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In situ rates of DNA damage and abnormal development in Antarctic and non-Antarctic sea urchin embryos

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ABSTRACT: To understand the *in situ* effects of ultraviolet radiation (UV-R) on the development of planktonic embryos from a range of latitudes, we quantified rates of DNA damage (cyclobutane pyrimidine dimer [CPD] production) and abnormal development in embryos of 4 sea urchin species: *Sterechinus neumayeri* (Antarctica), *Evechinus chloroticus* (New Zealand), *Diadema savignyi* and *Tripneustes gratilla* (Cook Islands). Quantifications were made using *in situ* experimental techniques that were standardised to allow direct comparisons among species. Captive embryos were held on moored experimental racks for 2 to 5 d and exposed to one of 3 light treatments: (1) full ambient light; (2) visible light but no UV-R; or (3) visible light and UV-A but no UV-B. Each treatment was repeated at 3 depths (0.5, 1.0, and 4 or 5 m). Ambient UV-R irradiance was highest during the tropical exposures, although UV-R dose during experiments was greatest in Antarctica. DNA damage (CPD concentration) was significantly higher in Antarctic embryos (up to 30.8 CPDs Mb^{-1} DNA) compared to New Zealand (16.5 CPDs Mb^{-1} DNA) and tropical species (2.2 and 3.0 CPDs Mb^{-1} DNA, respectively), with damage decreasing with depth. DNA damage was positively related to total UV-B dose accumulated during each experiment. Exposure to UV-R increased abnormal development, with rates generally higher in Antarctic embryos. High abnormality rates were associated with both UV-B and UV-A exposure. The greater sensitivity of Antarctic embryos to UV-R is consistent with their slow metabolism, low concentrations of sunscreens, and slow rates of DNA repair compared with temperate and tropical species.

KEY WORDS: UV-R · Climate change · Cyclobutane pyrimidine dimers · CPDs · Antarctica · Echinoid · Embryo

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INTRODUCTION

The marine environment of coastal Antarctica has experienced increases in UV-B radiation (290 to 320 nm) as a result of stratospheric ozone depletion (Smith 1989, Smith et al. 1992, Madronich et al. 1998). In addition, marine environments in polar regions may experience future increases in UV radiation (UV-R: 290 to 400 nm) penetration if sea ice, which is relatively opaque and highly reflective (Lesser et al. 2004), decreases in spatial and temporal extent as a result of global warming (Holland et al. 2006).

Understanding how increases in UV-R exposure will affect polar marine ecosystems requires quantifying the relative sensitivity of Antarctic marine species to UV-R. This is particularly true for the planktonic larval stages of marine species that can be particularly sensitive to UV-R due to their small size, high transparency, rapid cell division (Johnsen & Widder 2001), and distribution near the sea surface. Lower survival rates in these key life-history larval stages will reduce the long-term viability of affected populations.

Previous research has suggested that increased UV-B in the Antarctic may cause increased DNA damage in a range of marine zooplankton species (Malloy et al.

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1997). Previous research also suggests that Antarctic marine invertebrate larvae may be particularly sensitive to UV-R in terms of survival and rates of DNA damage, specifically cyclobutane pyrimidine dimer (CPD) production[1]. Embryos of the Antarctic sea urchin *Sterechinus neumayeri* exposed to ambient UV-R under a sea ice canopy of 3 to 4 m have decreased survival rates and elevated levels of DNA damage despite exposure to low UV-R irradiances (<0.0028 W m^{-2}; Lesser et al. 2004). Additionally, Karentz et al. (2004) quantified the *in situ* effects of UV-B and UV-A on *S. neumayeri* embryos and larvae in open water along the Antarctic Peninsula, observing CPD concentrations as high as 272 CPDs Mb^{-1} DNA after 6 d of exposure, with UV-R inducing up to 100% developmental abnormalities in samples at the sea surface. Biological weighting functions generated from laboratory exposures to UV-R for the embryos of *S. neumayeri* and 2 temperate sea urchin species (Lesser et al. 2006) also suggest a greater sensitivity in the Antarctic species in terms of CPD generation and developmental times.

There are good reasons to suspect that Antarctic embryos may be particularly sensitive to UV-R. For example, comparisons of DNA photorepair of CPDs in the pluteus stages of *Sterechinus neumayeri* with temperate and tropical counterparts indicate that repairing UV-R induced damage takes significantly longer in the polar sea urchin (Lamare et al. 2006). There are a number of factors that could contribute to a higher UV-R sensitivity in polar larvae, such as slower metabolic rates, and the effects of cold on enzymes (e.g. photolyase) involved in mitigating or repairing UV-induced damage (Lamare et al. 2006). Compounding these factors is the likelihood that polar species have experienced relatively low levels of UV-R in the past several million years, which may have reduced selective pressures to maintain high activities of DNA repair enzymes.

There have been few direct comparisons of *in situ* rates of DNA damage between polar and non-polar species, and most of those have been conducted on bacteria and phytoplankton (summarised in Karentz et al. 2004). To date, only 4 studies have measured *in situ* CPD concentrations in complex metazoan animals (Malloy et al. 1997, Vetter et al. 1999, Karentz et al. 2004, Lesser et al. 2004), and although they are informative, comparisons among the species in terms of latitudinal differences are confounded by differences in habitats, phylogenies, and experimental techniques.

[1] CPDs are the dominant form of DNA damage, the result of UV-R induced dimerisation of adjacent pyrimidine nucleotide bases (cytosine and thymine, C and T) to form either the predominant (≈70 to 80%) CPD, or to a lesser extent (≈20 to 30%), 6-4 photoproducts (6-4PP) (Friedberg et al. 1995)

To study the effects of present and future increases in UV-R in polar environments, we quantified the relative UV-R sensitivity of embryos from sea urchins inhabiting Antarctic, temperate and tropical regions by measuring CPD production in the embryos. These organisms are more closely related phylogenetically than those used in previous studies, and share similar life histories (i.e. free-swimming, surface-inhabiting, small, planktotrophic embryos that occur in the water column in spring and summer). We also replicated experimental techniques among the 3 habitats to reduce confounding effects of methodology.

The research involved experimentally assessing the effects of ambient UV-R on embryos *in situ* for each species in its natural environment. While UV-R has a range of detrimental effects on biological systems, in this study we quantified the detrimental effects in terms of DNA damage, specifically CPD lesions (the key UV-R impact on aquatic organisms, Hader & Sinha 2005), and on developmental biology in terms of morphological abnormalities.

MATERIALS AND METHODS

Species and study sites. Experiments were carried out at 3 locations: McMurdo Sound, Antarctica (77.06° S, 164.42° E), Otago Harbour, New Zealand (45.80° S, 170.71° E), and Aitutaki Lagoon, Cook Islands (18.85° S, 159.75° W). Embryos were studied in 4 echinoid species, the Antarctic *Sterechinus neumayeri* Meissner (Echinidae), the New Zealand *Evechinus chloroticus* Valenciennes (Echinometridae), and the tropical species *Diadema savignyi* Mechelin (Diadematidae) and *Tripneustes gratilla* L. (Toxophneustidae). Light measurements and *in situ* experiments on the embryos of each species were undertaken at sites where their adult populations were present. Ambient sea temperatures at the sites at the time of collection were –1.9°C for *S. neumayeri*, 17°C for *E. chloroticus*, and 26.5°C for *T. gratilla* and *D. savignyi*.

