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Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms

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Abstract Growth rate, survival, and stimulation of the production of UV-B (280 to 320 nm) absorbing compounds were investigated in cultures of five commonly occurring Antarctic marine diatoms exposed to a range of UV-B irradiances. Experimental UV-B exposures ranged from 20 to 650% of the measured peak surface irradiance at an Antarctic coastal site (0.533 J m^{-2} s^{-1}). The five diatom species (*Nitzschia lecointei*, *Proboscia alata*, *P. inermis*, *Thalassiosira tumida* and *Stellarima microtrias*) appear capable of surviving two to four times this irradiance. In contrast to *Phaeocystis* cf. *pouchetii*, another major component of the Antarctic phytoplankton, the concentrations of pigments with discrete UV absorption peaks in diatoms were low and did not change significantly under increasing UV-B irradiance. Absorbance of UV-B by cells from which pigments had been extracted commonly greatly exceeded that of the pigments themselves. Most of this absorbance was due to oxidisable cell contents, with the frustule providing the remainder. Survival of diatoms did not correlate with absorption by either pigments, frustules or oxidisable cell contents, indicating that their survival under elevated UV-B irradiances results from processes other than screening mechanisms.

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Introduction

The seasonal depletion of stratospheric ozone over the Antarctic and the Southern Ocean is a major ecological issue, as it has been suggested that the resultant increase in UV-B (280 to 320 nm) reaching the biosphere may adversely effect Antarctic marine ecosystems (Bidigare 1989; El-Sayed et al. 1990; Vosjan et al. 1990; Voytek 1990; Karentz 1991). Numerous studies indicate that productivity of marine phytoplankton in surface waters is reduced under ambient and elevated levels of UV (e.g. Worrest et al. 1981; Bühlmann et al. 1987; Smith et al. 1992). However, Hobson and Hartley (1983) and Gala and Giesy (1991) reported only limited inhibition of primary production in lake and fjord phytoplankton assemblages by UV radiation.

UV-B penetrates to depths in excess of 50 m in Antarctis waters (Gieskes and Kraay 1990; Karentz and Lutze 1990; Smith et al. 1992). Antarctic phytoplankton bloom in the high-light, high-nutrient regime of the marginal ice zone (MIZ) where the depth of the pycnocline can be 10 m or less for periods of up to 6 d (Veth 1991). This springtime bloom in the MIZ accounts for up to 67% of primary production in Antarctic waters (Smith and Nelson 1986). The coincidence of stratospheric ozone depletion with this near-surface seasonal algal bloom may have important consequences for phytoplankton and higher trophic levels if survival and/or primary production are affected (El-Sayed et al. 1990; Voytek 1990; Karentz 1991; Marchant et al. 1991). The impact of further increases in UV-B on phytoplankton in the MIZ will depend on the residence time of organisms in this shallow mixed zone, their present tolerance, and their ability to adapt to higher levels of UV-B (Bidigare 1989; Karentz 1991).

The effect of increasing solar UV-B flux on Antarctic phytoplankton and higher trophic levels is equivocal (El-Sayed et al. 1990; Karentz 1991). Calkins and Thordardottir (1980) found that the tolerance of six high-latitude marine diatoms to UV-B was similar and concluded that most organisms would adapt to enhanced solar UV through in-

creased protective pigmentation, repair, or avoidance mechanisms. Other authors have proposed a shift in species composition favouring those species with greater tolerance of UV-B (Häder and Worrest 1991; Karentz 1991; Marchant and Davidson 1991). Such changes would be likely to affect trophic interactions and vertical carbon flux.

Information on the effects of UV-B on growth and survival of Antarctic marine phytoplankton is integral to an understanding of the impact of elevated UV-B exposure on the Southern Ocean ecosystem. The mechanisms of UV-B protection in Antarctic phytoplankton species are largely unknown, although UV-B absorbing compounds and DNA repair-mechanisms have recently been reported for some species (Karentz 1988; Bidigare 1989; Mitchell et al. 1989; Karentz et al. 1991 a, b; Marchant et al. 1991). Species-specific investigations on the impact of UV-B are necessary to predict the effect of ozone depletion on these primary producers and the consequent impact on the ecosystems for which they constitute the trophic base (Karentz 1991). Here we report the effect of UV-B exposure on the growth rates and survival of five species of Antarctic marine diatoms and whether their production of UV-B absorbing compounds is promoted by UV-B exposure.

Materials and methods

Light measurements

All measurements of irradiance were made with an International Light IL 1700 Radiometer equipped with detectors to measure photosynthetically available radiation (PAR), UV-A (320 to 400 nm) and UV-B (Fig. 1). A National Institute of Standards and Technology intercomparison package (NIST Test #534/240436-88) was used to calibrate each light sensor.

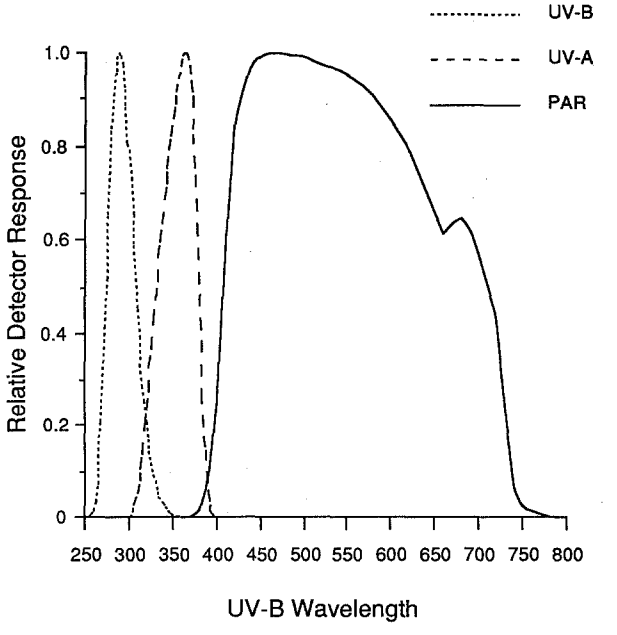

Fig. 1 Wavelength (nm) response of detectors used to measure PAR, UV-A (320 to 400 nm) and UV-B (280 to 320 nm) (redrawn from instrument specifications)

