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DNA photorepair in echinoid embryos: effects of temperature on repair rate in Antarctic and non-Antarctic species

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Summary

To determine if an Antarctic species repairs DNA at rates equivalent to warmer water equivalents, we examined repair of UV-damaged DNA in echinoid embryos and larvae. DNA repair by photoreactivation was compared in three species *Sterechinus neumayeri* (Antarctica), *Evechinus chloroticus* (New Zealand) and *Diadema setosum* (Tropical Australia) spanning a latitudinal gradient from polar (77.86°S) to tropical (19.25°S) environments. We compared rates of photoreactivation as a function of ambient and experimental temperature in all three species, and rates of photoreactivation as a function of embryonic developmental stage in *Sterechinus*. DNA damage was quantified from cyclobutane pyrimidine dimer (CPD) concentrations and rates of abnormal embryonic development. This study established that in the three species and in three developmental stages of *Sterechinus*, photoreactivation was the primary means of removing CPDs, was effective in repairing all CPDs in less than 24 h, and promoted significantly higher rates of normal development in UV-exposed embryos. CPD photorepair rate constant (*k*) in echinoid embryos ranged from 0.33 to 1.25 h^{-1}, equating to a time to 50% repair of between 0.6 and 2.1 h and time to 90% repair between 3.6 and 13.6 h. We observed that experimental temperature influenced photoreactivation rate. In *Diadema* plutei, the photoreactivation rate constant increased from *k*=0.58 h^{-1} to 1.25 h^{-1}, with a Q_{10}=2.15 between 22°C and 32°C. When compared among the three species across experimental temperatures (–1.9 to 32°C), photoreactivation rates vary with a Q_{10}=1.39. Photoreactivation rates were examined in three developmental stages of *Sterechinus* embryos, and while not significantly different, repair rates tended to be higher in the younger blastula and gastrula stages compared with later stage embryos. We concluded that photoreactivation is active in the Antarctic S*terechinus*, but at a significantly slower (non-temperature compensated) rate. The low level of temperature compensation in photoreactivation may be one explanation for the relatively high sensitivity of Antarctic embryos to UV-R in comparison with non-Antarctic equivalents.

Key words: UV-R, photoreactivation, photorepair, photolyase, cyclobutane pyrimidine dimers, CPDs, Antarctica, echinoid, embryo, temperature compensation.

Introduction

Over recent geological time the Antarctic marine system has been a relatively low ultraviolet radiation (UV-R) environment because of its polar location, high atmospheric ozone concentrations, and extensive seasonal covering by relatively opaque, and highly reflective, sea ice. Recent increases in ultraviolet-B radiation (280–315 nm) due to stratospheric ozone depletion have highlighted the susceptibility of marine ecosystems to this important abiotic factor (Smith et al., 1992; Karentz, 1994; Smith and Cullen, 1995). The occurrence of the spring *ozone hole* coincides with the development of embryos of many Antarctic marine invertebrates that may be susceptible to the effects of UV-R because of their small size, lack of a protective tegument, and high rate of cell division (Johnsen and Widder, 2001). Antarctic embryos may be especially vulnerable because they have unique physiological adaptations to survive the cold, food-poor Antarctic waters (cf. Marsh et al., 2001).

Our recent observations confirm that Antarctic marine larvae are very sensitive to UV-R (Lesser et al., 2004). Echinoid embryos exposed to ambient UV-R under annual Antarctic sea ice (a low UV-R environment with irradiances ⩽1% of surface irradiances), exhibited significantly higher rates of mortality, abnormal development (≈30–50%), and DNA damage than embryos exposed concurrently but under a UV-R filter. Biological weighting functions for DNA damage and survival

in Antarctic and temperate sea urchin embryos confirm that the Antarctic species (*Sterechinus neumayeri*) is significantly more sensitive to UV-R than temperate counterparts (Lesser et al., 2006). Similar research on the Antarctic Peninsula demonstrated that *Sterechinus neumayeri* embryos were harmed *in situ* to a depth of 5 m under a depleted ozone column (Karentz et al., 2004), although the damage was not attributed solely to UV-B, but to wider solar effects such as UV-A (315–400 nm).

Ultraviolet radiation is directly detrimental to biological systems by damaging photosensitive molecules (such as DNA, RNA, proteins and lipids), and indirectly through the production of reactive oxygen species within cells (Lesser, 2006). Marine organisms can prevent this damage by *passive* methods with relatively low energetic costs such as sunscreens and non-enzymatic anti-oxidants (Dunlap et al., 2000), or by *active*, energetically costly, mechanisms such as antioxidant enzymes, photoreactivation and dark-excision DNA repair (Kim and Sancar, 1993; Malloy et al., 1997; Mitchell and Hartman, 1990). Three important points relevant to Antarctic embryos with very slow metabolism are: (i) the metabolic costs of active UV-R mitigation could result in reduced rates of repair; (ii) the continuously cold Antarctic waters would result in reduced activity of enzymes involved in UV-R mitigation if no cold-adaptation has occurred (Somero, 1995; Marshall, 1997) and; (iii) the low UV-R environment in the past may not have imposed selective pressures to evolve efficient UV-R mitigation strategies.

To gain a greater understanding of the influence of UV-R damage and repair in Antarctic embryos, and how it is influenced by temperature and larval physiology, DNA repair was examined in the embryos and larvae of the Antarctic sea urchin, *Sterechinus neumayeri.* For comparison, DNA repair rates were also examined in embryos of a counterpart temperate species *Evechinus chloroticus* and a tropical species *Diadema setosum* by quantifying the concentration of DNA lesions, cyclobutane pyrimidine dimers (CPDs), and their repair. 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 (≈85%) CPD or to a lesser extent (≈15%), 6–4 photoproducts (6–4PP). Repair of these dimers can either be by excision repair associated with proof-reading enzymes, or by photorepair mediated by photolyase enzymes (EC 4.1.99.3). Photorepair is a light-dependent (and ATP independent) reaction that utilises the energy in light of wavelengths between 320 and 500 nm to resolve CPD into the constituent nucleotides in a largely error-free reaction (Sancar, 2003).

This research involved two laboratory experiments. The first examined rates of DNA repair in the embryos of the three species to quantify the degree of cold-temperature compensation in DNA repair. This was established by exposing embryos to UV-R and examining the rate of CPD changes over the following 24-h period, and in response to changes in temperature. The second experiment established the importance of light-dependent and light-independent repair