Environmental UV radiation measurements. Spectral irradiances were measured using a LiCor Li1800UW submersible spectroradiometer, which was factory calibrated twice (16 October 2001, 4 May 2005) during the study using US National Institute of Standards and Technology traceable standards. During the times of *in situ* embryo experiments, spectral irradiance was measured at the surface, and at 0.5, 1.0, 2.0, 3.0, and 5.0 m depth in Aitutaki Lagoon and Otago Harbour; and at the surface, 0.5, 1.0, 5.0, and 10.0 m depth in McMurdo Sound. Scans of spectral irradiance (W m^{-2} nm^{-1}) were made between 300 and 750 nm at 2 nm intervals, with 3 consecutive replicate scans

made for each depth (replicates averaged). All scans were made between 12:00 and 14:00 h, and when the sky was cloudless and the sea calm.

Optical properties of the water column were quantified by calculation of bulk spectral attenuation coefficients (K_d m^{-1}) at each wavelength using the equation:

$$I_D(\lambda) = I_0(\lambda)e^{-kD} \quad (1)$$

where $I_D(\lambda)$ = irradiance at wavelength (λ) at depth (D), $I_0(\lambda)$ = irradiance at wavelength (λ) at the surface, and k = light extinction coefficient. For each scan, UV-B (300 to 320 nm, W m^{-2}), UV-A (320 to 400 nm, W m^{-2}), and photosynthetically active radiation (PAR, 400 to 700 nm, µmol quanta m^{-2}) were also calculated. The attenuation of total UV-B, UV-A, and PAR was then calculated using Eq. (1).

The depth (D) at which 50% and 10% of surface UV-B, UV-A and PAR irradiance occurred at each site was modeled as:

$$D = \frac{\ln\left(\frac{I_D}{I_0}\right)}{-k} \quad (2)$$

where I_D/I_0 is either 0.5 or 0.10 for 50% and 10% irradiance, respectively. The ambient levels of UV-B, UV-A and PAR at the sea surface were monitored hourly during the periods of *in situ* field exposures using the spectroradiometer. The exception was during the McMurdo experiments, where it was not possible to leave the instrument unattended. In this case, hourly data on ambient UV-B, UV-A and PAR (400 to 600 nm), as well a spectral irradiance (300 to 400 nm) was obtained from data collected by the National Science Foundation UVR Monitoring Program using a SUV-100 spectroradiometer (Biospherical Instruments).

Spawning and larval rearing. Spawning and experimentation was carried out during the period when each species was ripe. Gametes were obtained from individuals that were induced to spawn by an intercoelomic injection of 0.5 M KCl. Eggs were fertilised by adding several drops of dilute sperm, and only batches of eggs with a fertilisation rate >95% were used.

Biologically effective irradiance and dose. Spectral irradiances between 300 and 400 nm were converted to biologically effective irradiances using the Setlow DNA weighting function (Setlow 1974). Surface spectral measurements were converted to spectral irradiances at our experimental depths using our bulk spectral attenuation coefficients (K_d m^{-1}) measured for each wavelength (Eq. 1). Using the weighted spectral irradiances, biologically effective UV-B (300 to 320 nm) and UV-A (320 to 400 nm) doses during *in situ* experiments were calculated for each species.

***In situ* embryo exposures.** *In situ* exposures of embryos to ambient solar radiation were undertaken by out-planting bagged embryos of each species onto experimental PVC racks (31 × 26 cm horizontal dimensions) at various depths down a moored rope (Lesser et al. 2004). The depths were 0.5, 1.5, and 5 m, with the exception of the Aitutaki exposures of *Diadema savignyi* and *Tripneustes gratilla*, where the deepest rack was at 4.0 m depth. At each depth, embryos were subjected to one of 3 light treatments: (1) PAR but no UV-R ('UV-0' treatment); (2) PAR+UV-A but no UV-B ('UV-A' treatment); and (3) PAR+UV-A+UV-B ('UV-T' treatment). The 3 treatments were achieved using plexiglass filters (170 × 170 mm) that varied in their transmission of different wavelengths. The UV-transparent filter (Treatment UV-T) transmitted PAR (84.5%), UV-A (84.6%) and UV-B (80.6%); the UV-opaque filter (Treatment UV-0) transmitted PAR (81.0%) but minimal UV-A (5.2%) and UV-B (0.0%); and the UV-B filter (Treatment UV-A) transmitted PAR (77.9%) and UV-A (46.5%) but minimal UV-B (0.1%).

For out-planting, embryos were bagged in 125 ml whirl pack bags that were transparent to both UV-R and visible light. Densities of embryos in the bags were 5 to 10 ind. ml^{-1} for *Sterechinus neumayeri* and *Evechinus chloroticus*, and 100 ind. ml^{-1} for *Diadema savignyi* and *Tripneustes gratilla*. The differences in density accounted for differences in egg size among species, and were low enough to eliminate any self-shading. For each treatment at each depth, 3 replicate bags of embryos were used. To compensate for differences in the development rate of the various species, *in situ* exposures were run for 2 d for *D. savignyi* and *T. gratilla*, 4 d for *E. chloroticus* and 5 d for *S. neumayeri*. This length of time normally allowed the embryos to reach the late-gastrula to prism stage by the termination of the experiment. At termination, the bags of embryos were removed from the racks and immediately placed in a dark, thermally insulated container containing 10 l of ambient temperature seawater until preservation (within 2 h). Care was taken to avoid exposure to direct sunlight during removal of the bags. Processing of each replicate involved counting the number of normal and abnormal embryos in three, 1 ml random sub-samples, with the remaining embryos concentrated, fixed in DNA preservative (Seutin et al. 1991), frozen and kept in the dark pending DNA extraction and CPD quantification.

DNA extraction and CPD quantification. Genomic DNA was isolated from each sample using a commercially available extraction kit (DNAeasy Kits, Qiagen). DNA concentrations and purity were measured spectrophotometrically. Quantification of CPDs in each sample was determined using ELISA (enzyme-linked immunoabsorbent assay-based system) carried out in

protamine sulphate (0.003%) coated 96-well PVC microtiter plates, with 3 replicate wells assayed for each sample. Methods are described in detail in Lamare et al. (2006). TDM-2 primary monoclonal antibodies specific to CPDs were supplied by Medical and Biological Laboratories, Nagoya, Japan. To express CPD concentrations as CPDs per Mb DNA, a standard curve was generated using calf thymus DNA standards containing known CPD concentrations (2.4, 8.7, 22.5, 36.4, 78.9 CPDs Mb^{-1} DNA).

Larval morphology. The effects of the light treatment on the stage of larval development and morphology was quantified by examining embryos (N > 100) in each treatment immediately at the end of the *in situ* experimental period. For each replicate we classified embryos as either having normal or abnormal development (Fig. 1). Abnormality of embryos was determined by comparison with control embryos kept in the laboratory and not exposed to UV-R.

Statistical analyses. Statistically significant differences in CPD concentrations and abnormal development rates in embryos from *in situ* experiments were tested among depths and treatments using a 2-way ANOVA. For the analysis of *Evechinus chloroticus* experiments (where the deep UV-0 and UV-A treatments were lost), we carried out a 2-way ANOVA on the 2 shallow depths, and a 1-way ANOVA for effect of depth on CPD concentration in the UV-T treatment. All CPD concentrations were $\ln(x+1)$ transformed for statistical analysis, while percentage abnormalities were arcsine square-root transformed prior to analyses. Post-hoc tests of differences among treatments and species were made with Tukey's HSD test.

