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Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin *Strongylocentrotus droebachiensis*

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Abstract Experiments were performed to determine how ultraviolet radiation (UVR) in the environmentally relevant range affects development of the sea urchin *Strongylocentrotus droebachiensis* (Müller) and whether mycosporine-like amino acids (MAAs), present in the early life stages, reduce UV-induced damage. Eggs, embryos, and larvae contained five MAAs having absorption maxima ranging from 320 to 334 nm. Eggs contained principally shinorine and porphyra-334, which absorb maximally at 334 nm and half-maximally at 312 and 348 nm, spanning much of the environmental range of biologically effective UVR. Concentrations of MAAs remained constant in unirradiated embryos through the gastrula stage, but decreased significantly in two-armed pluteus larvae. Daily exposure to combined photosynthetically active radiation (PAR, 400–700 nm) and UVR did not affect the concentration of MAAs in these embryos up to the two-armed pluteus stage. Prism larvae of sea urchins and the sand dollar *Echinarachnius parma* (Lamarck) did not accumulate shinorine from the surrounding seawater. Daily exposure of embryos to UVA (320–400 nm) and UVB (295–320 nm) radiation in the presence of PAR induced delays and abnormalities during development, and removing UVB eliminated this effect. Abnormalities in embryos included thickening of the blastoderm wall, filling of the blastocoel by abnormal cells, exogastrulation, and formation of abnormal spicules. The percentage of embryos that developed normally was lower in batches of embryos exposed to PAR + UVA + UVB, except in embryos from urchins maintained on MAA-rich diets. In all cases, the percentage of PAR + UVA + UVB-exposed embryos that developed normally was positively related to the concentration of MAAs in eggs from which the embryos developed. Thus, the MAAs found in *S. droebachiensis* embryos protect them against UVB-induced abnormalities during their development to at least the four-armed pluteus larval stage.

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Introduction

Owing to decreases in stratospheric ozone, levels of unweighted ultraviolet B (UVB, 295–320 nm) radiation reaching the Earth's surface increased in mid-latitudes of the Northern Hemisphere by an estimated 3% (summer/fall) to 6% (winter/spring) between the 1970s and 1998. In comparison, UVB increased by 50% in Antarctica and by 15% in the Arctic under seasonally ozone-depleted conditions. Atmospheric scientists predict it will be another decade before stratospheric ozone levels begin to recover (Madronich et al. 1998; WMO 2000). With or without such a recovery, organisms will be exposed to potentially damaging levels of ultraviolet radiation (UVR). It is important to understand how organisms are affected by UVR and whether and how they are able to defend themselves against its damaging effects.

Marine organisms living in shallow water are exposed to potentially damaging levels of solar UVB and UVA (320–400 nm) radiation that penetrate to at least 20 m in highly transparent seawater (Jerlov 1950), 20–30 m in Antarctic waters (Karentz and Lutze 1990; Smith et al. 1992), and from one to several meters depth in organically rich coastal seawater (Smith and Baker 1979; Lesser 1995; Booth and Morrow 1997; Adams et al. 2001). Most UVR reaching the earth is in the UVA range. UVA indirectly causes cellular damage by acting on biomolecular photosensitizers to form reactive

oxygen species (ROS) that can oxidize proteins, DNA, and membrane lipids (Tyrrell 1991). UVB radiation is more detrimental and directly damages DNA and proteins by forming pyrimidine dimers, photoadducts, and DNA–protein crosslinks (Harm 1980; Mitchell and Karentz 1993; Tevini 1993). Detrimental effects of UVR on aquatic organisms are well studied (reviewed by Worrest 1982; Shick et al. 1996; Häder et al. 1998), but not entirely characterized.

Because of their small size (optical radius), rapid rates of replication and morphogenesis, and lack of protective coverings, planktonic embryos and larvae of marine invertebrates are seemingly at risk of damage by UVR. Fertilized eggs of echinoderms (including sea urchins and sand dollars) have long served as a model for testing effects of UVR, because they develop normally in seawater in the laboratory, they undergo synchronous development, and UV-exposed echinoderm eggs and embryos exhibit a UV-dose-dependent delay in cell division compared with unirradiated eggs from the same batch (Giese 1964; Rustad 1971; Adams and Shick 1996).

Sea urchin gametes and zygotes also exhibit a host of morphological and physiological abnormalities in response to UVR (Akimoto and Shiroya 1986; Amemiya et al. 1986; Yabe et al. 1998). UV-irradiation decreases the overall size of the blastocoel, and causes it to become filled with non-dividing primary mesenchyme cells and cell fragments. These abnormalities interfere with development and create a permanent blastula, or cause the archenteron to evaginate in a process called exogastrulation, and can eventually inhibit their morphogenesis into prism and pluteus larvae (Akimoto and Shiroya 1986). Unfortunately, these and many earlier studies of development were performed using UVC radiation at 254 nm, which is not environmentally relevant because it is filtered out by atmospheric oxygen and ozone and never reaches the Earth's surface (Madronich et al. 1998).

In situ studies show that incident solar UVR induces similar abnormalities (Anderson et al. 1993; Henderson et al. 1999). However, more work is necessary to identify effects of UVB and UVA on cellular targets such as DNA and the signaling mechanisms that control morphogenesis during development, and to determine whether embryos or larvae are protected against such damage. Sensitivity to UVR appears to depend on developmental stage, with earlier stages being most vulnerable (Amemiya et al. 1986; Yabe et al. 1998).

Green sea urchins spawn in the spring (Cocanour and Allen 1967), during the period of ozone depletion in the Arctic (Madronich et al. 1998). The eggs sink, but the hatched embryos and larvae are negatively geotropic and may remain in surface waters like other echinoid larvae (Pennington and Emlet 1986; Miller and Emlet 1997) and so be exposed to UVR. Echinoid larvae are negatively phototactic (Pennington and Emlet 1986), although water turbulence keeps most slow swimming planktonic larvae from controlling their position in the water column (Denny and Shibata 1989). This may expose embryos and larvae to UVR for extended periods.

Marine phytoplankters show a size-dependent balance between UV-sunscreening and damage-repair defenses. Larger cells, by virtue of their longer optical radii, can derive effective UV-protection from measured concentrations of intracellular mycosporine-like amino acids, MAAs (Raven 1991; Garcia-Pichel 1994; Neale et al. 1998). Eggs, embryos, and larvae of marine invertebrates are sufficiently large that they, too, appear to receive such protection (Gleason 1993; Adams and Shick 1996). MAAs maximally absorb UVR between 309 and 360 nm and are ubiquitous among marine organisms (reviewed by Dunlap and Shick 1998). MAAs correlate with UV exposure in some taxa (Shick et al. 1999), and the concentration of MAAs varies among tissues, often being highest in the eggs (Chioccara et al. 1986; Adams and Shick 1996) and the epidermis (Shick et al. 1992) of echinoderms. Adult urchins acquire MAAs from macroalgae and selectively sequester them in their eggs (Carroll and Shick 1996), which reduces UV-induced delays in cleavage (Adams and Shick 1996). Thus, investiture of eggs with MAAs may decrease the chances of cellular damage and later developmental abnormalities.

