) and freshly isolated cultures. Apparently, resistance against high UVBR levels is not readily lost: it is a very stable characteristic.

It is not known what causes the high UVBR resistance in these microalgae. In future work we will analyse defense mechanisms such as pigmentation and DNA damage repair (Buma et al. 1996a). Indeed, it is possible that they form pigments that absorb specifically in the UVBR region of the spectrum (mycosporine-like amino acids, Carreto et al. 1990). Alternatively, the species may have acquired the very efficient UV induced damage repair system described for planktonic microalgae by Buma et al. (1995), possibly through rapid synthesis of enzymes involved in photoenzymatic repair. The acquisition of highly efficient repair mechanisms of benthic diatoms is probably related to the natural exposure to intense UVBR.

The UVBR response found here differs greatly from the UVBR response found in pelagic algae (Table 1). The pelagic diatoms and dinoflagellates and the prymnesiophyte *Emiliania huxleyi* that we tested under similar conditions showed much higher UVBR vulnerability; *E. huxleyi* showed complete growth inhibition already at 0.5 kJ m^{-2} d^{-1} (Buma pers. comm.). In Table 1, the UVBR sensitivity is expressed as the daily weighted UVBR dose where 50% growth reduction occurs (calculated from semi-log linear regression analysis). Although these are very rough estimates, because dose-response relationships are non-linear, the differences are large enough to make the figures reliable.

UVBR has probably been a natural selective factor in these microalgal communities; it has, in this aspect con-

Table 1 Comparison of UVBR sensitivity, expressed as the estimated daily UVBR dose where 50% growth rate reduction occurs, in several benthic species and algae isolated from pelagic environments (data of pelagic algae taken from own experiments under similar UVBR and PAR conditions)

Species	50% growth reduction (J m^{-2} d^{-1})
Benthic species	
Navicula salinarum	8000
Navicula salinarum 2	7000
Navicula flanatica	11000
Nitzschia thermalis	>20000
Amphiprora paludosa	10500
Amphiprora sp.	9500
Stauroneis constricta	>20000
Nitzschia closterium (b1)	5600
Nitzschia closterium (b2)	2000
Pelagic species	
Nitzschia closterium (p1)	800
Cyclotella nana	660
Thalassiosira nordenskioldii	1660
Prorocentrum micans	>1600
Alexandrium tamarense	1250
Emiliania huxleyi	150

tributed to the dominance of these species in algal mats because of their ability to outcompete more UVBR-sensitive species. The specific environmental conditions that determine the density and species composition of benthic estuarine diatom populations are not easy to determine since they are only one constituent of an ecosystem; this makes it impossible to predict if and how the composition of the system will change with an increase in UVBR levels. Moreover, very little is known about species-specific effects of UVAR and visible light on the repair of benthic diatoms. However, we suggest here that it is unlikely that UVBR levels will cause more UVBR stress in these communities even when ozone reduction continues at its present rate during the coming decades: in the worst case scenarios of ozone depletion the increase in daily UVBR exposure will certainly remain below the irradiance of 3.5 kJ m^{-2} s^{-1} and higher at which we started to observe negative effects on cell number increase.

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