RESULTS

Irradiance

Ambient surface irradiances

Ambient surface UV-R was highest at Aitutaki (Fig. 2), with maximum UV-B and UV-A recorded as 4.8 and 68.5 W m^{-2} (Table 1). Compared with Aitutaki, maximum UV-B and UV-A were approximately 60 and 40% lower, respectively in McMurdo Sound, while Otago Harbour maximal UV-B and UV-A were 38 and 28% lower, respectively (Table 1). Maximum PAR was greatest in Otago Harbour (892.9 W m^{-2}), although the site had low ambient irradiances (i.e. ≤198.1 W m^{-2} PAR) during the first day of *in situ* experiments due to thick cloud cover. Due to the differences in the length of the *in situ* embryo experiments and day length, total ambient UV-dose was greatest during the McMurdo experiments (Table 1).

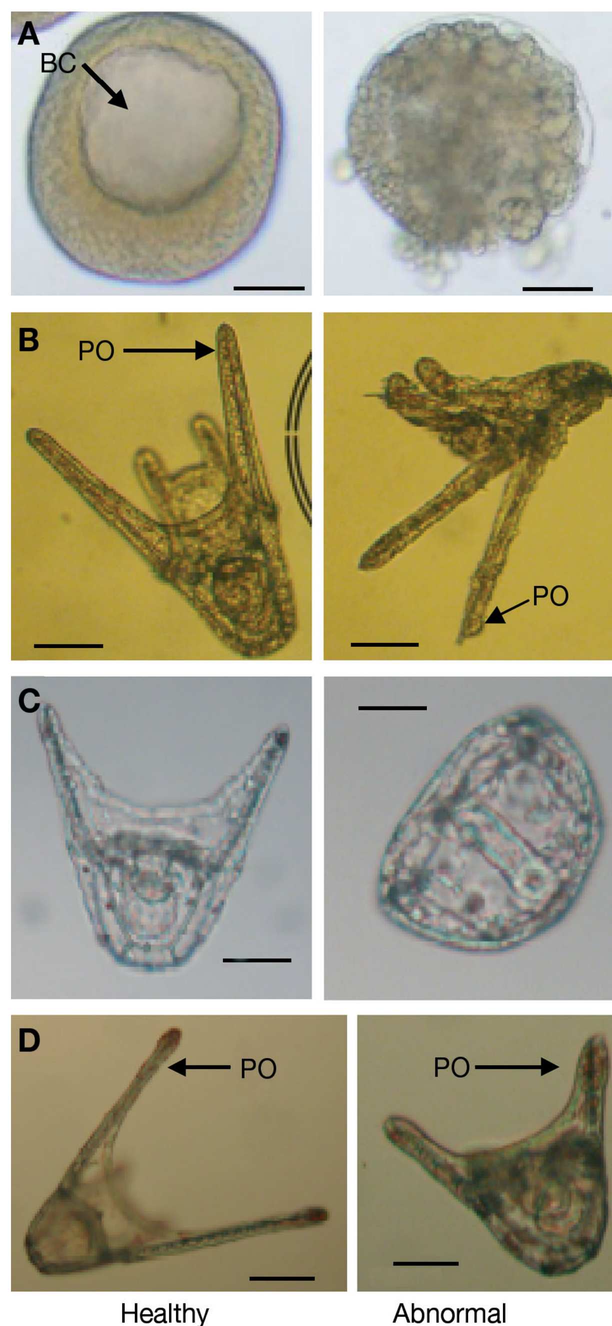

Fig. 1. (A) *Sterechinus neumayeri.* Healthy (left) and abnormally (right) developed embryos after 5 d exposure to ambient UV-R. Normally developed blastula has a clearly defined blastocoel (BC), a flattened vegetal pole, and the ingression of 1° mesechyme. In contrast, cell division rate and organization is highly retarded in the UV-R exposed abnormal blastula. (B) *Evechinus chloroticus.* Abnormal development included death or no development past the gastrula stage, or development to the pluteus stage but with absent, or shortened postoral arms (PO) with necrosis of tissue frequently observed. Abnormal development of (C) *Tripneustes gratilla* and (D) *Diadema savignyi* included death or no development past the gastrula stage, or development to the pluteus stage but with absent or shortened postoral arms (PO). Scale bars = 100 µm

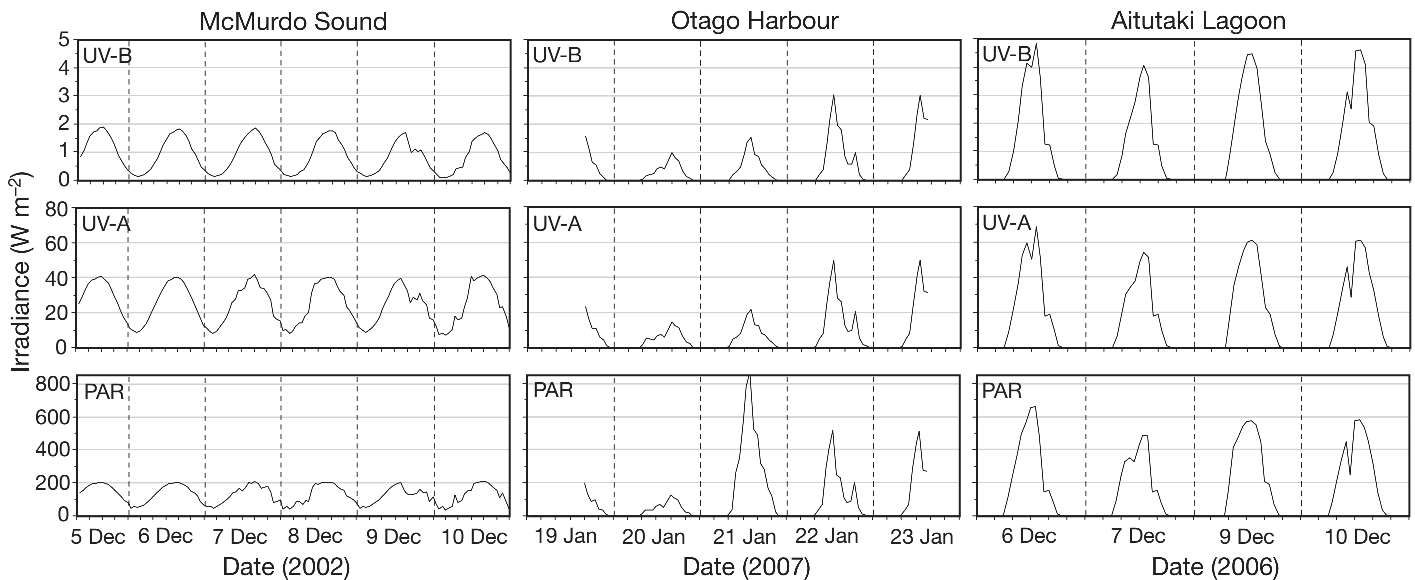

Fig. 2. UV-B, UV-A and PAR irradiances at Otago Harbour, McMurdo Sound and Aitutaki Lagoon during the respective periods of *in situ* embryo exposure assays. For Otago and Aitutaki, UV-B, UV-A and PAR were measured as 300–320, 320–400 and 400–700 nm, respectively; while Antarctica measurements were 290–320, 320–400 and 400–600 nm, respectively

Water column irradiances

Attenuation of light through the water column was greatest in the Otago Harbour, with the depth of 50% of surface irradiance calculated at 0.64, 1.05, and 1.48 m for UV-B, UV-A and PAR, respectively (Fig. 3).