Marine organisms also appear to receive protection against UVR through antioxidants including uric acid, carotenoids, tocopherols, glutathione, ovothiols, mycosporine-glycine, gadusols, and ascorbic acid (reviewed by Shick et al. 1996; Dunlap and Shick 1998; Dunlap et al. 2000). Minimization of initial damage by accumulating a dietarily derived UV-sunscreen seems less risky and perhaps less energetically expensive. Therefore, we tested whether daily exposure to UVA and UVB affected development of embryos and larvae of *Strongylocentrotus droebachiensis* and whether intracellular MAAs and one small molecule antioxidant present in embryos, ascorbic acid, protect against UV-induced damage during development of sea urchins, from fertilization through the pluteus stage, under conditions allowing photorepair. Additional experiments tested whether daily UV-exposure altered concentrations of MAAs in embryos and larvae of sea urchins and whether later larvae accumulated dissolved shinorine from seawater.

Materials and methods

Collection of sea urchins and sand dollars

Adult sea urchins, *Strongylocentrotus droebachiensis* (Müller), were collected at Pemaquid Point, Maine, USA (43°50.47′N; 69°30.96′W) and transported to the University of Maine, Orono, in March 1998 and 1999. They were held in a system of recirculating seawater at 4 °C. Eggs from these adults were used to determine MAA concentration during development, and to study whether sea urchin larvae accumulate MAAs from the surrounding media, how urchin embryos and larvae are affected by UVR, and whether MAAs protect against UV-induced damage.

Adult sand dollars, *Echinarachnius parma* (Lamarck), were collected at Fraser Point, Maine (44°20′N; 68°00′W) and transported to the University of Maine, Orono, in June 1997. They were held in a system of recirculating seawater at 15 °C. Embryos from these adults were used in studies examining whether larvae accumulate MAAs from the surrounding medium.

Collection and maintenance of sea urchins on controlled diets

Adult sea urchins were collected by SCUBA from depths >10 m at Crow Island, Maine (43°50.18′N; 69°32.79′W), in September 1997 and 1998, and were held at the Darling Marine Center in indoor aquaria containing flowing seawater at ambient seasonal temperatures (−1 to 15 °C) for 9 months, under ambient light conditions controlled by an astronomical clock with no UV-irradiation (Adams et al. 2001; this issue). Sea urchins were fed the kelp *Laminaria saccharina* or a combination diet of *L. saccharina* and the red alga *Chondrus crispus*. MAAs are undetectable in *L. saccharina* (Carroll and Shick 1996; Adams et al. 2001), but *C. crispus* contains the MAAs shinorine ($\lambda_{max} = 334$ nm), usujirene ($\lambda_{max} = 357$ nm), palythine ($\lambda_{max} = 320$ nm), and asterina-330 ($\lambda_{max} = 330$ nm) (Adams et al. 2001). In the combination diet, urchins were fed primarily *C. crispus* but given supplements of *L. saccharina* every third week to eliminate diet-related differences in egg biochemistry other than MAA content.

Spawning and handling of gametes

Spawning of adult sea urchins and sand dollars was induced by intracoelomic injections of 0.55 M KCl. Eggs and sperm were collected as previously described (Adams and Shick 1996). Eggs were washed three times with 0.22-μm-filtered artificial seawater (FASW) (Adams and Shick 1996). Sperm suspensions of 1:10,000 (sperm:FASW) were used to inseminate eggs.

Each batch of eggs used in experiments reached at least 90% fertilization within 5 min. FASW was used in UV-exposure experiments to avoid development-inhibiting byproducts formed by oxidation of dissolved carbon in natural seawater (Gjessing and Kälquist 1991) and to avoid possible contamination with dissolved MAAs in experiments testing accumulation of MAAs. Sea urchin larvae reared in ASW without the addition of food will develop only to the early four-armed pluteus stage (Ettenson et al. 1997), so development was observed only up to that stage in the present experiments. A 1–4 ml sample was taken of each batch of eggs, embryos, or larvae. Samples were centrifuged, frozen, lyophilized, and stored at −80 °C.

Analysis of MAAs and ascorbic acid

MAAs were removed from lyophilized eggs and embryos by three serial 60-min extractions in 80%. High performance liquid chromatography (HPLC) grade aqueous methanol at 4 °C in the dark. MAAs were separated by reverse-phase HPLC, identified, and quantified as in Adams et al. (2001) using quantitative standards prepared by Dr. W.C. Dunlap (Australian Institute of Marine Science, Townsville). Extraction efficiencies for individual MAAs in each type of sample (eggs and embryos) were calculated (Adams and Shick 1996), and used to correct the concentration of MAAs. Ascorbic acid was quantified in the extracts of eggs as in Adams et al. (2001).

UV-absorption of shinorine extract

Shinorine was partially purified from extracts of the red alga *Mastocarpus stellatus* by HPLC. Extracts were injected (50 μl) onto the HPLC, and the shinorine fraction of the extract was collected at the outflow from the detector cell. The shinorine fractions were combined and scanned on the spectrophotometer to determine the absorbance of the pooled sample and injected onto the HPLC to determine the concentration of shinorine in the sample. The extinction coefficient was calculated for this sample using Beer's Law ($A = \varepsilon \cdot c \cdot l$), where A is the absorbance, c is concentration (M), ε is the extinction coefficient of a compound (M^{-1} cm^{-1}), and l is the path length of the sample cell (cm).

UV-photostability of MAAs in vivo

A comparison was made among MAA concentrations in embryos that were either exposed to PAR alone, or to PAR + UVR during development. Sea urchins were induced to spawn and eggs were fertilized and reared in FASW in a coldroom at 5 ± 2 °C with periodic stirring and water changes. Within 20 min after fertilization, each batch of embryos was halved to create two treatment groups (5% suspension). Each half was placed in a 20 cm diameter culture bowl on a 12 h light:12 h dark photocycle provided by Coolwhite F40 bulbs (Sylvania), and exposed to PAR alone or PAR and 6 h day^{-1} of full UVR (UVA + UVB) ($n = 4$) in the middle of the 12 h light period. Four UV-340 lamps (Q-Panel Lab Products) that were placed 20 cm above the layer of embryos provided UVR (see Shick et al. 1999 for emission properties). Culture bowls containing embryos were covered with UV-opaque Plexiglas G (50% transmission at 400 nm) that transmits only PAR, or UV-transparent Plexiglas G UF-3 (50% transmission at 295 nm) to provide the same level of PAR as the control treatment while exposing embryos to full UVR.

PAR (400–700 nm) was measured using a Li-Cor LI-185B quantum photometer and LI-190SB quantum sensor. UVR was detected using an International Light IL1400A radiometer, and model SEL033 UVA and SEL240 UVB sensors having peak sensitivities at 350 and 295 nm, respectively. Embryos exposed to full UVR experienced an irradiance of 19.3 μmol m^{-2} s^{-1} PAR, 7.04 W m^{-2} UVA, and 0.07 W m^{-2} UVB for 6 h. Embryos exposed to PAR, but not to UVR, experienced a fluence of 18.7 μmol m^{-2} s^{-1} PAR, 0.001 W m^{-2} UVA, and 0 W m^{-2} UVB.

Unirradiated developing sea urchins were sampled as newly fertilized embryos, 2-day hatched blastulae, 4-day gastrulae, 8-day two-armed plutei, and 10-day non-feeding plutei. UV-irradiated embryos and larvae were sampled up to 8 days only. Samples were frozen and lyophilized, and their MAA concentration determined using HPLC. The mean concentrations of MAAs were compared among controls and UV-treated embryos over time using a split-plot analysis of variance (ANOVA), and followed by Student–Newman–Keuls (SNK) multiple comparison tests at $\alpha = 0.05$. Statistical analyses were performed using StatView 5.0 (SAS Institute).

