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Biological weighting functions for DNA damage in sea urchin embryos exposed to ultraviolet radiation

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Abstract

Laboratory experiments examining the effects of ultraviolet radiation (UVR, 290–400 nm) on DNA damage were carried out using the embryos of three species of sea urchins from different habitats; *Strongylocentrotus droebachiensis* from the Gulf of Maine, *Sterechinus neumayeri* from the Antarctic, and *Evechinus chloroticus* from New Zealand. All three species exhibited significant amounts of accumulated DNA damage, measured as cyclobutane pyrimidine dimers (CPD) photoproducts, when exposed to UVR in the laboratory. Biological weighting functions (BWFs) revealed that *S. neumayeri* has significantly higher sensitivity to UVR-induced DNA damage across most of the UVR spectrum compared to the other two species, and all species were observed to have weightings in the ultraviolet-A (UVA, 320–400 nm) portion of the spectrum. The increased sensitivity to ultraviolet-B (290–320 nm) and UVA in *S. neumayeri* is correlated with the lowest concentration of UVR absorbing compounds observed in the embryos of the three species of urchin used in this study. Sea urchin embryos and larvae in the respective habitats of the species tested are known to occur within 5 m of the surface of the ocean where both UVB and UVA wavelengths occur. Solar irradiances of UVR at a depth of 5 m, weighted using the urchin DNA damage BWFs, show that *E. chloroticus* receives the greatest amount of biologically effective UVR despite having the lowest wavelength dependent weightings for DNA damage when compared to the other two species.

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1. Introduction

The biological effects of exposure to UVR, especially UVB, continue to be a significant global concern. Recent assessments of future stratospheric ozone depletion predict that the irradiances of unweighted UVB radiation will not recover to 1980 levels by 2050 (McKenzie et al., 2003). When the

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irradiance of UVB increases due to ozone depletion there is no proportional increase in longer UVA and blue wavelengths involved in photoreactivation and photorepair and causes a net increase in the amount of DNA damage (Sancar et al., 2004). The transmission of UVR in marine environments varies significantly with changes in the stability and optical properties of the water column due to seasonal variation in solar irradiation, phytoplankton blooms, or increases in dissolved organic matter (Smith et al., 1992; Kuhn et al., 2000; Lesser, 2000). This variability in the attenuation of UVR has been shown to be a significant determinant of the biological effects on the planktonic life-history phases of marine organisms from a variety of taxa (Smith et al., 1992; Herndl et al., 1993; Jeffrey et al., 1996; Malloy et al., 1997; Lesser and Barry, 2003).

Exposure to UVB and UVA can cause direct structural damage to DNA and indirectly by UVA wavelengths. The CPD photoproduct is caused by direct exposure to UVB radiation while UVA wavelengths cause damage both directly, and indirectly through the photodynamic production of hydroxyl radicals causing strand breaks and DNA crosslinks (Setlow, 1974; Imlay and Linn, 1988; Peak and Peak, 1990, Kuluncsics et al., 1999). The amount of damage to DNA is dependent on the equilibrium between damage, which is dependent on the absorbed dose of UVR, and rates of repair. DNA damage caused by oxidative stress can lead to programmed cell death or apoptosis in metazoans such as sea urchin embryos if not repaired (Lesser et al., 2003).

Defenses against solar UVR include blocking potentially harmful wavelengths by using UVR absorbing compounds. Many of these compounds have been identified as mycosporine-like amino acids (MAAs), and are found in a wide variety of marine organisms (Shick and Dunlap, 2002). The differential survival of planula larvae from broadcast spawning corals has been attributed to the differences in MAAs translocated from adult tissues to the eggs (Gleason and Wellington, 1995). Eggs, embryos, and larvae from temperate sea urchins contain MAAs (Adams and Shick, 1996, 2001; Banaszak et al., 1998), and for sea urchin embryos and larvae a protective role for these compounds has been experimentally demonstrated (Adams and Shick, 1996, 2001). In addition to these “sunscreen” compounds developing embryos

and larvae employ photorepair mechanisms (e.g., photolyase), or use sacrificial layers of cells, and antioxidant defenses to protect themselves from the effects of UVR (Epel et al., 1999; Lesser et al., 2003).

When considering the effects of absorbed UVR and how those effects may vary with differences in irradiance or wavelength it is important to construct BWFs that provide quantitative information on the wavelength dependency for the process of interest. Acclimatization processes, such as the accumulation of MAAs, may also have a significant effect on the shape or magnitude of the BWF (Cullen and Neale, 1997). Weighting functions provide the biologically effective irradiance or dose of UVR and have been described for a number of marine organisms (Neale, 2000). The functions generally have a steep exponential decline through the UVB portion of the spectrum with the spectral slope being a strong determinant of the sensitivity to changes to ozone depletion (Cullen and Neale, 1997; Neale, 2000). Polychromatic weighting functions have recently been favored for ecologically relevant BWFs because they provide information on the balance between damage and repair processes (Caldwell et al., 1986). In this approach, PAR, which is important for light mediated repair processes, is kept constant and UVR is varied by sequential increases in UVR wavebands (Cullen et al., 1992; Cullen and Neale, 1997; Neale, 2000). Computational methods must also consider whether reciprocity holds, or if the effects of UVR are solely dependent on cumulative exposure, and independent of rate. Analytical approaches for constructing polychromatic BWFs have been described and include principal components analysis and non-linear regression which results in robust BWFs in absolute units (Cullen et al., 1992; Cullen and Neale, 1997; Neale, 2000). A simpler approach was described by Rundel (1983) and uses the differential irradiance and differential effects between filter treatments and assumes a specific shape for the BWF that is constructed using iterative non-linear regression. This approach is simpler but the resolution of the shape of the BWF is dependent on sample size (Neale, 2000).