Table 1. Maximum (Max.) irradiance, mean daily dose and total dose of UV-B, UV-A and PAR for Aitutaki Lagoon, Otago Harbour and McMurdo Sound during the period of *in situ* embryo exposures

	Max. irradiance ($W\ m^{-2}$)	Mean daily dose ($kJ\ d^{-1}$)	Total dose ($kJ\ m^{-2}$)
Aitutaki (6 & 7 Dec)[a]			
UV-B	4.57	87.8	175.6
UV-A	60.9	1338.4	2676.9
PAR	576.4	13016.5	26033.1
Aitutaki (9 & 10 Dec)[a]			
UV-B	4.81	86.6	173.2
UV-A	68.5	1315.8	2631.6
PAR	658.2	12768.5	25537.0
Otago[a]			
UV-B	3.02	38.2	152.8
UV-A	49.72	640.3	2561.3
PAR	892.9	5936.1	23744.2
McMurdo[b]			
UV-B	1.88	73.0	438.1
UV-A	41.93	2031.9	12191.6
PAR	209.23	10531.9	63101.7

[a]UV-B: 300–320 nm, UV-A: 320–400 nm, PAR: 400–700 nm
[b]UV-B: 290–320 nm, UV-A: 320–400 nm, PAR: 400–600 nm

Extinction coefficients (K_d) calculated for UV-B and UV-A were: Otago, 1.084 and 0.66 m^{-1}; Aitutaki, 0.234 and 0.129 m^{-1}; and McMurdo, 0.316 and 0.171 m^{-1}, respectively. There was greater penetration of light through the Aitutaki and McMurdo water columns, with the depth of 50% of surface irradiance 2.19 m and 2.96 m, respectively, for UV-B, and 4.05 m and 5.37 m, respectively, for UV-A (Fig. 3). Percentage of UV-B and UV-A at 5 m depth was greatest at Aitutaki (26.9 and 48.9%, respectively), compared with McMurdo (14.4 and 28.0%) and Otago (0.09 and 1.31%).

Maximum irradiances at the depths at which embryos were exposed *in situ* were highest at all depths for the Aitutaki site, with UV-B modelled as 4.11, 2.99 and 1.62 W m^{-2} for 0.5, 1.5 and 4 m depths, respectively, and UV-A modelled as 64.23, 56.46 and 40.89 W m^{-2}. At the shallowest depth (0.5 m), UV-B and UV-A were 2.86 and 35.8 W m^{-2}, respectively, in Otago Harbour and 1.61 and 38.5 W m^{-2} in McMurdo Sound, but much less at 1.5 and 5 m depths in the Otago Harbour (0.59 and 0.01 W m^{-2}) compared with McMurdo (1.17 and 0.38 W m^{-2}). In Otago Harbour, UV-A at 1.5 and 5 m depths was 18.5 and 1.83 W m^{-2} and in McMurdo Sound 45.4 and 15.0 W m^{-2}.

Total UV-B and UV-A doses at each experimental depth were greatest for McMurdo, with total UV-B dose estimated as 374.0, 272.7, and 90.2 kJ m^{-2} at 0.5, 1.5 and 5.0 m depths, respectively, and total UV-A dose estimated as 3109.1, 2620.4, and 1440.3 kJ m^{-2} for the same depths. Total UV-B doses at Aitutaki for 0.5, 1.5 and 4.0 m depths were 149.97, 109.33 and 49.62 kJ

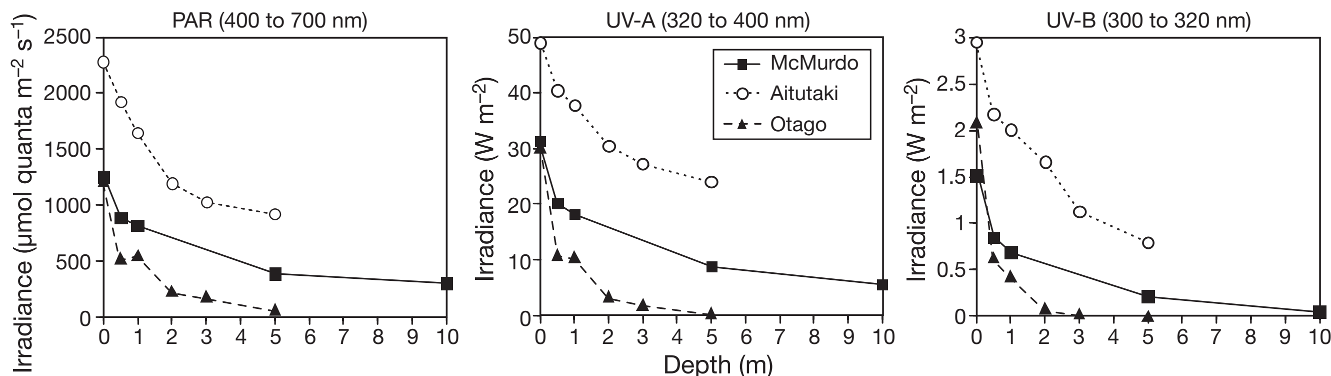

Fig. 3. PAR, UV-A and UV-B irradiance at Otago Harbour, McMurdo Sound, and Aitutaki Lagoon

m^{-2}, and for UV-A 2509.7, 2206.0 and 1597.9 kJ m^{-2}, respectively. For Otago, total UV-B doses at 0.5, 1.5 and 5.0 m depths were 144.76, 30.1 and 0.67 kJ m^{-2}, respectively, and for UV-A 1841.36, 951.71 and 94.46 kJ m^{-2}.

Wavelength specific attenuation coefficients (Fig. 4) were similar in the Aitutaki Lagoon and McMurdo Sound water columns, although the attenuation of the shortest wavelengths (<320 nm) was marginally higher at the latter site. In comparison, attenuation coefficients at each wavelength in the Otago Harbour were 3 to 4 times higher, especially in the shorter UV-R wavelengths, where attenuation was disproportionately higher than in visible wavelengths.

Biologically effective irradiance

Spectral irradiances at each depth were estimated from the product of hourly surface spectral measurements and bulk attenuation coefficients for the water column of each site (Fig. 4). Biological weighting of spectral irradiances at each experimental depth was made using the Setlow DNA weighting function (Setlow 1974). Set-