Uptake of MAAs by *S. droebachiensis* and *E. parma*

M. stellatus, collected at Schoodic Point, Maine (44°22′N; 68°00′W), was ground, frozen, lyophilized, extracted for 2 h at room temperature using 5 ml NanoPure (Barnstead) water per gram of dry tissue, and then centrifuged. Accessory pigments were removed from extracts by two passes over C18 Sep-Pak (Waters Corp.) cartridges. Extracts were heated in a boiling water bath and mixed with acid-washed charcoal for 5 min to remove any remaining pigments. Extracts were then clarified by centrifugation, any charcoal remaining in solution was removed using a 0.7 μm glass fiber filter, and extracts were sterilized by filtration through a 0.2 μm Acrodisk filter (Gelman). The concentration of shinorine was determined using HPLC. Calculated amounts of extracts or Nanopure water were added to 5 ml of FASW to achieve the desired final concentration of shinorine in each 2-l (FASW) culture.

To determine whether larvae of *S. droebachiensis* can accumulate MAAs from the surrounding seawater, sea urchins were spawned, their eggs were fertilized, and four batches of embryos were reared in FASW at 5 ± 2 °C with gentle stirring until they reached the gastrula stage (3 days). Each batch was divided into thirds, and the embryos cultured in FASW containing 0, 2.5, or 25 μM shinorine and allowed to develop at 5 ± 2 °C with constant stirring for 3 days until they became prism larvae. Half of the

larvae were rinsed three times with fresh FASW devoid of MAAs and retained on 80 μm Nitex mesh. Prism larvae were centrifuged, the seawater decanted, and the larvae then immediately frozen in liquid nitrogen. The remaining larvae were rinsed once with FASW and then placed in fresh beakers of FASW containing shinorine at the same concentration as that of their prior treatment. Larvae were allowed to develop for two more days to the pluteus stage. These plutei were rinsed, frozen, lyophilized, stored at −80 °C, and analyzed for MAAs using HPLC as described above. The concentrations of all MAAs were compared among 0, 2.5, and 25 μM incubations using a randomized complete block design (RCBD), one-way ANOVA (blocked by batch of eggs).

Additional experiments were performed to determine whether larvae of *E. parma* could accumulate MAAs dissolved in seawater because they have no detectable MAAs in their eggs. Sand dollars were induced to spawn, their eggs were fertilized, and four batches of embryos were reared in FASW at 15 ± 2 °C with gentle stirring until they reached the two-armed pluteus stage (4 days). Each batch was divided into thirds, cultured in seawater containing 0, 1, or 100 μM shinorine, and allowed to develop for an additional 2 days. Plutei were processed as described for sea urchin larvae.

Differential effects of UVA and UVB on development

Sea urchins collected from Pemaquid Point, Maine, in 1998 and 1999 were induced to spawn. Within 20 min of fertilization, each of four replicate batches of embryos was divided among three treatments in separate culture dishes at 5% embryo suspension: (1) PAR (covered with UV-opaque Plexiglas G); (2) PAR + UVA (covered with Mylar Type D fluoropolymer film, 50% transmission at 320 nm); or (3) PAR + UVA + UVB (covered with UV-transparent Plexiglas G UF-3, 50% transmission at 290 nm). Embryos were reared in FASW at 5 ± 2 °C on a 12 h light:12 h dark PAR schedule with UV-exposure for 6 h in the middle of the PAR light cycle. Survivorship and developmental abnormalities of embryos and larvae were documented daily for at least 10 days or until PAR-irradiated embryos died. Embryos exposed nominally to PAR alone experienced an irradiance of 18.7 μmol m^{-2} s^{-1} PAR, 0.001 W m^{-2} UVA, and 0 W m^{-2} UVB. Embryos exposed to PAR and UVA experienced an irradiance of 19.3 μmol m^{-2} s^{-1} PAR, 6.95 W m^{-2} UVA, and 0 W m^{-2} UVB. Embryos exposed to PAR and full UVR (UVA + UVB) experienced an irradiance of 19.3 μmol m^{-2} s^{-1} PAR, 7.04 W m^{-2} UVA, and 0.07 W m^{-2} UVB for 6 h. These fluences are similar to UVR levels measured in the water column where the adult sea urchins were collected (Adams et al. 2001). PAR levels are approximately 100× lower than ambient levels due to the limitation of Coolwhite fluorescent lamps, which may result in limited photorepair of UV-induced damage in these embryos.

Specific abnormalities were identified and counted. These were classified as abnormal blastulae [blastocoels filled with cell fragments or abnormal primary mesenchyme cells (PMCs)], abnormal gastrulae (exogastrulae), or abnormal prism larvae (larvae having no gut and supernumerary and abnormal spicules).

The percentage of normal development in each UV-treatment was compared by age of the embryos using a RCBD, three-way ANOVA (blocked by batch), and followed by SNK multiple comparison tests. Planned (a priori) comparisons were used to characterize further significant interactions among UV-treatments and the age of embryos. Percentage values were arcsine-transformed for statistical analysis.

Changes in the percentage of normal development as a function of the MAA concentration at day 4 in each batch of embryos that were exposed to the full complement of UVR were analyzed by linear regression (StatView). This day was chosen in advance because MAA concentrations were measured for eggs of these batches, and the MAA concentration in embryos remains the same as that in eggs up until at least day 4 (Adams and Shick 1996).

Embryos from each batch were fixed in 1% formalin in buffered seawater at 48 h. The diameter of whole embryos and of the blastocoel, as well as the thickness of the blastoderm, were measured at 100× using a compound microscope and an ocular micrometer. The thickness of the blastoderm was compared among UV treatments using an RCBD one-way ANOVA (blocked by batch), and followed by SNK multiple comparison tests.

Photoprotection by MAAs in sea urchin embryos and larvae

To determine whether MAAs protect against UV-induced damage during development, adult urchins were first maintained on a controlled diet of *L. saccharina* or a combination diet in 1997–1998, as described in "Collection and maintenance of sea urchings on controlled diets". Aliquots of eggs were removed and frozen for analysis of MAAs. Within 20 min of fertilization, each of three batches of embryos was separated into two groups, and replicates were exposed under the previously mentioned Coolwhite and UVA lamps to one of the following treatments: (1) PAR (covered with UV-opaque Plexiglas G) or (2) PAR + full UVR (covered with UV-transparent Plexiglas G UF-3). Embryos were reared in FASW at 5 ± 2 °C on a 12 h light:12 h dark PAR schedule. Embryos exposed to PAR alone experienced an irradiance of 19.3 μmol m^{-2} s^{-1} PAR, 0.001 W m^{-2} UVA, and 0 W m^{-2} UVB. Embryos exposed to PAR + full UVR experienced an irradiance of 18.7 μmol m^{-2} s^{-1} PAR, 5.53 W m^{-2} UVA, and 0.052 W m^{-2} UVB for 6 h in the middle of the PAR light cycle. Survivorship and developmental abnormalities of embryos and larvae were documented daily for at least 10 days or until PAR-irradiated embryos died.

This experiment was repeated in 1998–1999 with an additional comparison among the three treatments PAR, PAR + UVA, and PAR + full UVR, as described in "Differential effects of UVA and UVB on development". To examine whether embryos having exceptionally low concentrations of MAAs were susceptible to UVA in the absence of UVB, embryos were exposed to the same treatments described for embryos of sea urchins collected from the field in 1999.