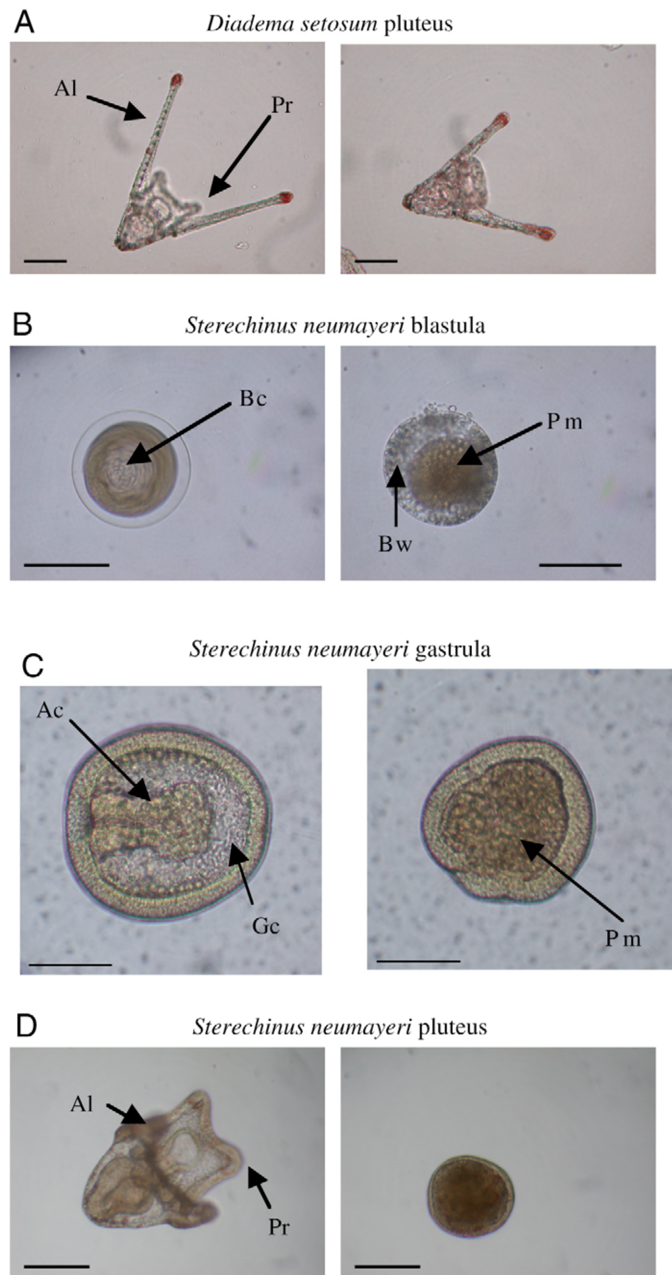

Fig. 1. Normal (left panels) and abnormal (right panels) development in embryos and larvae of (A) *Diadema setosum* and (B–D) *Sterechinus neumayeri*. For *Diadema*, the abnormal plutei (A) were characterised by shortened anterolateral arms (Al), and postoral arms (Pr) appeared to be absent or reduced. For *Sterechinus*, abnormal blastulae (B) had an abnormally thickened blastula wall (Bw) with loosely connected blastomeres. Primary mesenchyme (Pm) appeared to fill the blastocoel (Bc). Abnormal *Sterechinus* gastrulae (C) had no invagination of an archenteron (Ac) with the coelom (Gc) packed with primary mesenchyme cells (Pm). Abnormal *Sterechinus* did not develop beyond the gastrula stage (D). In both *Diadema* and *Sterechinus,* abnormal embryos were either immobile or showed retarded swimming ability compared with swimming in normal embryos. Scale bars, 100 μm.

processes within the three species, both for CPD repair in the embryos, and also for normal embryonic development. This involved exposing embryos to UV-R, followed by a light or dark treatment for 24 h, after which time the degree of abnormal development and DNA damage as a function of light or dark dependent processes was inferred.

Materials and methods

Species and collection sites

Rates of CPD production and repair in DNA were examined for the embryos and larvae of three species, the Antarctic *Sterechinus neumayeri* Meissner (Family: Echinidae), the New Zealand *Evechinus chloroticus* Valenciennes (Family: Echinometridae) and the widespread tropical species *Diadema setosum* Leske (Family: Diadematidae). *Sterechinus* and *Evechinus* were collected from their normal habitats by scuba divers, and *Diadema* were obtained from a 2.5×10^{6} l tank maintained in a public aquarium. The length of time the *Diadema* had been in captivity was unknown. Ambient water temperatures were –1.9°C, 15°C and 27°C for the Antarctic, New Zealand and Australian collection sites, respectively (Table 1).

Spawning and larval rearing

Spawning and experimentation was carried out during the period when each species was ripe, namely October/November for *Sterechinus*, February for *Evechinus* and December for *Diadema.* Ripe individuals were induced to spawn by an inter-coelomic injection of 0.5 mol l^{-1} KCl. Injected animals were inverted over appropriate sized beakers containing filtered ambient temperature seawater, ensuring that the gonopores were underwater. After sufficient gametes were collected (usually ≈15 min), animals were removed and the gametes cleaned by serial partial water changes. Eggs were fertilised by adding several drops of dilute sperm. Fertilisation rate was determined from the appearance of a fertilisation envelope, and only batches of eggs with a fertilisation rate >90% were used in experiments. Fertilised eggs were washed by serial partial water changes, and reared through the blastula and gastrula to the late four-armed pluteus stages (Fig. 1). For this, embryos were diluted to a density of 5–10 individuals ml^{-1}, and transferred to 3 l plastic culture jars. Cultures were kept in motion by gentle agitation with plastic paddles with temperature maintained at the environmental ambient. Embryos and larvae were not fed, and water quality in cultures was maintained by periodic water changes. Development schedules for each species are given in Table 1.

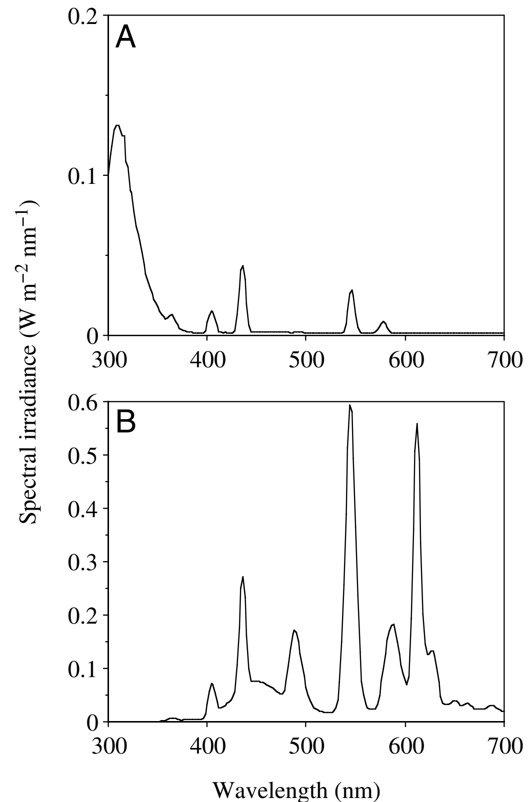

Fig. 2. Spectral irradiances of laboratory light treatments. (A) UV-R bulbs; (B) full spectrum bulbs.

Ultraviolet radiation exposure and repair experiments

Rates of UV-R-induced DNA CPD production and repair was determined in a series of *in vitro* experiments that involved inducing CPDs in embryos and larvae by exposure to UV-B radiation and subsequently monitoring their disappearance (repair) over a 24 h period. Embryos and larvae were subjected to 1 h exposure to UV-radiation emitted from two FS20 lamps (General Electric, Schenectady, NY, USA) suspended 20 cm above the larval cultures. Bulbs were covered with a sheet of

Table 1. *Sampling location and embryonic development schedule for Sterechinus neumayeri, Evechinus chloroticus and Diadema setosum*

Species	Sample location	Latitude/longitude	Sea temperature (°C)	Time to reach developmental stage		
				Blastula	Gastrula	Four-armed pluteus
Sterechinus	McMurdo Sound, Antarctica	77.86°S/166.676°E	–1.9	6 days	12 days	19 days
Evechinus	Doubtful Sound, New Zealand	45.447°S/167.001°E	13–17	–	–	7 days
Diadema	Townsville, Australia	–	25–30	–	26 h	41 h

Table 2. *Ultraviolet and visible light irradiances, Setlow DNA-weighted irradiances and hourly dose in UV-R and visible light treatments administered during laboratory experiments*

	Unweighted irradiance ($W m^{-2}$)			Dose ($J m^{-2}$)		
	UV-B	UV-A	PAR†	UV-B	UV-A	PAR†
UV-lights	1.936	2.557	1.246	6.97	9.208	4.489
Full-spectrum light*	0.003	0.215	30.961	0.014	0.773	111.461

Setlow‡ DNA weighted irradiances

	Setlow weighted irradiance ($W m^{-2}$)		
	UV-B	UV-A	PAR†
UV-lights	0.016	0.000041	–
Full-spectrum light*	0.000005	0.0000003	–

*Full-spectrum lights used in conjunction with Lexan filters to filter all UV-R emissions during 24 h light treatments.
†400–700 nm.
‡(Setlow, 1974).

cellulose acetate to absorb any emitted UV-C and short wavelength UV-B (Cullen and Lesser, 1991). The spectral output, UV-B (300-315 nm), UV-A (315–400 nm) and PAR (400–700 nm) irradiances was measured using a LiCor Li-1800UW spectroradiometer and total dose for the UV bulbs are given in Fig. 2 and Table 2. For the exposures, 5 l of well-mixed larval culture (5–10 larvae ml^{-1}) were divided among five 1 l glass beakers. After 1 h of exposure, the larvae were pooled, well mixed and divided in to 18 aliquots of 250 ml. The pooling ensured that the initial concentration of CPDs were equal in each aliquot. Each aliquot of embryos was allocated to one of three water temperatures. For *Sterechinus* these temperatures were –1.9°C, 0°C and 2°C, and for *Diadema* they were 22°C, 27°C and 32°C. *Evechinus* embryos were only subjected to 15°C. Within each temperature, three of the replicate aliquots were kept in the dark by wrapping each beaker with aluminium foil, while the other three replicate aliquots were exposed to full spectrum artificial light provided by four fluorescent bulbs (Vita-Lite 72, Duro-Test, Philadelphia, PA, USA) suspended 15 cm above the beakers. Each of these light treatments was covered with a Lexan plastic lid that removed any incident UV-R. The spectral output, UV-B, UV-A and PAR irradiance, and total dose for the full spectrum bulbs are given in Fig. 2 and Table 2. Replicates exposed to full spectrum light (light treatment) tested whether embryos were able to repair CPDs through both light-dependent processes (i.e. photoreactivation) and light-independent mechanisms (i.e. dark excision repair), whereas those aliquots wrapped in foil (dark treatments) tested whether embryos could only repair CPDs through light-independent mechanisms.

