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RESEARCH ARTICLE

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Limited effects of Antarctic ozone depletion on sea urchin development

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Abstract The sea urchin, *Sterechinus neumayeri*, has a circumpolar distribution and is an abundant species in benthic communities of the Antarctic. Reproduction occurs during austral spring, when ozone concentrations over the past 25 years have been reduced by 50% or more, potentially exposing the planktonic embryos and larvae to elevated levels of UVB. During spring of 1996, cultures of *S. neumayeri* embryos incubated under ambient and partitioned sunlight (minus UVB) at static depths between 0 and 7 m were analyzed for DNA damage [cyclobutane pyrimidine dimers (CPDs)] and morphological abnormalities. At 0-m and 1-m depths, nearly 100% of embryos developed abnormally, even under UVB-shielded conditions where little or no DNA damage accumulated. At depths >3 m, reduced or no abnormality was evident and DNA damage was negligible. Although UVB contributed to 0–65% of solar-induced abnormalities, the mean contribution was $11 \pm 17\%$ and UVB was not primarily responsible for observed defects in urchin development. Moreover, developmental responses were not linearly related to ambient UVB gradients as might be expected, but are better characterized relative to threshold levels of total UVB exposure. Accumulated exposures of ≤ 25 kJ m^{-2} ambient UVB caused minimal DNA damage and allowed normal embryological development to proceed. Higher UVB exposures (especially ≥ 80 kJ m^{-2}) precluded normal development. An ancillary threshold limit of 17 CPDs mb^{-1} has been identified as the level of DNA damage that proscribes abnormal development. While higher wavelengths of UVA and visible light are not affected by ozone concentration and do not initiate significant CPD DNA damage, they did interfere significantly with the embryological development of *S. neumayeri*. It is concluded that exposure to increased UVB during recent Antarctic ozone-depletion cycles probably has only a small degree of impact relative to the magnitude of other solar effects on the developmental success of *Sterechinus* embryos, or compared to spawning seasons before ozone depletion (i.e., years prior to 1978).

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Introduction

Antarctic ozone depletion and biological effects of UV exposure

Springtime ozone depletion over Antarctica has been occurring for nearly 25 years and a definitive assessment of the ecological impact is still elusive (Weiler and Penhale 1994; Vernet and Smith 1997; Karentz and Bosch 2001; Karentz 2003). While modeled predictions based on compliance with Montreal Protocol standards for release of ozone-destroying compounds provide an optimistic view of ozone layer recovery, recurrent springtime depletion and accompanying increases of ultraviolet B radiation [UVB (280–320 nm)] in Antarctic environments are expected to continue for at least several decades (Montzka et al. 1999). Potential links between ozone depletion and global warming suggest an even longer protraction of austral ozone-depletion cycles (Shindell et al. 1998; Hartmann et al. 2000).

The detrimental effects of UV exposure to organisms has been recognized since at least the late 1800s (Downes and Blunt 1877). UV is absorbed by many biological

molecules and causes molecular damage that can impair vital physiological functions. DNA damage in particular is a ubiquitous result of UV exposure and a variety of DNA lesions can be caused by UV radiation (Harm 1980; Mitchell and Karentz 1993; Friedberg et al. 1995; Mitchell 1996). Cyclobutane pyrimidine dimers (CPDs) are the most common form of UV-induced DNA damage and unrepaired CPDs contribute to debilitation, mutagenesis and death. Numerous reviews detailing the biological effects of UV exposure on aquatic organisms are available (e.g., Calkins 1982; Young et al. 1993; Karentz et al. 1994; Weiler and Penhale 1994; De Mora et al. 2000; Häder et al. 2003). Historically, research on the effects of increased UVB on Antarctic marine organisms has focused on the productivity of marine phytoplankton and results have consistently indicated that low levels (typically $<15\%$) of UVB inhibition of photosynthesis occur (Smith et al. 1992; Arrigo 1994; Neale et al. 1998). Studies of Antarctic bacterioplankton have shown similarly small UVB effects (Helbling et al. 1995; Huot et al. 2000; Buma et al. 2001). However, adequate evaluation of ecosystem response has not been possible with these data sets, in part because quantitative linkages between primary and secondary production in the Southern Ocean are not known. Also, the full assessment of the ecological aspects of ozone depletion and related increases in UVB stress must include investigations of species at higher trophic levels (Bothwell et al. 1994).

Ecology of *Sterechinus*

The sea urchin, *Sterechinus neumayeri*, has a circumpolar distribution and is an abundant species in Antarctic shelf (<450 m) benthic communities (Dell 1972; Brey and Gutt 1991; Pawson 1994). A calcareous body wall and the over-lying water column provide substantial UV protection to adults (Karentz 1994; Karentz et al. 1997). *S. neumayeri* spawns during the austral spring and early summer with planktonic embryos and larvae developing for over 2 months before reaching a stage that is competent to settle in a benthic habitat and metamorphose into an adult (Bosch et al. 1987; Stanwell-Smith and Peck 1998). These early reproductive events overlap with the annual ozone-depletion cycle (Farman et al. 1985; Solomon 1999) and gametes, embryos and larvae are potentially exposed to elevated UVB. Generally, early life history stages are considered to be more vulnerable to UV exposure because of their small size and lack of UV-shielding outer layers (Mitchell and Hartman 1990; Dring et al. 1996; Huovinen et al. 2000; Cordi et al. 2001; Browman 2003). For *Sterechinus*, adults differentially accumulate UV-absorbing mycosporine-like amino acids (MAAs) in various tissues, with highest concentrations in ovaries and eggs (Karentz et al. 1997). Zygotes and embryos retain MAAs and thus may be provided with some level of UV protection during development. In order to assess the extent to which ambient UV exposure might interfere with sea urchin development and eventual recruitment into adult populations, a series of in situ experiments were conducted in two austral spring seasons during annual ozone-depletion cycles. Results from a subset of experiments from one season are presented here and indicate that while sunlight severely affects embryos in shallow surface waters, ozone depletion probably has a minimal effect on the development and survival of Antarctic urchin populations.

Materials and methods

Location

This study was conducted in October and November 1996 in Hero Inlet, Anvers Island, Palmer Archipelago, Antarctic Peninsula (64° 46′ S, 64° 3′ W). Hero Inlet is adjacent to Palmer Station, a US research facility in the vicinity of Arthur Harbor and Bonaparte Point.

Ozone

Daily images of column ozone concentrations during the study period were available at the U.S. National Aeronautics and Space Administration (NASA) web site (http://jwocky.gsfc.nasa.gov) and these were accessed routinely during the field season. A compilation of daily values for stratospheric ozone concentrations over the study area were provided from the Total Ozone Mapping Spectrometer (TOMS) mounted on the Earth Probe Satellite.

Solar radiation

Intensities of incident UVB, UVA and the 400–600 nm subset of visible (VIS) radiation (designated $VIS_{400–600}$ as the full range of VIS is 400–750 nm) were obtained from the NSF Polar Programs UV Radiation Monitoring Network operated by Biospherical Instruments, Inc. (Booth et al. 1994). The network spectroradiometer at Palmer Station performed hourly scans of incident solar radiation. Data are available at the Biospherical Instruments, Inc. web site (http://www.biospherical.com). Instantaneous values were integrated across each hour to calculate total incident light within the UVB, UVA and $VIS_{400–600}$ wavebands for each experimental period. Biological weighting functions for DNA (Setlow 1974) and UV damage to early developmental stages of the northern anchovy, *Engraulis mordax* (Hunter et al. 1979; Smith and Baker 1982), were also obtained from the Monitoring Network databases.

There is not a consistent demarcation of UVB and UVA wavebands in the literature. In 1999 the International Commission on Illumination reaffirmed a 1930's definition of UVB as 280-315 nm (CIE 1999). However, in reports describing UV effects on organisms, the waveband of 280–320 nm is most commonly cited for UVB, with UVA ranging from 320–400 nm. In the experiments described here, polyester film (Mylar D, E.I. Dupont de Nemours) was used as a cutoff filter to eliminate a large proportion of UVB selectively from test exposures in an effort to determine UVB effects. Mylar D has a 50% cutoff at 318 nm (Karentz and Lutze 1990); therefore, to quantify the UV exposures most accurately and interpret UV effects observed in our experiments, all calculations of UVB indicated here are for the wavelength range 290–318 nm and UVA is equivalent to 318–400 nm.

Attenuation coefficients (k_λ, where λ indicates the wavelength in nm used to calculate k) for the water column were determined from data obtained with a Biospherical PUV-501 underwater profiling spectroradiometer with sensors for 308 nm, 320, nm, 340 nm,

380 nm and $VIS_{400–600}$ (Biospherical Instruments). Underwater measurements were taken periodically during each experiment and mean values for k across the incubation period were used to estimate the total exposure at each depth during each experiment relative to the concurrent incident light values from the NSF UV Radiation Monitoring Network mentioned above. Calculations of exposures at each depth were made for UVB using k_{308}; UVA using the mean of k_{320} and k_{340} for the 318–360 nm waveband, and k_{380} for the 360–400 nm waveband; and $VIS_{400–600}$ using $k_{400–600}$.

Weather

Sky-cover and sea-ice data were provided by Palmer Station weather operations, a component of the World Meteorological Organization World Weather Watch.

Experimental protocol

Adult urchins were collected by SCUBA diving from subtidal areas in the immediate vicinity of Palmer Station (Hero Inlet and Arthur Harbor). Urchins were placed in indoor flowing-seawater tanks plumbed directly with water from Arthur Harbor and animals were spawned within a few days of collection. For each experiment, eggs from two or more females were pooled and fertilized with sperm from a single male. Zygotes were used immediately for in situ incubations in Hero Inlet or maintained in stirred 20-l UV-transparent culture chambers (UVT Plexiglas, Rohm Hass) under 50% ambient incident light for 1–2 days until used for experiments. Shading to 50% ambient radiation was provided by one layer of neutral density fiberglass screen.

For each experiment, cultured zygotes or early-cleavage embryos from the same fertilization batch were sealed into UV-transparent polyethylene bags (Whirlpak, Nasco) that were then fastened to PVC frames (Karentz and Lutze 1990). The frames were suspended vertically at five discrete depths (0, 1, 3, 5 and 7 m) in the water column of Hero Inlet. Surface floats were attached to an overhead guard line to prevent cultures from drifting away and a lead weight at the bottom of the assembly provided stability and kept the array vertical. Frames were protected as much as possible from light exposure during deployment and retrieval. Incubations lasted 3–6 days (Table 1).

At each depth, three exposure treatments with four replicate cultures each were deployed: (1) unshielded bags received full-spectrum sunlight (UVB + UVA + VIS), (2) polyester film filters (0.002-mil Mylar D) wrapped around bags attenuated nearly all UVB wavelengths (< 318 nm) so that cultures were exposed only to UVA + VIS, and (3) bags enclosed in aluminum foil provided dark control conditions. For absorbance spectra of the polyethylene and polyester materials used see Karentz and Lutze (1990).

Nine experiments were successfully completed, two under "normal" ozone levels of 319 and 339 Dobson units (DU), with incident UVA to UVB ratios of 36 and 44, respectively; and seven experiments were conducted under depleted ozone columns of 200–224 DU, with an incident ratio of UVA to UVB of 23–25. After incubation in situ, cultures were subdivided into samples for microscopic analyses of developmental abnormalities and for molecular analyses of DNA damage as explained below.