For three ecologically important species of sea urchin from diverse habitats, we present here the effects of UVR on survivorship, and DNA damage. We then construct BWFs for net DNA damage on the early embryos of these sea urchins and, from these,

examine the potential effects of UVR in their respective habitats.

2. Materials and methods

2.1. Experimental animals

Adult sea urchins from the Isles of Shoals, Gulf of Maine (42°59.29' N, 70°37.01' W), *Strongylocentrotus droebachiensis* (Müller), Cape Armitage, Antarctica (77° 51.62' S, 166° 40.63' E), *Sterechinus neumayeri* (Meissner), and New Zealand (45°16.60' S, 166°50.60' EW), *Evechinus chloroticus* (Valenciennes), were collected at depths of 10–15 m using SCUBA. All animals were maintained at 5 °C, –1.8 °C, and 15 °C respectively in flowing seawater before spawning. Animals were not fed and all were used within 1 week of collection. Reproductively mature animals were brought into the laboratory and induced to spawn by intracoelomic injection of 0.5 M KCl. Eggs from at least five females were combined and washed three times with 0.22 µm filtered seawater and collected using a 100-µm Nitex filter after each wash. The size of the eggs was ~155 µm, 150 µm, and 120 µm for *S. droebachiensis*, *S. neumayeri*, and *E. chloroticus*, respectively. Sperm were left “dry” until used

and the sperm of at least three males combined and diluted in 0.22 µm filtered seawater at ~1: 10,000 to fertilize eggs at a density of 500 individuals ml⁻¹ in 2 l sterile plastic containers. After 5 min freshly fertilized embryos (FFE) were washed to remove excess sperm and maintained at a density of 20–30 individuals ml⁻¹ until used for experiments. All species exhibited fertilization success rates of over 95%.

2.2. Embryo experiments

Freshly fertilized embryos were exposed to artificial visible and UVR using three different sets of fluorescent bulbs; for *S. droebachiensis* four UV-340 lamps (Q-Panel, Cleveland, OH) and four F40 Sun lamps (General Electric, Schenectady, NY) suspended ~20 cm from the top of the filters, for *S. neumayeri* two FS20 lamps (General Electric, Schenectady, NY) and two VitaLite lamps (Duro-Lite Inc, Alsip, IL) were suspended ~15 cm from the top of the filters, and for *E. chloroticus* two Cleo Professional Sunlamp (Phillips, Baltimore, MD) and two F40 Sun lamps (General Electric, Schenectady, NY) were suspended ~20 cm from the top of the filters to provide a mixed field (visible and UVR) down-welling exposure. Representative spectra from the UVR portion of the spectrum are shown in Fig. 1. The PAR/UVR ratios (on a

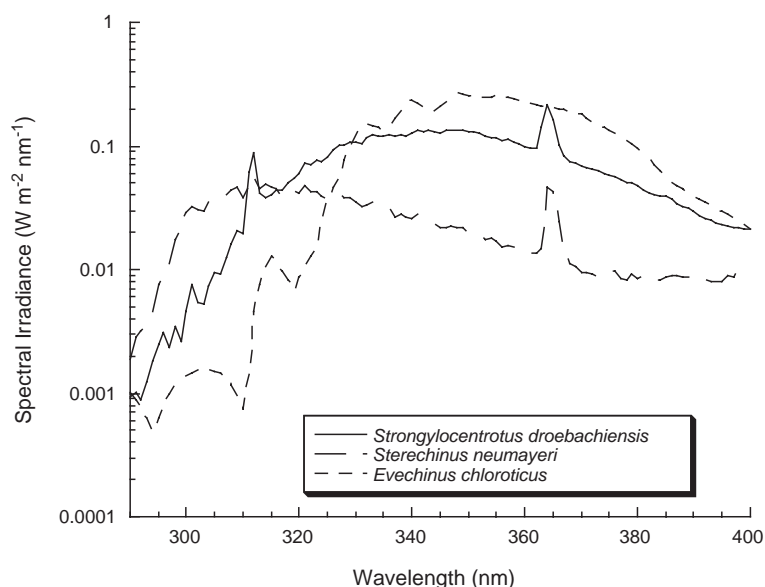


Fig. 1. Spectral irradiance of laboratory UVR (290–400 nm) exposures (see description in Materials and methods).