Samples from each of the aliquots were taken at a number of times during the experiment. Three samples were initially taken from pooled embryos prior to UV-R exposure, and a further three were taken immediately after UV-R exposure. These two sampling times represented a control (no UV damage) and time 0 (maximum UV damage immediately after UV-R exposure and with no repair). For the light treatments, subsequent samples were taken from each aliquot at 1 h, 2 h, 6 h, 12 h and 24 h post-UV exposure. For the dark treatments, similar sampling was undertaken but only the 24 h samples were analysed for CPDs. Samples were spun down (15 s at 3578 g), resulting in a 100–200 μl pellet of packed embryos or larvae. The supernatant was immediate replaced with DNA preservative (Seutin et al., 1991) and the pellet re-suspended by vortex mixing. Samples were then frozen and kept in the dark pending further analysis. All sampling was made under yellow light to ensure that dark treatments were not able to photorepair during sampling.

DNA extractions and CPD quantification

Genomic DNA was isolated from each sample using commercially available extraction kits (DNAeasy Kits, Qiagen Inc., Valencia, CA, USA). DNA concentrations and purity were determined 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 polyvinyl chloride microtiter plates, with three replicate wells assayed for each sample.

For each well, 10 ng of single stranded DNA in PBS (phosphate-buffered saline) (denatured by boiling at 100°C for 10 min followed by 10 min on ice) was added and incubated overnight. Plates were washed five times with 100 μl PBS-T (0.05% Tween-20 in PBS), then 100 μl of 1% milk powder in PBS-T added and incubated for 30 min, after which time wells were washed five times with 100 μl of PBS-T.

To each well, 100 μl of 0.001% TDM-2 primary monoclonal antibody (produced in mice, Medical and Biological Laboratories, Nagoya, Japan) was added, and incubated for 30 min, after which time wells were washed five times with 100 μl PBS-T. 100 μl of 0.002% biotin-F(ab′)$_2$

goat anti-mouse IgG (H+L) (Zymed Laboratories, San Francisco, CA, USA) was added to each well and incubated a further 30 min, at which time wells were washed five times with 100 μl of PBS-T. To each well, 100 μl of 0.0001% streptavidin horseradish peroxidase conjugate (Zymed Laboratories, San Francisco, CA, USA) was added and incubated for 30 min, after which time wells were washed five times with 100 μl of PBS-T. Plates were then washed once with 150 μl of a 0.05 mol l^{-1} solution of citrate phosphate buffer, after which 100 μl of ABTS substrate solution (8.2 mg 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and 3 μl of H_2O_2 in 10 ml of citrate phosphate buffer) was added to each well and incubated for 2 h. Absorption at 405 nm was measured using a microplate reader. For each plate, the absorption readings were adjusted for background absorption, where background absorption was measured in six replicate blank wells in which identical assays were carried out (as above) but without the addition of DNA as in the initial step. All incubations were carried out at 37°C.

For determining rates of CPD repair, a standard curve was generated for each plate, where absorption at 405 nm was measured in wells with 10 ng calf thymus DNA standards that had been exposed to known UV-C doses (0, 2.5, 5, 7.5,10 and 15 J m^{-2} at 254 nm wavelength). The concentrations of CPDs in DNA standards were unknown, but served as internal standards to: (1) ensure that each assay was successful; (2) allow comparisons among assays made on different plates and at different times; and (3) to correct for a non-linear relationship between absorption and CPD concentration. Standard curves (linear) were generated by software (Revelation QuickLink V5, Dynex Technologies, VA, USA) running the microplate reader, and absorption measurements converted to effective dose units (*EDU*) for direct comparison between assays run at different times and on different plates. For comparing light and dark CPD concentrations, we used raw absorption data, although internal standards were used to ensure the assays were successful and plates were comparable.

Repair modelling

Rates of DNA repair were calculated from the decrease in CPDs over time, and modelled as first order exponential decay using the equation:

$$Y = A_0 e^{-kt} ,$$

where Y=concentration of CPDs at time t, A_0=the initial concentration of CPDs, and $-k$ is the rate constant. To allow for the fact that the exponential decay did not tend to zero (due to non-specific binding in the assay resulting in background absorption), we added an extra parameter (c):

$$Y = (A_0 - c)e^{-kt} + c .$$

Parameters for the equation were fitted using a non-linear regression modelling function (Simplex procedure, SYSTAT V5.1, Systat Software Inc., CA, USA). An example of the modelling is shown for *Sterechinus* plutei (Fig. 3). Time taken for 50% and 90% of the CPDs to be repaired (T_{50} and T_{90}) was estimated as:

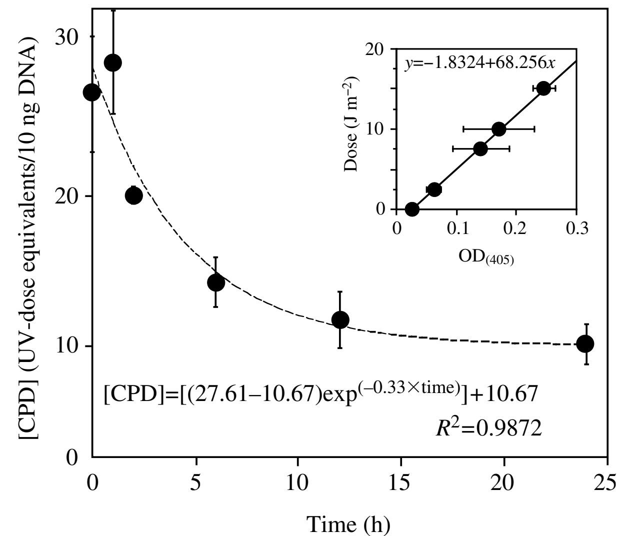

Fig. 3. Change in CPD concentration in *Sterechinus neumayeri* plutei at 0°C. The exponential decay model [$Y=(A_0-c)e^{-kt}+c$] is fitted to the data, from which the rate constant (k) was calculated. The inset is the standard curve of OD_{405} *versus* UV dose [calf thymus DNA exposed to known UV-C doses (0, 2.5, 7.5, 10 and 15 J m^{-2} at 254 nm wavelength)].

$$T = \ln(100\%/b) / k ,$$

where b is the percentage of CPDs remaining (i.e. 50% or 90%).

We examined the relationship between temperature and repair rate by calculating a Q_{10} using the equation:

$$Q_{10} = (k_2/k_1)^{[10/(t_2-t_1)]} ,$$

where k_2 and k_1 are rates at temperature 2 (t_2) and temperature 1 (t_1) ($t_2>t_1$).

Larval morphology

The effects of UV-R exposure and post-UV treatment on stage of larval development and morphology was quantified by examining embryos in each treatment immediately at the end of the 24 h experimental period. For each replicate we examined 20 embryos and classified them as either having normal or abnormal development (Fig. 1). Abnormality of embryos was determined by comparison with control embryos from the same culture not exposed to UV-R.