Morphological observations

The number of dead and abnormally developing embryos, blastulas or gastrulas in each replicate of each treatment was determined within a few hours of the termination of an experiment by direct microscopic observation at magnifications of 100× and 400×. Three or four replicate counts were made from each of the four replicate cultures under each light regime and depth. Abnormal embryos were identified by anomalies in the size and arrangement of blastomeres. Blastulas and gastrulas were counted as abnormal if they showed one or more of the following developmental aberrations: pronounced thickening of the blastodermis in combination with reduction of the blastocoel, abnormal development of primary mesenchyme cells, occlusion of the blastocoel by cellular debris, exogastrulation or other forms of aberrant archenteron development.

DNA damage analyses

Embryos were analyzed for DNA damage by radioimmunoassay to quantify the frequencies of CPDs (Mitchell 1999). Briefly, embryos were concentrated by centrifugation and immediately frozen in a dry ice/methanol bath followed by storage at −70° C. DNA was extracted with a standard sequence of Tris-saturated phenol, phenol/chloroform/isoamyl alcohol and chloroform/isoamyl alcohol (Maniatis et al. 1982). Separation of aqueous and organic phases was facilitated with Phase-lock Gel I (light) (5 Prime-3 Prime, Colo., USA). Damage in extracted DNA was quantified by competitive binding of radio-labeled antibodies for CPDs. The concentration of CPDs was calculated per megabase of DNA (CPDs mb^{-1}).

Calculations and comparisons

Unless otherwise indicated, data presented on embryo morphology (normal versus abnormal development) and DNA damage (CPDs mb^{-1}) have been standardized against dark controls. In order to evaluate the contribution of UVB to the biological responses monitored, the percentage enhancement of normality ($\%_{enh}$) and percentage reduction in CPDs mb^{-1} ($\%_{red}$) resulting from the removal of UVB wavelengths by Mylar filters were calculated as follows:

$$\%_{enh} = ((value_{UVA+VIS}/value_{UVB+UVA+VIS}) * 100) - 100$$
$$\%_{red} = 100 - ((value_{UVA+VIS}/value_{UVB+UVA+VIS}) * 100)$$

where, $value_{UVA+VIS}$ is the response observed under the polyester filter (i.e., no UVB exposure) and $value_{UVB+UVA+VIS}$ is the response observed under full solar exposure. (Note that $\%_{enh}$ has no upper limit, but the maximum value for $\%_{red}$ is 100%.)

Table 1 Experiment number, developmental stage at start of each incubation, dates of experiments and mean ozone column concentrations during each in situ incubation of *Sterechinus neumayeri* embryos in Hero Inlet, Anvers Island, Antarctica

Experiment number	Initial stage	Start date	End date	Mean $[O_3]$ (Dobson units)
1[a]	Morulas	29 October	2 November	319 ± 40
2	Zygotes	2 November	7 November	339 ± 58
3	Zygotes	8 November	13 November	203 ± 15
4	8–32 Cells	9 November	14 November	200 ± 13
5	Zygotes	9 November	14 November	200 ± 13
6	Zygotes	18 November	24 November	221 ± 23
7	4–8 Cells	18 November	24 November	221 ± 23
8	Zygotes	29 November	5 December	224 ± 18
9	Zygotes	29 November	5 December	224 ± 18

[a]No DNA damage data are available for experiment number 1

To evaluate possible fit to existing action spectra relating to DNA damage and development of the northern anchovy, values for $\%_{enh}$ normality and $\%_{red}$ DNA damage for each incubation period were plotted against the mean daily values of the Setlow DNA (1974) and Hunter et al. anchovy (1979) weighting functions as calculated in the NSF UV Radiation Monitoring Network database. Surface data (0 m) were analyzed for DNA damage, and the 3-m values were used for morphological comparisons to the modeled action spectra, because these depths exhibited the greatest differentials between light treatments for the two endpoints measured.

Results

Ozone, solar radiation and weather

During the experimental incubation periods, ozone levels ranged from a minimum of 186 DU to a maximum of 405 DU over the study area (Fig. 1, Table 1). Mean values for individual experiments ranged from 200 to 339 DU. The ellipsoidal shape and the rotational pattern of the polar vortex and variable cloud cover resulted in local daily fluctuations of UVB intensities over the Palmer Archipelago (Figs. 1, 2, 3).

Total incident radiation during the incubation periods varied from 77 to 325 kJ m^{-2} for UVB, 2,772 to 7,991 kJ m^{-2} for UVA, and 13,161 to 39,994 kJ m^{-2} for $VIS_{400–600}$ (Table 2). Day lengths increased and minimum daily solar-zenith angles decreased as the summer solstice approached. The majority (75%) of incubation days were overcast with ≥80% cloud cover typical of spring weather along the Antarctic Peninsula (Fig. 3) (Karentz et al. 1997). Lowest instantaneous incident UVB intensities (< 100 μW cm^{-2} s^{-1}) occurred in late October and early November during experiments number 1 and number 2 when ozone concentrations were the highest (Fig. 1). UVA and $VIS_{400–600}$ levels were also lowest at this time ($< 3{,}000$ and $< 12{,}000$ μW cm^{-2} s^{-1}, respectively).

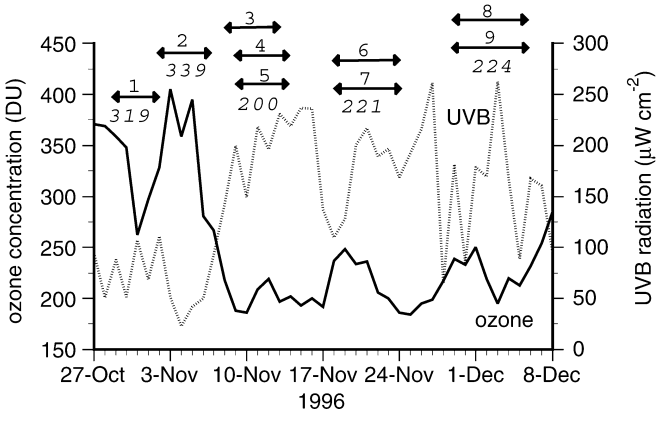

Fig. 1 Variation in column ozone concentration and noontime incident UVB (290–318 nm) during spring 1996 over Hero Inlet, Anvers Island, Antarctic Peninsula. *Horizontal arrows* indicate time periods for individual in situ incubation experiments (see Table 1); *numbers above arrows* identify the experiment number; *italicized numbers below arrows* indicate the mean ozone concentration during each incubation period

Mean attenuation coefficients for UVB in Hero Inlet ranged from 0.39 to 0.64 across the experimental incubation periods (Table 2 and Fig. 4A). Mean attenuation coefficients for 320 nm ranged from 0.27 to 0.51, for 340 nm from 0.15 to 0.44, for 380 nm from 0.11 to 0.31 and for VIS from 0.09 to 0.28. During all experimental incubations, the ratio of UVA to UVB increased exponentially with depth (Fig. 4B). This trend was more pronounced for experiments number 1 and number 2 when ozone levels were highest (> 300 DU). In experiment number 1, the ratio increased from 36 at the surface to 424 at a depth of 7 m. In later experiments (numbers 3–9) when ozone levels were lower (200–224 DU), the mean ratio of UVA to UVB was 23 ± 1 at the surface and increased to 154 ± 2 at 7 m.

Biological responses

In the dark control cultures, the mean percentage of normally developing embryos across all depths was $77 \pm 9\%$ [$n = 163$ (note that some samples were lost in the

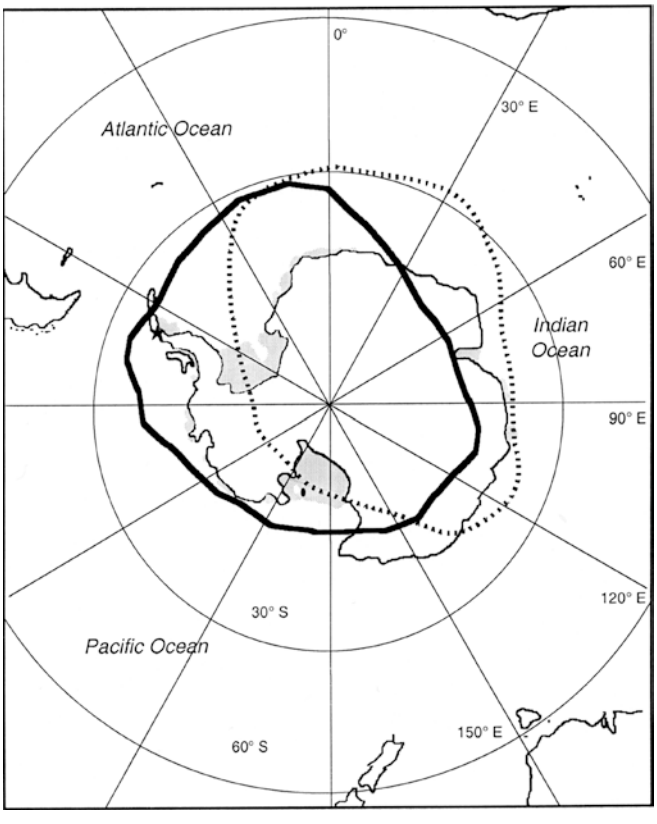

Fig. 2 Orientation of the polar vortex and 200-DU (Dobson unit) isolume of column ozone concentrations over Antarctica on 3 November 1996 when the study site was "outside" of the area of ozone depletion (*broken line*) and on 2 December 1996 when a depleted ozone column was overhead (*solid line*). The *star* symbol indicates the location of Palmer Station along the Antarctic Peninsula; *shaded coastal areas* designate permanent ice shelves. Data are from the NASA web site for the Earth Probe Satellite (http://toms.gsfc.nasa.gov)

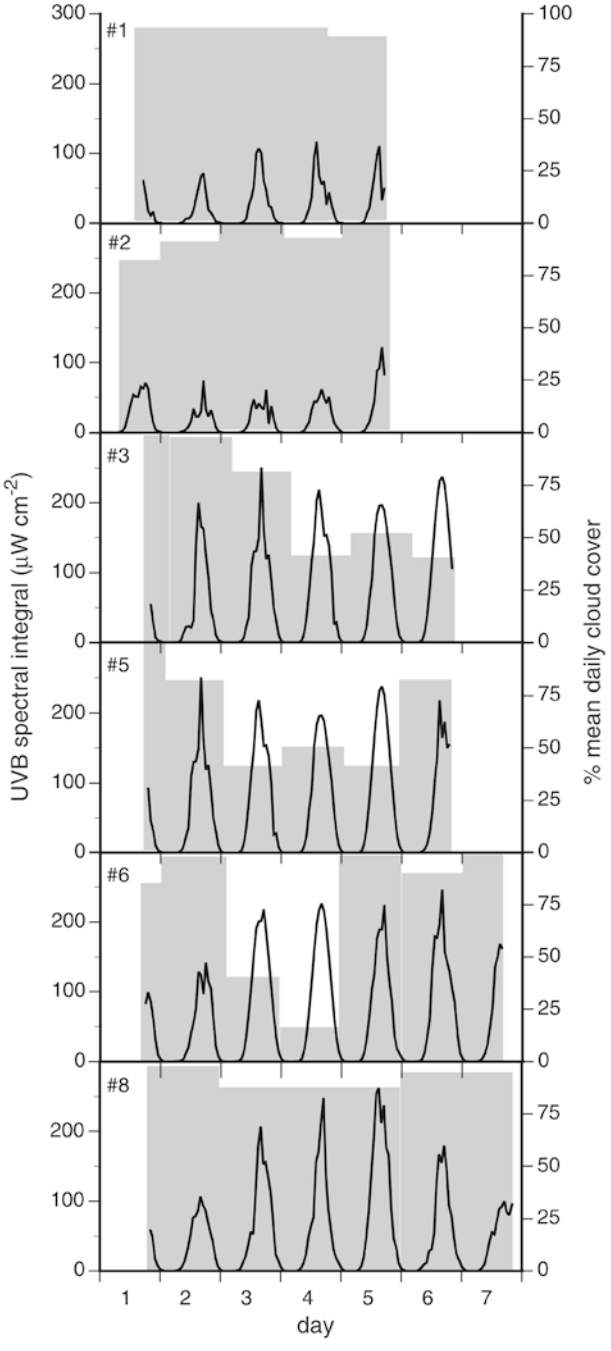

Fig. 3 Hourly (not integrated) incident UVB (290–318 nm) intensities during each incubation experiment (see Table 1). *Experiment numbers* are indicated on each panel. Incubation times for experiments number 4 and number 5 differed by only 0.5 h and only data for experiment number 5 are shown; experiments number 6 and number 7 differed by 6 h and only data for experiment number 6 are shown; experiments number 8 and number 9 had identical incubation periods. *Shaded areas* represent the percentage mean cloud cover relative to clear sky for each day