W m^{-2} basis) for the experimental set-ups are 2.18 (*S. droebachiensis*), 2.60 (*S. neumayeri*), and 1.86 (*E. chloroticus*). For each species of sea urchin, an experiment was conducted using glass beakers (400 ml) containing 5–7 individuals ml^{-1} to test the effects of UVR on embryos. Five treatments were used to partition the effects of UVB from UVA and visible radiation with three replicates per treatment for a total of 15 beakers in each experiment. The spectral treatments were created using Schott WG and GG long pass filters (6" × 6") with nominal cutoffs (50% T) in the UVB (280, 305, 320), UVA (375), and visible (400 nm) portions of the spectrum. All experiments on embryos were conducted at the respective temperatures of collection for the adult urchins and were 3 days in length on a 12:12 light/dark cycle for *S. droebachiensis* and *E. chloroticus*, and 5 days of constant illumination for *S. neumayeri*. At the end of the experiments, the number of surviving embryos were counted. Sub-samples (10 ml) from each replicate in each treatment were fixed in 3% glutaraldehyde and filtered (0.22 μm) seawater and counts of normally developed stages of development were recorded as survivors using light microscopy. The remaining embryos were harvested for the analysis of DNA damage as described below.

2.3. Measurements of ultraviolet radiation

For laboratory experiments UVR (UVB and UVA) and photosynthetically active radiation (PAR, 400–700 nm) were measured using a wavelength and radiometrically calibrated (National Institutes of Standards and Technology [NIST] traceable standards) CCD spectrometer and fiber optics (Ocean Optics, Inc., Orlando, FL). Three scans were taken and the mean reported in units of $\text{W m}^{-2} \text{nm}^{-1}$. Integrated values of unweighted UVR (W m^{-2}) were calculated for each treatment. Spectral irradiance measurements of UVR and PAR in the field were made simultaneously with scanning spectroradiometers (LiCor 1800UW, Lincoln, NE). The spectroradiometers were programmed to scan three times every hour (total scan time approximately 45 s) at 2-nm intervals from 300 to 700 nm and the hourly mean reported in units of $\text{W m}^{-2} \text{nm}^{-1}$. The instruments use a cosine-corrected sensor, a single monochromator, and a filter wheel to reduce stray light by

isolating and measuring different portions of the spectrum, and are calibrated using NIST traceable standards. The spectral width reaching the detector is ± 8 nm and minimum excitation energies are $10^{-4} \text{W m}^{-2} \text{nm}^{-1}$ and can be further lowered by time averaging multiple scans as described above. The temperature dependence of the detector varies from -0.1% to 0.5% over the spectral range of measurements. Comparisons of this instrument to other commercial instruments have shown that the errors associated with their measured irradiances overlap (Kirk et al., 1994, Figs. 6 and 7). All spectral data were scrutinized for signals approaching the noise level of the instrument's detector. In all cases where low signal-to-noise was observed at a particular wavelength, all data from that wavelength, and all shorter wavelengths, were eliminated from the data set. Spectra from the Gulf of Maine were made at the Isles of Shoals in March 2000, for New Zealand at the mouth of Doubtful Sound in November 2001, and at Cape Armitage, Antarctic (under the 2–3 m of annual ice) during the Austral Spring in 2002 all from a depth of 5 m. This depth is one where, in all three habitats, both UVB and UVA wavelengths penetrate during the time that embryos are in the water column. Biologically effective irradiances (W m^{-2}) and doses (kJ m^{-2}) for laboratory experiments and biologically effective field irradiances (W m^{-2}) were calculated by multiplying the unweighted irradiance with the DNA weighting function of Setlow (1974) as parameterized by Bernhard and Seckmeyer (1997) and by using the DNA biological weighting functions derived for each species of urchin as described below.

2.4. Quantification of DNA damage

CPDs were measured using a monoclonal antibody (TDM-2) in an enzyme-linked immunoabsorbent assay (ELISA) based system (Mori et al., 1991). Genomic DNA was isolated using commercially available kits (Qiagen, Inc., Valencia, CA) and 100 ng of DNA from each sample was used in the assay. The DNA was denatured at 100°C for 10 min followed by rapid cooling in ice (4°C) for 10 min. The single-stranded DNA was then adhered to flat-bottomed 96-well microtiter plates with 0.3% protamine sulfate in phosphate-buffered saline (PBS, pH 7.5). Blocking

buffer (100 μl of 1% BSA in PBS) was placed in each well for 1.5 h, then emptied and rinsed. The primary antibody (100 μl of TDM-2), at a dilution of 1:1000, was placed in each well and incubated at 37 °C for 1.5 h. After rinsing, 100 μl of a goat anti-mouse IgG secondary antibody (1:3000 dilution), conjugated with horseradish peroxidase, was placed in each well and incubated at room temperature for 2.0 h. The wells were rinsed and the final color development was carried out with Sigma Fast reagents (Sigma, Inc., St. Louis, MO) and read using a plate reader (Bio-Rad, Inc., Hercules, CA) at 490 nm after 30 min and the addition of 50 μl 2 mol l^{-1} H_2SO_4 to stabilize the final color development. All rinse steps consisted of three rinses with 0.05% Tween-20 in PBS. The CPD assay is a relative measurement of DNA damage based on the optical density of the ELISA assay normalized to a specific concentration of DNA and expressed as OD_{490} 100 ng DNA^{-1} . All measurements were corrected for background level of color using appropriate controls (e.g., no primary antibody) for the ELISA.