Statistical analysis

Rate constants of DNA repair (k) were statistically compared among species, developmental stages and experimental temperatures using ANOVA. Our dependent variable, rate constant (k), was $\ln(x+1)$ transformed to ensure normality of data and homogeneity of variances. Fixed factors were species, developmental stage, and experimental temperature. Differences in CPD concentrations among light

Table 3. *Rates of cyclobutane pyrimidine dimer removal in Sterechinus neumayeri, Evechinus chloroticus and Diadema setosum embryos and larvae*

Species	Stage	Experimental temperature (°C)	Rate (k)	EDU a	EDU c	R^2	T_{50} (h)	T_{90} (h)
Sterechinus	Blastula	–1.9	0.83±0.10	131.64	83.93	0.996	0.8	5.4
Sterechinus	Blastula	0	0.48±0.05	41.05	21.78	0.995	1.4	9.4
Sterechinus	Blastula	2	0.81±0.11	126.01	56.19	0.991	0.9	5.6
Sterechinus	Gastrula	–1.9	0.97±0.44	29.18	9.38	0.981	0.7	4.6
Sterechinus	Gastrula	0	0.64±0.30	49.48	17.36	0.988	1.1	7.0
Sterechinus	Gastrula	2	0.93±0.25	28.65	11.21	0.98	0.7	4.8
Sterechinus	Pluteus	–1.9	0.57±0.25	57.51	27.70	0.995	1.2	7.9
Sterechinus	Pluteus	0	0.33±0.10	27.61	10.67	0.987	2.1	13.6
Sterechinus	Pluteus	2	0.34±0.11	26.38	13.13	0.979	2.0	13.2
Evechinus	Pluteus	15	0.93±0.66	13.33	5.34	0.995	0.7	4.8
Diadema	Pluteus	22	0.58±0.12	4.19	0.83	0.983	1.2	7.8
Diadema	Pluteus	27	0.84±0.05	19.37	7.08	0.989	0.8	5.4
Diadema	Pluteus	32	1.25±0.30	18.82	7.47	0.998	0.6	3.6

Rate constants (k h^{-1}; mean ± s.e.m.) were calculated from the exponential decay model [$Y=(A_0-c)e^{-kt}+c$] for three replicates. Time to remove 50% (T_{50}), and 90% (T_{90}) of CPDs are calculated from the equation $T=\ln(100\%/b)/k$, where b=50% or 90%.

EDU, effective dose units.

and dark treatment at 0 h and 24 h after UV-R exposure, and for a no-UV control was examined among species, developmental stages and temperatures. Our dependent variable, CPD concentration, was $\ln(x+1)$ transformed to ensure normality of data and homogeneity of variances. Fixed factors were species, developmental stage, and experimental temperature. Statistical comparison of the effect of UV-R treatment on the ratio of normal and abnormal embryo development among species, developmental stages and temperatures was made using ANOVA. Percentages were arcsine($\sqrt{x}$) transformed prior to analysis. Homogeneity of variances and normality of data were examined using Cochrane's C-statistic and visual inspection of data respectively, and data transformed when required.

Results

DNA CPD repair rates

Exposure to UV-R resulted in the formation of CPDs (expressed as *EDU*) per 10 ng DNA based on DNA standards) in the DNA of embryos and larvae of the three species that we examined (Table 3). Initial CPD concentration after UV-R exposure was highest in *Sterechinus* blastula (41.05–131.64 *EDU*/10 ng DNA), and lower in gastrula (28.65–49.48 *EDU*/10 ng DNA) and pluteus stages (26.38–57.51 *EDU*/10 ng DNA). CPD concentrations after UV-R exposure tended to be lower in *Evechinus* and *Diadema* plutei, ranging from 4.19–19.37 *EDU*/10 ng DNA.

Concentrations of CPDs decreased exponentially over time in all species and stages, with the mean rate constant of CPD removal ranging from k=0.33 h^{-1} in *Sterechinus* to k=1.25 h^{-1} in *Diadema* (Table 3). Within *Sterechinus*, rate constants of CPD repair among developmental stage (Fig. 4, Table 3) tended to be higher in blastula (k=0.48–0.83 h^{-1}) and gastrula stages (k=0.64–0.97 h^{-1}), and lower in the pluteus stage (k=0.33–0.57 h^{-1}), although two-way ANOVA indicated that the differences were not significantly different ($F_{(2,18)}$=3.08, P=0.07). Within *Sterechinus* stages, there was no effect of temperature on repair rate ($F_{(2,18)}$=1.49, P=0.25). For *Diadema* plutei, there was a significant difference among temperatures ($F_{(1,7)}$=7.91, P=0.03) between temperatures and CPD repair rate, with the rate constant increasing from k=0.58±0.12 h^{-1} to 1.25±0.3 h^{-1} over the range of experimental temperatures (22–32°C). The change in repair rate between 22°C and 32°C equates to a Q_{10}=2.15.

Inter-specific comparisons of the rate constant of CPD repair in plutei of *Sterechinus*, *Evechinus* and *Diadema* as a function of temperature (Fig. 5) indicate a significant ($F_{(1,19)}$=7.75, P=0.01) relationship between the two variables, with the rate of repair varying threefold over the –1.9°C to 32°C range of experimental temperatures. The inter-specific relationship between temperature and repair rate was best described by the exponential curve rate=$0.416e^{0.033\times\text{temperature}}$, which equates to a Q_{10}=1.39 over the range of temperature we examined.

Estimated time to repair 50% of CPDs was from 0.6 h in *Diadema* plutei at 32°C to 2.1 h in *Sterechinus* plutei at 0°C (Table 3), whereas time to 90% was from 3.6 h to 13.6 h, respectively. Inter-specific comparisons in estimated time to 90% repair of DNA in plutei stages ranged from 3.6 to 7.8 h in *Diadema* to 7.9 to 13.6 h in *Sterechinus* plutei. For the plutei, inter-specific differences in the time to complete repair decreased 3.8-fold with increasing experimental temperature (Fig. 6).

Effects of light and dark treatment on CPD concentration

The concentration of CPDs among treatments (UV-R exposure, 24-hours light, 24-hour dark treatment, no-UV

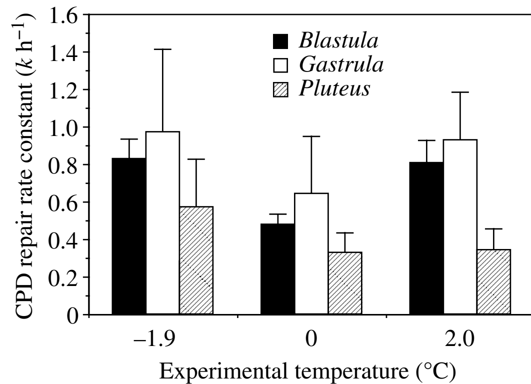

Fig. 4. Relationship between experimental temperature and CPD removal rate constant in the blastula, gastrula and pluteus stages of *Sterechinus neumayeri* at three experimental temperatures (–1.9°C, 0°C, 2°C). Values are means ± s.e.m. (N=3).

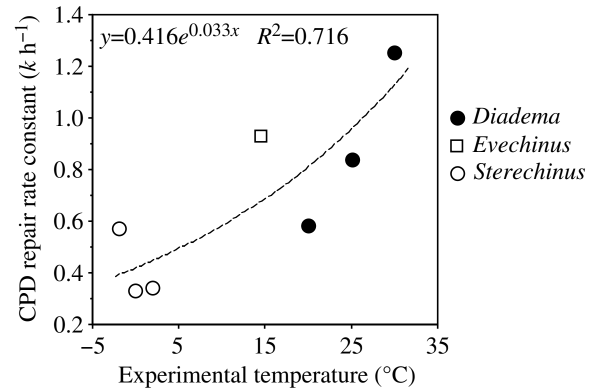

Fig. 5. Relationship between experimental temperature and CPD removal rate constant in pluteus stage of *Sterechinus neumayeri*, *Evechinus chloroticus* and *Diadema setosum.* The relationship between the two variables is expressed by the exponential equation $y=ae^{bt}$.