field or the laboratory)] and the mean for background DNA damage was 6 ± 5 CPDs mb^{-1} ($n = 146$); one anomalous value of 118 CPDs mb^{-1} was eliminated from this calculation. There were no differences between the 0-m and 7-m dark control samples; therefore, there is no apparent effect of water pressure on embryo developmental patterns or the amount of non-UV-induced CPD formation. Embryological development of *S. neumayeri* was strongly influenced by both full-spectrum solar exposure and UVB-filtered solar radiation to depths of 3–5 m.

Surface intensities of full-spectrum or UVB-filtered solar radiation caused obvious morphological aberrations, temporal delays in the developmental sequence and death. The highest UV exposures caused death or inhibited early cleavage events, while lower-level exposures were more likely to cause irregular cleavages and abnormal morphologies of blastulas and gastrulas (Fig. 5). The most frequently observed abnormalities in these stages were aberrations in the development of primary mesenchyme, thickening of the blastoderm and occlusion of the blastocoel by abnormal cells and cell debris. A small proportion of UV-altered blastulas and gastrulas developed into four-arm plutei. However, these larvae showed gross morphological and mesenchyme abnormalities that most likely would preclude successful completion of development to the adult stage.

While UVB contributed to developmental abnormalities to a depth of 5 m, quantifiable UVB damage to DNA was limited to the upper 3 m of the water column. A comparison of vertical profiles of normal embryos and concentrations of CPDs in full sunlight and UVB-shielded exposures indicates the contribution of UVB to developmental irregularities in whole embryos and DNA damage to cells (Figs. 6, 7). At the surface under full sunlight, abnormal development occurred in nearly all embryos (only 2% were normal in surface samples from experiment number 1, 0% were normal in all other incubations) (Fig. 6). Only in experiments number 1 and number 2 did shielding with polyester to remove UVB wavelengths enhance the normal development of embryos (from 2% and 0% to 39% and 55%, respectively). In experiments numbers 3–9 exposure to surface levels of UVA + VIS alone caused 100% abnormal development.

At a depth of 1 m, development was still greatly affected by sunlight exposure, with or without UVB. Under the full solar spectrum, seven out of nine 1-m incubations resulted in $< 4\%$ normal embryos; and even in the absence of UVB, four of nine had 100% abnormally developing embryos. In the upper meter of the water column, the largest proportions of normal embryos were observed in experiments number 1 and number 2 where incubations were made under the highest ozone and lowest UVB fluences. These incubation periods also had the lowest light levels for UVA and VIS because of sun angle and cloud cover.

The most marked developmental differences between full and filtered sunlight occurred at 3 m, although there was wide variation in the percentage enhancement of normal development across the experiments. For example, in experiment number 3 the percentage of normal embryos in the 3-m incubations increased from 6% under full sunlight to 71% with removal of UVB. In

Table 2 Integrated incident light values and associated mean attenuation coefficients (k_λ) for specific wavelengths ($\lambda = 308$ nm, 320 nm, 340 nm, 380 nm and 400–600 nm) for each experimental incubation period (see Table 1 for more details). Note that total incident UVB is calculated as the waveband 290–318 nm (see text for explanation)

Experiment number	Integrated incident light (kJ m^{-2})			Attenuation coefficients				
	UVB	UVA	$VIS_{400-600}$[a]	k_{308}	k_{320}	k_{340}	k_{380}	$k_{400-600}$
1[b]	77	2,772	13,161	0.64 ± 0.06	0.27 ± 0.01	0.19 ± 0.00	0.12 ± 0.00	0.09 ± 0.00
2	80	3,485	17,436	0.39 ± 0.03	0.26 ± 0.01	0.19 ± 0.01	0.11 ± 0.01	0.08 ± 0.01
3	274	6,327	33,262	0.43 ± 0.02	0.29 ± 0.01	0.15 ± 0.05	0.13 ± 0.01	0.11 ± 0.02
4	285	6,635	34,396	0.43 ± 0.02	0.29 ± 0.01	0.15 ± 0.05	0.13 ± 0.01	0.11 ± 0.02
5	289	6,746	35,003	0.43 ± 0.02	0.29 ± 0.01	0.15 ± 0.05	0.13 ± 0.01	0.11 ± 0.02
6	325	7,991	39,994	0.60 ± 0.04	0.51	0.44	0.28	0.23
7	312	7,574	37,880	0.60 ± 0.04	0.51	0.44	0.28	0.23
8	277	6,347	31,797	0.64 ± 0.03	0.51 ± 0.01	0.44 ± 0.01	0.31 ± 0.02	0.28 ± 0.04
9	277	6,347	31,797	0.64 ± 0.03	0.51 ± 0.01	0.44 ± 0.01	0.31 ± 0.02	0.28 ± 0.04

[a] *VIS* Visible light
[b] No DNA damage data are available for experiment number 1

other cases, such as experiment number 2, UVB fluences at 3 m were not very harmful and 96% of the embryos developed normally under full sunlight at this depth. At 5 and 7 m across all incubations, 68–100% of embryos develop normally under full sunlight and 89–100% show normal development with removal of UVB.

Differences in DNA damage were much more distinct than morphological abnormalities when comparing exposure under full sunlight to only the UVA + VIS portion of the solar spectrum (Fig. 7). UVB was the primary cause of DNA damage in the urchin embryos, with hardly any damage induced by higher wavelengths. At the surface, full solar exposure caused 13–273 CPDs mb^{-1} while only 1–18 CPDs mb^{-1} were observed when UVB wavelengths were removed. At 1-m depths, DNA damage was reduced to 9–79 CPDs mb^{-1} in full sunlight and 0–3 CPDs mb^{-1} under UVA + VIS. Below 3 m, CPD concentrations were low under both radiation treatments (0–2 CPDs mb^{-1} in all 5-m and 7-m samples).

Embryo responses within the water column do not readily correlate with ozone levels, nor are developmental irregularities or DNA damage incremented along gradients of UV intensity. These responses of the urchin embryos are best characterized relative to threshold levels of total radiation exposure (Figs. 8, 9). Similarly, morphological abnormalities are not directly correlated to levels of DNA damage, but are also best described by a threshold of cumulative CPDs mb^{-1} (not corrected against dark controls) (Fig. 10). When sunlight exposure (regardless of depth) included ≤ 25 kJ m^{-2} UVB ($n = 16$), 96 ± 10% of embryos developed normally. With UVB exposures > 25 and < 80 kJ m^{-2} ($n = 13$), normality decreased to 61 ± 33%. There is an apparent threshold exposure level of ≥80 kJ m^{-2} UVB beyond which embryos cannot achieve normal development. At these exposures, 99 ± 1% abnormality was observed ($n = 15$).

With the exception of surface data for experiment number 1, UVB-filtered exposures of < 3,000 kJ m^{-2} UVA ($n = 25$) did not greatly affect development (97 ± 5% of embryos are normal). When UVB-filtered treatments had UVA exposures > 3,000 and < 5,000 kJ UVA, morphological developmental responses were more variable ($n = 6$), ranging from 19% to 87% abnormality. With cumulative exposure to UVA > 5,000 kJ nearly all embryos exhibited abnormal development ($n = 12$). The single exception was experiment number 4 (1-m depth) where 27 ± 10% of embryos were normal. In all other UVA exposures > 5,000 kJ, normal development was 0%.

Under full-spectrum sunlight, dark-corrected DNA photoproduct concentrations had a maximum of 272 ± 34 CPDs mb^{-1} (experiment number 7, 0 m; Fig. 7). In the duplicate sample exposed to UVB-filtered radiation, the number of residual DNA photoproducts was reduced to 18 ± 5 CPDs mb^{-1}. The increase in accumulated DNA damage between these two treatments of the same culture (254 CPDs mb^{-1}) was associated with a 172 kJ m^{-2} cumulative UVB exposure under an ozone column of 221 ± 23 DU. In full-sunlight incubations at depths below 1 m ($n = 24$), the net yield of DNA damage was extremely low (0–6 CPDs mb^{-1}, mean = 1 ± 2 CPDs mb^{-1}). With the exception of the 18 ± 5 CPDs mb^{-1} mentioned above, photoproduct induction under exposures without UVB ($n = 39$) ranged from 0–7 CPDs mb^{-1} (mean = 2 ± 4 CPDs mb^{-1} with ≤ 1 CPD mb^{-1} in 75% of UVB-shielded samples).

The levels of abnormality and the amounts of accumulated DNA damage in different developmental stages (zygotes, 4–8 cells, 8–32 cells or morulas) do not show markedly different responses to sunlight exposure (Fig. 8). When morphological data were examined relative to the percentage change resulting from the removal of UVB wavelengths (i.e., the percentage enhancement of normality under UVB shielding relative to full-spectrum sunlight exposure), the more UVB removed, the higher the percentage of observed enhancement of normality in all samples across all stages (Fig. 9A). The curves have an exponential fit with

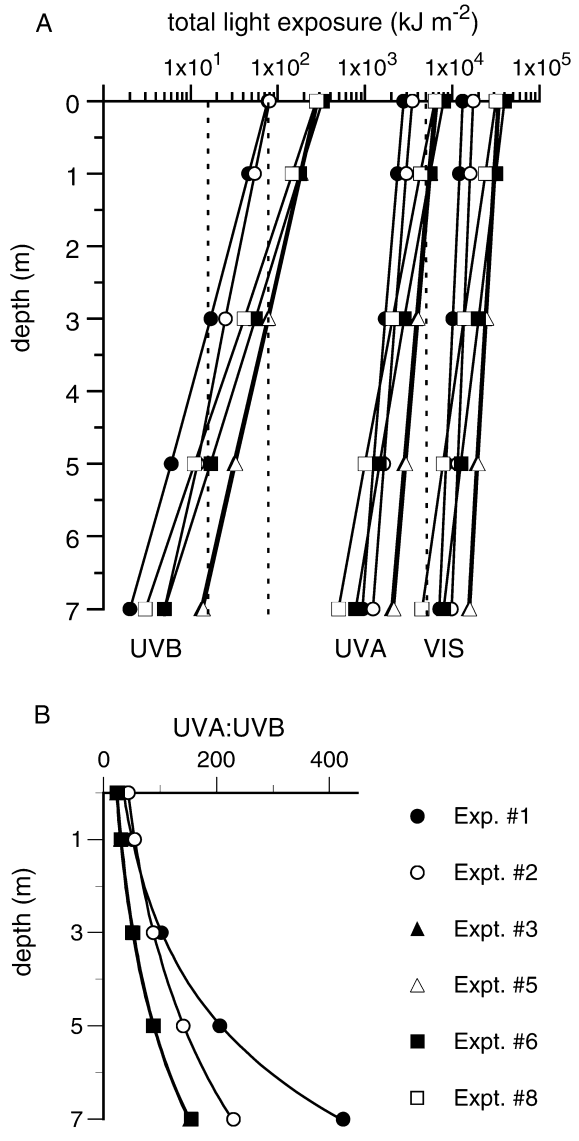

Fig. 4 **A** Total exposures for UVB, UVA and $VIS_{400–600}$ at each depth during the incubation periods of individual experiments. *Vertical dashed lines* indicate 25, 80 and 3,000 kJ m^{-2} values. Incubation times for experiments number 4 and number 5 differed by only 0.5 h and only data for experiment number 5 are shown; experiments number 6 and number 7 differed by 6 h and only data for experiment number 6 are shown; experiments number 8 and number 9 had identical incubation periods. **B** Changing ratios of UVA fluence to UVB at individual depths during each incubation period (data for experiments numbers 3–9 are nearly identical and appear as a single curve with *square symbols*)