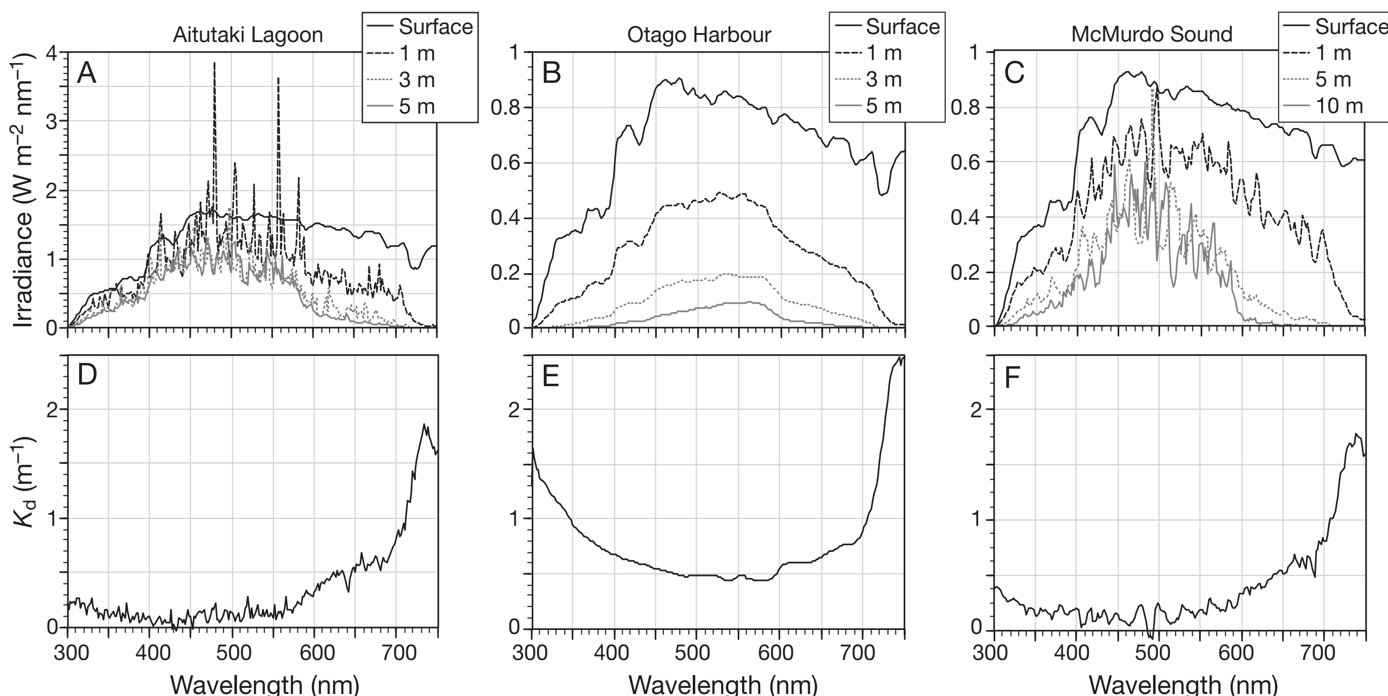

Fig. 4. (A–C) Spectral irradiance vs. wavelength between 300 and 750 nm for Aitutaki Lagoon, Otago Harbour and McMurdo Sound. (D–F) Bulk attenuation coefficient (K_d m^{-1}) versus wavelength between 300 and 750 nm through the water column at Aitutaki Lagoon (between 0.5 m and 5 m depth), Otago Harbour (between 1 and 3 m depth) and McMurdo Sound (between 1 and 5 m depth)

Table 2. Setlow DNA-weighted UV-R doses at the three experimental depths at Aitutaki Lagoon, Otago Harbour and McMurdo Sound during the period of *in situ* embryo exposures

Depth (m)	Weighted UV-R doses (kJ m^{-2})			
	Aitutaki 6 & 7 Dec	Aitutaki 9 & 10 Dec	Otago Harbour	McMurdo Sound
0.5	0.2211	0.2672	0.1003	0.1850
1.5	0.1794	0.2168	0.0233	0.1323
4 to 5	0.1069	0.1292	0.0001	0.0422

low-weighted doses during *in situ* experiments were highest for the 2 tropical species, ranging from 0.26 to 0.10 kJ m^{-2} over the depth range (Table 2). Setlow-weighted UV-R doses during the McMurdo experiments were lower, ranging from 0.18 to 0.04 kJ m^{-2}, while Setlow-weighted doses during the Otago experiments were between 0.001 and 0.1 kJ m^{-2}.

In situ embryo exposures

CPD concentration

Concentrations of CPDs after *in situ* UV-R exposures varied from 0 to 30.8 CPDs Mb^{-1} DNA, depending on species, depth and UV treatment (Fig. 5). Concentrations were highest in *Sterechinus neumayeri* embryos, decreasing from an average of 30.8 CPDs Mb^{-1} DNA in the shallow UV-T treatments, to 0.49 CPDs Mb^{-1} DNA in the shallow UV-0 treatments. For *S. neumayeri*, there was a significant ($F_{2,18}$ = 79.43, p < 0.001) effect of light treatment, with CPD concentrations significantly higher in the UV-T compared with UV-0 and UV-A treatments, but no significant difference between the UV-A and UV-0 treatment (Fig. 5D). The light treatment effect was consistent for all depths (Filter × Depth, $F_{4,18}$ = 0.85, p = 0.57), with CPD concentrations reduced by between 87.0 and 90.6% when UV-R is removed, and by 87.6 to 96.2% when UV-B is removed compared with UV-T treatments. CPD concentrations decreased significantly ($F_{2,18}$ = 4.03, p = 0.03) with increasing depth, with concentrations in the UV-T treatment 46% higher at the shallowest treatment (30.8 CPDs Mb^{-1} DNA) compared with the deep UV-T treatment (16.9 CPDs Mb^{-1} DNA).

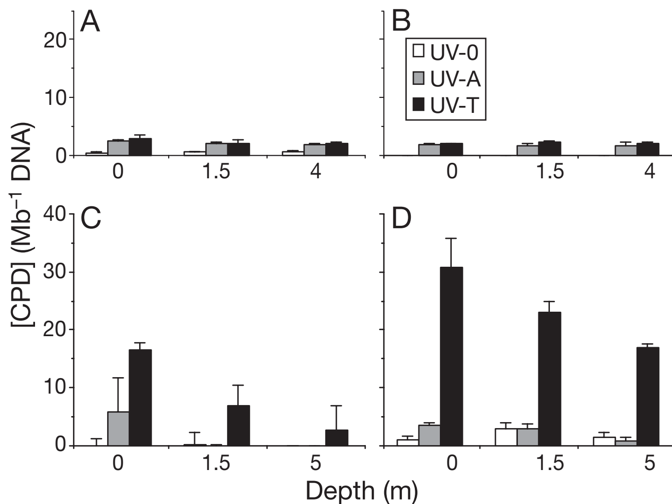

Fig. 5. (A) *Diadema savignyi*, (B) *Tripneustes gratilla*, (C) *Evechinus chloroticus* and (D) *Sterechinus neumayeri*. Cyclobutane pyrimidine dimer (CPD) concentrations in embryos as a function of UV-treatment (UV-minus [UV-0], UV-B minus [UV-A], UV-transparent [UV-T]) at the 3 experimental depths (0.5, 1.5, and 4.0 or 5.0 m) for 4 sea urchin species. N = 3 for each bar; error bars: +SE

Evechinus chloroticus embryos had concentrations of CPDs ranging from 16.5 CPDs Mb^{-1} DNA to 0.7 CPDs Mb^{-1} DNA (Fig. 5C). For the 0.5 and 1.5 m depths, concentrations of CPDs were significantly ($F_{2,12}$ = 8.25, p = 0.005) different among light treatments. The effect of light treatment was consistent at both experimental depths (Depth × Light; $F_{2,12}$ = 1.56, p = 0.249), with concentrations higher in the UV-T treatment compared with the UV-A and UV-0 treatments, although the later 2 treatments were not significantly different from one another. CPD concentrations were between 64.1 and 89.7% lower at 0.5 and 1.5 m depths respectively when UV-B was removed, and between 97.3 and 82.5% lower at 0.5 and 1.5 m depths, respectively, when UV-R was removed. CPD concentrations were 87% higher in the shallow UV-T treatment compared with the deep treatment, although the differences among the depths were not significantly different ($F_{2,6}$ = 2.422, p = 0.169).

Lowest concentrations of CPDs were measured in the 2 tropical species (Fig. 5A,B), with concentrations of CPDs <3.0 CPDs Mb^{-1} DNA in *Diadema savignyi* and <2.2 CPDs Mb^{-1} DNA in *Tripneustes gratilla.* For *D. savignyi,* there was no significant ($F_{2,18}$ = 0.859, p = 0.44) effect of depth on CPD concentration. The effect of filter was significant ($F_{2,18}$ = 9.46, p = 0.001), with the removal of UV-B reducing CPD concentration by 17.1 and 9.4% at 0.5 and 4 m depths, respectively (concentrations were 6.2% higher in the UV-A treatment at 1.5 m). Blocking of total UV-R reduced CPD concentrations by 84.1, 72.5 and 73.3% at the 0.5, 1.5 and 4 m depths, respectively. For *T. gratilla,* CPD concentrations in each filter treatment did not differ significantly ($F_{2,18}$ = 0.089, p = 0.91) among depths, with CPD concentrations in the UV-T treatment ranging from 2.16 to 2.24 CPDs Mb^{-1} DNA. Concentrations of CPDs were significantly ($F_{2,18}$ = 79.471, p < 0.001) lower in the UV-0 treatment compared with the UV-T and UV-A treat-

ments, which in turn, were not significantly different. Removal of UV-B reduced CPD concentrations by 10.8, 27.8 and 17.6 % at 0.5, 1.5 and 4 m depths, respectively, while blocking of all UV-R, resulted in 100, 99.1 and 98.9 % CPD concentration reductions for the same depths, respectively.