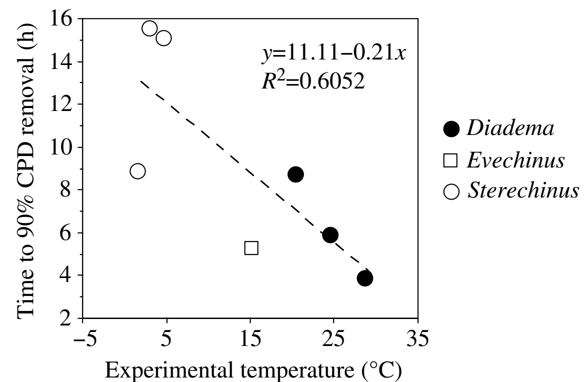

Fig. 6. Relationship between experimental temperature and time to repair 90% of CPDs in plutei of *Sterechinus neumayeri*, *Evechinus chloroticus* and *Diadema setosum.*

control) for embryos and larvae of *Sterechinus*, and *Diadema* is shown in Fig. 7. Two-way ANOVA indicated that CPD concentration was significantly different among experimental treatments ($F_{(3,136)}$=34.97, P<0.01), but was not significantly different among species ($F_{(1,136)}$=3.51, P=0.06). The effect of treatment was not significantly different between species ($F_{(3,136)}$=0.67, P=0.57). Within each species, two-way ANOVA indicated that the effect of treatment on CPD concentration was not significantly influenced by experimental temperature (P>0.05).

Tukey's HSD pairwise comparisons indicated that in all cases, the concentration of CPDs was significantly lower (P<0.01) in embryos and larvae after a 24 h light treatment than immediately after UV-R exposure. In addition, CPD concentrations in the 24 h light treatment were not significantly different (P=0.88) to control concentrations. Concentrations of CPDs in embryos and larvae in the 24 h dark treatment were not significantly different (P=0.09) to concentrations immediately after UV-R exposure, but were significantly higher (P<0.01) than concentrations in the controls. In all cases, the concentration of CPDs were significantly lower (P<0.01) in the 24 h light treatment than in the 24 h dark treatment.

Two-way ANOVA of the effect of light and dark treatment on CPD concentration among developmental stages indicated a statistically significant interaction between the two variables ($F_{(6,132)}$=2.68, P=0.02). Inspection of the data suggest the interaction is due to the variations in concentrations of CPDs in the 24 h dark treatment, which ranged from 82% lower than initial CPD concentrations (i.e. *Sterechinus* gastrulae at 0°C) to 225% higher (i.e. *Sterechinus* plutei at –1.9°C), depending on the stage.

For *Evechinus chloroticus* plutei at 15°C, we found a similar pattern in the effects of light and dark treatments on CPD concentration (Fig. 7), with CPDs lower in light treatments than in dark treatment. We did not however, have a control in this experiment, therefore *Evechinus* was not included in the statistical comparisons with *Sterechinus* and *Diadema.*

Effects of light and dark treatment on larval development

The effect of light and dark post-UV-R treatment on *Sterechinus* and *Diadema* embryo and larval development is shown as in relation to experimental temperature (Fig. 8). The percentage abnormal development was significantly higher ($F_{(1,68)}$=36.65, P<0.01) in dark-treated embryos, ranging from a mean of 16.4% to 79.4%, compared with light treatments which ranged from a mean of 0% to 39.7%. The effects of treatment on development was not a function of species ($F_{(1,68)}$=2.14, P=0.14). Within each species, two-way ANOVA indicated that the effect of treatment on percentage abnormality was not significantly influenced by experimental temperature (P>0.05). Percentage abnormality was significantly different ($F_{(2,66)}$=12.51, P<0.01) among developmental stages. A significant statistical interaction ($F_{(2,66)}$=6.00, P<0.01) between developmental stage and treatment indicates the effects of treatment differed among developmental stages.

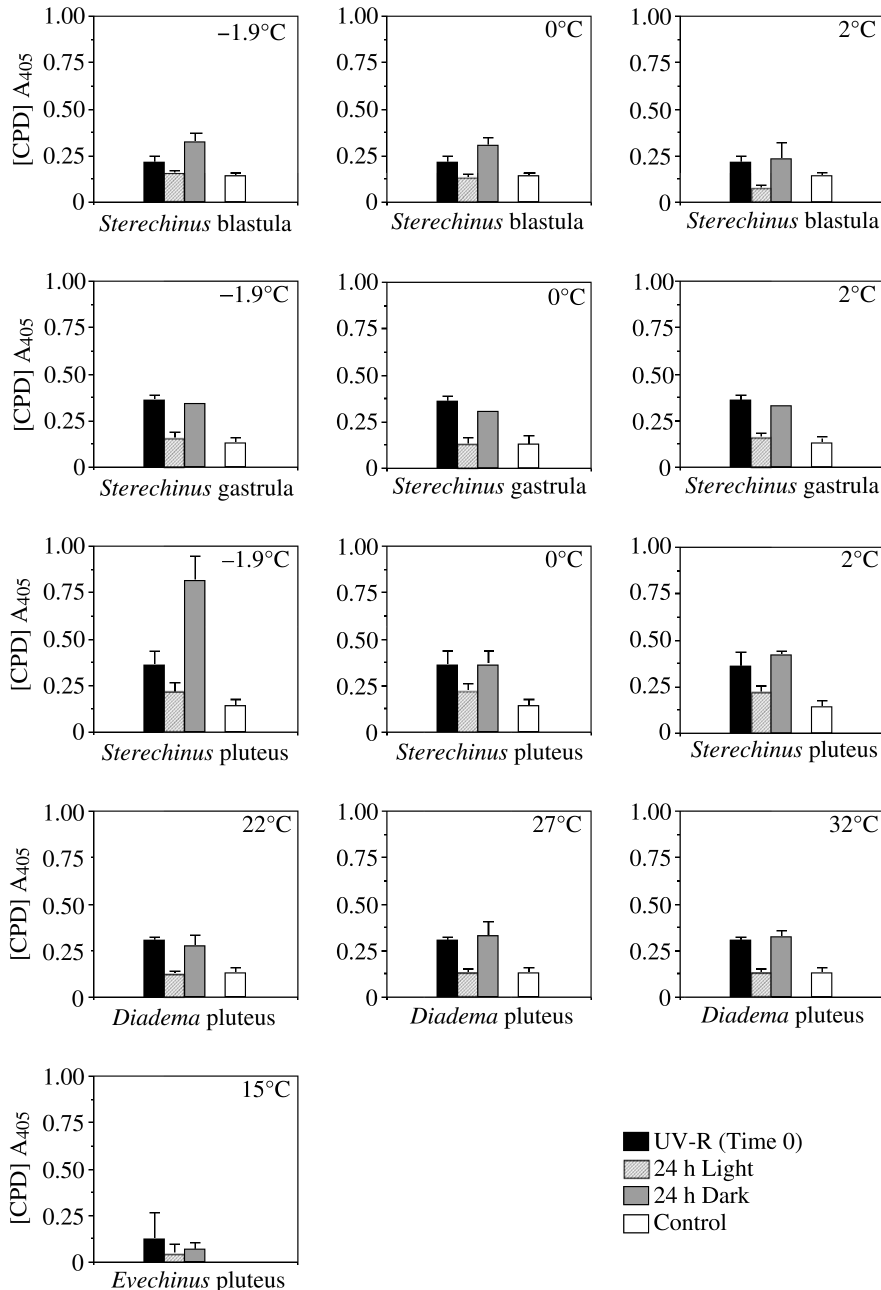

Fig. 7. CPD concentration (expressed as absorbance at 405 nm) immediately after exposure to UV-R for 1 h (time 0), UV-R exposure plus 24 h full spectrum light (24 h Light), UV-R exposure plus 24 h dark (24 h Dark), and no UV-R exposure (Control) in three stages of *Sterechinus* at three experimental temperatures (–1.9°C, 0°C, 2°C) in *Diadema* plutei at three experimental temperatures (22°C, 27°C, 32°C) and in *Evechinus* plutei at 15°C (no control). Values are means ±1 s.e.m. (N=3).