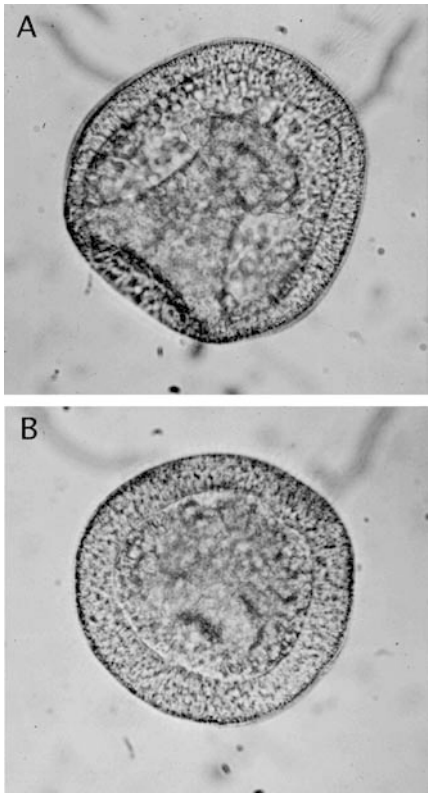

Fig. 5A, B Changes in embryo morphology caused by UV exposure. **A** Normal embryo (gastrula) of *Sterechinus neumayeri* incubated in the dark. **B** Typical abnormal morphology of embryos of same age after exposure to ≥80 kJ m^{-2} ambient UVB or >2,000 kJ m^{-2} UVA. Abnormal embryos have thickened blastoderm, reduced blastocoel diameter, proliferation of mesenchyme or cell debris in the blastocoel and show no evidence of gastrulation

regression coefficients of 0.72 for all data, 0.79 for zygotes, 0.65 for stages with 4–8 cells, 1.00 for stages with 8–32 cells and 0.93 for morulas. When levels of DNA damage under UVB shielding were examined, only zygotes exhibited a gradient change in percentage reduction of CPDs mb^{-1} with UVB removed (Fig. 9B). The regression coefficient was 0.73. The concentrations of CPDs were lower for zygotes under partitioned sunlight compared to full sunlight and UVA did induce some damage in this developmental stage. In experiments initiated with multicellular embryos (4–8 and 8–32 cells), there was nearly a 100% reduction (97 ± 3%) in DNA damage when UVB was removed.

At <80 kJ m^{-2} UVB or <5,000 kJ m^{-2} UVA (exposures without UVB), ≤ 17 CPDs mb^{-1} accumulated in embryos (Fig. 8A). At higher exposures and when absolute (not dark corrected) photoproduct concentrations were >17 CPDs mb^{-1}, 100% abnormal development or lethality occurred, regardless of whether damage was incurred from total sunlight or filtered exposures (Fig. 10).

Attempts to gain meaningful insight into morphological and DNA damage responses by correlating UVB exposures and weighting functions to the integral differences between vertical profile curves in Figs. 6 and 7 were unsuccessful. However, when data were selected from specific depths where maximum differences in responses to full and UVB-filtered sunlight were exhibited, both embryo morphology (3 m) and DNA damage (0 m) have close fits to the mean weighted exposure values calculated from the Setlow (1974) DNA and Hunter et al. (1979) anchovy larvae action spectra models (Fig. 11). The correlation coefficient for morphology was 0.92 to the DNA weighted dose and 0.93 to

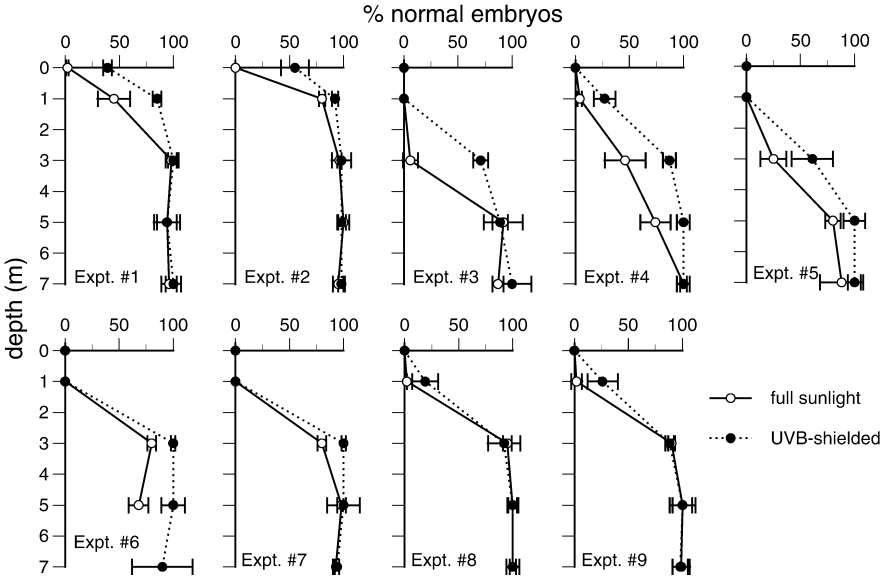

Fig. 6 Vertical profiles of percentages of normal embryos under full sunlight and UVB-shielded exposures. All values are standardized against dark controls. *Experiment numbers* are indicated in each panel

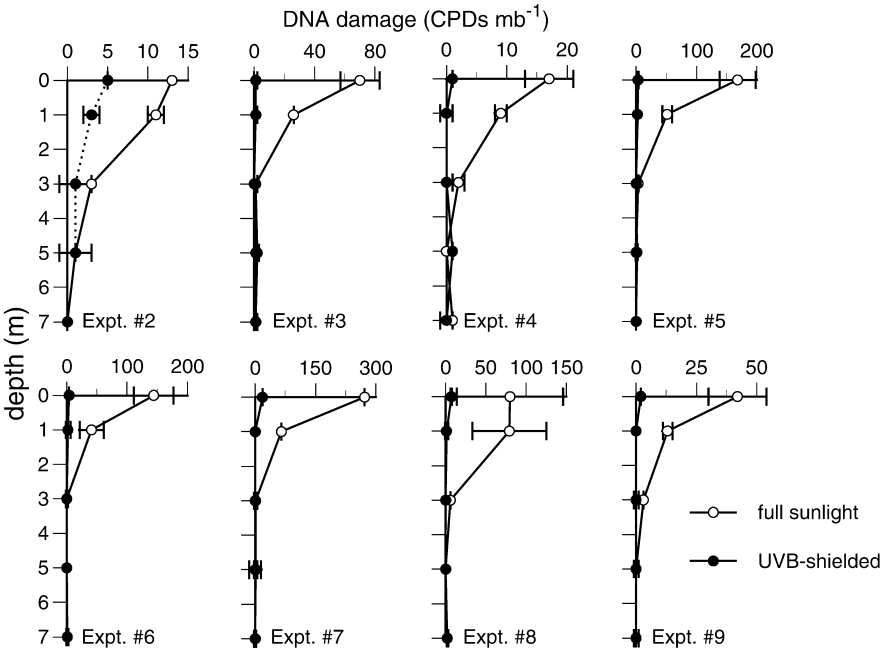

Fig. 7 Vertical profiles of DNA damage under full sunlight and UVB-shielded exposures. All values are standardized against dark controls. *Experiment numbers* are indicated in each panel. The scales of the x-axes are not uniform, in order to optimize presentation of differences in treatments and viewing of error bars. There are no DNA damage data for experiment number 1

the anchovy larval model, and correlations with DNA damage were 0.95 and 0.96, respectively.

Discussion

Summary

The results of this study indicate that (1) development in *Sterechinus* embryos is adversely affected by solar radiation to a maximum depth of 5 m under a depleted ozone column (<225 DU); (2) inhibition of normal development is not limited to UVB stress, but is initiated to a great extent by exposure to higher wavelengths of UVA and VIS; (3) accumulated exposures of <80 kJ m^{-2} UVB cause minimal DNA damage and allow normal embryological development to proceed ($81 \pm 29\%$ normal embryos observed), while UVB exposures >80 kJ m^{-2} preclude normal development ($1 \pm 1\%$ normal embryos observed); (4) there is a threshold limit of DNA damage (17 CPDs mb^{-1}) that prohibits normal development; and (5) *Sterechinus* could serve as a model system for studying UV effects in Antarctic invertebrate populations.

UV effects on development

Sea urchin development has been intensely studied over the past century and a great deal is known and understood about the controlled fate of the fertilized egg and

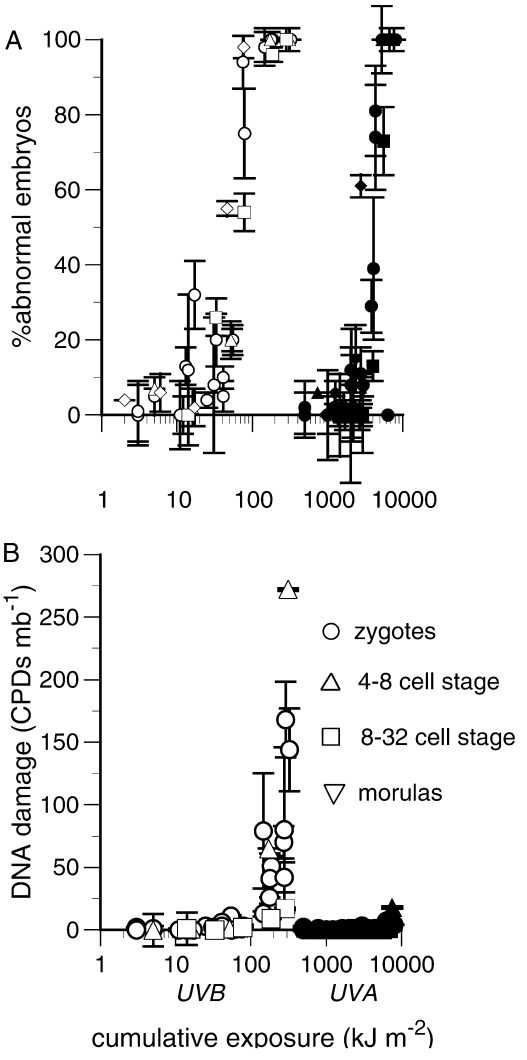

Fig. 8A, B Relationships of thresholds to cumulative exposures to UVB and UVA. **A** Embryo development. **B** DNA damage. In order to identify thresholds for individual wavebands, responses to full-spectrum sunlight (*open symbols*) are plotted against UVB values only and responses under UVB-filtered (UVA + VIS) radiation (*filled symbols*) are plotted against UVA intensities only. *Symbol shapes* (as shown) indicate development stage at start of individual experiments. There are no DNA damage data for experiment number 1