Cell isolation and culture

Unialgal cultures of the diatoms *Nitzschia lecointei* V.H., *Proboscia* (*Rhizosolenia*) *alata* (Brightwell) Sundström, *P.* (*Rhizosolenia*) *inermis* (Castracane) Jordan and Ligowski, *Thalassiosira tumida* (Jan.) Hasle, *Stellarima* (*Coscinodiscus*) *microtrias* (Ehrenberg) Hasle and Sims, *Odontella weisflogii* (Janisch) Grunow, *Nitzschia curta* (V.H.) and *Chaetoceros simplex* Ostenfeld were isolated from sea ice collected in Prydz Bay, Antarctica, during the 1990/1991 austral summer. Cultures were maintained in 250 ml glass flasks using *f*/2 growth medium (Guillard and Ryther 1962) at a temperature of 4 °C±2 C°. Cool-white fluorescent lights provided a PAR intensity of 11.80 m^{-2} s^{-2} (58.85 µE m^{-2} s^{-1}), with no UV-B enhancement, on a 12 h light:12 h dark cycle.

UV-B-enhanced treatments

50 ml Lux tissue-culture flasks (which completely absorbed wavelengths below 295 nm) were filled from a single parental culture in exponential growth phase and irradiated for 24 h in a 48 h experimental period (6 h light:12 h dark:12 h light:12 h dark:6 light). Day 0 in data calculations occurs at the end of this irradiance period. Exposures were conducted in a Thermoline controlled-environment cabinet at 4 °C±2 C° with cool-white fluorescent tubes to provide PAR and UV-A, with UV-B provided by FS20T 12 UV-B Westinghouse sunlamps. PAR and UV-A irradiances were 12.13±2.13 W m^{-2} (60.5±10.4 µE m^{-2} s^{-1}) and 1.19±0.68 W m^{-2}, respectively. The spectral distribution and UV-B irradiance were varied by attenuation with glass filters (Marchant et al. 1991). The zero UV-B irradiance treatment was screened by Mylar which excluded light below 320 nm. Sensors were each covered by an attenuating glass screen and a single layer of Lux culture flask to measure the experimental irradiances to which the diatoms were exposed. UV-B irradiances of 0.10 to 3.40 W m^{-2} were chosen to span the range of 20 to 650% of peak UV-B exposure as measured at an Antarctic coastal site (Casey station; 66° S) in the 1989 summer (C. Roy unpublished data). A single culture of each species was incubated at each of these irradiances and a control culture of each species was returned to culture maintenance conditions and received only PAR.

Calculation of viable cell concentration

Immediately after irradiation (Day 0), 5 or 10 ml (depending on cell concentration) of the control culture was sediment with Lugol's iodine and the concentration of cells with cytoplasmic contents (live cells) was calculated from counts over 15 replicate fields, using an Utermöhl settling tube and inverted microscope. The mean cell concentration in the control culture at Day 0 was then calculated ($N_{0\ \text{control}}$). Also on Day 0, a 5 ml aliquot of each irradiated culture and the control were inoculated into 30 ml of *f*/2 medium in a glass flask and returned to the culture maintenance conditions described above. These subcultures were incubated for up to 10 d and the concentration of live cells was counted at 2 to 4 d, intervals depending on their growth rate. The growth rate of the control culture of each species (K control) was calculated using the equation of Verity et al. (1988) (Eq. 1 below). Eq. (2) was then used to calculate the viable cell concentration on Day 0 ($N_{0\ \text{irradiated}}$) using the cell concentration for each of 15 replicate fields after ongrowth (N_t), the growth rate of the control (K control), and the time of culture ongrowth (t)

$$K = 1/t \times \log_2 \frac{N_t}{N_0}, \tag{1}$$

$$N_0 \text{ irradiated} = \frac{N_t \text{ irradiated}}{{}_2K \text{ control} \times t}, \tag{2}$$

$$S\% = \frac{N_0 \text{ irradiated}}{N_0 \text{ control}} \times 100, \tag{3}$$

where K = growth rate, t = number of days of growth, N_t = number of cells at time t, N_0 = number of cells immediately after irradiation (Day 0) and $S\%$ = percent survival.

The calculated viable cell concentration of each replicate field at Day 0 ($N_{0\ \text{irradiated}}$) was then converted to percent survival ($S\%$) in

comparison with the unirradiated control at Day 0 ($N_{0\ control}$) using Eq. (3). In cases where the cell number in irradiated cultures was greater than that in the control culture, computed survival could not exceed 100%. The percent survival in each replicate field was arcsine square-root transformed, the mean and standard error of the replicate fields were computed, and the mean and upper and lower confidence intervals were sine-squared to revert the data to percentages (Zar 1984).

Eq. (1) was then used to calculate the growth rate of all ongrown irradiated cultures. Growth rates were calculated for each species from the day at which the cell concentration in the culture had reached a sufficient concentration to allow statistically acceptable mean estimates (N_0) and from the concentration 4 d later (N_t). The t_0s for each species were: *Nitzschia lecointei*, Day 4; *Proboscia alata*, Day 6; *P. inermis*, Day 6; *Thalassiosira tumida*, Day 8; *Stellarima microtrias*, Day 8.

Removal of dark period from irradiance cycle

Nitzschia lecointei and *Stellarima microtrias* were exposed to the same experimental light irradiances as described above but with the dark period of the cycle removed, giving 24 h of constant illumination. These two species were chosen as they exhibited different responses to treatments which included a dark period. The same procedures were followed as described above for determining survival and growth rate.