Discussion

Antarctic embryos have a relatively high sensitivity to UV-R, for which slow DNA repair may be a contributor. To address this issue, DNA repair by photoreactivation was compared in sea urchin embryos of three sea urchin species (*Sterechinus neumayeri*, *Evechinus chloroticus* and *Diadema setosum*) that span a latitudinal gradient from 77.86S to 19.25S. The species are from different echinoid families so we cannot rule out phylogenetic effects on photoreactivation. Ideally we would examine photoreactivation in more closely related Antarctic and non-Antarctic species, but this is problematical given the lack of a suitable suite of animals. Despite these limitations however, researchers often make useful comparisons of the physiology of distantly related Antarctic and non-Antarctic species (i.e. Hoegh-Guldberg et al., 1991; Viarengo et al., 1994; Shilling and Manahan, 1994; Marsh et al., 2001), with the comparisons yielding significant insights into the biology of cold-adapted organisms. With these limitations in mind, we compared rates of photoreactivation as a function of ambient and experimental temperature in all three species, and rates of photoreactivation as a function of developmental stage in *Sterechinus.* DNA damage was quantified from CPD concentrations and embryo development.

Previous research has shown that CPD repair is primarily carried out by photoreactivation (Malloy et al., 1997; Häder and Sinha, 2005) and occurs in the embryos of a number of sea urchin species (Marshak, 1949; Wells and Giese, 1950; Ejima et al., 1984; Akimoto and Shiroya, 1986). In our study, we established that in the three species and in three developmental stages of *Sterechinus*, photoreactivation was the primary means of removing CPDs, and was effective in repairing all CPDs in less than 24 h (Fig. 7). In this respect, concentrations of CPDs exposed to UV-R in the laboratory were not significantly different from control concentrations (no UV-R exposure) after 24 h light treatment (i.e. photoreactivation-induced), whereas those embryos kept in the dark (photoreactivation-inhibited) had CPD concentrations that were not significantly lower than initial post-UV exposure CPD concentrations (Fig. 7). At a developmental level (Fig. 8), embryos that were photoreactivation-inhibited after UV-R exposure has significantly higher levels of abnormal development (16.4 to 79.4%) compared with embryos where photoreactivation was induced (0 to 39.7%).

Given the primary importance of photoreactivation repair of CPDs, it was possible to make direct comparisons in photoreactivation rates among species, developmental stages and experimental temperatures without substantial,

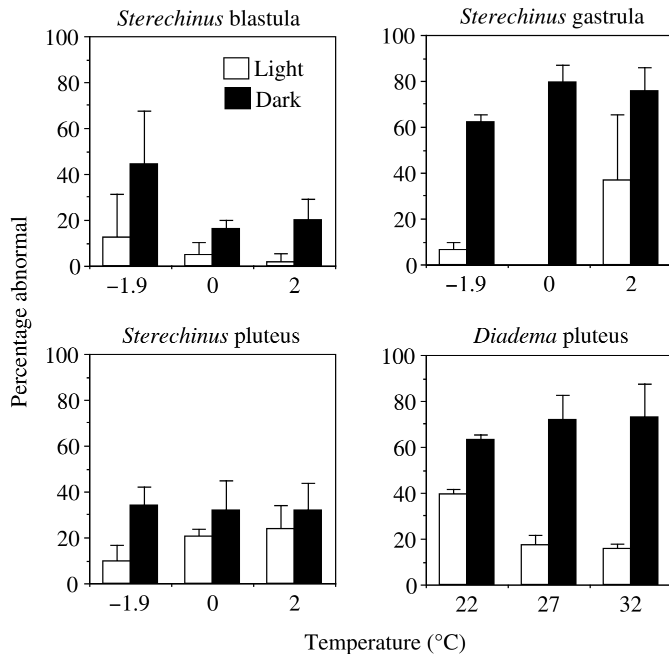

Fig. 8. Percentage rates of abnormality (mean ± s.e.m.; N=3) as a function of temperature in embryos exposed to UV-R followed by a 24 h light or 24 h dark treatment. Shown are rates for three *Sterechinus* stages (blastula, gastrula, pluteus) and *Diadema* pluteus.

confounding effects of alternative repair mechanisms (i.e. NER). We estimated CPD photorepair rate constants (k) in echinoid embryos of between k=0.33 and 0.125 h^{-1}, equating to a time to 50% repair of 0.6 to 2.1 h and time to repair 90% of 3.6 to 13.6 h. These rates encompass previous estimates of CPD repair rate constants (reported as relative photorepair rate, R) in Antarctic [R=0.57 and 0.93 (Malloy et al., 1997)] and temperate killifish *Fundulus heteroclitus* (R=0.68 to 0.91), krill *Euphausia superba* [R=0.96 (Malloy et al., 1997)] and cultured frog cells [$R\approx$0.75 (Mitchell et al., 1986)], but lower than rates observed in the variable platyfish, *Xiphophorous variatus* [$R\approx$1.6 (Mitchell et al., 1993)]. Time to repair CPDs expressed as a percentage for these organisms as well as for *E. coli* and mammalian cells (Table 4) are variable, with time to repair 50% ranging from 25 to 40 min in *E. coli* (Koehler et al., 1996), 1 h in the vascular plant *Arabidopsis thaliana* (Pang and Hays, 1991), to 6 h in mammalian cells (Mellon et al., 1986). Percentage repaired at 24 h ranged from 70 to 95.5%, whereas 90% of CPDs were removed by 6 h in *Arabidopsis thaliana* (Pang and Hays, 1991).

Among these organisms, photoreactivation repair rates cannot be related to phylogenetic differences, with rates in fishes and echinoids sharing similar ranges. Furthermore, repair rate could not be differentiated between embryonic cells (i.e. echinoid embryos) and non-embryonic cells (i.e. Antarctic krill adults, skin of the species). The comparison suggests instead, that rate is more strongly influenced by life history or environment. Indeed, Malloy et al. concluded from their comparison of repair in Antarctic fish that repair rate might be related to vertical distribution in the water column and ambient UV-R (Malloy et al., 1997).

We examined photorepair rate in echinoid embryos, organisms that are more closely related phylogenetically, 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 found that experimental temperature influenced photoreactivation rate. In *Diadema setosum* plutei, the photoreactivation rate constant increased from k=0.58 to 1.25 h^{-1}, with a Q_{10}=2.15 between 22 and 32°C. This is within the range expected for an enzyme-mediated response, and is consistent with previous research on the effects of temperature on photolyase activity and photoreactivation response. The photoreactivation rate increases with increasing temperature (warming) in a wide range of organisms including ciliates (Sanders et al., 2005), freshwater crustaceans *Daphnia pulicaria* (Macfadyen et al., 2004), marine macroalgae *Palmaria palmata* (Pakker et al., 2000) and tobacco cells (Li et al., 2002). An increase in photoreactivation is expected based on the understanding of photolyase enzyme structure and kinetics. Whereas photoreactivation is a largely light-dependent reaction process (namely the monomerisation of the CPD while held within the enzyme), previous work has shown the temperature-dependent nature of the quantum yield of dimer repair (Sancar, 2003). The decrease in quantum yield at lower temperatures has been

Table 4. *Published estimates of photoreactivation repair rates of cyclobutane pyrimidine dimers*

Species	Temperature (°C)	Rate of repair (k)	Time to 50% repair	Time to 80% repair	% at 24 h
Arabidopsis thaliana[5]	22	–	1 h	90% in 6 h	
Human cells[3,4]	37	–	30–50% in 6 h	–	70–90
Antarctic rockcod[1]	–1	0.57	2.37 h	15.84 h	84.0
Antarctic icefish[1]	1	0.93	1.11 h	4.64 h	95.0
Antarctic krill[1]	1	0.96	1.06 h	4.35 h	95.5
Killifish[1]	6	0.68	1.77 h	9.66 h	88.8
	10	0.74	1.55 h	7.80 h	90.8
	25	0.91	1.14 h	4.86 h	94.7
Escherichia coli[2]	–	–	25–40 min	–	–

[1](Malloy et al., 1997); [2](Koehler et al., 1996); [3](van Zeeland et al., 1981); [4](Mellon et al., 1986); [5](Pang and Hays, 1991).

attributed to the polypeptide chain providing some of the activation energy, with the reaction not simply a "photon-powered DNA repair factory" (Sancar, 2003). Consistent with the temperature-dependent nature of the process is the idea that the actual rate-determining step is the bond-breaking that results from the strain imposed on the dimer by the photolyase polypeptide chain (Sancar, 2003).