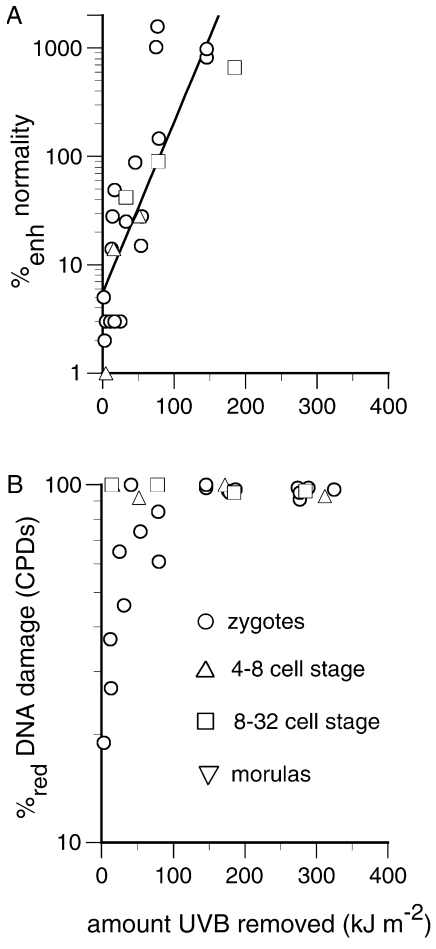

Fig. 9 **A** Percentage enhancement of normality relative to amount of UVB removed by Mylar filters. **B** Percentage reduction of CPDs relative to amount of UVB removed by Mylar filters. (Data that included zero values for CPDs under full sunlight or UVB-shielded treatments are not included in this figure)

subsequent embryonic cells (for recent reviews see Davidson et al. 1998; Angerer and Angerer 2000). Numerous gene expression markers have been identified for individual blastomeres and complex models have been developed for describing patterning mechanisms and signaling interactions across the landscape of the developing embryo (Davidson et al. 2002). It is well recognized that the precise choreography of gene regulation, apoptosis and directed cell movements for progression through the sequential stages of development and morphogenesis can be disrupted by a myriad of environmental factors such as temperature, mechanical pressure, chemicals (e.g., mitogens, teratogens, toxic compounds) and solar radiation.

The most obvious result of exposure to sunlight is an increase in morphological abnormalities during early development compared to dark controls. In the current study, the majority of embryos ($92 \pm 21\%$) incubated at depths of ≤ 1 m had aberrant morphologies that indicate severe impacts on cellular processes. UV exposure has been identified as the cause of a variety of disruptions to embryonic development in many organisms, including invertebrates, ascidians, amphibians and fish (e.g., Aboul-Ela 1958; Rustad 1975; Akimoto et al. 1983; Amemiya et al. 1986; Jeffery 1990; Adams and Shick 1996, 2001; Blaustein et al. 1997; Heasman 1997; Ankley et al. 2000). UV can initiate temporal delays in cell cycle kinetics, cleavage irregularities, deformities, defective post-embryonic development and ultimately cause death. The absorption of UV by cellular molecular targets, notably proteins and nucleic acids, can induce sufficient damage to disrupt or disable mechanisms that are essential in developmental processes. For example, UV has been found to incapacitate the molecular motors that drive microtubule assembly and cellular movement

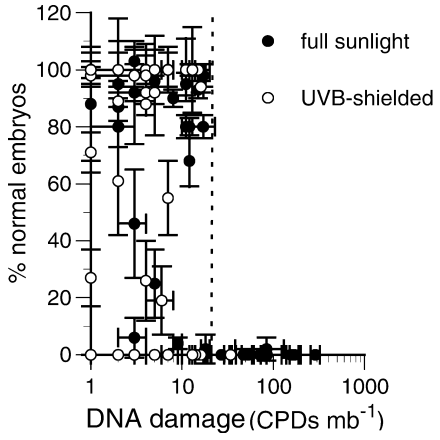

Fig. 10 The amount of DNA damage induced under full-spectrum sunlight and UVB-shielded exposures in relation to normal embryo development. (DNA damage data in this figure is not corrected for dark treatments to account for the absolute damage concentrations present in cells.) The *vertical dashed line* indicates the apparent developmental threshold level of 17 CPDs mb^{-1}

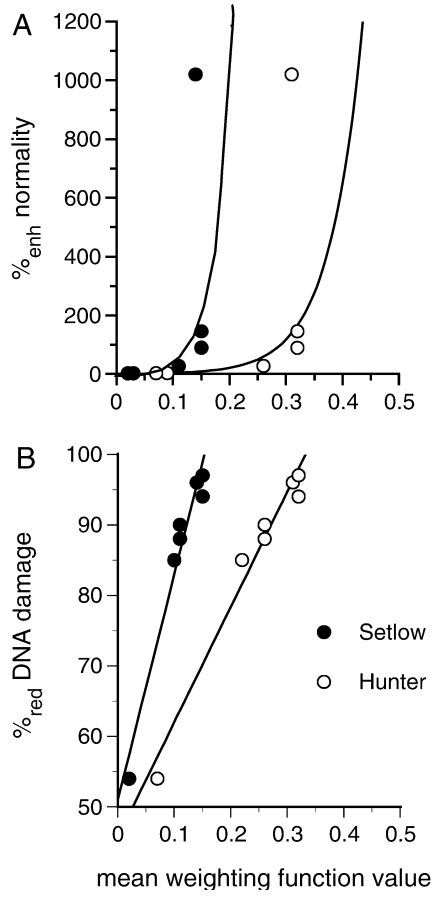

Fig. 11 **A** Relationship between mean daily Setlow (1974) DNA and Hunter et al. (1979) fish larval UVB weighting functions for each experimental period and percentage enhancement of normality of *Sterechinus* embryos at 3 m depths with removal of UVB. **B** Relationship between these weighting functions and percentage reduction of CPDs in embryos from 0-m incubations with removal of UVB

during epiboly in zebrafish embryos, thereby preventing migration of cells at the proper time to designated locations and rendering embryos defunct (Strähle and Jesuthasan 1993).

While *Sterechinus* embryos incubated at deeper depths (≥5 m) appeared relatively unaffected by full-spectrum solar exposure (92 ± 10% normal), the lack of teratological symptoms does not necessarily mean that development is not affected by UV exposure. There are very many genes that are sequentially expressed and down-regulated during development such that a gastrula that appears normal could still be unable to transition into a pluteus larva. Since exposed embryos were not routinely monitored after the in situ incubation periods, it is not possible to comment definitively on the ability of exposed "normal-looking" embryos to complete a full life cycle successfully. Cursory observations of the fate of UV-exposed embryos in culture did indicate that blastulas and gastrulas that exhibit morphological anomalies do not develop successfully beyond the early pluteus stage (Bosch and Karentz, personal observations).

UVA effects on sea urchin development

Morphological deformities were induced in *Sterechinus* embryos regardless of the spectral quality of the light treatment (with or without UVB). Exposure to UVB-filtered light at 0-m and 1-m depths most often resulted in 100% abnormality, indicating that higher wavelengths in the UVA band that are nominally affected by ozone depletion and do not cause excessive induction of CPDs (1 ± 3 CPDs mb^{-1}) are nonetheless capable of initiating severe developmental defects. Consequently, at these shallow depths, it is not possible to discern or distinguish the UVB contribution to observed morphological aberrations.

It is presumed that the impairment of development in embryos shielded from UVB is the result of UVA wavelengths and not exposure to VIS. While UVA is not a primary cause of DNA damage, UVA radiation is involved in photochemical reactions with water to generate reactive oxygen species (ROS) that can cause significant intra- and extracellular oxidative damage. Such damage can compromise structural components (e.g. membranes, spindle fibers); alter DNA, RNA and protein molecules involved in replication, transcription, translation and other enzymatic functions; interfere with gene expression; and result in retarded growth during crucial early developmental stages (Blondel and Favre 1988; Pizarro and Orce 1988; Ryter and Tyrrell 1998). In DNA molecules, ROS can induce photo-oxidation (e.g. formation of 8-oxodeoxyguanosine) and photo-hydration products (e.g., production of cytosine hydrates and thymine glycol) that were not quantified in the present study. UVA exposure has also been specifically implicated in debilitating cells of insect larvae by interfering with cell-cycle checkpoint mechanisms (Toyoshima et al.

2002). Conversely, there are beneficial aspects of UVA exposure (e.g., it is required for photoreactivation of CPD DNA damage) and so species responses to exposure vary considerably.

The observations of strong detrimental UVA effects on *Sterechinus* embryos are similar to most reports of UVA responses in marine and freshwater invertebrate and fish embryos and larvae. Negative UVA effects have been reported for embryos of the copepod, *Calanus finmarchicus* (Browman et al. 2000; Rodriguez et al. 2000a); American lobster, *Homarus americanus* (Rodriguez et al. 2000b); Japanese medaka, *Oryzias latipes* (Bass and Sistrun 1997); yellow perch, *Perca flavescens* (Williamson et al. 1997); and cichlid fish, *Cichlasoma nigrofasciatum* (Winckler and Fidhiany 1996a, b). UVA has been shown to have greater influence on inhibiting settlement of coral larvae than UVB (Baker 1995). Negative impacts of UVA on bacteria and phytoplankton in whole water samples have also been documented (Buma et al. 2001) and UVA causes lethality in the freshwater cladoceran *Daphnia pulivaria* (Zellmer 1998). Similar observations have been reported for amphibian development where UVB shielding had no ameliorating effect on hatching success of embryos (Blaustein et al. 1999). In contrast, while UVB exposure can be lethal for larvae of the Atlantic cod, *Gadus morhua*, UVA exposure has little or no significant effect on codfish development (Béland et al. 1999; Kouwenberg et al. 1999a).

It is of interest to note that the large majority (90%) of MAAs absorb maximally in the UVA waveband (Karentz 2001; Shick and Dunlap 2002). MAAs are UV-absorbing compounds that are generally believed to serve as UV sunscreens for aquatic organisms. While most studies have focused on UVB effects, the observations of high levels of negative UVA impacts and UVA-induced mortality may provide some insight into the adaptive significance of MAAs. The results of the present study further substantiate the importance of UVA protection in freshwater and marine species.

The spectral distribution of UVB and UVA radiation in incident sunlight is an important factor in determining the relative biological effects of exposure to these wavebands (Williamson et al. 2001). UVA fluences reaching the incubated samples in this study were at least an order of magnitude higher than the concomitant fluences of UVB. During periods of highest ozone (339 DU), incident UVB made up 2.2% of the full UV spectrum. Under depleted ozone (221 DU) the proportion nearly doubled to 3.9%. While this is a relatively small contribution to the total incident solar energy, the potential biological inactivation capacity of UVB wavelengths can be up to five orders of magnitude greater than that for equivalent quanta of UVA (Tyrrell and Pidoux 1987); therefore, small increases in UVB can have disproportionate biological effects. At shallow depths, this is apparently not an issue for *S. neumayeri* embryos as UVA levels alone were sufficiently high enough to cause excessive harm and usually complete mortality. In deeper water, the ratio of UVA to UVB increases and the overall impact of UVB within the water column is reduced. Full-spectrum solar-exposed embryos showed moderate UVB-induced abnormality compared to UVA teratogenesis with depth, and there was minimal UVB-induced DNA damage below 3 m.