Measurement of UV absorption

A known volume of culture was filtered through 2.5 cm diam Whatman GF/F filters. Filters were cut up into an homogeniser and 1.5 ml of 4:1, methanol:tetrahydrofuran (MeTHF) were added. The sample was then homogenised using a glass tube and teflon grinder for 30 s at ≃1000 rpm and decanted into a centrifuge tube. A further 0.5 ml of MeTHF was added to rinse the homogeniser; this was again decanted into the centrifuge tube, and the sample was centrifuged at 480×*g* for 10 min at 0 °C. The absorbance of the supernatant was measured between 250 and 800 nm using a Hewlett Packard 8450A spectrophotometer. When measurements were not carried out immediately, the extracts were stored at −120 °C for no longer than 4 wk. The wavelength of maximum UV absorbance was identified and the peak absorption height above the adjacent minima was measured for each extract. Data were then averaged over all cultures that received sublethal irradiances. Average absorbance was then normalised to chlorophyll *a* peak height at 665 nm. Cell carbon content was calculated for each species using cell concentration, volume and carbon-conversion equations of Eppley et al. (1970), and the absorbance was normalised to cell carbon concentrations (C). The amount of UV-absorbing pigment was calculated per unit C to allow comparison between species that varied in volume from ~7.90×10^2 μm^3 to 1.92×10^5 μm^3 for *Nitzschia lecointei* and *Proboscia inermis*, respectively. UV absorbance was also normalised to cell concentration. Regression analysis of log absorbance per cell for each species was used to ascertain whether the concentration of UV-B-absorbing compounds was promoted by increased UV-B irradiance.

Absorption by UV-B pigments, extracted cell contents and frustules was measured in exponentially growing cultures of *Nitzschia lecointei*, *Proboscia alata*, *Thalassiosira tumida*, *Odontella weisflogii*, *N. curta*, and *Chaetoceros simplex* grown in *f*/2 medium under culture maintenance conditions (as above). Seven hundred ml of each culture was centrifuged at 200×*g* for 40 min at 0 °C to concentrate the cells, and the supernatant was discarded. Two ml of 4:1, MeTHF was then added, the cells were resuspended and the intracellular pigments were allowed to extract overnight at 0 °C. The centrifugation was repeated and absorption of the supernatant was measured as above. To remove any contamination by intracellular UV-absorbing pigments, the extracted material was rinsed three times with 2.0 ml of MeTHF followed by resuspension and centrifugation at 200×*g* for 10 min at 0 °C. The material was then resuspended in a further 2.0 ml of MeTHF and the absorbance was measured as above.

To clear diatom frustules of organic contents, a known volume of the above MeTHF-extracted cell concentrate was centrifuged at 480×*g* for 10 min and the MeTHF supernatant was discarded. The sample was then digested for 24 h in 5 ml of 30% H_2O_2, and 25 g of $K_2Cr_2O_7$ were added to oxidise and clean the frustales. The solution was diluted to 15 ml with Milli Q water and centrifuged at 480×*g* for 1 h, and the supernatant again discarded. Microscopic examination of samples showed that this was sufficient to remove the cell contents from all species except *Thalassiosira tumida*; two treatments were necessary to clear the frustales of this species. Samples were resuspended twice in 15 ml of Milli Q, centrifuged at 480×*g* for 1 h, and the supernatant discarded. Finally, the cleared frustales were resuspended in a volume of MeTHF equal to that of the initial MeTHF extract, and the absorbance was measured as described above.

Results

UV-B absorbance

Only compounds with absorption >290 nm were investigated. Pigment extracts of *Nitzschia lecointei*, *Proboscia alata*, *P. inermis*, *Thalassiosira tumida*, *Stellarima microtrias* and *N. curta* had chlorophyll *a* absorbance peaks at 665 nm and chlorophylls and carotenoids at ≃440 nm (Fig. 2). None of the species investigated had any pronounced absorbance peaks in the UV-B region of the spectrum. There was, however, increasing background absorption in the UV region of the spectrum and distinct absorbance peaks between 325 and 342 nm for each species (Table 1), the shoulder of which absorbed at UV-B wavelength (Fig. 2). The ratio of the UV-absorbing compound peak-height to that of chlorophyll *a* at 665 nm for the diatoms was ≤2.1:1. Most of the absorption was at UV-A wavelengths, and absorption in the UV-B region at the shoulder of these peaks was much less.

As the concentration of chlorophyll *a* can change in response to UV-B exposure (Bidigare 1989), it was not used in normalising UV-B induced changes. Log absorbances per unit cell C for each species over the range of UV-B irradiances is shown in Fig. 3. Data from Antarctic *Phaeocystis* (Marchant et al. 1991) using similar methods is included for comparison. Regression analysis of the UV absorbance peak-height per cell against sublethal irradiance showed that increased UV-B flux elicited no significant response in UV absorbance in any diatom, and the *F*-test showed that the regression slopes were not significantly different from zero (Table 2).

Absorption of MeTHF-insoluble material by *Nitzschia lecointei*, *Proboscia alata*, *Odontella weisflogii*, *N. curta* and *Chaetoceros simplex* (Fig. 4 A–B, D–F) gradually decreased with increasing wavelength while that of *Thalassiosira tumida* remained approximately constant (Fig. 4C). Absorption by cleared frustules of each species also decreased with increasing wavelength, but only accounted for between 13 and 29% of the total UV-B absorption by the cells (Figs. 4 and 5). Total cellular UV-B absorption per μg cell carbon varied between 3.4×10^{-5} for *T. tumida* to 8.2×10^{-4} for *N. lecointei* (Fig. 5). MeTHF-soluble pigments comprised between 12 and 26% of this absorption. The exception was *T. tumida*, in which it accounted for 45% of UV-B absorption. The majority of UV-B absorp-