Given the temperature-dependent nature of photoreactivation, the maintenance of photoreactivation rates across environmental temperature gradients would require temperature compensation in enzyme activity. Temperature compensation in enzymes can be achieved through a number of mechanisms such as increasing enzyme concentrations or structural modification to alter kinetic properties (Somero, 1995), and has been demonstrated in DNA enzymes such as uracil DNA glycosylase (Olufsen et al., 2005). Our inter-specific comparisons of photoreactivation rates in echinoid embryos suggest that there has been minimal temperature compensation. When photoreactivation rate is compared among the three species across experimental temperatures (32°C to –1.9°C), photoreactivation rates decrease with a Q_{10}=1.39 (Fig. 5). Observations by Malloy et al., however (Malloy et al., 1997), suggest that there has been temperature compensation across broader phylogenetic comparisons, with rates in Antarctic krill and pelagic fish comparable with warmer water fishes. Photolyases are an ancient enzyme evolutionarily, and have been shown to be highly conserved structurally and functionally amongst distantly related organisms (Sancar, 1990). Therefore, the mechanism for maintaining photoreactivation rates at the physiological level (be it through structure modification of the enzyme or increased gene expression) is an interesting question.

Although decreasing photoreactivation with lowering ambient temperatures is consistent with the suggestion that the lower temperature will slow down photoreactivation through decreasing enzyme activity (as discussed earlier), other important differences exist between the Antarctic, temperate and tropical embryos and need to be considered. Metabolic rates in Antarctic embryos are very low (Peck, 2002), with respiration orders of magnitude lower than in temperate counterparts (Hoegh-Guldberg et al., 1991). Growth rates and developmental times are similarly slower at lower temperatures (Clarke, 1992), with time to complete development ranging from 115 days in *Sterechinus* (Bosch et al., 1987), 30–60 days in *Evechinus* (Lamare and Barker, 1999) and 42 days in *Diadema setosum* (Onoda, 1936; Mortensen, 1937). Slow physiology as a general feature of Antarctic invertebrate embryos has been attributed to both low temperatures and nutrient constraints (low phytoplankton concentrations). Consequently, the slow rates of photoreactivation observed in *Sterechinus* may not necessarily reflect a lack of temperature compensation in this enzymatic process in isolation, but a symptom of general hypometabolism and excessive metabolic constraints across of suite of physiological process.

Interestingly, we did not see the same relationship between photoreactivation rate and temperature within *Sterechinus* plutei, with the rate decreasing with increasing temperature. Similarly, in *Sterechinus* blastulae and gastrulae rates were either similar or decreased with increasing temperature. This apparent inconsistency may reflect the stenothermal nature of *Sterechinus.* Estimates of thermal tolerance in this species varies, with Tyler et al. (Tyler et al., 2000) finding that embryos and plutei survived well at 2.5°C, whereas Stanwell-Smith and Peck (Stanwell-Smith and Peck, 1998) observed greater survival at temperatures less than ≈1.7°C and an optimal development rate at 0.2°C. In this respect, although our experiments on *Sterechinus* were conducted at physiologically appropriate temperatures, we cannot rule out the slower photoreactivation at experimental temperatures above ambient (0°C and 2°C) being the result of physiological stress. Other echinoid larvae are more eurythermal, and indeed the optimal early developmental temperatures for *Diadema setosum* has been reported between 22°C and 29°C (Fujisawa and Shigei, 1990). Similarly, *Evechinus* has a large latitudinal range and spawns in water temperatures between 12°C and 22°C (M. F. Barker, personal observation). Most other studies have shown an increase in photoreactivation rate with increasing temperature, but there are examples of PER rate decreasing. Photoreactivation in the vascular plant *Arabidopsis thaliana* was found to be temperature sensitive and decreased (Pang and Hays, 1991).

Photoreactivation rates were examined in three developmental stages of *Sterechinus* embryos, and although not significantly different, repair rates tended to be higher in the younger blastula and gastrula stages compared with later stage embryos. Photoreactivation rate has been related to developmental age in other organisms. In the copepod *Acartia omorii*, eggs had more efficient photoreactivation than older stages (Lacuna and Uye, 2001). Similarly, rotifer juveniles showed photoreactivation activity whereas adults showed no evidence of photoreactivation following UV-R exposure (Grad et al., 2003). It appears that photoreactivation rate is more efficient in developing embryos than in differentiated cells (Mitchell and Hartman, 1990), which would be consistent with our observations of greater repair in the earlier less differentiated embryonic stages.

An interesting observation in our results was that 24 h after our UV-R exposures, neither the concentrations of CPDs or the percentage of abnormal development was significantly different among species or temperatures that were photoreactivation induced, despite differences in photoreactivation rates. Therefore, it might be reasonable to ask whether DNA repair rate is important. In this respect, it is important to note that our experimental method involved giving embryos a pulse of UV-R, which would be in contrast to the longer time UV-R exposure experienced *in situ* (i.e. hours). In reality, the accumulation of CPDs is a kinetic process that integrates rate of CPD production and repair over time (Lesser et al., 1994). Therefore, a slow rate of repair, such as in the Antarctic, may be significant if the rate of repair is slower than the rate of CPD accumulation. In that scenario, a net detrimental accumulation of CPDs over time would occur.

The influence of CPD repair rate on normal development may be a function of cell cycle rate. Our results show a positive relationship between the two variables, with both embryonic development rates and photoreactivation rates increasing with increasing ambient temperature. This raises the suggestion that slow DNA repair rates may not contribute to higher UV-R sensitivity by being compensated by slower cell division rates. This idea, however, is not consistent with the results of our field experiments described earlier, where we observed significant CPD accumulation in *Sterechinus* embryos over 5 days despite low ambient UV-R levels. To fully understand the relationship between photorepair rate and *in situ* DNA damage, we are presently undertaking comparative field experiments on *Evechinus* and *Diadema* embryos.

In conclusion, this study found that photoreactivation is active in the Antarctic echinoid *Sterechinus*, but at significantly slower (non-temperature compensated) rates compared with non-Antarctic equivalents. The low level of temperature compensation in photoreactivation may be one explanation for the relatively high sensitivity of Antarctic embryos to UV-R in comparison with non-Antarctic equivalents. Polar regions face three important physical environmental changes: ozone depletion, reductions in sea ice (already evident in the Arctic), and increases in temperature, of which, the first two should significantly increase the amount of UV-R entering the marine system. If slower rates of DNA repair and heightened sensitivity to UV-R are common in Antarctic invertebrate embryos, increased intensities of UV-R within high latitude marine environments may have detrimental influences on the future viability of such Antarctic species. In addition, the stenothermal nature of Antarctic organisms may mean that any increase in sea temperature may reduce and not enhance the activity of enzymes such as photolyase.

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References

Akimoto, Y. and 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.

Bosch, I., Beauchamp, K. A., Steele, M. E. and Pearse, J. S. (1987). Development, metamorphosis, and seasonal abundance of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri*. *Biol. Bull.* **173**, 126-135.

Clarke, A. (1992). Reproduction in the cold – Thorson revisited. *Inv. Reprod. Develop.* **22**, 175-184.

Cullen, J. J. and Lesser, M. P. (1991). The Inhibition of phytoplankton photosynthesis by UV-B radiation: Photoinhibition as a function of dose and dosage. *Mar. Biol.* **111**, 183-190.

Dunlap, W. C., Shick, J. M. and Yamamoto, Y. (2000). UV protection in marine organisms. 1. Sunscreens, oxidative stress and antioxidants. In *Free Radicals in Chemistry, Biology and Medicine* (ed. T. Yoshikawa, S. Toyokuni, Yamamoto and Y. Naito). London: OICA International.

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

Fujisawa, H. and Shigei, M. (1990). Correlation of embryonic temperature sensitivity of sea urchins with spawning season. *J. Exp. Mar. Biol. Ecol.* **136**, 123-139.

Grad, G., Burnett, B. J. and Williamson, C. E. (2003). UV damage and photoreactivation: timing and age are everything. *Photochem. Photobiol.* **78**, 225-227.