Comparative ranges of DNA damage

CPD DNA damage in *Sterechinus* embryos was mostly limited to UVB exposure, as observed in other organisms (e.g., Tyrrell 1973; Buma et al. 1997). Also, the DNA photoproduct frequencies measured in *Sterechinus* are well within the range of values reported for DNA dosimeters and marine organisms studied under ambient conditions in Antarctica and at other latitudes (Table 3). However, unlike results from laboratory studies of diatoms where DNA damage (and therefore, UVB) is a primary determinant for inhibiting growth (Karentz et al. 1991; Buma et al. 1995, 1997), non-UVB solar stress is strongly debilitating for urchin development.

Table 3 Representative cyclobutane-pyrimidine dimer (CPD) concentrations reported for naked DNA molecules and marine organisms or communities under ambient sunlight exposures from collections and in situ experiments at various latitudes as indicated

Sample type	Source	Region	DNA damage (CPD mb^{-1})	Reference
DNA dosimeter	Incubation in situ for 1 day	Subtropics	Approx. 1,800	Aas et al. 1996
DNA dosimeter	Incubation in situ for 1 day	Polar[a]	Approx. 800	Meador et al. 2001
DNA dosimeter	Incubation in situ for 3 h	Tropics	Approx. 90	Visser et al. 1999
DNA dosimeter	Incubation in situ for 1 day	Polar	53	van de Poll et al. 2002
Bacterioplankton	Field collection	Subtropics	Approx. 800	Jeffrey et al. 1996b
Bacterioplankton	Field collection	Polar[a]	Approx. 200	Jeffrey et al. 1997
Bacterioplankton	Incubation in situ for 3 h	Tropics	Approx. 17	Visser et al. 1999
Picoplankton	Field collection	Tropics	20	Boelen et al. 2000
Phytoplankton	Field collection	Subtropics	Approx. 500	Jeffrey et al. 1996b
Phytoplankton	Field collection	Polar[a]	Approx. 250	Meador et al. 2001
Macroalgae (5 spp.)	Incubation in situ for 4 h	Polar	1–14	van de Poll et al. 2002
Fish larvae	Field collection	Polar[a]	350	Malloy et al. 1997
Fish larvae	Field collection	Temperate	9	Vetter et al. 1999

[a]From Antarctica

Rates of damage induction and repair in the incubated urchin embryos are not known, although there is evidence that developing embryos generally have more efficient DNA repair than differentiated cells of adults (Mitchell and Hartman 1990). In addition to the ubiquitous pathway of nucleotide excision repair, the DNA repair mechanism of photoreactivation has been observed in eggs and embryos of several sea urchin species: *Hemicentrotus pulcherrimus* (Ejima et al. 1984; Akimoto and Shiroya 1986), *Arbacia punctulata* (Marshak 1949) and *Strongylocentrotus purpuratus* (Wells and Giese 1950). Although highly probable, it has not yet been determined whether *Sterechinus* can also remove CPDs using the photoenzymatic repair process.

A maximum mean concentration of 273 ± 34 accumulated CPDs mb^{-1} was measured in full sunlight-exposed samples [at the surface (experiment number 7)] and a level of 17 CPDs mb^{-1} is identified as the threshold of damage beyond which all *Sterechinus* embryos develop with abnormal morphology. Very few other data are available for comparison to these results relative to evaluating biological endpoints and all are from artificial UVB exposures. Antarctic diatoms have species-specific lethal limits of 2–25 CPDs mb^{-1} (Karentz et al. 1991); a threshold of approximately 90 CPDs mb^{-1} has been determined for death of cultured goldfish cells (Shima and Setlow 1984); and approx. 20–23 CPDs mb^{-1} cause 10% mortality in fathead minnow embryos (Applegate and Ley 1988). In *Xenopus laevis* (frog), teratogenesis occurs in 20-60% of larvae with approx. 30 CPD mb^{-1} and unlike *Sterechinus,* the proportion of malformed embryos has a significant, but low ($r^2 = 0.51$), correlation with CPD concentration (Bruggeman et al. 1998). Identification of species-specific lethal limit loads for DNA damage would provide a convenient standard measure for evaluating relative tolerances to UV and assessing population responses to ambient UV levels.

Life history of *Sterechinus neumayeri* and distribution of embryos in the water column

S. neumayeri spawns in November–December and the relatively non-pigmented embryos and larvae are believed to develop in the water column for over 2 months before settling to undergo metamorphosis into juveniles. During this planktonic phase of the life cycle, eggs, embryos and larvae are potentially exposed to increased UVB under ozone-depleted conditions. Developing embryos and larvae of *Sterechinus* have been collected in coastal areas at depths ranging from just below the surface to < 1 m above the substrate at a 30-m depth (Bosch et al. 1987; Stanwell-Smith et al. 1999). Reported densities of early developmental stages in the plankton are ≤ 0.4 m^{-3} (Shreeve and Peck 1995; Stanwell-Smith et al. 1999); however, no urchin embryos or larvae were collected during extensive plankton sampling of the study area (Arthur Harbor) from August to December 1996 or August 1977 to February 1998 (Bosch and Karentz, personal observations).

There has been some suggestion that the negatively buoyant *Sterechinus* eggs might remain demersal for the pre-hatching period of development (5 days) and that as older embryos become motile they move into the water column (Marsh and Manahan 2000). If so, fertilized eggs and early (smaller) developmental stages that might be more vulnerable to UV would be exposed to minimum levels of solar radiation by remaining in the benthic habitat during initial cleavage events. The first few days after fertilization have been found to be the most vulnerable for copepod (*Calanus finmarchicus*) embryos (Kuhn et al. 2000) and the embryos of a temperate sea urchin (*Strongylocentrotus droebachiensis*) (Adams and Shick 2001). Several studies have determined that early smaller stages of development of invertebrates and fish are more sensitive to UV than older larger embryos and larvae (Karanas et al. 1979; Steeger et al. 1999; Leech and Williamson 2000), although this may not hold for all species (e.g., Kim et al. 2000). In Atlantic cod (*G. morhua*), embryos are most damaged by UVB exposure during gastrulation and later during hatching (Kouwenberg et al. 1999a). For *Sterechinus*, there does not seem to be any marked stage-specific difference in morphological responses to solar exposure; however, zygotes appear to be more susceptible to UVA-induced DNA damage than older embryos.

The depth-dependent effect of UV on planktonic organisms is an important ecological consideration that has been most difficult to evaluate. The photoproduct frequencies quantified in *Sterechinus neumayeri* embryos represent the net balance of rates of damage induction and repair at the time of collection (after 3–6 days of solar exposure). In contrast, the developmental abnormalities observed reflect the integrated response of embryos to the total period of radiation exposure. Unlike the static incubations utilized in our experiments, urchin embryos in nature are vertically transported through the water column on varying (and undetermined) time scales during the course of development and exposed to variable intensities and spectral qualities of light. Thus, under normal environmental conditions, the balance between UV-induced damage and repair may be quite different from what we are reporting.

Water column mixing has been shown to have a definite effect on accumulated DNA damage in bacterioplankton. On calm days that promote a stable water column, more DNA photoproducts accumulate in cells that are held near the surface, as opposed to windy days when cells are more rapidly and more frequently transported to deeper dimmer depths where repair can proceed under lower rates of damage induction (Jeffrey et al. 1996a, b; Buma et al. 2001). For phytoplankton and some zooplankton species (e.g., *C. finmarchicus*) rapid mixing rates increase exposure and cause more physiological inhibition and mortality than might occur on calm days (Neale et al. 1998; Zagarese et al. 1998; Kuhn et al. 2000). The static-depth incubations in our

experimental design do not account for accumulation or mitigation of damage by accelerated mixing regimes, but provide a closer proxy for calm days with little turnover. The experimental design employed provided a "worst case" scenario from the shallow incubations and a "best case" from the deeper low light exposures. Similarly, diel patterns of DNA damage and repair, where maximal damage is concurrent with highest midday irradiances, have been observed in bacterio- and icthyoplankton populations (Jeffrey et al. 1996b; Vetter et al. 1999), but such short-term changes were not accounted for in the present study. Since both dose rate (as affected by mixing and diurnal patterns) and cumulative dose are important parameters in determining the biological response (Damkaer et al. 1981; Kouwenberg et al. 1999b; Saito and Taguchi 2003), the data presented here allow for limited interpretation regarding the biology of ambient UV effects, but do provide for insights in evaluating the ecological impact of ozone depletion on Antarctic urchin populations.

Attenuation of UVB and impact of ozone depletion

While UVB wavelengths have been detected to 60 m in the Southern Ocean (Smith et al. 1992), observable biological effects have been limited to much shallower depths as seen in this and other studies (e.g., Karentz and Lutze 1990; Boucher and Prézelin 1996; Buma et al. 2001). In temperate areas where light attenuation is higher, mortality of invertebrate and fish eggs and embryos in the plankton occurs mostly in the upper 1 m of the water column (Béland et al. 1999; Browman et al. 2000; Aarseth and Schram 2002). Incident solar fluences in Hero Inlet were quite variable on short time scales (hours) and resulted in fluctuating radiation exposures during the considerably longer incubation periods (days) because of the diurnal progression of sun angles and dynamic weather conditions. Hydrographic factors that affect water column attenuation also vary unpredictably and atmospheric factors significantly modulate incident intensities of UV during the springtime ozone-depletion cycle. During this period, there is persistent cloud cover over most of the coastal regions of Antarctica, with the average number of cloudy days during August–November ranging from 23 to 28 per month (Karentz et al. 1997). The persistence of variable cloud cover obviates attempts to correlate biological effects of UVB with ozone depletion as day-to-day variation in biologically effective UV doses can be as high as 285% (Sobolev 2000). The occurrence of Antarctic ozone depletion also coincides with the presence of extensive sea ice covering the ocean surface, further minimizing the penetration of light into the water column. Therefore, there are a number of environmental parameters that can greatly reduce increased UV stress that might otherwise result from ozone depletion.

There are also many biological factors that can further mitigate the potential impact of UV exposure on planktonic organisms, including avoidance of light by negative phototaxis or positive geotaxis (Barile et al. 1994), presence of UV-opaque outer coverings or UV-absorbing compounds (Karentz 2001), DNA repair mechanisms (Mitchell and Karentz 1993), or timing of egg release and larval settlement (Gleason and Wellington 1995). While cumulative UV exposure is determined by the dose rate as regulated by clouds, ice and water column attenuation, the tolerance of an individual organism as determined by its inherent genetic capabilities is crucial to its survival, reproductive potential and maintenance of the population.

Conclusions

The restriction of solar effects on *Sterechinus* embryos to the upper 5 m of the water column and the strong relative impact of UVA wavelengths on the developmental process are major findings of this study. The identification of threshold levels of radiation exposure and CPD concentrations for defining morphological aberrations is also significant. While the extremely low observed densities of *Sterechinus* embryos and larvae in the water column make it difficult to assess the natural exposure regime, the observations reported here suggest that increased UVB during recent Antarctic ozone-depletion cycles may have had only a minor, if not negligible, impact relative to the magnitude of non-ozone-related solar effects on the developmental success of *Sterechinus* embryos. The impact of ozone depletion on the trophic dynamics of later larval development or recruitment to benthic adult populations is not known and these issues remain an important challenge for assessing ecosystem responses to ozone depletion on a global scale.