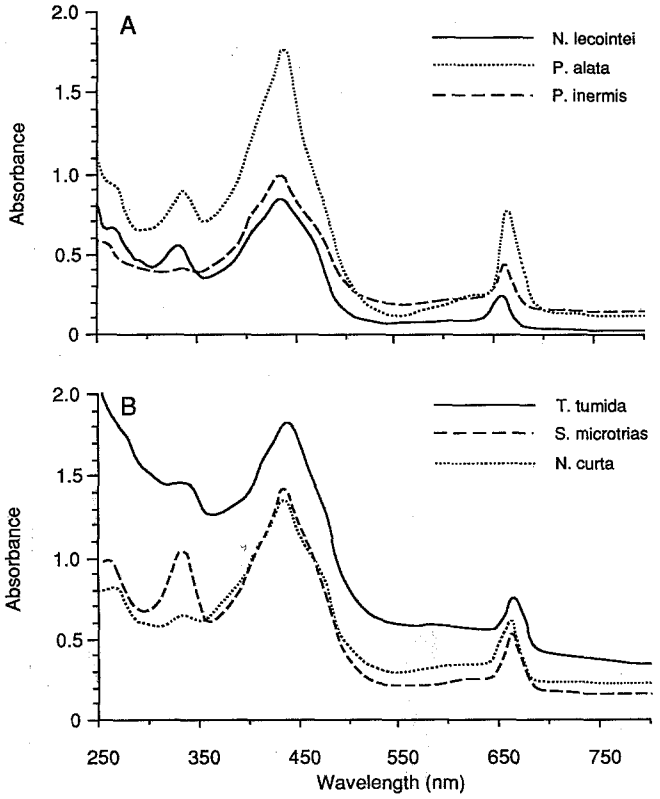

Fig. 2 **A** *Nitzschia lecointei*, *Proboscia lata* and *P. inermis*; **B** *Thalassiosira tumida*, *Stellarima microtrias* and *N. curta*. Absorbance spectra of extracts in 80% menthanol:20 tetrahydrofuran between 250 and 800 nm for control cultures

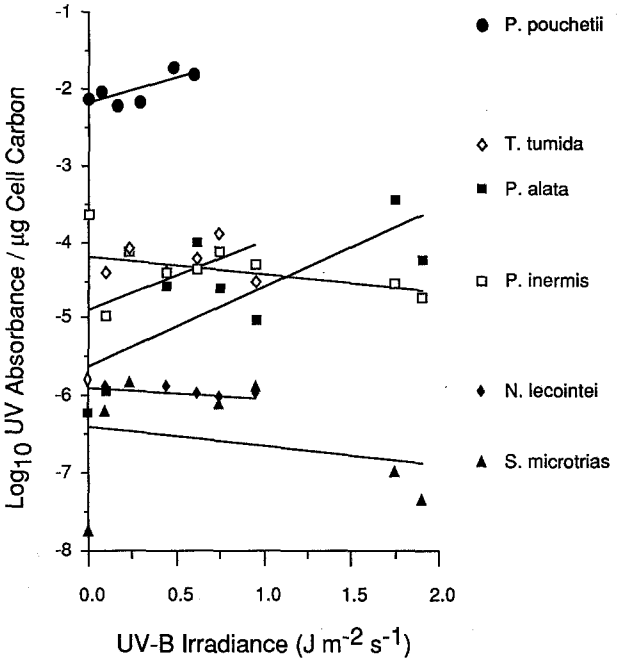

Fig. 3 *Proboscia inermis*, *Nitzschia lecointei*, *Thalassiosira tumida*, *Proboscia alata*, *Stellarima microtrias* and *Phaeocystis* cf. *pouchetii*. Log peak UV absorbance per unit cell carbon as a function of sublethal UV-B irradiances. (Data for *Phaeocystis* cf. *pouchetti* from Marchant et al. 1991)

Table 1 UV-absorbing compounds in Antarctic marine diatoms and *Phaeocystis* cf. *pouchetti* (*Phaeocystis*), showing wavelength of peak UV-absorbance, ratio of UV-absorbing compound peak-height to chlorophyll *a* peak-height at 665 nm, and UV absorbance per µg cell carbon. *Phaeocystis* calculated from data in Marchant et al. (1991). Data are mean values of all sublethal irradiances

Species	Peak absorbance (nm)	UV abs : chl *a* ratio	UV abs μg^{-1} cell C
Nitzschia lecointei	325	0.9	1.10×10^{-6}
Proboscia alata	336	1.7	6.86×10^{-5}
Proboscia inermis	340	2.1	6.17×10^{-5}
Thalassiosira tumida	342	1.2	5.08×10^{-5}
Stellarima microtrias	342	1.8	5.91×10^{-7}
Phaeocystis	323	27.5	1.04×10^{-2}

Table 2 Regression statistics obtained by linear regression of UV absorbance per cell against sublethal UV-B irradiance for Antarctic marine diatoms

Species	*P*(*r*)	*P*(*F*)
Nitzschia lecointei	0.2 >×>0.1	0.1955
Proboscia alata	0.2>×>0.1	0.1009
Proboscia inermis	0.5 >×>0.2	0.2732
Thalassiosira tumida	0.5 >×>0.2	0.2542
Stellarima microtrias	>0.50	0.5851

tion was due to MeTHF-insoluble cell contents, except in *T. tumida* where the proportion was slightly less than that of the MeTHF-soluble material (Fig. 5).