The MAA concentrations in batches of eggs from adults maintained on different diets were compared using a one-way ANOVA, where the fixed effect was adult diet. The percentage of embryos showing normal development in each UV-treatment was compared by diet of the adults and by age of the embryos using a RCBD, three-way ANOVA (blocked by batch of eggs), and followed by SNK multiple comparison tests. Planned comparisons were used to characterize further significant interactions among UV-treatments, age of embryos, and diet of adults. Percentage values were arcsine-transformed for analysis. Changes in the percentage of normal development in each batch of embryos at day 4 as a function of the MAA concentration were analyzed by linear regression.

The diameters of whole embryos and of the blastocoel, as well as the thickness of the blastoderm, were measured at 48 h for each batch of embryos, as described in "Differential effects of UVA and UVB on development". The thickness of the blastoderm was compared among UV treatments and adult diets using an RCBD two-way ANOVA (blocked by batch of eggs), and followed by SNK multiple comparison tests.

Results

UV-photostability of MAAs in vivo

Figure 1 shows representative UV-visible light absorption spectra of chromatographically purified shinorine and of methanolic extracts of sea urchin eggs. Eggs of *Strongylocentrotus droebachiensis* contained five MAAs – shinorine ($\lambda_{max} = 334$ nm), porphyra-334 ($\lambda_{max} = 334$ nm), mycosporine-2 glycine ($\lambda_{max} = 331$ nm), palythine ($\lambda_{max} = 320$ nm), and asterina-330 ($\lambda_{max} = 330$ nm).

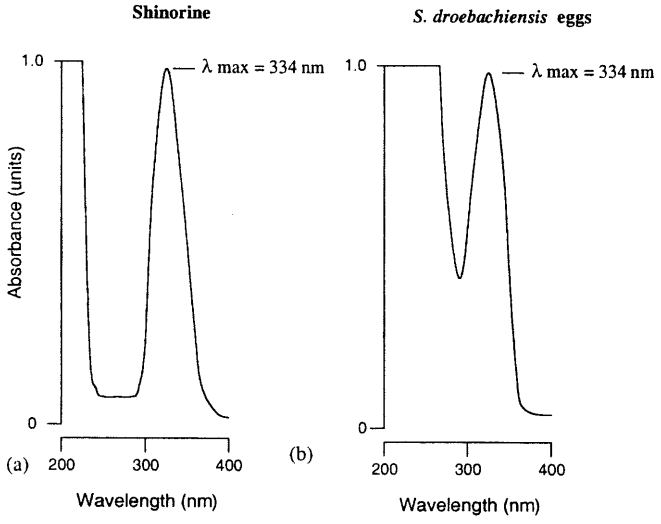

Fig. 1a, b *Mastocarpus stellatus* and *Strongylocentrotus droebachiensis*. UV spectra of (**a**) partially purified shinorine from aqueous methanolic extracts of *M. stellatus* and (**b**) methanolic extracts of eggs from *S. droebachiensis*

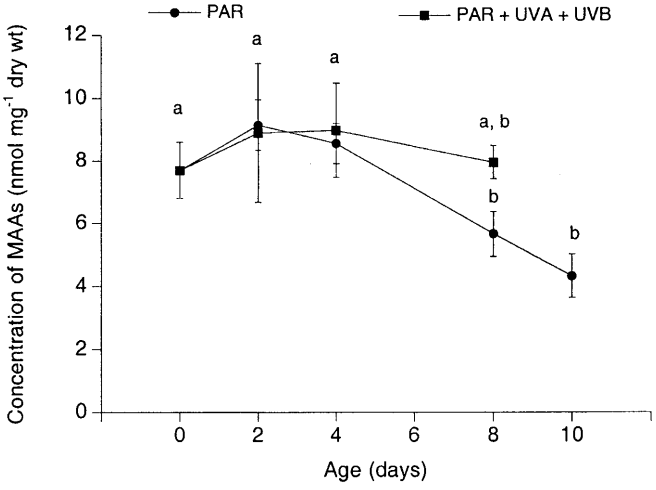

Fig. 2 *Strongylocentrotus droebachiensis*. Concentration of MAAs (mean ± SE) in embryos and larvae exposed to, or protected from, full UVR; eggs from urchins freshly collected from the field. An "a" or "b" indicates groupings of concentrations that were the same ($P > 0.05$, planned comparisons, $n = 4$ batches of embryos in each treatment)

Extracts of eggs absorbed UVR broadly between 300 and 400 nm, and maximally at 334 nm owing to the predominance of shinorine and porphyra-334 (Adams and Shick 1996) and only small amounts of the other three MAAs. Partially purified shinorine absorbed between 280 and 400 nm, with an absorption maximum of 334 nm. Absorption by extracts of sea urchin eggs and of shinorine was 50% of maximal at 312 and 348 nm, well within the range of biologically effective UVR. The calculated extinction coefficient for the semi-pure preparation of shinorine is 44,568 M^{-1} cm^{-1} at 334 nm. The calculated extinction coefficient for shinorine at 320 nm is 31,670 M^{-1} cm^{-1}, 87.5% of the published extinction coefficient of palythine at its absorption maximum of 320 nm (36,200 M^{-1} cm^{-1}). The calculated extinction coefficient for shinorine at 310 nm is 16,178 M^{-1} cm^{-1}, 60% of the published extinction coefficient for mycosporine-glycine at its absorption maximum of 310 nm (28,100 M^{-1} cm^{-1}).

The concentration of MAAs in sea urchin embryos remained constant until the gastrula stage (4 days) in embryos irradiated with PAR only, but thereafter decreased (Fig. 2; $P = 0.001$). MAA concentrations were not affected by UV-irradiation of the embryos and larvae ($P > 0.05$), nor was there an interaction between UV-treatment and time ($P > 0.05$). The concentration of MAAs remained constant in embryos exposed to PAR + UVA + UVB for the duration of this experiment (Fig. 2; $P > 0.05$). The MAA concentration in 8-day early plutei was significantly lower than in all earlier stages of unirradiated embryos ($P < 0.05$), but did not differ from UV-irradiated embryos of the same age ($P > 0.05$). UV-irradiated embryos died on day 10, although unirradiated embryos survived and their concentration of MAAs on day 10 was equal to MAA concentrations in 8-day unirradiated larvae ($P > 0.05$).

MAA uptake by *S. droebachiensis* and *Echinarachnius parma*

Sea urchin plutei developing from eggs spawned by adults from the field contain five MAAs – shinorine, porphyra-334, mycosporine-2 glycine, palythine, and asterina-330 (Table 1). Neither prism nor pluteus larvae of sea urchins accumulated shinorine that was dissolved in the culture water in concentrations of up to 25 μM ($P > 0.05$), well above that of the free amino acids (FAAs) they acquire from seawater (as low as 250 nM). Similarly, plutei of *E. parma*, which, as eggs, had no detectable endogenous MAAs ($n = 10$), did not accumulate MAAs from the surrounding medium at concentrations up to 100 μM.

Differential effects of UVA and UVB on development

The diameters of the blastulae and the blastocoel of *S. droebachiensis* embryos were smaller in the PAR + UVA + UVB treatment compared with blastulae exposed to either PAR or PAR + UVA (Fig. 3a, b and data not shown; $P < 0.05$), which did not differ from one another ($P > 0.05$). The blastoderm in blastulae exposed to PAR + UVA + UVB was significantly thicker compared with blastulae exposed to either PAR or PAR + UVA (data not shown; $P < 0.05$), which did not differ from one another ($P > 0.05$). Thus, irradiation of embryos with UVB, or a combination of UVB and UVA, thickened cells in the blastodermal wall and decreased the volume of the blastocoel.