2.5. Construction of the biological weighting functions

The BWF for net DNA damage in embryos exposed to UVR (5 treatments, 3 replicates per treatment for an $N=15$) was developed for each species by fitting a simple exponential function $\varepsilon_{\text{H}}(\lambda) = \exp(a_0 + a_1\lambda + a_2\lambda^2)$ using the differential irradiance approach originally described in Rundel (1983) with the relative DNA damage described as OD_{490} 100 ng DNA^{-1} and the cumulative UVR energy exposure under each filter treatment during the species specific experimental protocol. The parameters were varied to minimize mean-squared error χ^2 using iterative non-linear fitting in the JMP 5.01a statistical package. To see if the original BWFs were significantly different from each other in magnitude, we calculated and plotted the 95% confidence intervals for all BWFs using the variance of the estimates and the correlation matrix of the fitted estimates in an asymptotic linear approximation routine in JMP 5.01a. The $\varepsilon_{\text{H}}(\lambda)$, plotted as a function of wavelength, is the BWF. The product of the weighting coefficient for each wavelength ($\varepsilon_{\text{H}}(\lambda)$ in $(\text{J m}^{-2})^{-1}$) and the cumulative energy exposure at each wavelength, integrated over the specific waveband of inter-

est (e.g., UVR, UVB, or UVA), is the net biologically weighted exposure.

We present the weighted UVR exposures as a function of irradiance and total dose of UVR for the laboratory exposures and as a function of irradiance for field exposures. The biological weighting functions resulting from this polychromatic approach (Caldwell et al., 1986) are more ecologically relevant when examining UVR-induced DNA damage since the measured damage is the net result of damage and repair processes during exposures to PAR and UVR on time scales similar to those experienced by these species in their native habitats.

2.6. UV absorbing compounds (mycosporine-like amino acids)

Samples of embryos ($N=3$) from the beginning of the experiment were analyzed for MAAs before experimentation using the methods described in Lesser (2000). Individual MAAs were separated with reverse-phase isocratic high-performance liquid chromatography (HPLC) on a RP-8 column (Spheri-5, 4.6 mm ID \times 25 cm) protected with an RP-8 guard column (Spheri-5, 4.6 mm ID \times 3 cm) using an aqueous mobile phase of 40–55% methanol (v/v) and 0.1% acetic acid at a flow rate of 0.6 ml min^{-1} . Detection of peaks is by UV absorbance at 313 and 340 nm. Identity of peaks is accomplished using co-chromatography with known primary and secondary standards. Quantification of individual peaks is accomplished using response factors calculated from known amounts of standard. Soluble protein composition was assessed using Coomassie Brilliant Blue, and bovine serum albumin as a standard (Bio-Rad, Inc., Hercules, CA) to normalize data.

2.7. Statistical analysis

DNA damage was statistically analyzed using a one-way analysis of variance (ANOVA) at a significance level of 5%. No unequal variances were detected using the F_{max} test, and individual treatment differences were assessed using the (Student–Neuman–Keuls) SNK multiple comparison test. Where appropriate, ratios and percentages were arcsine or log transformed for analysis and back transformed for presentation.

3. Results

Table 1 shows the unweighted and integrated UVR and PAR irradiances for each experimental set-up in the laboratory and the respective field locations at a depth of 5 m where the planktonic embryos and larvae of these species are known to occur. When you apply the urchin DNA damage BWFs to weight the 5-m spectral data for all cases, the weighted irradiance in the field is dominated by the UVA component. The Setlow function, however, which is heavily weighted in the UVB portion of the spectrum shows that the weighted underwater spectrum at 5 m in the Antarctic and New Zealand is dominated by UVB radiation but for the Gulf of Maine, the weighted irradiance is dominated by UVA radiation (Table 1).

The UVB portion of the spectrum dominates the laboratory irradiances, when weighted by the Setlow BWF. When the laboratory spectral regimes are weighted by the species specific urchin BWFs for DNA damage, the total weighted doses of UVR reflect the irradiance regime and the specific experimen-

tal design for each species of sea urchin which a priori was a function of differences in photoperiod and time to reach the blastula stage from FFE.

For each individual experiment on the urchin species used in this study, the number of surviving embryos at the end of the experiment was significantly related to UVR exposure (Fig. 2A). For *S. droebachiensis* there was a significant effect of filter treatment (ANOVA, $P=0.0002$) on embryos survival and multiple comparison testing revealed that all short wavelength UVB treatment groups were significantly different from all other groups but that there was a significant effect of UVA as well (SNK, $P<0.05$, Fig. 2A). For *S. neumayeri* there was also a significant effect of filter treatment on embryo survival (ANOVA, $P=0.0003$) and multiple comparison testing revealed that the UVB filter treatments were significantly different from UVA treatments with the visible radiation group having the greatest survivorship (SNK, $P<0.05$, Fig. 2A). In *E. chloroticus* we also observed a significant effect of UVR on embryo survival (ANOVA, $P=0.0019$) and multiple comparison testing revealed that the UVB treatments were signifi-