UV-B response: survival and growth rate

Survival of diatoms exposed to UV-B differed between species (Fig. 6). Diatoms screened with Mylar received no UV-B (irradiance 0; Fig. 6), but did receive unattenuated UV-A and exhibited low survival (9 to 32%). Survival of *Nitzschia lecointei*; *Proposcia alata* and *P. inermis* at sublethal irradiances approximated 100% survival (Fig. 6 A–C) but that of *Thalassiosira tumida* and *Stellarima microtrias* at sublethal irradiances ranged from 51 to 85% and 59 to 75%, respectively (Fig.6 D, E). At 1.75 J m^{-2} s^{-1}, the survial of *N. lecointei*, and *P. alata* fell to 17 and 14% respectively and survival was negligible at 3.4 J m^{-2} s^{-1} (Fig. 6 A, B). No significant change in survival of *P. inermis*, *T. tumida* and *S. microtrias* occurred until a UV-B irradiance

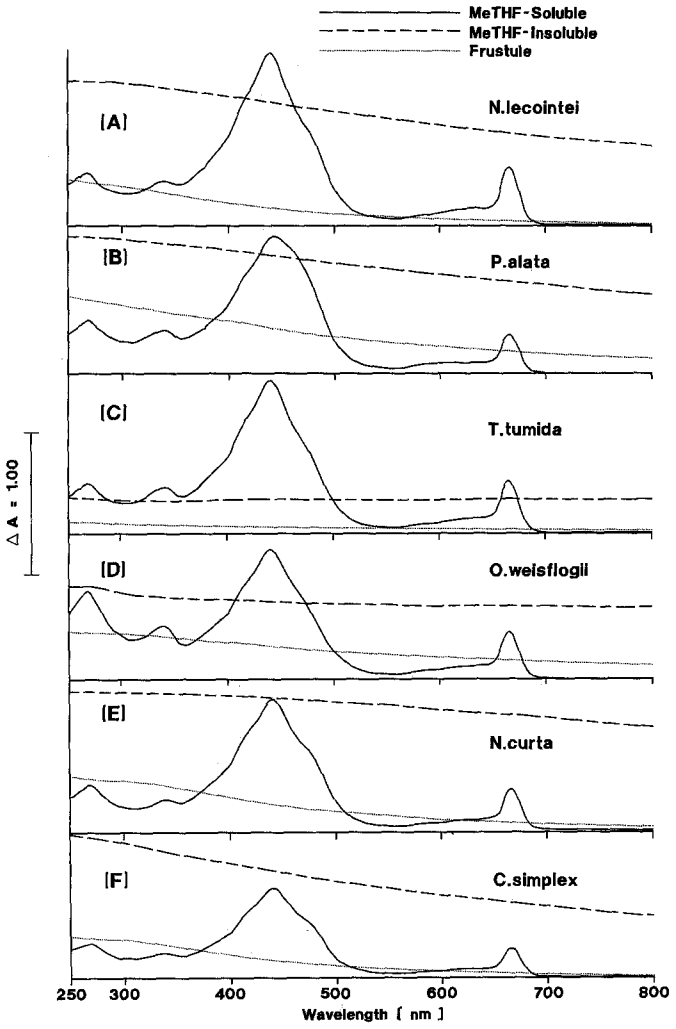

Fig. 4 **A** *Nitzschia lecointei*, **B** *Proboscia alata*, **C** *Thalassiosira tumida*, **D** *Odontella weisflogii*, **E** *Nitzschia curta*, **F** *Chaetoceros simplex*. Absorbance spectra of extracts in 80% methanol:20 tetrahydrofuran for cultures grown under maintenance conditions. Cells were extracted with MeTHF and insoluble material was oxidised to clear frustules. Absorbance by MeTHF-soluble compounds, MeTHF-insoluble matter and cleared frustules and absorbance between 250 and 800 nm are shown

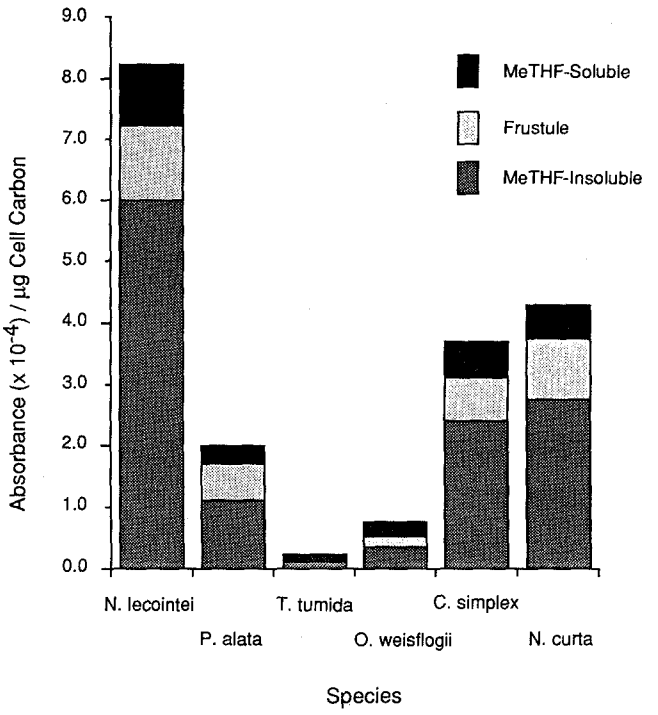

Fig. 5 *Nitzschia lecointei*, *Proboscia alata*, *Thalassiosira tumida*, *Odontella weisflogii*, *Chaetoceros simplex* and *Nitzschia curta*. Average UV-B absorbance of MeTHF-soluble compounds, MeTHF insoluble matter and cleared frustules per µg cell carbon

of 3.4 J m^{-2} s^{-1}, at which irradiance their survival fell to 25% (Fig. 6C, D, E). In contrast, the survival of Antarctic colonial *Phaeocystis* cf. *pouchetti* (Fig. 6F) (Thereafter referred to as *Phaeocystis*) was reduced to 30% at a UV-B irradiance of 1.0 J m^{-2} s^{-1}, with survival reduced to 0% at a UV-B irradiance of 2.1 J m^{-2} s^{-1} (Marchant et al. 1991).