Additional abnormalities induced by UVR are depicted in Fig. 3b, d, f and compared with control (PAR irradiated) embryos (Fig. 3a, c, e). Embryos that were exposed to PAR only underwent normal development

Table 1 *Strongylocentrotus droebachiensis* and *Echinarachnius parma*. Mycosporine-like amino acids (*MAAs*) in larvae exposed to different concentrations of dissolved shinorine from the surrounding media. Values are mean (±SE) concentrations of MAAs (nmol mg^{-1} dry wt) in larvae of sea urchins collected from Pemaquid Point, Maine, or sand dollars collected from Frasier Point, Maine

Species	Stage	Shinorine in medium (μM)	Concentration of MAA				
			Shinorine	Porphyra-334	Mycosporine-2 glycine	Palythine	Asterina-330
S. droebachiensis	Prism	0	1.67 ± 0.54	1.37 ± 0.46	0.17 ± 0.06	0.13 ± 0.06	0.02 ± 0.01
	Prism	2.5	1.98 ± 0.67	1.55 ± 0.54	0.22 ± 0.08	0.18 ± 0.11	0.03 ± 0.02
	Prism	25	1.92 ± 0.87	1.44 ± 0.68	0.16 ± 0.08	0.20 ± 0.10	0.03 ± 0.02
S. droebachiensis	Pluteus	0	1.67 ± 0.81	1.29 ± 0.68	0.16 ± 0.08	0.10 ± 0.06	0.02 ± 0.02
	Pluteus	2.5	1.93 ± 0.93	1.38 ± 0.73	0.16 ± 0.08	0.13 ± 0.03	0.01 ± 0.01
	Pluteus	25	1.90 ± 1.07	1.28 ± 0.53	0.15 ± 0.07	0.13 ± 0.07	0.02 ± 0.01
E. parma	Pluteus	0	0	0	0	0	0
	Pluteus	1	0	0	0	0	0
	Pluteus	100	0	0	0	0	0

from the blastula (Figs. 3a, 4) to the gastrula (Figs. 3c, 4), prism (the stage between the pictured gastrula and pluteus stages), and two-armed pluteus stages (Figs. 3e, 4). After 1 day of development at 5 ± 2 °C, embryos were at the blastula stage, but did not hatch until the next day (Figs. 3a, 4). The archenteron started invaginating by the third day of development, when embryos were classified as early gastrulae (Fig. 4), and was almost fully extended by the fourth day (Figs. 3c, 4). Embryos developed into early prism larvae by the fifth day (Fig. 4) and became two-armed plutei by the seventh day of development (Figs. 3e, 4). Embryos exposed to PAR + UVA developed similarly to these controls (Fig. 4).

In contrast, although most embryos exposed to PAR + UVA + UVB appeared normal on the first day of development (Fig. 4), a few developed into blastulae that were filled with abnormal primary mesenchyme cells or cell fragments (Fig. 3b). By the third day, some embryos were arrested at the normal blastula stage, others had become abnormal, "packed" blastulae, but most became early gastrulae (Fig. 4). On the fourth day, the surviving embryos were arrested as abnormal blastulae, or normal gastrulae, or were abnormal gastrulae having stunted, curled, or everted archenterons (Fig. 4). This pattern continued until the seventh day of development, when embryos formed abnormal blastulae, normal gastrulae, or abnormal larvae that arose when the archenterons of exogastrulae started to pinch off from the embryos. Abnormal larvae swam slowly, had no digestive tract, and often had three small spicules forming in their blastocoel (Figs. 3f, 4), compared with controls that became pluteus larvae having a well-developed digestive tract and two normal tri-radiate spicules supporting their arms (Figs. 3e, 4). Thus, irradiation of embryos with PAR + UVA + UVB delayed development of embryos, and none of these irradiated embryos developed into normal prism or pluteus larvae (Fig. 4). The percentage of abnormalities increased with cumulative exposure to full UVR.

The percentage of embryos developing normally from eggs of field-collected females is presented as a function of embryonic age and treatment (Fig. 5). There was a significant effect of UVR ($P < 0.05$) but not of time ($P > 0.05$) on development, although there was a significant interaction between UVR and time (Fig. 5a; $P < 0.05$). On the fourth day of development, a significantly lower percentage of embryos exposed to PAR + UVA + UVB were normal compared with embryos exposed to PAR or to PAR + UVA ($P < 0.05$), which did not differ from one another ($P > 0.05$). This trend was consistent throughout the remainder of the experiment.

The percentage of embryos on day 4 that had developed normally while exposed to PAR + UVA + UVB was positively related to the concentrations of MAAs in the eggs from which these embryos developed (Fig. 5b; $P < 0.05$). Ascorbic acid in eggs ranged from 0.66 to 1.16 nmol mg^{-1} dry wt and did not affect the response of embryos to UVR ($P > 0.05$, $r^2 = 0.001$).

Photoprotection by MAAs in sea urchin embryos and larvae

Similar experiments were performed to expand the range of concentration of MAAs by using eggs from sea urchins maintained on controlled diets. In 1998, the mean concentration of MAAs in eggs from *Laminaria saccharina*–fed adults, 1.68 ± 0.86 (SE) nmol mg^{-1} dry wt, was significantly lower than the concentration of MAAs in eggs from sea urchins fed a combination diet of *L. saccharina* and *Chondrus crispus*, 10.54 ± 1.28 nmol mg^{-1} dry wt ($P < 0.01$, $n = 3$). UV-treatment significantly affected the percentage of embryos developing normally (Fig. 6a; $P < 0.05$), but there was