Häder, D. P. and Sinha, R. P. (2005). Solar ultraviolet radiation-induced DNA damage in aquatic organisms: potential environmental impact. *Mutat. Res.* **571**, 221-233.

Hoegh-Guldberg, O. H., Welborne, J. R. and Manahan, D. T. (1991). Metabolic requirements of Antarctic and temperate asteroid larvae. *Antarctic J.* **26**, 163-165.

Johnsen, S. and Widder, E. A. (2001). Ultraviolet absorption in transparent zooplankton and its implications for depth distribution and visual predation. *Mar. Biol.* **138**, 717-730.

Karentz, D. (1994). Ultraviolet tolerance mechanisms in Antarctic marine organisms. *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects. Antarct. Res. Ser.* 62 (ed. C. S. Weiler and P. A. Penhale), pp. 93-110. Washington, DC: American Geophysical Union.

Karentz, D., Bosch, I. and Mitchell, D. M. (2004). Limited effects of Antarctic ozone depletion on sea urchin development. *Mar. Biol.* **145**, 277-292.

Kim, S. T. and Sancar, A. (1993). Photochemistry, photophysics, and mechanism of pyrimidine dimer repair by DNA photolyase. *Photochem. Photobiol.* **57**, 895-904.

Koehler, D. R., Courcelle, J. and Hanawalt, P. C. (1996). Kinetics of pyrimidine (6-4) pyrimidone photoproduct repair in *Escherichia coli*. *J. Bacteriol.* **178**, 1347-1350.

Lacuna, D. G. and Uye, S.-I. (2001). Influence of mid-ultraviolet (UVB) radiation on the physiology of the marine planktonic copepod *Acartia omorii* and the potential role of photoreactivation. *J. Plankton Res.* **23**, 143-156.

Lamare, M. D. and Barker, M. F. (1999). *In situ* estimates of larval development and mortality in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). *Mar. Ecol. Prog. Ser.* **180**, 197-211.

Lesser, M. P., Cullen, J. J. and Neale, P. J. (1994). Carbon uptake in a marine diatom during acute exposure to ultraviolet B radiation: relative importance of damage and repair. *J. Phycol.* **30**, 183-192.

Lesser, M. P., Lamare, M. D. and Barker, M. F. (2004). Transmission of ultraviolet-B radiation through the Antarctic annual sea ice and its biological effects on sea urchin embryos. *Limnol. Oceanogr.* **49**, 1957-1963.

Lesser, M. P., Barry, T. M., Lamare, M. D. and Barker, M. F. (2006). Biological weighting functions for DNA damage in sea urchin embryos exposed to ultraviolet radiation. *J. Exp. Mar. Biol. Ecol.* **328**, 10-21.

Li, S. S., Paulsson, M. and Björn, L. O. (2002). Temperature-dependent formation and photorepair of DNA damage induced by UV-B radiation in suspension-cultured tobacco cells. *J. Photochem. Photobiol.* **66**, 67-72.

MacFadyn, E. J., Williamson, C. E., Grad, G., Lowery, M., Jeffrey, W. D. and Mitchell, D. L. (2004). Molecular response to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria*. *Global Climate Change* **10**, 408-416.

Malloy, K. D., Holman, M. A., Mitchell, D. and Detrich, H. W., III (1997). Solar UVB-induced DNA damage and photoenzymatic DNA repair in Antarctic zooplankton. *Proc. Natl. Acad. Sci. USA* **94**, 1258-1263.

Marsh, A. G., Maxson, R. E. and Manahan, D. T. (2001). High macromolecular synthesis with low metabolic cost in Antarctic sea urchin embryos. *Science* **291**, 1950-1952.

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.

Marshall, C. J. (1997). Cold-adapted enzymes. *Trends Biotech*. **15**, 359-364.

Mellon, I., Bohr, V. A., Smith, C. A. and Hanawalt, P. C. (1986). Preferential DNA repair of an active gene in human cells. *Proc. Natl. Acad. Sci. USA* **83**, 8878-8882.

Mitchell, D. L., Clarkson, J. M., Chao, C. C.-K. and Rosenstein, B. S. (1986). Repair of cyclobutane dimmers and (6-4) photoproducts in ICR 2A frog cells. *Photochem. Photobiol.* **43**, 595-597.

Mitchell, D. L., Scoggins, J. T. and Morizot, D. C. (1993). DNA repair in the variable platyfish *(Xiphophorus variatus)* irradiated *in vivo* with ultraviolet B light. *Photochem. Photobiol.* **58**, 455-459.

Mitchell, D. L. and Hartman, P. S. (1990). The regulation of DNA repair during development. *BioEssays* **12**, 74-79.

Mortensen, T. (1937). Contributions to the study of the development and larval forms of echinoderms III. *Kongelige Danske Videnskabernes Selskab Skrifter, Naturvidenskabelig og Mathematisk Afdeling* (Ser. 9) **7**, 1-65.

Olufsen, M., Smalas, A. O., Moe, E. and Brandsdal, B. O. (2005). Increased flexibility as a strategy for cold adaptation. *J. Biol. Chem.* **280**, 18042-18048.

Onoda, K. (1936). Notes on the development of some Japanese echinoids with special references to the structure of the larval body. *Jap. J. Zool.* **6**, 637-654.

Pakker, H., Martins, R. S. T., Boelen, P., Buma, A. G. J., Nikaido, O. and Breeman, A. M. (2000). Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). *J. Phycol.* **36**, 334-341.

Pang, Q. S. and Hays, J. B. (1991). UV-B-inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*. *Plant Physiol.* **95**, 536-543.

Peck, L. S. (2002). Ecophysiology of Antarctic marine ectotherms: limits to life. *Polar Biol.* **25**, 31-40.

Sancar, G. B. (1990). DNA photolyases: physical properties, action mechanism, and roles in dark repair. *Mutat. Res.* **236**, 147-160.

Sancar, A. (2003). Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chem. Rev.* **103**, 2203-2237.

Sanders, R. W., Macaluso, A. L., Sardina, T. J. and Mitchell, D. L. (2005). Photoreactivation in two freshwater ciliates: differential responses to variations in UV-B flux and temperature. *Aquat. Micro. Ecol.* **40**, 283-292.

Setlow, R. B. (1974). The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proc. Natl. Acad. Sci. USA* **71**, 3363-3366.

Shilling, F. M. and Manahan, D. T. (1994). Energy metabolism and amino acid transport during early development of Antarctic and temperate echinoderms. *Biol. Bull.* **187**, 398-407.

Smith, R. C., Prézelin, B. B., Baker, K. S., Bidigare, R. R., Boucher, N. P., Coley, T., Karentz, D., MacIntyre, S., Matlick, H. A., Menzies, D., et al. (1992). Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* **255**, 952-959.

Smith, R. C. and Cullen, J. J. (1995). Effects of UV radiation on phytoplankton. *Rev. Geophys.* **33**, 1211-1223.

Somero, G. N. (1995). Proteins and Temperature. *Ann. Rev. Physiol.* **57**, 43-68.

Seutin, G., White, B. N. and Boag, P. T. (1991). Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool*. **69**, 82-90.

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

Tyler, P. A., Young, C. M. and Clarke, A. (2000). Temperature and pressure tolerances of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri* (Echinodermata: Echinoidea): potential for deep-sea invasion from high latitudes. *Mar. Ecol. Prog. Ser.* **192**, 173-180.

van Zeeland, A. A., Smith, C. A. and Hanawalt, P. C. (1981). Sensitive determination of pyrimidine dimers in DNA of UV-irradiated mammalian cells. Introduction of T4 endonuclease V into frozen and thawed cells. *Mutat. Res.* **82**, 173-189.

Viarengo, A., Accomando, R., Roma, G., Benatti, U., Damonte, G. and Orunesu, M. (1994). Differences in lipid composition of cell membranes from Antarctic and Mediterranean Scallops. *Comp. Biochem. Physiol.* **109B**, 579-584.

Wells, P. H. and Giese, A. C. (1950). Photoreactivation of ultraviolet light injury in gametes of the sea urchin *Strongylocentrotus purpuratus. Biol. Bull.* **99**, 163-172.