Table 1

Comparison of laboratory and field values of UVR (290–400 nm), UVB (290 or 300–320 nm^a), UVA (320–400), and photosynthetically active radiation (PAR: 400–700 nm) for DNA weighted and unweighted exposures for the three species of urchins

	<i>Strongylocentrotus droebachiensis</i>		<i>Sterechinus neumayeri</i>		<i>Evechinus chloroticus</i>	
	Laboratory	Field (5 m)	Laboratory	Field (5 m)	Laboratory	Field (5 m)
<i>Ambient</i>						
UVR ^b	7.462	1.0274	2.502	0.132	8.770	4.000
UVB ^b	0.573	0.0003	0.879	0.001	0.087	0.0015
UVA ^b	6.889	1.0271	1.623	0.131	8.683	3.985
PAR ^c	75	275	75	15	75	545
<i>Setlow</i>						
UVR ^b	0.08974	0.000036	0.33430	0.000036	0.02840	0.000501
UVB ^b	0.08921	0.000001	0.33410	0.000030	0.02798	0.000352
UVA ^b	0.00053	0.000035	0.00020	0.000006	0.00042	0.000149
<i>Urchin BWFs^d</i>						
UVR ^c	0.191	0.013021	1.10	0.012651	0.166	0.043530
UVB ^c	0.058	0.000018	0.704	0.000825	0.005	0.000693
UVA ^c	0.133	0.013003	0.396	0.011826	0.161	0.042837

NA=not applicable.

^a Instrument dependent, laboratory measurements were from 290 to 320 nm whereas field measurements were from 300 to 320 nm.

^b W m⁻².

^c μmol quanta m⁻² s⁻¹.

^d Biological weighting functions from this study.

^e kJ m⁻².

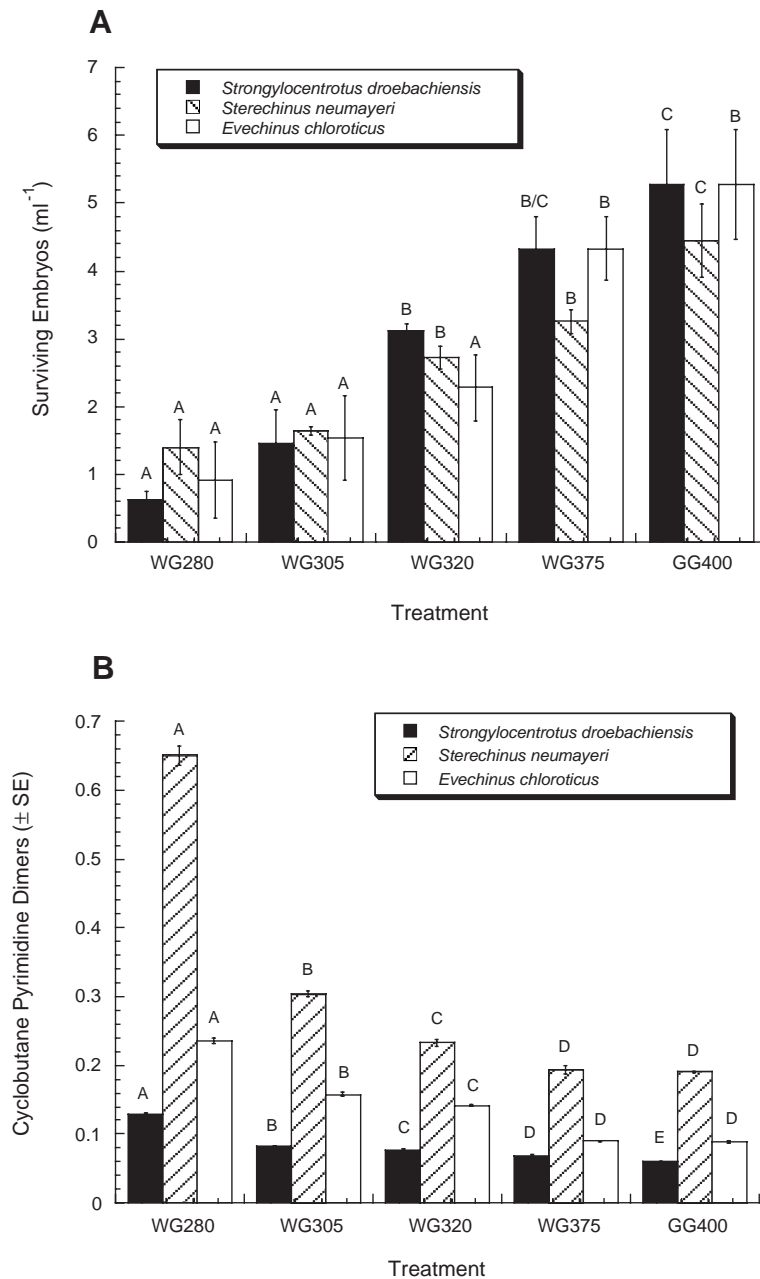


Fig. 2. (A) Embryo survivorship (mean \pm SE) of separate experiments on urchin embryos exposed to UVR under the filter treatment groups. Treatment groups with common superscripts are not significantly different from one another using multiple comparisons testing (SNK) at a significance level of 0.05%. (B) DNA damage measured as CPDs (mean OD_{490} $100 \text{ ng DNA}^{-1} \pm$ SE) for the individual experiments on urchin embryos exposed to UVR under the filter treatment groups. Treatment groups with common superscripts are not significantly different from one another using multiple comparisons testing (SNK) at a significance level of 0.05%.

cantly different than the UVA treatments (SNK, $P < 0.05$, Fig. 2A) but that the visible radiation treatment (GG400), exhibiting the highest survivorship formation, was only distinguishable from the UVB treatments.