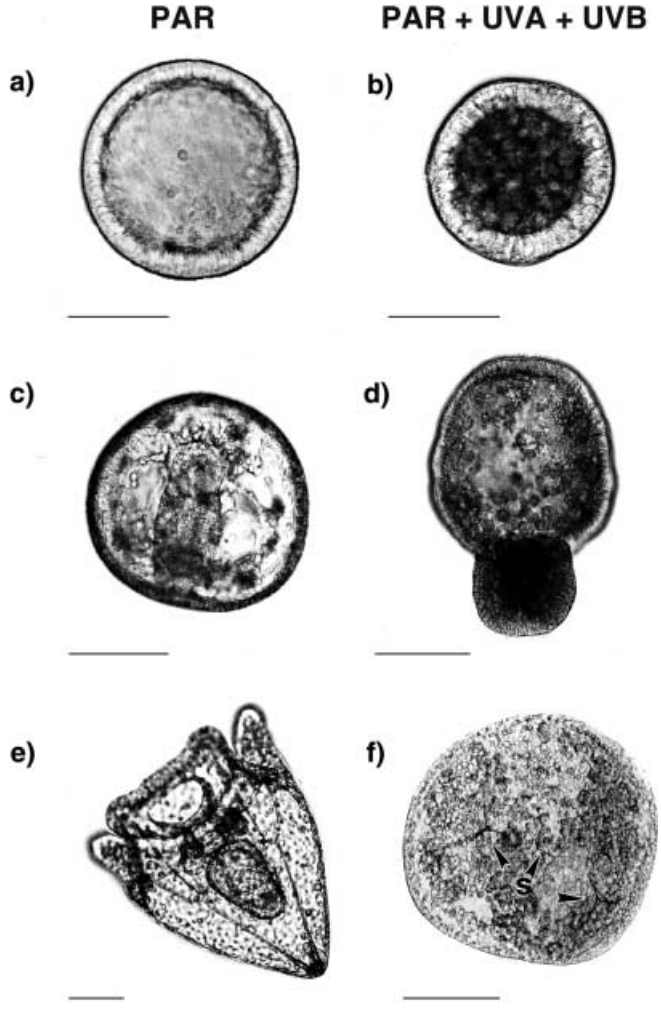

Fig. 3a–f *Strongylocentrotus droebachiensis*. Comparison of control (PAR only) and UV-irradiated (PAR + UVA + UVB) embryos and larvae: **a** Normal blastula, **b** UV-irradiated abnormal blastula, **c** normal gastrula, **d** UV-irradiated abnormal gastrula (exogastrula), **e** normal two-armed pluteus larva, and **f** UV-irradiated abnormal larva (*b* blastoderm; *a* archenteron; *s* spicules). *Scale bar* 100 μm

no effect of time on the percentage of embryos developing normally (Fig. 6a; $P > 0.05$). There was a significant interaction among diet, UV-treatment, and duration of the experiment (Fig. 6a; $P < 0.05$). On day 4 of exposure to PAR + UVA + UVB of embryos from adults fed *L. saccharina*, there was a significant decline in the percentage of embryos showing normal development compared with UV-exposed embryos from adults fed a combination diet (Fig. 6a; $P < 0.05$), which did not differ from controls (Fig. 7a; $P > 0.05$). This trend was consistent throughout the remainder of the experiment. The percentage of embryos that developed normally to day 4 while exposed to combined PAR + UVA + UVB was positively related to the MAA concentration in eggs from which these embryos developed (Fig. 6b; $P < 0.05$).

This experiment was repeated in 1999 with the addition of a PAR + UVA treatment to determine whether there were separate effects of UVA and UVB, or an effect of low MAA concentrations on development of UV-exposed embryos from adults fed *L. saccharina*. The mean concentration of MAAs in eggs for *L. saccharina*–fed adults, 0.759 ± 0.31 (SE) nmol mg^{-1} dry wt, was significantly lower than concentrations of MAAs in eggs from urchins fed a combination diet, 3.30 ± 0.87 nmol mg^{-1} dry wt ($n = 3$, $P < 0.05$). Eggs from adults fed a combination diet had a lower concentration of MAAs than in previous experiments (see above).

There were significant effects of UVR, time, and diet on development ($P = 0.001$ for each). There was a significant interaction between UV-treatment and time ($P = 0.001$) and UV-treatment and diet ($P = 0.001$), but not between diet and time ($P = 0.267$) or among the three factors combined ($P = 0.746$). The percentage of embryos that was exposed to PAR + UVA and that developed normally did not differ from all control embryos at any time during development (Fig. 7a; $P > 0.05$). By the second day of development, a lower percentage of embryos exposed to PAR + UVA + UVB and originating from adults fed *L. saccharina* developed normally compared with all other PAR or PAR + UVA treatments ($P < 0.05$). The percentage of normal development in these embryos was equal to that in embryos from adults reared on a combination diet that were exposed to PAR + UVA + UVB ($P > 0.05$), which did not differ from controls (Fig. 7a; $P > 0.05$). On the third, fourth, fifth, and sixth days, the percentage of normal embryos from adults fed *L. saccharina* that were exposed to PAR + UVA + UVB continued to be significantly lower than all other treatments (Fig. 7a; $P < 0.05$). Over the same time period, embryos from adults reared on a combination diet showed a significantly lower percentage of normal development compared with unirradiated controls (Fig. 7a; $P < 0.05$), but a higher percentage of normal development than embryos from adults fed *L. saccharina* ($P < 0.05$). Importantly, the percentage of embryos developing normally while exposed to combined UVA and UVB was significantly and positively related to the concentration of MAAs in the eggs from which these embryos developed (Fig. 7b; $P < 0.05$). Pooled data for all experiments give the widest range of MAA concentrations and clearly indicate that MAAs in embryos reduced UV-induced abnormalities (Fig. 8; $P = 0.001$).

Concentrations of ascorbic acid in eggs from adults fed *L. saccharina* and the combination diet, 0.56 ± 0.36 nmol mg^{-1} dry wt and 0.67 ± 0.57 nmol mg^{-1} dry wt, respectively, were not significantly different ($P > 0.05$, $n = 3$). Ascorbic acid in all eggs ranged from 0.02 to 1.8 nmol mg^{-1} dry wt, but did not affect the sensitivity of embryos to UVR ($P > 0.05$, $r^2 = 0.021$). These results demonstrate that, regardless of the diet of the adults, the MAAs that they sequestered in their eggs reduced UV-induced abnormalities during development of their offspring, whereas ascorbic acid did not.