Inter-specific comparisons of CPD concentrations within each filter treatment indicated significant differences among species. For the UV-T treatment, there was a significant difference between shallow and deep CPD concentrations ($F_{2,24} = 4.29$, $p < 0.025$), and among species ($F_{3,24} = 22.68$, $p < 0.001$), with the exception of the 2 tropical species that did not differ in CPD concentration. For the UV-0 treatment, there was no difference in concentrations among depths ($F_{2,24} = 0.416$, $p = 0.66$), but there was a significant ($F_{3,24} = 13.39$, $p < 0.001$) difference among species, with concentrations in *Sterechinus neumayeri* significantly higher than in the other 3 species. For the UV-A treatment, there was no significant difference in CPD concentrations among depth and species.

CPD concentrations were positively correlated with total UV-B dose (Fig. 6A) for *Sterechinus neumayeri* and *Evechinus chloroticus*, but not in the 2 tropical species. The rate of increase in CPD concentration with increasing UV-B dose was similar in *S. neumayeri* and *E. chloroticus* (i.e. described by a common linear relationship), however, concentrations in the 2 tropical species were significantly lower at equivalent doses, and did not follow the same linear relationship. There was no relationship between CPD concentration and total *in situ* UV-A dose when UV-B was removed (Fig. 6B). Concentrations of CPDs in *S. neumayeri* and *E. chloroticus* increased with increasing Setlow-weighted total *in situ* UV-R doses (Fig. 6C), but the same relationship could not be found in the 2 tropical species. Furthermore, CPD concentrations in *S. neumayeri* and *E. chloroticus* were significantly higher than for the 2 tropical species, despite the later 2 receiving similar or higher Setlow-weighted UV-R doses.

Embryo development

In *Tripneustes gratilla* (Fig. 7A), percentage abnormality did not differ significantly among depths ($F_{2,18} = 1.41$, $p = 0.271$) or irradiance treatments ($F_{2,18} = 1.32$, $p = 0.293$). In *Diadema savignyi* (Fig. 7B), a significant effect of irradiance treatment ($F_{2,18} = 7.80$, $p = 0.004$) was observed at 0.5, 1.5 and 4 m, but there was no effect of depth ($F_{2,18} = 3.16$, $p = 0.066$) on abnormality rate. Post-hoc comparisons indicate significantly higher abnormality in the UV-T treatment, but no difference between the UV-A and UV-0 treatments. For *Evechinus chloroticus* (Fig. 7C), at 0.5 and 1.5 m depth, abnormality rates were significantly different among irradiance treatments ($F_{2,12} = 7.42$, $p = 0.008$) but not depth ($F_{1,12} = 3.242$, $p = 0.097$). Post-hoc comparisons indicated significantly higher abnormality in the UV-T treatment, but no difference between the UV-A and UV-0 treatments. In *Sterechinus neumayeri* (Fig. 7D), the percentage abnormality was significantly different among irradiance treatment ($F_{2,18} = 262.78$, $p < 0.001$) and depths ($F_{2,18} = 27.21$, $p < 0.001$), although the effect of irradiance treatment was dependent on depth (Depth × Irradiance; $F_{4,18} = 19.02$, $p < 0.001$) and specifically, the equivalent percentage abnormality in the UV-A and UV-T treatment at 0.5 m compared with 1.5 and 5 m depth.

Inter-specific comparisons of percentage abnormality within each irradiance treatment indicate significant differences among species. For the UV-T treatment, the difference among species was depth dependent (Species × Depth, $F_{6,24} = 2.53$, $p = 0.048$). For the shallowest exposure, average percentage

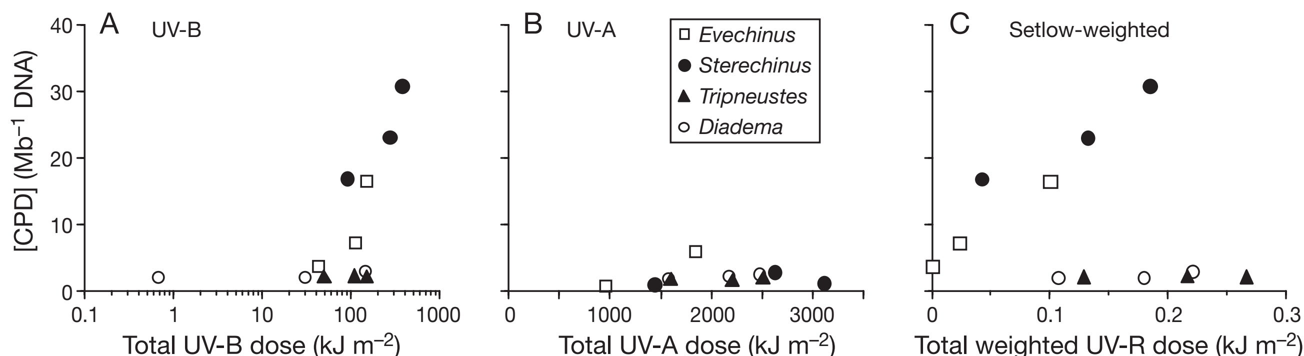

Fig. 6. *Evechinus chloroticus*, *Sterechinus neumayeri*, *Tripneustes gratilla* and *Diadema savignyi.* (A) Cyclobutane pyrimidine dimer (CPD) concentration vs. total UV-B dose (log scale) for embryos exposed to ambient UV-R *in situ* (UV-T treatment). (B) CPD concentration vs. total UV-A dose for embryos after *in situ* exposures where UV-B is removed (UV-A treatment). (C) CPD concentration vs. total Setlow-weighted UV-R dose for embryos exposed to ambient UV-R *in situ.* N = 3 for each data point

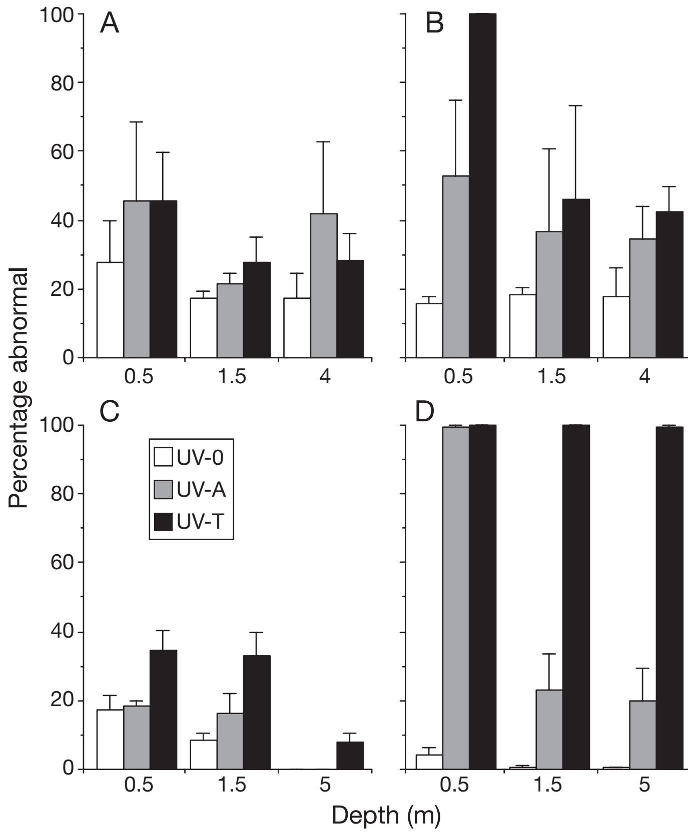

Fig. 7. (A) *Tripneustes gratilla*, (B) *Diadema savignyi*, (C) *Evechinus chloroticus* and (D) *Sterechinus neumayeri*. Percentage of abnormally developed embryos as a function of UV-treatment (UV-minus [UV-0], UV-B minus [UV-A], UV-transparent [UV-T]) at the 3 experimental depths (0.5 m, 1.5 m, and 4.0 or 5.0 m) for 4 sea urchin species. N = 3 for each bar; error bars: +SE