At the end of each experiment, CPD photoproducts were measured as described above with all urchin species exhibiting significant treatment effects of UVR on CPD formation (Fig. 2B). For *S. droebachiensis* there was a significant effect of filter treatment

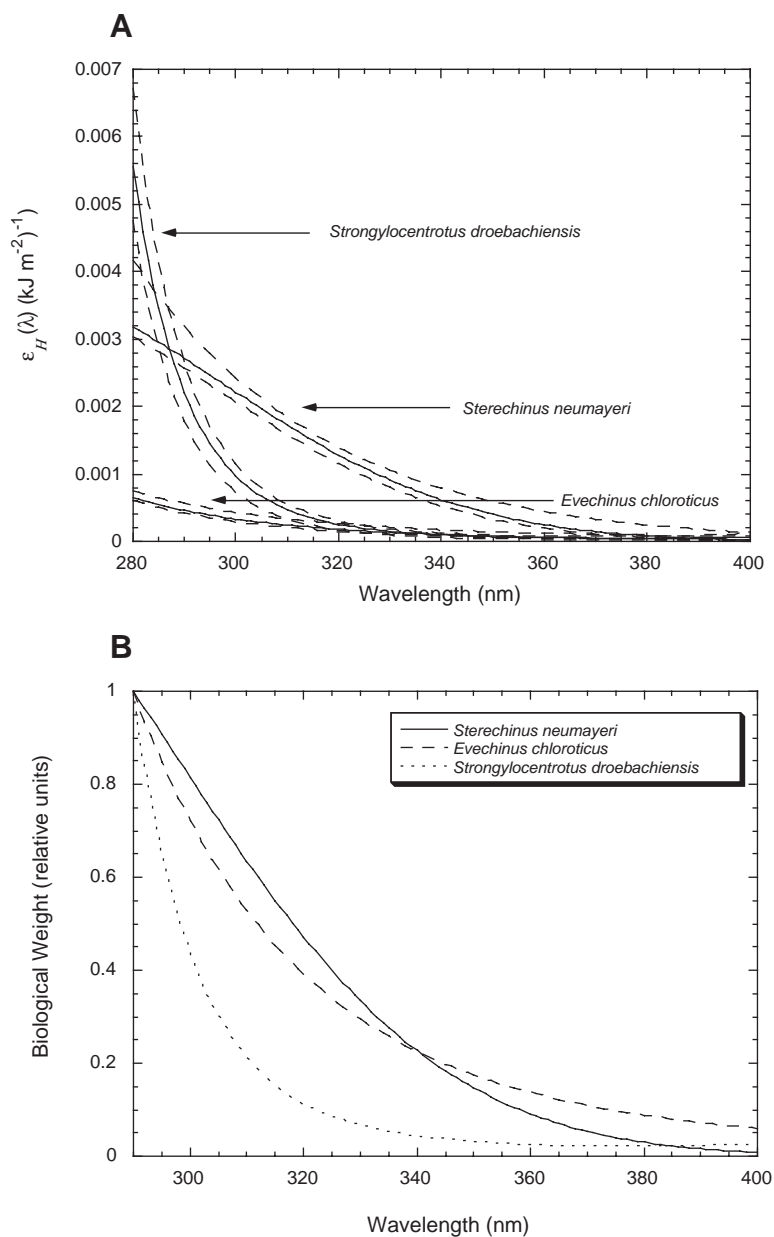


Fig. 3. (A) Calculated biological weighting functions ($\pm 95\%$ CI) for DNA damage in the urchin embryos exposed to UVR. (B) Biological weighting functions for DNA damage in embryos exposed to UVR normalized to 1 at 290 nm.

(ANOVA, $P < 0.001$) on CPD formation and multiple comparison testing revealed that all treatment groups were significantly different from one another (SNK, $P < 0.05$, Fig. 2B). For *S. neumayeri* there were greater levels of CPD formation during exposures to UVR and also a significant effect of filter treatment (ANOVA, $P < 0.001$) with multiple comparison testing showing that all treatment groups were significantly different from one another (SNK, $P < 0.05$, Fig. 2B) except for the WG375 and the visible radiation treatment (GG 400) which were not significantly different from each other. In *E. chloroticus* we also observed a significant effect of UVR on CPD formation (ANOVA, $P = 0.039$) and the same treatment-specific pattern as observed for *S. neumayeri* (SNK, $P < 0.05$) and intermediate amounts of CPD formation (Fig. 2B).