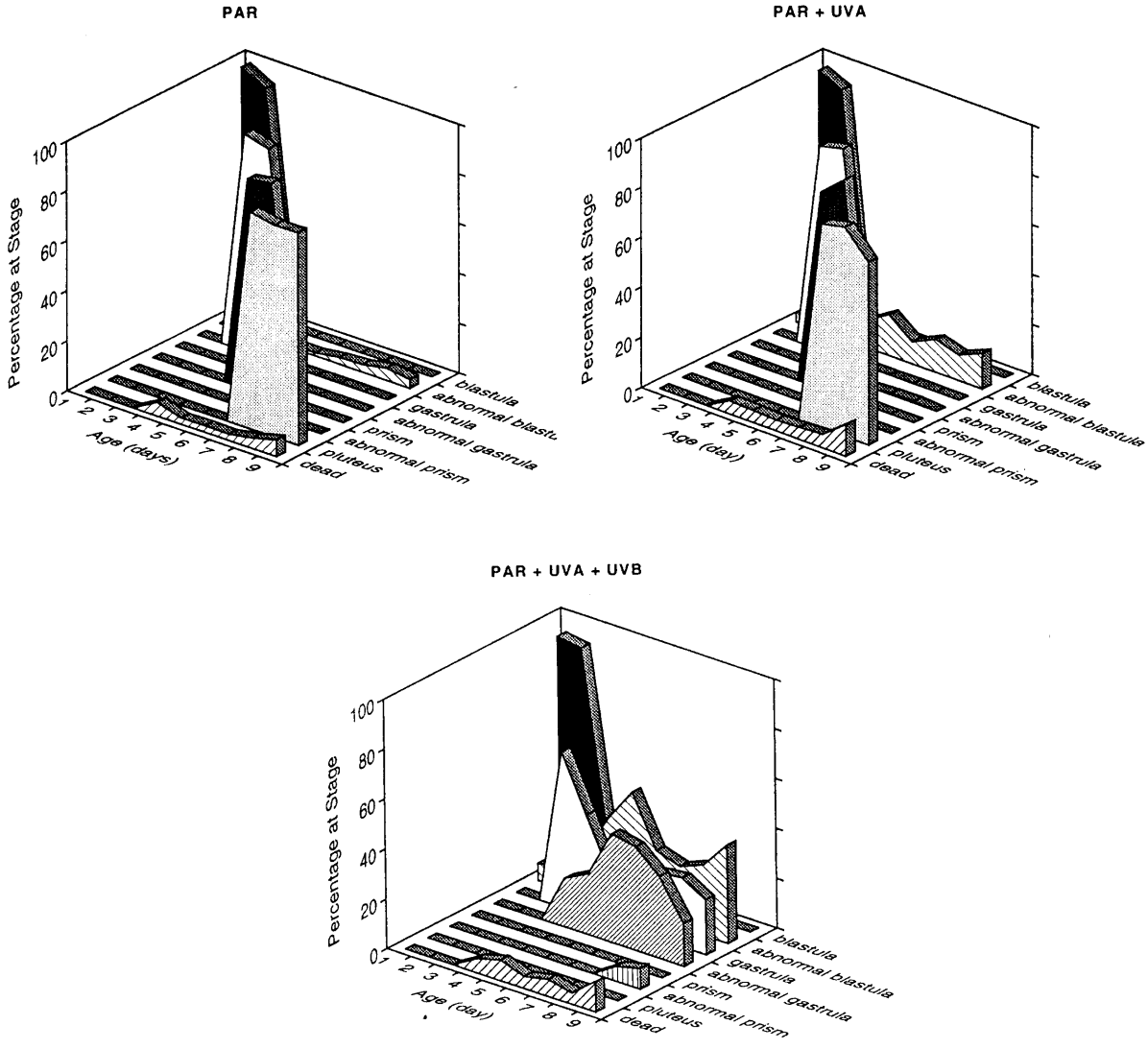

Fig. 4 *Strongylocentrotus droebachiensis*. Comparison of UVA + UVB radiation-induced delays and abnormalities during development. Values are means for the percentage of embryos at a specific developmental stage at each age that were exposed to PAR, PAR + UVA, or PAR + UVA + UVB (> 100 embryos counted from $n = 4$ batches of embryos in each treatment)

Comparisons were also made of the thickness of the blastoderm among UV-treatments and dietary groups. The blastoderm walls in blastulae from adults fed either an MAA-poor or an MAA-rich diet and exposed to PAR + UVA + UVB were 71.0 ± 1.0 μm and 67.4 ± 1.6 μm, respectively, and were significantly greater than in blastulae exposed to PAR + UVA (52.2 ± 0.9 μm and 45.8 ± 1.1 μm, respectively) or exposed to PAR alone (49.2 ± 1.4 μm and 45.0 ± 1.3 μm, respectively). The thickness of the blastoderm of embryos exposed to PAR + UVA was not different from controls regardless of diet ($P > 0.05$). Therefore, irradiation by UVA + UVB thickened the blastoderm of embryos, but there was no interaction between UV treatment or dietary group ($P > 0.05$). There was a significant correlation between MAA concentration and thickness of the blastodermal wall ($P < 0.05$, $r^2 = 0.04$, $n = 6$).

Discussion

Exposure of *Strongylocentrotus droebachiensis* embryos to levels of PAR and full UVR (UVA + UVB) similar to those measured in seawater in the field causes them to develop abnormally, although the incidence of UV-induced developmental abnormalities is reduced by intracellular MAAs in embryos and larvae of this sea urchin. The significantly lower percentage of abnormalities caused by UVR in MAA-rich eggs compared with MAA-poor ones from different dietary groups is evidence for a UV-protective role of these compounds. Moreover, the positive relationship between MAA concentrations among individual batches of eggs and the percentage of UV-exposed embryos that develop normally indicates there is concentration-dependent protection, regardless of its dietary origins. This extends the finding that MAAs protect against UV-induced

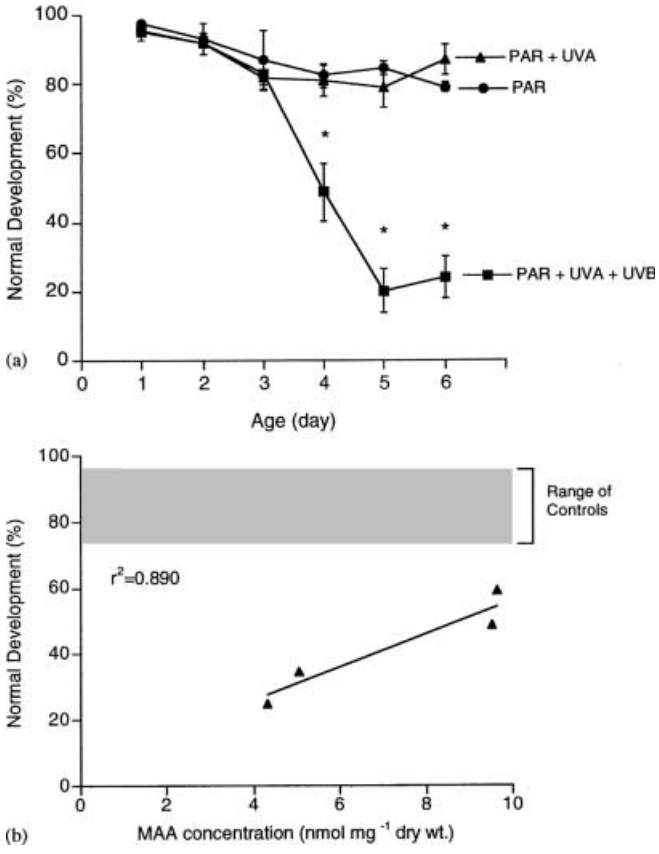

Fig. 5a, b *Strongylocentrotus droebachiensis*. Effects of UVB on development of embryos from adults freshly collected from Pemaquid Point, Maine. **a** Percentage of embryos developing normally (mean ± SE) by day that were exposed to PAR, PAR + UVA, or PAR + UVA + UVB. *Asterisks* indicate a significant decline in the percentage of normal development ($P < 0.001$, planned comparisons, $n = 4$). **b** The percentage of individuals from each batch of eggs showing normal development on day 4 in embryos exposed to PAR + UVA + UVB correlates linearly with total MAA concentration ($r^2 = 0.890$, $P = 0.037$). *Shaded area* denotes range of percentage of normal development in PAR-irradiated (control) embryos from the same batches on day 4

delays in the first cleavage in eggs of this sea urchin (Adams and Shick 1996), and for the first time demonstrates that MAAs reduce the incidence of UV-induced abnormalities in sea urchin embryos up to the pluteus stage.

Exposure of embryos to PAR + full UVR resulted in significantly more abnormalities than did exposure of embryos to PAR alone or to PAR + UVA only, suggesting that UVB or a combination of UVB and UVA caused the abnormal development observed. There was little difference in the total irradiance (W m^{-2}) of exposures between the PAR + UVA and PAR + UVA + UVB treatments because UVB irradiance was ~100 times lower than UVA or PAR. Thus, UVB wavelengths cause more damage per quantum, and it appears that UVB, specifically, and not overall intensity of irradiation induced the damage in our experiments.

UVA + UVB, but not UVA alone, caused an increase in the thickness of the blastoderm and a decrease in the volume of the blastocoel in *S. droebachiensis*, as described previously for other sea urchins (Akimoto and Shiroyo 1986; Henderson et al. 1999). In addition, the overall size of *S. droebachiensis* blastulae was decreased by exposure to UVA + UVB. These endpoints were inversely correlated with concentrations of MAAs, although < 5% of the change in the thickness of the blastoderm could be explained by a change in the concentration of MAAs. It appears that a larger range of MAA concentrations in eggs is needed to test this relationship.