Regression analysis showed that there was no significant relationship between growth rate after irradiation of the diatoms and the UV-B irradiance they received (Table 3). The growth rate of *Nitzschia lecointei* appeared to decline as UV-B irradiance increased, but, this was not observed until the highest irradiance (3.4 J m^{-2} s^{-1}; Fig. 7). Growth rates for irradiated cultures of *Proboscia alata*, *P. inermis*, *Thalassiosira tumida*, and *Stellarima microtrias* were comparable to those occurring in the PAR control.

Dark-period removal

Removal of the dark period from the irradiance of both *Nitzschia lecointei* and *Stellarima microtrias* elicited a different survival response (Fig. 8) from those treatments incorporating a dark period. *N. lecointei* cells survived an irradiance incorporating dark periods of 1.75 J m^{-2} s^{-1} (Fig. 6A), but survived all but the maximum irradiance when exposed without dark periods (Fig. 8A). In contrast to *N. lecointei*, survival of *S. microtrias* during irradiation including a dark period did not decline significantly until an irradiance of 3.2 J m^{-2} s^{-1}. When the dark period was removed, survival of *S. microtrias* declined to 23% at an irradiance of 1.75 J m^{-2} s^{-1}. In addition, survial of *S. microtrias* over the lower range of UV-B irradiances was ≃ 20% lower in exposures with a dark period (Fig. 6E) than in those without (Fig. 8B).

Discussion

This study was structured to approach natural conditions, so that some insights into the basic responses of diatoms

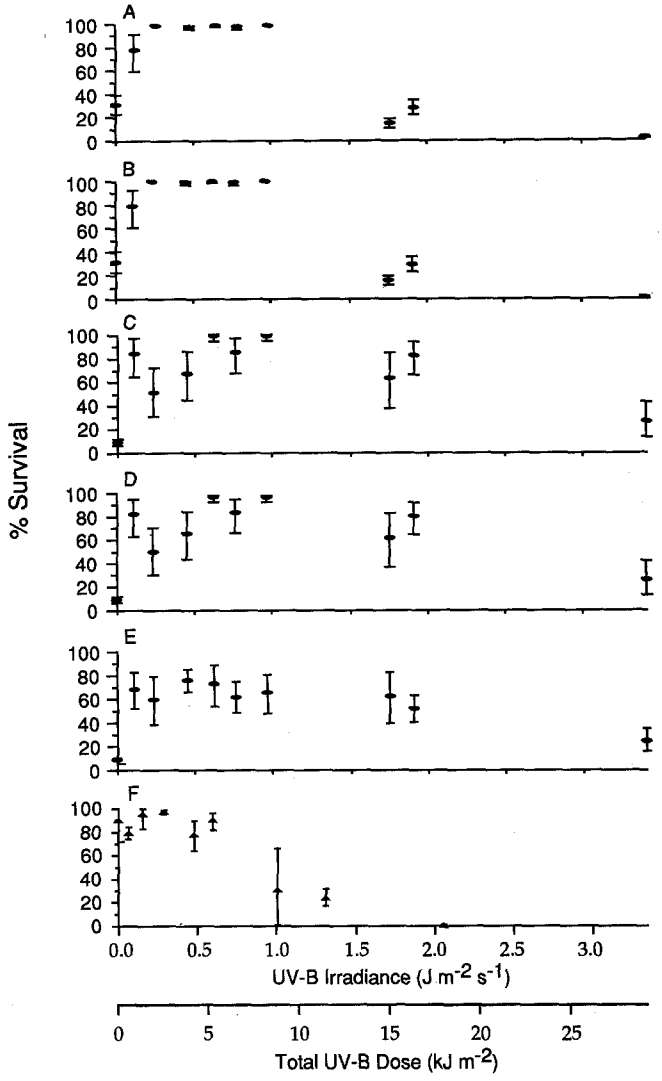

Fig. 6 **A** *Nitzschia lecointei*, **B** *Proboscia alata*, **C** *P. inermis*, **D** *Thalassiosira tumida*, **E** *Stellarima microtrias*, **F** *Phaeocystis* cf. *pouchetti*. Percent survival of diatoms irradiated for 24 h of a 48 h period as a function of UV-B irradiance. (Data for *Phaeocystis* from Marchant et al. 1991). Errors bars represent standard errors calculated from Zar (1984)

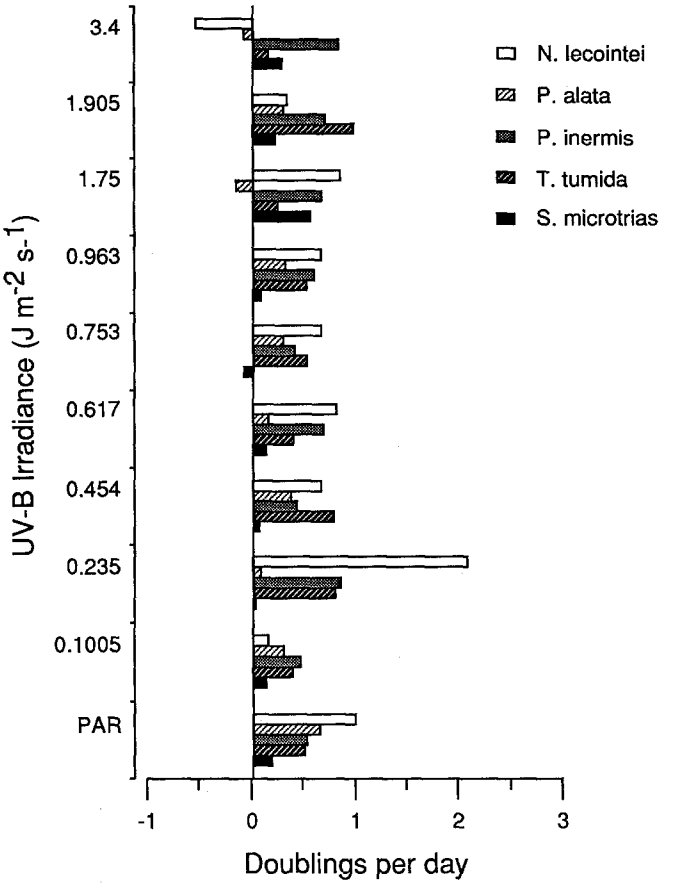

Fig. 7 *Nitzschia lecointei*, *Proboscia alata*, *P. inermis*, *Thalassiosira tumida* and *Stellarima microtrias*. Growth rate of exponentially growing culture after exposure to 12.13±2.13 W m^{-2} (60.5±10.6 µE m^{-2} s^{-1}) PAR, 1.19±0.68 W m^{-2} UV-A, and various UV-B irradiances