Biological weighting functions were derived as described (Fig. 3A). The non-linear regression parameter values for the derived weighting function, using the exponential equation $\varepsilon_H(\lambda) = \exp(a_0 + a_1\lambda + a_2\lambda^2)$, were $a_0 = 61.054$, $a_1 = -0.376002159 \text{ nm}^{-1}$, $a_2 = 0.0004978907 \text{ nm}^{-2}$, $r^2 = 0.981$ for *S. droebachiensis*, $a_0 = -20.054$, $a_1 = 0.1156565895 \text{ nm}^{-1}$, $a_2 = -0.000230613 \text{ nm}^{-2}$, $r^2 = 0.982$ for *S. neumayeri*, and $a_0 = 7.947$, $a_1 = -0.074236762 \text{ nm}^{-1}$, $a_2 = 0.0000706312 \text{ nm}^{-2}$, $r^2 = 0.979$ for *E. chloroticus*. Note the steeper slope for the *S. droebachiensis*

BWF compared with the other species. This predicts more sensitivity in the UVB portion of the spectrum that is known to be affected by ozone depletion. The *S. neumayeri* BWF has greater weightings across the UVR spectrum from $\sim 295 \text{ nm}$, and including the entire UVA portion of the spectrum while *S. droebachiensis* and *E. chloroticus* are not significantly different from each other starting at $\sim 310 \text{ nm}$. When the BWFs are normalized to one at 290 nm they have the same general shape and no indication of species-specific changes due to the presence of UV absorbing compounds because of the low resolution of these BWFs (Fig. 3B). All of these BWFs approach, but never reach, zero in the UVA portion of the spectrum revealing a UVA component for DNA damage, measured as CPDs, for these embryos. The absolute BWFs were then multiplied by the total dose of UVR for an embryo at 5 m as described for each species in the laboratory protocol (Table 1; Fig. 4) to obtain a biologically effective dose for exposure of embryos to UVR in the field. The biologically effective irradiances ($290\text{--}400 \text{ nm}$) are 0.0130 for *S. droebachiensis*, 0.0126 for *S. neumayeri*, and 0.4035 kJ m^{-2} for *E. chloroticus*.

There was a significant effect of species on MAA concentration (ANOVA: $P < 0.001$) and the total concentration of MAAs was significantly different be-

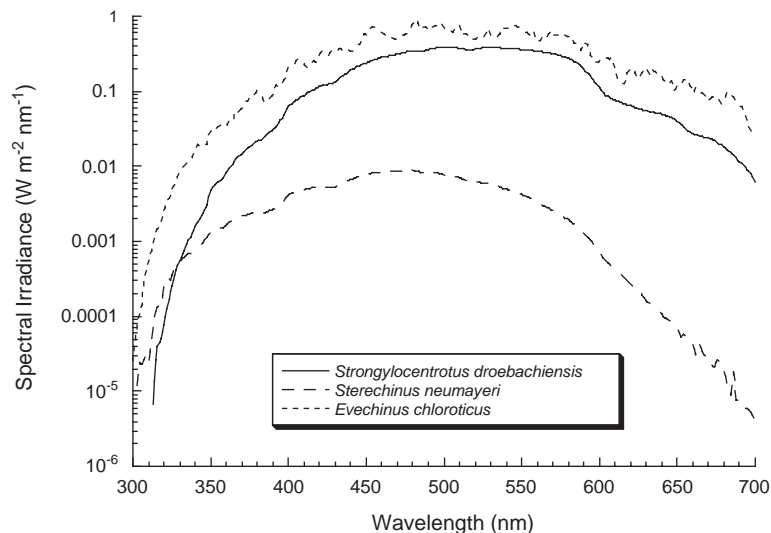


Fig. 4. Spectral irradiance ($300\text{--}700 \text{ nm}$) for the habitat of each individual species of sea urchin at a common depth of 5 m . Spectral irradiance was recorded using a LiCor LI-1800UW scanning spectroradiometer (LiCor, Lincoln, Nebraska) and each spectrum represent the means of three scans.

tween all species (SNK: $P < 0.05$). Embryos of *S. droebachiensis* contained the highest concentration of total MAAs ($650.54 \text{ nmol mg protein}^{-1} \pm 82.25$ [SE]), with shinorine, and porphyra-334 being quantified. Embryos of *S. neumayeri* contained the lowest concentration of total MAAs ($6.20 \text{ nmol mg protein}^{-1} \pm 4.15$ [SE]), with shinorine, porphyra-334, and palythine being quantified. Lastly, embryos of *E. chloroticus* contained intermediate concentrations of total MAAs ($155.66 \text{ nmol mg protein}^{-1} \pm 47.75$ [SE]), with mycosporine-glycine and small amounts of shinorine being quantified.