abnormality was significantly higher ($F_{3,8}$ = 38.25, p < 0.001) in *Sterechinus neumayeri* (100%) and *Diadema savignyi* (99.9%) compared with *Evechinus chloroticus* (34.3%) and *Tripneustes gratilla* (45.5%), while at 1.5 and 5 m depths, *S. neumayeri* had significantly greater percentage abnormality (99.3 to 100%) among the 4 species. Difference in percentage abnormality in the UV-A treatments among species was depth dependent (Species × Depth, $F_{3,16}$ = 3.29, p = 0.047), with the percentage significantly higher ($F_{3,8}$ = 6.03, p = 0.018) in *S. neumayeri* at 0.5 m depth (99.6%), while there was no significance difference ($F_{3,8}$ = 2.21, p = 0.174) among species at 1.5 m depth. For the UV-0 treatment at 0.5 and 1.5 m depths, there was a significant difference in the percentage of abnormally developing embryos among species ($F_{3,16}$ = 16.96, p < 0.001), but not depths ($F_{1,16}$ = 3.71, p = 0.072). Among the species, the percentage of abnormally developing embryos was significantly lower in *S. neumayeri* (0.6 and 3.9%), while *T. gratilla* had significantly higher percentage abnormalities (17.2 and 27.5%) than the other species.

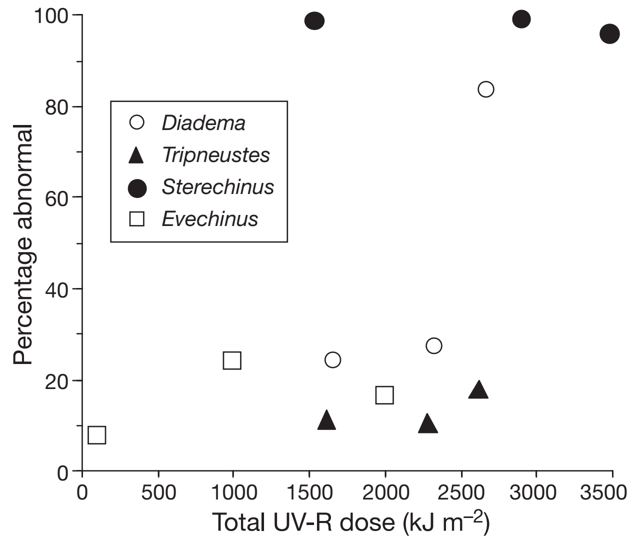

Fig. 8. *Diadema savignyi*, *Tripneustes gratilla*, *Sterechinus neumayeri* and *Evechinus chloroticus*. Percentage of abnormally developed embryos as a function of total UV-R dose (300 to 400 nm) for embryos of 4 sea urchin species during *in situ* exposures. To account for non-UV related abnormal development, the percentage abnormality was expressed as percentage abnormal in the UV-T treatment minus percentage abnormal development in the UV-0 treatment

Among species, percentage abnormality was not closely correlated with total UV-R dose experienced during *in situ* experiments (Fig. 8), although there is some suggestion that abnormal development rates are relatively low (<27.7%) when doses are <2300 kJ m^{-2}, but approach 100% above this level. The exception was the 5 m depth *Sterechinus neumayeri* embryos that experienced 98.8% abnormal development despite receiving 1530 kJ m^{-2} UV-R during the experiment. There was no relationship between percentage abnormality and Setlow-weighted UV-R dose experienced during *in situ* experiments.

DISCUSSION

This study quantified rates of DNA damage in echinoid embryos exposed to ambient UV-R. *In situ* UV-R irradiances varied among the 3 locations examined, both as a function of incident UV-R levels and differences in the optical properties of the water column. Penetration of UV-R at each site can be put into context by a wider review of UV-R penetration in the marine environment (Tedetti & Sempéré 2006). Light penetration in Aitutaki Lagoon is comparable with open ocean conditions, with the depth of UV-B ($Z_{10\%}$ 305 nm) and UV-A ($Z_{10\%}$ 340 nm) measured as 10.16 and 18.87 m, respectively, within the range of open water measurements reported in Tedetti & Sempéré (2006) of 5 to

15.5 m for UV-B, and 8 to 37 m for UV-A. Penetration in McMurdo Sound is within the range of previous estimates made for Antarctic waters. We estimated $Z_{10\%}$ 305 and $Z_{10\%}$ 340 nm as 6.01 and 15.98 m, respectively, which is similar to previous estimates made for Antarctic open water of 5 to 11.5 m for $Z_{10\%}$ 305 nm and 7 to 17 m for $Z_{10\%}$ 340 nm (Tedetti & Sempéré 2006). Our UV-B penetration is at the lower end of these estimates, which is expected given our study was undertaken under relative high atmospheric ozone conditions (286 to 344 DU) compared to reported Antarctic measurements that were generally under ozone hole conditions. In Otago Harbour, $Z_{10\%}$ at 305 and 340 nm were measured at a depth of 1.53 and 2.12 m, respectively, which is at the lower end of estimates reported by Tedetti & Sempéré (2006) for coastal regions of $Z_{10\%}$ 305 nm ranging from 0.5 to 6.7 m and $Z_{10\%}$ 340 nm ranging from 0.7 to 8.2 m.

Tedetti & Sempéré (2006) compared estimates of the penetration of DNA effective doses (using DNA dosimeters) with penetration of light at 305 nm, and concluded that the degree of 305 nm penetration measured at noon can provide an estimate of DNA CPD production in the water column. Based on these observations, potential DNA damage in the Aitutaki Lagoon water column ($Z_{10\%}$ 305 nm = 10.16 m) should be higher than in McMurdo Sound ($Z_{10\%}$ 305 = 6.01 m), and considerably higher than in the Otago Harbour ($Z_{10\%}$ 305 = 1.53 m). Our direct observations of DNA damage in echinoid embryos at the 3 sites do not, however, correspond with the expected amount of damage. CPD production in the Antarctic embryos was an order of magnitude higher than for embryos of the 2 tropical species exposed at Aitutaki, while Otago embryos had intermediate levels of CPD concentrations. Karentz et al. (2004) also recorded high CPD concentrations in *Sterechinus neumayeri* embryos, with concentrations of up to 272 CPDs Mb^{-1} DNA, although these were accumulated at embryos exposed at the sea surface.