Table 3 Regression statistics obtained by linear regression of post-irradiation growth-rate against UV-B irradiance for Antarctic marine diatoms

Species	$P(r)$
Nitzschia lecointei	0.1 <×<0.05
Proboscia alata	0.2 <×<0.1
Proboscia inermis	0.1 <×<0.05
Thalassiosira tumida	0.5 <×<0.2
Stellarima microtrias	0.2 <×<0.1

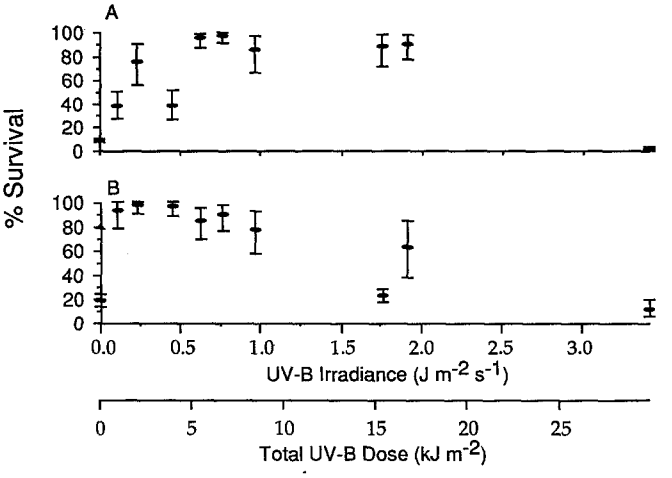

Fig. 8 **A** *Nitzschia lecointei*, **B** *Stellarima microtrias*. Percent survial after 24 h continuous UV-B irradiation. Errors bars represent standard errors calculated from Zar (1984)

to UV-B exposure could be revealed. Experimental limitations included the inability to replicate the dynamic nature of the light climate of the Antarctic marine ecosystem in the laboratory, and the relatively short period over which the UV-B irradiation treatments took place (48 h period with 24 h exposure to UV-B). However, a UV-B exposure of 24 to 48 h reportedly produces significant changes in phytoplankton photosynthetic (Bidigare 1989) and UV-absorbing (Marchant et al. 1991) pigmentation. Numerous Antarctic marine organisms have been shown to produce UV-B-absorbing compounds (Mitchell et al. 1989; Vernet et al. 1989; Karentz et al. 1991 b; Marchant et al. 1991), and there is now a substantial literature indicating that such compounds, principally mycosporine-like amino acids (MAAs), can provide protection against UV-B damage (e. g. Vernet et al. 1989; Carreto et al. 1990; Karentz et al. 1991 b). Marchant et al. (1991) reported a high concentration of UV-B-absorbing compounds in the colonial stage of the Antarctic prymnesiophyte *Phaeocystis*, and demonstrated that the colonial stage of this alga survived higher levels of UV-B irradiation than either the motile stage, or the colonial stage of temperate strains which lacked or contained much lower concentrations of these compounds.

Only compounds with absorption at wavelengths of >290 nm were considered in this study, since shorter wavelengths are not encountered in the marine environment (Smith and Baker 1979; Baker et al. 1980) and would be of no ecological significance in UV protection. The diatoms we investigated have compounds that absorb at UV-A wavelengths, with only low absorbance at the shoulders of these peaks at UV-B wavelengths. The concentrations of UV-absorbing compounds in the diatoms were ≃2 to 5 orders of magnitude less per unit cell C than concentrations in *Phaeocystis*, and – unlike *Phaeocystis* – the concentration of UV-absorbing compounds did not increase significantly as irradiance increased. Therefore, it appears that these diatoms are not using pigments as protection from UV-B to the same extent as does *Phaeocystis*. It remains possible that absorbance at these wavelengths is an incidental consequence of possessing certain cell proteins or metabolites which constitute a target rather than a protective mechanism. Thus, the significance of UV-absorbing compounds in the diatoms as a screen remains uncertain but appears low.

On the basis of the high concentrations of UV-absorbing compounds in *Phaeocystis* and their relative absence in diatoms, Marchant and Davidson (1991) proposed the possibility of a change in the species composition of Antarctic phytoplankton to favour *Phaeocystis* at the expense of diatoms in the marginal ice edge zone. However, the data of the present study demonstrate that diatoms are capable of surviving higher levels of UV-B exposure than *Phaeocystis*. Smith et al. (1992) also found that the rate of cell division of another diatom (*Chaetoceros socialis*) in the Southern Ocean was less affected by a given solar irradiance (including UV-B) than was *Phaeocystis*.

The reports of substantial levels of UV-B-absorbing compounds in mixed phytoplankton from Antarctic waters by Mitchell et al. (1989), Vernet et al. (1989), and Gieskes and Kraay (1990) do not conflict with our data. Their unidentified samples could have contained *Phaeocystis*, an abundant component of the Antarctic phytoplankton community which produces high concentrations of such compounds.