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References

Aarseth KA, Schram TA (2002) Susceptibility to ultraviolet radiation in *Calanus finmarchicus* and *Lepeophtheirus salmonis* and the adaptive value of external filtering (Crustacea: Copepoda). J Plankton Res 24:661–680

Aas P, Lyons MM, Pledger R, Mitchell DL, Jeffrey WH (1996) Inhibition of bacterial activities by solar radiation in nearshore waters and the Gulf of Mexico. Aquat Microb Ecol 11:229–238

Aboul-Ela IA (1958) Effects of ultraviolet radiation on oysters. Nature 181:1013

Adams NL, Shick JM (1996) Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin *Strongylocentrotus droebachiensis*. Photochem Photobiol 64:149–158

Adams NL, Shick JM (2001) Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin *Strongylocentrotus droebachiensis*. Mar Biol 138:267–280

Akimoto Y, Shiroya T (1986) UV-induced morphological abnormality and abnormal protein pattern, and their photoreversion in sea-urchin embryos (*Hemicentrotus pulcherrimus*). J Radiat Res 27:26–26

Akimoto Y, Shiroya T, Egami N (1983) Abnormal morphogenesis of sea urchin embryo induced by UV partial irradiation given at cleavage stage. J Radiat Res 24:197–202

Amemiya S, Yonemura S, Kinoshita S, Shiroya T (1986) Biphasic stage sensitivity to UV suppression of gastrulation in sea urchin embryos. Cell Differ 18:45–49

Angerer LM, Angerer RC (2000) Animal-vegetal axis patterning mechanisms in the early sea urchin embryo. Dev Biol 218:1–12

Ankley GT, Tietge JE, Holcombe GW, De Foe DL, Diamond SA, Jensen KM, Degitz SJ (2000) Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. Can J Zool 78:1092–1100

Applegate LA, Ley RD (1988) Ultraviolet-induced lethality and repair of pyrimidine dimers in fish embryos. Mutat Res 198:85–92

Arrigo KR (1994) Impact of ozone depletion on phytoplankton growth in the Southern Ocean: large-scale spatial and temporal variability. Mar Ecol Prog Ser 114:1–12

Baker A (1995) Solar UV-A inhibition of planula larvae in the reef-building coral *Pocillopora damicornis*. In: Gulko D, Jokiel PL (eds) Ultraviolet radiation and coral reefs. Technical report 41, Sea Grant Publication UNIHI-SEAGRANT-CR-95-03. Hawaii Institute of Marine Biology, University of Hawaii, pp 149–163

Barile PJ, Stoner AW, Young CM (1994) Phototaxis and vertical migration of the queen conch (*Strombus gigas linne*) veliger larvae. J Exp Mar Biol Ecol 183:147–162

Bass EL, Sistrun SN (1997) Effect of UVA radiation on development and hatching success in *Oryzias latipes*, the Japanese medaka. Bull Environ Contam Toxicol 59:537–542

Béland F, Browman HI, Rodriguez CA, St-Pierre J-F (1999) Effect of solar ultraviolet radiation (280–400 nm) on the eggs and larvae of Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 56:1058–1067

Blaustein AR, Kiesecker JM, Chivers DP, Anthony RG (1997) Ambient UV-B radiation causes deformities in amphibian embryos. Proc Natl Acad Sci USA 94:13735–13737

Blaustein AR, Hays JB, Hoffman PD, Chivers DP, Kiesecker JM, Leonard WP, Marco A, Olson DH, Reaser JK, Anthony RG (1999) DNA repair and resistance to UV-B radiation in western spotted frogs. Ecol Appl 9:1100–1105

Blondel MO, Favre A (1988) $tRNA^{Phe}$ and $tRNA^{Pro}$ are near-ultraviolet molecular targets triggering the growth delay effect. Biochem Biophys Res Commun 150:979–986

Boelen P, de Boer MK, Kraay GW, Veldhuis MJW, Buma AGJ (2000) UVBR-induced DNA damage in natural marine picoplankton assemblages in the tropical Atlantic Ocean. Mar Ecol Prog Ser 193:1–9

Booth CR, Luca TB, Morrow JB, Weiler CS, Penhale PA (1994) United States National Science Foundation's polar network for monitoring ultraviolet radiation. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation and biological research in Antarctica. American Geophysical Union, Washington, D.C., pp 17–37

Bosch I, Beauchamp KA, Steele ME, Pearse JS (1987) Development, metamorphosis and seasonal abundance of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri*. Biol Bull 173:126–135

Bothwell ML, Sherbot DMJ, Pollock CM (1994) Ecosystem response to solar ultraviolet-B radiation: influence of trophic level interactions. Science 265:97–100

Boucher NP, Prézelin BB (1996) Spectral modeling of UV inhibition of *in situ* Antarctic primary production using a field-derived biological weighting function. Photochem Photobiol 64:407–418

Brey T, Gutt J (1991) The genus *Sterechinus* (Echinodermata, Echinoidea) on the Weddell Sea shelf and slope (Antarctica)—distribution, abundance and biomass. Polar Biol 11:227–232

Browman HI (2003) Assessing the impacts of solar ultraviolet radiation on the early life stages of crustacean zooplankton and ichthyoplankton in marine coastal systems. Estuaries 26:30–39

Browman HI, Rodriguez CA, Béland F, Cullen JJ, Davis RF, Kouwenberg JHM, Kuhn PS, McArthur B, Runge JA, St-Pierre J-F, Vetter RD (2000) Impact of ultraviolet radiation on marine crustacean zooplankton and ichthyoplankton: a synthesis of results from the estuary and Gulf of St. Lawrence, Canada. Mar Ecol Prog Ser 199:293–311

Bruggeman DJ, Bantle JA, Goad C (1998) Linking teratogenesis, growth, and DNA photodamage to artificial ultraviolet B radiation in *Xenopus laevis* larvae. Environ Toxicol Chem 17:2114–2121

Buma AGJ, Vanhannen EJ, Roza L, Veldhuis MJW, Gieskes WWC (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. J Phycol 31:314–321

Buma AG, Engelen AH, Gieskes WWC (1997) Wavelength-dependent induction of thymine dimers and growth rate reduction in the marine diatom *Cyclotella* sp. exposed to ultraviolet radiation. Mar Ecol Prog Ser 153:91–97

Buma AGJ, de Boer MK, Boelen P (2001) Depth distributions of DNA damage in Antarctic marine phyto- and bacterioplankton exposed to summertime UV radiation. J Phycol 37:200–208

Calkins J (ed) (1982) The role of solar ultraviolet radiation in marine ecosystems. Plenum, N.Y.

CIE (1999) Standardization of the terms UV-A1, UV-A2 and UV-B. CIE (International Commission on Illumination) collection in photobiology and photochemistry number 134-1999. An abstract is available at http://www.cie.co.at. Cited 17 June 2003

Cordi B, Donkin ME, Peloquin J, Price DN, Depledge MH (2001) The influence of UV-B radiation on the reproductive cells of the intertidal macroalga, *Enteromorpha intestinalis*. Aquat Toxicol 56:1–11

Damkaer DM, Dey DB, Heron GA (1981) Dose/dose-rate responses of shrimp larvae to UV-B radiation. Oecologia 48:178–182

Davidson EH, Cameron RA, Ransick A (1998) Specification of cell fate in the sea urchin embryo: summary and some proposed mechanisms. Development 125:3269–3290

Davidson EH, Rast JP, Oliveri P, Ransick A, Calestani C, Yuh C-H, Minokawa T, Amore G, Hinman V, Arenas-Mena C, Otim O, Brown CT, Livi CB, Lee PY, Revilla R, Schilstra MJ, Clarke PJC, Rust AG, Pan Z, Arnone MI, Rowen L, Cameron RA, McClay DR, Hood L, Bolouri H (2002) A provisional regulatory gene network for specification of endomesoderm in the sea urchin embryo. Dev Biol 246:162–190

De Mora SJ, Demers S, Vernet M (eds) (2000) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge

Dell RK (1972) Antarctic benthos. Adv Mar Biol 10:1–216

Downes A, Blunt TP (1877) Researches on the effect of light upon bacteria and other organisms. Proc R Soc Lond 26:488–500

Dring M, Makarov V, Schoschina E, Lorenz M, Luening K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126:183–191

Ejima Y, Ikenaga M, Shiroya T (1984) Action spectrum for photoreactivation of ultraviolet-induced morphological abnormalities in sea urchin eggs. Photochem Photobiol 40:461–464

Farman JC, Gardiner BG, Shanklin JD (1985) Large losses of total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction. Nature 315:207–210

Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. American Society of Microbiology Press, Washington, D.C.

Gleason D, Wellington GM (1995) Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient. Mar Biol 123:693–703

Häder D-P, Kumar HD, Smith RC, Worrest RC (2003) Aquatic ecosystems: effects of solar ultraviolet radiation and interactions with other climatic change factors. Photochem Photobiol Sci 2:39–50

Harm W (1980) Biological effects of ultraviolet radiation. Cambridge University Press, Cambridge

Hartmann DL, Wallace JM, Limpasuvan V, Thompson DWJ, Holton JR (2000) Can ozone depletion and global warming interact to produce rapid climate change? Proc Natl Acad Sci USA 97:1412–1417

Heasman J (1997) Patterning the *Xenopus* blastula. Development 124:4179–4191

Helbling EW, Marguet ER, Villafañe VE, Holm-Hansen O (1995) Bacterioplankton viability in Antarctic waters as affected by solar ultraviolet radiation. Mar Ecol Prog Ser 126:293–298

Hunter JR, Taylor JH, Moser G (1979) Effect of ultraviolet irradiation on eggs and larvae of the northern anchovy, *Engraulis mordax*, and the Pacific mackerel, *Scomber japonicus*, during the embryonic stage. Photochem Photobiol 29:325–338

Huot Y, Jeffrey WH, Davis RF, Cullen JJ (2000) Damage to DNA in bacterioplankton: a model of damage by ultraviolet radiation and its repair as influenced by vertical mixing. Photochem Photobiol 72:62–74

Huovinen PS, Oikari AOJ, Soimasuo MR, Cherr GN (2000) Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. Photochem Photobiol 72:308–313

Jeffery WR (1990) Ultraviolet irradiation during ooplasmic segregation prevents gastrulation, sensory cell induction, and axis formation in the ascidian embryo. Dev Biol 140:388–400

Jeffrey WH, Aas P, Lyons MM, Coffin RB, Pledger RJ, Mitchell DL (1996a) Ambient solar radiation-induced photodamage in marine bacterioplankton. Photochem Photobiol 64:419–427

Jeffrey WH, Pledger RJ, Aas P, Hager S, Coffin RB, Von Haven R, Mitchell DL (1996b) Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation. Mar Ecol Prog Ser 137:283–291

Jeffrey WH, Miller RV, Mitchell DL (1997) Detection of ultraviolet radiation induced DNA damage in microbial communities of Gerlache Strait. Antarct J US 32:85–87

Karanas JJ, Dyke HV, Worrest RC (1979) Midultraviolet (UV-B) sensitivity of *Acartia clausii* Giesbrecht (Copepoda). Limnol Oceanogr 24:1104–1116

Karentz D (1994) Prevention of ultraviolet radiation damage in Antarctic marine invertebrates. In: Biggs RH, Joyner M (eds) Stratospheric ozone depletion/UVB radiation in the biosphere. Proceedings of NATO advanced research workshop. Springer, Berlin Heidelberg New York, pp 175–180