Direct interpretation of these observations would suggest that Antarctic embryos are more sensitive to UV-R, with higher rates of CPD accumulation despite being exposed to lower instantaneous irradiance levels. Alternatively, because detrimental effects of UV-R on biological systems (i.e. CPD accumulation) have been shown to be a kinetic process involving a balance between damage and repair (Lesser et al. 1994, Vetter et al. 1999, Browman & Vetter 2002), a time component or total accumulated dose may be more important. Indeed, while CPD concentrations could not be correlated with instantaneous irradiances, CPD concentrations in the 4 species can be related to total UV-B dose received during the experiments. In this respect, *Sterechinus neumayeri* received approximately 2 to 2.5 times the dose at 0.5 m than the 2 tropical species, and even greater doses at depth than *Evechinus chloroticus in* Otago Harbour. A similar observation was made by Karentz et al. (2004), who found that CPD production in *S. neumayeri* correlated well with total UV-B dose, but not with instantaneous irradiance. As with Karentz et al. (2004), we found no relationship between CPD accumulation and total UV-A dose over the course of the field exposures.

The kinetics of CPD accumulation in each species will be the product of CPD formation and repair (i.e. photoreactivation). We know that DNA photoreactivation rates are significantly slower in *Sterechinus neumayeri* compared with *Evechinus chloroticus* and the tropical species *Diadema setosum* (Lamare et al. 2006), with time to remove 90 % of UV-R-induced CPDs modelled as 13.6, 4.8, and 3.6 h for each species, respectively. These rates of DNA repair correspond to the observed differences in CPD concentration, and strongly suggest that DNA repair capacity is an important component of CPD accumulation by sea urchin embryos exposed to UV-R *in situ*. Whether or not the initial rates of CPD formation also differ among the species is unknown, but will primarily be a function of sunscreening capacity by compounds such as mycosporine-like amino acids (MAAs). Previous research has shown that UV-R induced developmental abnormalities in sea urchin embryos and larvae is reduced with increased MAA concentrations (Adams & Shick 1996, 2001), a result which almost certainly reflects a reduction in CPD formation (Adams & Shick 2001). In this respect, Lesser et al. (2006) noted that concentrations of MAA sunscreens were significantly lower in *S. neumayeri* (6.2 ± 4.15 nmol mg^{-1} protein) compared with *E. chloroticus* (155.6 ± 44 nmol mg^{-1} protein), which could result in differential rates of CPD formation between the 2 species. Concentrations of MAAs in the 2 tropical species were not quantified, although spectrophotometric analysis of MeOH extracts indicated the presence of UV-R absorbing compounds, and it is likely that MAAs are an important UV-R mitigating strategy for these 2 species. Indeed, Banaszak et al. (1998) noted a high ovarian MAA concentration of 133.3 nmol mg^{-1} protein in the closely related tropical sea urchin, *Tripneustes ventricosus.* Further support to the suggestion that physiological differences amongst the 4 species (such as sunscreening and DNA repair capacity) drive the observed differences in CPD formation *in situ* is the fact that no relationship existed between observed CPD concentration and Setlow-weighted irradiances among species. In this respect, *S. neumayeri* accumulated much higher CPD numbers than the tropical species, despite receiving a similar Setlow-weighted UV-R dose.

At the developmental level, *Sterechinus neumayeri* embryos showed the highest rates of abnormality

when exposed to UV-R *in situ,* with abnormality rates approaching 100% when exposed to both UV-B and UV-A. These rates are similar to those observed in the same species by Karentz et al. (2004) who observed up to 100% abnormality in shallow waters when embryos were exposed to either full sunlight or UV-A and PAR only. The high rates of abnormality observed in *S. neumayeri* are consistent with the suggestion that this species is the most sensitive to UV-R among the 4 examined. However, the lack of a correspondence between % abnormality and CPD concentration (both within and among species), and the fact that *Diadema savignyi* had relatively high rates of abnormality despite low CPD concentrations, suggests that other UV-R induced damage such as oxidative stress, is also important. This is not surprising given the significant indirect effects of UV-R, especially UV-A, in producing reactive oxygen species and being another source of DNA damage in addition to the direct effects and CPD formation (Lesser 2006).

Previous studies have examined CPD accumulation in marine organisms such as phytoplankton, viruses, and zooplankton from a range of latitudes (summarised in Karentz et al. 2004). In contrast to the present study, CPD accumulation in viruses, bacteria and phytoplankton was greater in sub-tropical or tropical latitudes (see Helbling et al. 2001, 2005, Meador et al. 2002). Patterns in complex metazoans are less clear. Antarctic fish larvae accumulated up to 350 CPDs Mb^{-1} DNA (Malloy et al. 1997), higher than concentrations recorded during *in vitro* exposures of temperate latitude anchovy larvae (*Engraulis mordax*) that accumulated up to 9 CPDs Mb^{-1} DNA (Vetter et al. 1999). CPD accumulation in a suite of invertebrate macrozooplankton collected from surface waters off the Antarctic Peninsula were low, ranging from 4 to 6 CPDs Mb^{-1} DNA (Malloy et al. 1997), contrasting more recent studies on *Sterechinus neumayeri* planktonic embryos which accumulated up to 272 CPDs Mb^{-1} DNA (Karentz et al. 2004).

The difference in the response of various groups (namely picoplankton and phytoplankton versus zooplankton) to latitudinal changes in UV-R is informative, and may reflect differences in the capacity to acclimatize to changes in the light environment. For example, marine algae are able to rapidly produce MAAs in response to increases in UV-R (Helbling et al. 1996, Riegger & Robinson 1997), thereby reducing any detrimental effects of UV-R. Sea urchins accumulate MAAs (through dietary assimilation), but do not increase MAA concentrations in response to increases in UV-R (Adams et al. 2001), an example of the lower capacity of embryos to adapt to changing UV-R levels. Antarctic marine embryos have other physiological characteristics that probably contribute to higher UV-R sensitivity such as very slow development and metabolic rates (Bosch et al. 1987, Hoegh-Guldberg et al. 1991, Marsh et al. 2001), and a low capacity to repair DNA compared with temperate and tropical species (Lamare et al. 2006).

CONCLUSIONS

Previous research has suggested that increased UV-B in the Antarctic may cause increased DNA damage in a range of marine zooplankton (Malloy et al. 1997). This study examined the effects of UV-R on sea urchin embryos *in situ,* with the aim of understanding the relative sensitivity of an ecologically important Antarctic species, *Sterechinus neumayeri,* to UV-R radiation. Antarctic embryos appear to have a low capacity to mitigate the effects of UV-R. In contrast to temperate and tropical species, the Antarctic embryos accumulated a higher number of CPDs during early development, and were also found to have the highest rates of abnormal embryo development when exposed to both UV-B and UV-A. Lamare et al. (2006) showed that DNA repair is slower and likely due to the lack of temperature compensation in the enzyme mediated photorepair process in *S. neumayeri.* The greater DNA damage in Antarctica may in essence be the result of cold sea temperatures, which while not effecting initial CPD formation (the rate of which should be relatively temperature independent), substantially slows metabolic processes such as DNA repair.

The marine environment of coastal Antarctica will likely have elevated ultraviolet-B radiation irradiances (UV-B: 290 to 320 nm) over the next 50 yr as a result of continued stratospheric ozone depletion (Madronich et al. 1998). In addition, marine environments in polar regions may experience future increases in ultraviolet radiation (UV-R: 290 to 400 nm) penetration if sea ice, which is relatively opaque and highly reflective (Lesser et al. 2004), decreases in thickness and spatial and temporal extent as a result of global warming (Holland et al. 2006). Given the observed sensitivity of *Sterechinus neumayeri* embryos to UV-R, these higher *in situ* irradiances have the potential to reduce survival rates in these key life-history stages in the future, and consequently reduce their long-term viability. In addition, such consequences of climate change may be more widespread if the high UV-R sensitivity observed in *S. neumayeri* is a feature of Antarctic marine invertebrate larvae in general (i.e. due to a slower metabolism and development).

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