With the exception of *Thalassiosira tumida*, absorption of UV-B by cell concentrations from which MeTHF-soluble pigments had been extracted was considerably greater than the maximum UV absorption by MeTHF-soluble pigments. Absorption of UV-B by the frustule was similar to or greater than absorption by the MeTHF-soluble pigments. Most of the UV-B absorbance in all species except *T. tumida* was due to oxidisable cell contents. The location of these absorbing compounds and structures such as membranes, proteins and carbohydrates within the cell in relation to UV targets within the cell would determine their value as an intracellular screen against UV-B damage. The low absorption by MeTHF-soluble pigments supports the argument that they are not primarily UV-B-protective compounds. Further, the UV-B irradiance at which each species showed a significant decrease in survival did not correlate with their absorption by MeTHF-soluble pigments, frustules or MeTHF-insoluble cell contents. This suggests that none of these fractions provide significant protection from UV-B radiation and that processes other than UV-B screening are responsible for the survival of diatoms at elevated UV-B irradiances.

The pycnocline in the MIZ may be 10 m or less for periods of up to 6 d (Veth 1991). Thus, phytoplankton in this environment may receive high UV-B irradiances for prolonged periods. Our results indicate that diatoms and *Phaeocystis* are able to survive and grow at UV-B irradiances approximately twice (and in the case of *Proboscia inermis* and *Stellarima microtrias* over three times) the peak surface irradiance currently experienced in Antarctic waters for at least 24 h. Their capacity to withstand UV exposure may reflect changes in species composition or selection of UV-resistant strains over the 15 yr of known existence of ozone depletion. Alternatively, high UV-B environments may have existed for substantial periods in their evolution, thus pre-adapting these organisms (Yentsch and Yentsch 1982). Smith et al. (1992) found that the growth rates of phytoplankton after irradiation were independent of the depth from which the samples had been taken, and depended only on the dose received at the depth of incubation. This evidence lends further weight to the idea that these organisms are pre-adapted to a relatively high UV-B environment. Our data also suggests that the impact upon diatoms of increased UV-B irradiance as a result of ozone depletion may be minimal.

The survival of *Thalassiosira tumida* and *Stellarima microtrias* was <100% at UV-B irradiances between 0 and 1.75 J m^{-2} s^{-1}. However, their survival at these irradiances did not appear to be correlated with UV-B irradiance, and *S. microtrias* did not show any significant decline in survival until the highest UV-B irradiance. Thus, the lower maximum survival probably reflects subculturing disturbance or overestimation of the Time 0 population of the PAR-irradiated control culture. The low survival observed

in all species under Mylar screens, which received no UV-B but unattenuated UV-A, indicates that UV-A is also potentially lethal to phytoplankton but that this damage is ameliorated by UV-B. While photorepair of UV-B-induced damage has been reported (Harm 1980; Karentz 1988; Karentz et al. 1991 a), UV-B-facilitated repair of UV-A-induced damage has not. UV-A is largely responsible for the inhibition of carbon fixation (Bühlmann et al. 1987), but it would appear unlikely that photoinhibition alone could be responsible for the observed mortality in Mylar-screened treatments. The short wavelength UV-A emitted from the UV lamps used in these experiments may have been affecting other cellular processes or constituents.

Ongrowth of cultures irradiated over 24 to 48 h indicated that the growth rate of most species was unaffected by the UV-B irradiance to which they were subjected. This was observed even at irradiances which resulted in high mortality. The results indicate that cells which survived sustained their metabolism during irradiation and were then able to resume normal growth, making their growth rate indistinguishable from both control cultures and those cultures which had received much lower UV-B irradiances. Thus, their contribution to the population after irradiance is dependent upon their survival rather than on any persisting consequence of the UV-B dose received.

Estimates of mixing times from the surface layer to a depth of 10 m range from 30 min to hundreds of hours (Denman and Gargett 1983; Karentz 1991). Because of the stability of the MIZ, phytoplankton are subjected to little darkness during late spring and summer (Sakshaug and Skjoldal 1989; Lizotte and Sullivan 1991; Veth 1991). Phytoplankton above the pycnocline will be exposed to changes in UV-B irradiances over periods greater those used in our experiments (24 h). Therefore, these experimental results can only be considered as indicative of natural conditions. The two diatom species that received continuous irradiation at various UV-B irradiances for a 24 h period showed a high tolerance to such exposure. Dark-dependent DNA repair-processes have been cited as fundamentally important to many organisms (prokaryotes, plants and animals) for repair of UV-B induced damage (Harm 1980). Over the 24 h duration of this experiment they appeared to play little part in the survival and reproduction of *Nitzschia lecointei* and *Stellarima microtrias* denied a dark period.

Karentz et al. (1991 a) found that smaller cells with a greater surface area:volume ratio were more sensitive to UV than larger cells. Our results are not consistent with this proposal. The survival of smaller species such as *Nitzschia lecointei* with a surface area to volume ratio of ~ 0.94 was equivalent to that of *Proboscia inermis* with a surface area to volume ratio of ~ 0.20. The observations of Karentz et al. (1991 a) may have been due to cell size and volume affecting sinking rates, which in turn would have affected their relative exposure to UV-B (Denman and Gargett 1983, Karentz 1991, Thompson et al. 1991, Veth 1991).

Conclusions

Our results show that over a 24 to 48 h period, at least some diatoms tolerate levels of UV-B that are considerably higher than the irradiances received in Antarctic surface waters in the austral spring of 1989. The amount of UV-absorbing compounds in the diatom species investigated was much less than that observed in the prymnesiophyte *Phaeocystis*, but their UV-B tolerance exceeds that of *Phaeocystis*. Thus, in constrast to the previously proposed scenario of Marchant and Davidson (1991), any pronounced increase in Antarctic UV-B levels may favour diatoms at the expense of *Phaeocystis*. There is, however, little direct evidence of changes in species composition in the Southern Ocean. The high tolerance of UV-B radiation by the phytoplankton species we have studied suggests that major changes in phytoplankton species composition as a result of extensive UV-B induced mortality are unlikely.

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