Karentz D (2001) Chemical defenses of marine organisms against solar radiation exposure: UV-absorbing mycosporine-like amino acids and scytonemin. In: McClintock J, Baker W (eds) Marine chemical ecology. CRC, Boca Raton, Florida, pp 481–520

Karentz D (2003) Environmental change in Antarctica: ecological impacts and responses. In: Huiskes AHL, Gieskes WWC, Rozema J, Schorno RML, van der Vies SM, Wolff WJ (eds) Antarctic biology in a global context. Backhuys, Leiden, pp 45–55

Karentz D, Bosch I (2001) Influence of ozone-related increases in ultraviolet radiation on Antarctic marine organisms. Am Zool 41:3–16

Karentz D, Lutze LH (1990) Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. Limnol Oceanogr 35:549–561

Karentz D, Cleaver JE, Mitchell DM (1991) Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation exposure. J Phycol 27:326–341

Karentz D, Bothwell ML, Coffin RB, Hanson A, Herndl GJ, Kilham SS, Lesser MP, Lindell M, Moeller RE, Morris DP, Neale PJ, Sanders RW, Weiler CS, Wetzel RG (1994) Impact of UVB radiation on pelagic freshwater ecosystems: report of working group on bacteria and phytoplankton. Arch Hydrobiol 43:31–69

Karentz D, Dunlap WC, Bosch I (1997) Temporal and spatial occurrence of UV-absorbing mycosporine-like amino acids in tissues of the Antarctic sea urchin *Sterechinus neumayeri* during springtime ozone depletion. Mar Biol 129:343–353

Kim GB, Lee RF, Mitchell DL (2000) Damage to grass shrimp (*Palaemonetes pugio*) embryo DNA by summer sunlight followed by DNA repair in the dark. Mar Biol 137:675–682

Kouwenberg JHM, Browman HI, Cullen JJ, Davis RF, St-Pierre J-F, Runge JA (1999a) Biological weighting of ultraviolet (280–400 nm) induced mortality in marine zooplankton and fish. I. Atlantic cod (*Gadhus morhua*) eggs. Mar Biol 134:269–284

Kouwenberg JHM, Browman HI, Runge JA, Cullen JJ, Davis RF, St-Pierre J-F (1999b) Biological weighting of ultraviolet (280–400 nm) induced mortality in marine zooplankton and fish. II. *Calanus finmarchicus* (Copepoda) eggs. Mar Biol 134:285–293

Kuhn PS, Browman HI, Davis RF, Cullen JJ, McArthur BL (2000) Modeling the effects of ultraviolet radiation on embryos of *Calanus finmarchicus* and Atlantic cod (*Gadus morhua*) in a mixing environment. Limnol Oceanogr 45:1797–1806

Leech DM, Williamson CE (2000) Is tolerance to UV radiation in zooplankton related to body size, taxon, or lake transparency? Ecol Appl 10:1530–1540

Malloy KD, Holman MA, Mitchell D, Detrich III HW (1997) Solar UVB-induced DNA damage and photoenzymatic DNA repair in Antarctic zooplankton. Proc Natl Acad Sci USA 94:1258–1263

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Marsh AG, Manahan DT (2000) Metabolic differences between "demersal" and "pelagic" development of the Antarctic sea urchin *Sterechinus neumayeri*. Mar Biol 137:215–221

Marshak A (1949) Recovery from ultra-violet light-induced delay in cleavage of *Arabacia* eggs by irradiation with visible light. Biol Bull 97:315–322

Meador J, Jeffrey WH, Kase JP, Pakulski JD, Chiarello S, Mitchell DL (2001) Seasonal fluctuation of DNA photodamage in marine plankton assemblages at Palmer Station, Antarctica. Photochem Photobiol 75:266–271

Mitchell DL (1996) Ultraviolet radiation damage to DNA. In: Meyers RA (ed) Encyclopedia of molecular biology and molecular medicine. Chernow, New York, pp 149–164

Mitchell DL (1999) Quantification of DNA photoproducts in mammalian cell DNA using radioimmunoassay. In: Henderson DS (ed) Methods in molecular biology DNA repair protocols. Humana, Totowa, N.J., pp 165–175

Mitchell DM, Hartman PS (1990) The regulation of DNA repair during development. Bioessays 12:74–79

Mitchell DL, Karentz D (1993) The induction and repair of DNA photodamage in the environment. In: Young AR, Björn LO, Moan J, Nultsch W (eds) Environmental UV photobiology. Plenum, New York, pp 345–377

Montzka SA, Butler JH, Lock LT (1999) Present and future trends in the atmospheric burden of ozone-depleting halogens. Nature 398:690–694

Neale PJ, Davis RF, Cullen JJ (1998) Interactive effects of ozone depletion and vertical mixing on photosynthesis of Antarctic phytoplankton. Nature 392:585–589

Pawson DL (1994) Antarctic echinoderms: history, distribution, ecology, 1968–1993. In: David B, Guille A, Féral J-P, Roux M (eds) Proceedings of the eighth international echinoderm conference, Dijon, France. Balkema, Rotterdam, The Netherlands

Pizarro R, Orce LV (1988) Membrane damage and recovery associated with growth delay induced by near-UV radiation in *Escherichia coli* K-12. Photochem Photobiol 47:391–397

Poll WH van de, Hanelt D, Hoyer K, Buma AGJ, Breeman AM (2002) Ultraviolet-B-induced cyclobutane-pyrimidine dimer formation in Arctic marine macrophytes. Photochem Photobiol 76:493–500

Rodriguez CA, Browman HI, Runge JA, St-Pierre J-F (2000a) Impact of solar ultraviolet radiation on hatching of a marine copepod, *Calanus finmarchicus*. Mar Ecol Prog Ser 193:85–93

Rodriguez CA, Browman HI, St-Pierre JF (2000b) High survival of neustonic zoea I larvae of American lobster *Homarus americanus* following short-term exposure to ultraviolet radiation (280–400 nm). Mar Ecol Prog Ser 193:305–309

Rustad R (1975) Irradiation, chemical treatment and cleavage-cycle and cleavage pattern alteration. In: Czihak G (ed) The sea urchin embryo: biochemistry and morphogenesis. Springer, New York Berlin Heidelberg, pp 345–363

Ryter SW, Tyrrell RM (1998) Singlet molecular oxygen ($^1O_2$): a possible effector of eukaryotic gene expression. Free Radic Biol Med 24:1520–1534

Saito H, Taguchi S (2003) Influence of UVB radiation on hatching success of marine copepod *Paracalanus parvus* sp. l. J Exp Mar Biol Ecol 282:135–147

Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Natl Acad Sci USA 71:3363–3366

Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protected functions in aquatic organisms. Annu Rev Physiol 64: 223–262

Shima A, Setlow RB (1984) Survival and pyrimidine dimers in cultured fish cells exposed to concurrent sun lamp ultraviolet and photoreactivating radiations. Photochem Photobiol 39:49–56

Shindell DT, Rind D, Lonergan P (1998) Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentrations. Nature 392:589–592

Shreeve RS, Peck LS (1995) Distribution of pelagic larvae of benthic marine invertebrates in the Bellinghausen Sea. Polar Biol 15:369–374

Smith RC, Baker KS (1982) Assessment of the influence of enhanced UV-B on marine primary productivity. In: Calkins J (ed) The role of solar ultraviolet radiation in marine ecosystems. Plenum, N.Y., pp 509–537

Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z, Waters KJ (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959

Sobolev I (2000) Effect of column ozone on the variability of biologically effective UV radiation at high southern latitudes. Photochem Photobiol 72:753–765

Solomon S (1999) Stratospheric ozone depletion: a review of concepts and history. Rev Geophys 37:275–316

Stanwell-Smith D, Peck LS (1998) Temperature and embryonic development in relation to spawning and field occurrence of larvae of three Antarctic echinoderms. Biol Bull 194:44–52

Stanwell-Smith D, Peck LS, Todd CD (1999) The distribution, abundance and seasonality of pelagic marine invertebrate larvae in the maritime Antarctic. Philos Trans R Soc Lond B 354:471–484

Steeger H-U, Wiemer M, Freitag JF, Paul RJ (1999) Vitality of plaice embryos (*Pleuronectes platessa*) at moderate UV-B exposure. J Sea Res 42:27–34

Strähle U, Jesuthasan S (1993) Ultraviolet irradiation impairs epiboly in zebrafish embryos: evidence for a microtubule-dependent mechanism of epiboly. Development 119:909–919

Toyoshima M, Takinami S, Hieda K, Furusawa Y, Negishi T (2002) The involvement of cell cycle checkpoint-mutations in the mutagenesis induced in *Drosophila* by a longer wavelength light band of solar UV. Photochem Photobiol Sci 1:178–183

Tyrrell RM (1973) Induction of pyrimidine dimers in bacterial DNA by 365 nm radiation. Photochem Photobiol 17:69–73

Tyrrell RM, Pidoux M (1987) Action spectra for human skin cells: estimates of the relative cytotoxicity of the middle ultraviolet, near ultraviolet, and violet regions of sunlight on epidermal keratinocytes. Cancer Res 47:1825–1829

Vernet M, Smith RC (1997) Effects of ultraviolet radiation on the pelagic Antarctic ecosystem. In: Häder D-P (ed) Effects of ozone depletion on aquatic ecosystems. Academic and Landes, Austin, Tex., pp 247–265

Vetter RD, Kurtzman A, Mori T (1999) Diel cycles of DNA damage and repair in eggs and larvae of northern anchovy, *Engraulis mordax*, exposed to solar ultraviolet radiation. Photochem Photobiol 69:27–33

Visser PM, Snelder E, Kop AJ, Boelen P, Buma AGJ, Van Duyl FC (1999) Effects of UV radiation on DNA photodamage and production in bacterioplankton in the coastal Caribbean Sea. Aquat Microb Ecol 20:49–58

Weiler CS, Penhale PA (eds) (1994) Ultraviolet radiation and biological research in Antarctica. American Geophysical Union, Washington, D.C.

Wells PH, Giese AC (1950) Photoreactivation of ultraviolet light injury in gametes of the sea urchin *Strongylocentrotus purpuratus*. Biol Bull 99:163–172

Williamson CE, Metzgar SL, Lovera PA, Moeller RE (1997) Solar ultraviolet radiation and the spawning habitat of yellow perch, *Perca flavescens*. Ecol Appl 7:1017–1023

Williamson CE, Neale PJ, Grad G, De Lange HJ, Hargreaves BR (2001) Beneficial and detrimental effects of UV on aquatic organisms: implications of spectral variation. Ecol Appl 11:1843–1857

Winckler K, Fidhiany L (1996a) Combined effects of constant sublethal UVA irradiation and elevated temperature on the survival and general metabolism of the convict-cichlid fish, *Cichlasoma nigrofasciatum*. Photochem Photobiol 63:487–491

Winckler K, Fidhiany L (1996b) Significant influence of UVA on the general metabolism in the growing cichlid fish, *Cichlasoma nigrofasciatum*. J Photochem Photobiol B 33:131–135

Young AR, Björn LO, Moan J, Nultsch W (eds) (1993) Environmental UV photobiology. Plenum, New York

Zagarese HE, Cravero W, Gonzalez P, Pedrozo F (1998) Copepod mortality induced by fluctuating levels of natural ultraviolet radiation simulating vertical water mixing. Limnol Oceanogr 43:169–174

Zellmer ID (1998) The effect of solar UVA and UVB on subarctic *Daphnia pulicaria* in its natural habitat. Hydrobiologia 379:55–62