4. Discussion

The sea urchins used in this study commonly inhabit benthic environments from shallow to deep-water, but large populations are found in shallow waters (<10 m) where their principal food source of macrophytes is available. Additionally, all of these species are broadcast spawners whose embryos and larvae are also found in the shallow waters of their respective habitats. Despite high within- and between-site variability in the optical properties of the water at these sites, UVR has been measured to depths of 7–10 m in these habitats (Banaszak et al., 1998; Lamare et al., 2004; Lesser et al., 2004). Buoyant, swimming, or vertically advected planktonic phases of these urchins are exposed to UVR at 10 m depth or less. There are important consequences for embryos and larvae in the plankton whose residence time within the upper portion of the water column is controlled by physical processes. An important component of the effect of a longer residence time in the upper portion of the water is whether reciprocity holds, that is, does a short-term exposure to high irradiances to UVR have the same effect as a longer-term, lower irradiance exposure where the total exposure is equivalent. Survival curves for these species of sea urchins suggests that reciprocity does hold when ecologically relevant time periods are used to quantify DNA damage (Lesser and Barry, 2003; Lesser, unpublished). DNA damage to developing urchin embryos in the water column should be strictly a function of the cumulative dose of UVR and the spectral distribution of that UVR. Experimentally, the duration of exposure, both in terms of photoperiod and total duration, to UVR chosen for the laboratory

studies was dependent on the species-specific developmental times from fertilized embryos to blastula in their respective habitats. The BWFs therefore represent the net wavelength dependent effects of UVR on unrepaired, or accumulated, DNA damage as embryos develop from fertilized embryos to blastula. The rate of development in these non-feeding stages is largely dependent on the temperature of the water at the time of this transition which is significantly different between the three species of urchins.

A distinctive feature of this study is the observation of UVA effects on DNA damage measured as CPD production and the calculated weightings in the UVA portion of the BWFs. Similar results have been reported for several developmental stages of sea urchins (Lesser and Barry, 2003; Lesser et al., 2003, 2004), and for the developmental stages of fish (Lesser et al., 2001; Browman et al., 2003). Importantly, despite the common perception that CPDs are caused only by UVB radiation, the detection of UVA induced CPDs has been reported by Setlow (1974) and in numerous other studies (Quaite et al., 1992; Kuluncsics et al., 1999; Rochette et al., 2003). However, while Karentz et al. (2004) did observe abnormal development and decreased survival of *S. neumayeri* embryos during exposure to UVA, they did not see significant UVA induced DNA damage in their experiments. We believe that the time of year for the experiments, and optical properties of the water around the Antarctic Peninsula compared to McMurdo Sound contributed significantly to the differences reported in their study compared to Lesser et al. (2004).

The differences in sensitivity to UVR reported here may reflect both the concentration of MAAs measured in each species and the types of MAA present in each species of urchin. The two dominant MAAs for *S. neumayeri* and *S. droebachiensis* were shinorine and porphyra-334 that absorb maximally at 334 nm while for *E. chloroticus* the dominant MAA is mycosporine-glycine that absorbs maximally at 310 nm (Shick and Dunlap, 2002). All of these MAAs have high molar extinction coefficients and generally broad absorption spectra extending into the UVB, and UVA portion of the spectrum (Shick and Dunlap, 2002). Previous work has shown that MAA concentrations in developing urchins are invariant when exposed to UVR and during development up to the pluteus stage (Adams

and Shick, 1996, 2001). Urchin larvae with high concentrations of MAAs can obtain as much as an 86% blocking efficiency at 334 nm (Adams and Shick, 1996, 2001). With the differences in total MAAs and the similar sizes of the eggs in these species, it is reasonable to assume that there would be significant differences in the shading capabilities of MAAs between these species. Assuming that the MAA concentrations reported for these experiments were constant for the duration of the experiment, the greater overall sensitivity of *S. neumayeri* to UVR is at least partially associated with the low concentrations of MAAs. Another important aspect of sensitivity to UVR is the ability to repair DNA damage. For CPDs light-dependent photorepair mediated by the enzyme photolyase, which requires long-wavelength UVA and visible radiation to be active, may be another factor determining differential sensitivity. For the embryos of *S. droebachiensis* and *E. chloroticus* active light dependent repair is present but for the embryos of *S. neumayeri* there is extremely low light mediated photorepair observed at $-1.8\text{ }^{\circ}\text{C}$ (Lamare and Lesser, unpublished).

In addition to MAAs, it is well known that UVR effects in the field are related to the optical properties of the water in which an embryo finds itself. Combining the urchin DNA BWFs with the spectral irradiances observed at 5 m for each urchin species shows that UVA is the dominant component of the weighted irradiance in the field for all of the species examined. The absolute BWF for *E. chloroticus* exhibits the lowest overall sensitivity to UVR, but the greater irradiances of UVR at the mouth of Doubtful Sound result in the highest biologically effective irradiances. Unlike most of Fiordland, the mouth of Doubtful Sound is not influenced significantly by dissolved organic matter and has optically clear oceanic water (Lamare et al., 2004). Further inshore of the sound little or no UVR is detected below the 3–5-m-thick layer of dissolved organic matter and embryos in those waters would be protected from UVB (Lamare et al., 2004). In conclusion, both UVR and UVA are important factors contributing to DNA damage, and low survival of sea urchin embryos in laboratory experiments. Biological weighting functions for the effects of UVR on DNA damage show species-specific differences in their sensitivity to UVR, and potentially high effects of UVA in the field. Here we have shown that some of the differ-

ences appear to be related to the concentration of MAAs but it is clear that rates of repair of DNA damage could be just as, if not more, important in determining sensitivity to UVR. The effects of UVR on sea urchin embryos in the field are related to both the species-specific sensitivity to UVR, the variability in the optical properties of the water, and the site-specific irradiance of UVR.

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