Embryos exposed to full UVR were delayed in development and rarely progressed to the pluteus larval stage. Instead, these embryos arrested at the thickened "packed blastula," exogastrula, or abnormal larval stages, the last of these having supernumerary spicules and no digestive tract. These abnormalities confirm that UVR damages the primary mesenchyme cells that form the spicules (Akimoto and Shiroya 1986). Abnormal embryos may survive for many days, but to develop further they need a fully developed skeletal system that allows the pluteus larva to orient itself for swimming and feeding (Pennington and Strathmann 1990). These abnormal embryos will eventually die.

UV-induced exogastrulation of embryos exposed to environmentally relevant wavelengths of UVR has not been reported previously. Exogastrulation suggests that the archenteron does not attach to the blastoderm, indicating that the secondary mesenchyme cells that attach the archenteron to the inner wall of the blastoderm or the blastoderm wall itself are altered (Marsden and Burke 1998). UV-irradiation of cells damages their cytoskeletal microtubules and actin filaments, leading to distortion and lysis of cells (Godar et al. 1993), and perhaps interferes with development. UVR may also damage the extracellular matrix (ECM) or receptor proteins that are involved in gastrulation. The inhibition of the assembly of the ECM, especially the integrins, causes thickening of the blastoderm and interferes with gastrulation and skeletal patterning-receptors (Marsden and Burke 1998). Similarly, the activation of protein kinase C and the disruption of signaling, mediated by a platelet-derived growth factor receptor (PDGF) affect early events of gastrulation and spiculogenesis in sea urchins (Livingston and Wilt 1989; Ramachandran et al. 1997). Thus, it is possible that UV-exposure causes abnormalities involving many signaling molecules and the ECM that control morphogenesis, and that MAAs protect these compounds during development.

The degree of UV-induced mortality in sea urchin embryos correlates with the number of pyrimidine dimers in the DNA of these embryos (Akimoto and Shiroya 1987a), suggesting that dimers could be the primary lesions that lead to the formation of the abnormal larva (Akimoto and Shiroya 1987b). To date there have been no studies to show whether MAAs lessen the formation of UVB-induced dimers in the DNA of developing sea urchins.

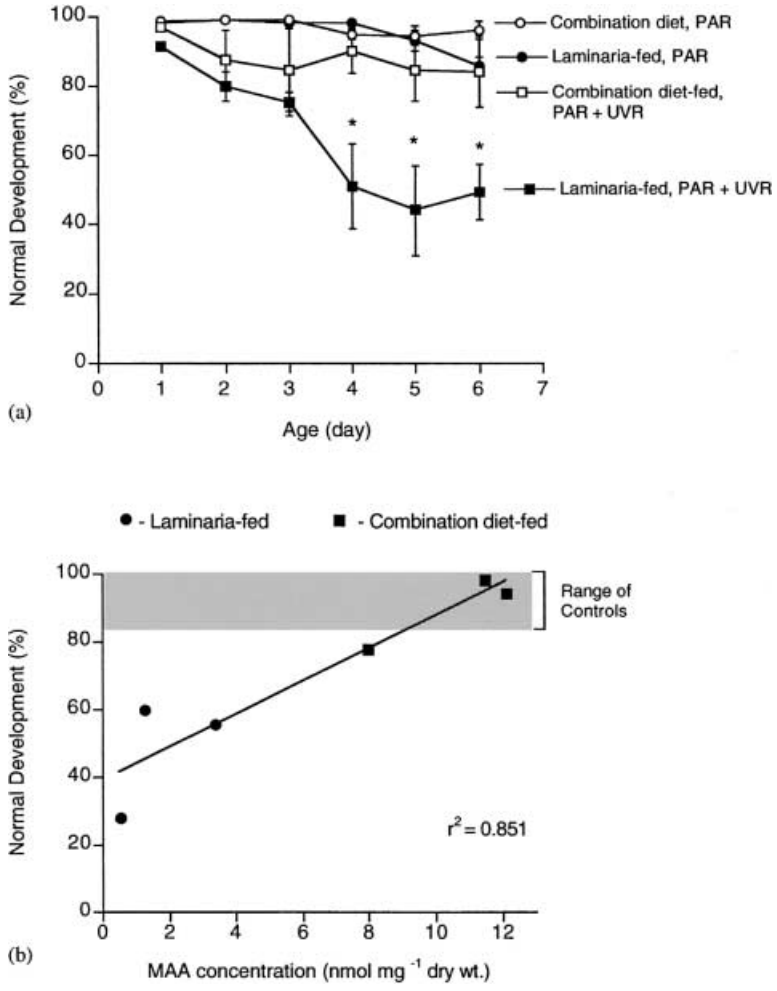

Fig. 6a, b *Strongylocentrotus droebachiensis*. Effects of UV radiation on development in sea urchin embryos. **a** Percentage of embryos developing normally (mean ± SE) by age and UV-treatment of embryos from adults fed either *Laminaria saccharina* (no MAAs) or a combination diet of *L. saccharina* and *Chondrus crispus* (high MAAs) from October 1997 to June 1998. *Asterisks* indicate a significant decline in the percentage of embryos developing normally ($P < 0.001$, planned comparisons, $n = 4$). **b** The percentage of individuals from each batch of eggs developing normally on day 4 in embryos exposed to PAR + UVA + UVB correlates linearly with total MAA concentration ($r^2 = 0.851$, $P = 0.005$). *Shaded area* denotes range of percentage of normal development of PAR-irradiated (control) embryos from the same batches on day 4

PAR or long-wavelength UVA radiation activates photolyase, which repairs pyrimidine dimers (Akimoto and Shiroya 1987a, b; Mitchell and Karentz 1993). Exposure of sea urchin embryos to low fluences of PAR (11 W m^{-2}) immediately after exposure to UVR leads to repair of dimers during the S phase of the cell cycle and partially reduces the developmental abnormalities (Akimoto and Shiroya 1987a, b). The photoreversibility is lost after the S phase when embryos enter the next cell cycle, suggesting that dimers must be repaired when DNA synthesis is taking place before base mismatching occurs. Our results show that the long-term exposure of embryos to UVR induces abnormalities despite the availability of photoreactivating wavelengths. This suggests that although dimers may be constantly repaired, some of the damage becomes permanent, leading to gross morphological abnormalities. Cellular components other than DNA, such as the structural or signaling proteins discussed above, may also have been damaged and thus be responsible for developmental abnormalities. Thus, the molecular targets of UVR that induce morphological abnormalities and the manner in which MAAs protect these targets need to be more fully characterized.

Because exposure to longer-wavelength UVA or PAR activates repair processes, there is an increasing biological effectiveness and damage associated with a successively higher ratio of UVR to PAR (Cullen and Neale 1997). From these differences in responses one can estimate a biological weighting function, which is essentially an action spectrum across polychromatic exposures, and quantify the biological effectiveness of groups of wavelengths (Cullen and Neale 1997). Biological weighting functions have not yet been calculated for effects of UVR on development and would require using narrower-pass filters than those used in this study.

In the present experiments the fluence of PAR was constant across treatments to allow equal photorepair. PAR fluence was ~10% of ambient solar levels, potentially decreasing the amount of repair during experiments compared with that expected at similar levels of UVB radiation in the field. If a lower level of repair occurred during these experiments, our results would then imply that MAAs protect against damage when repair is low, but experiments should be performed using natural ratios of PAR to UVR to determine whether damage still occurs and whether MAAs still protect embryos from UVR.

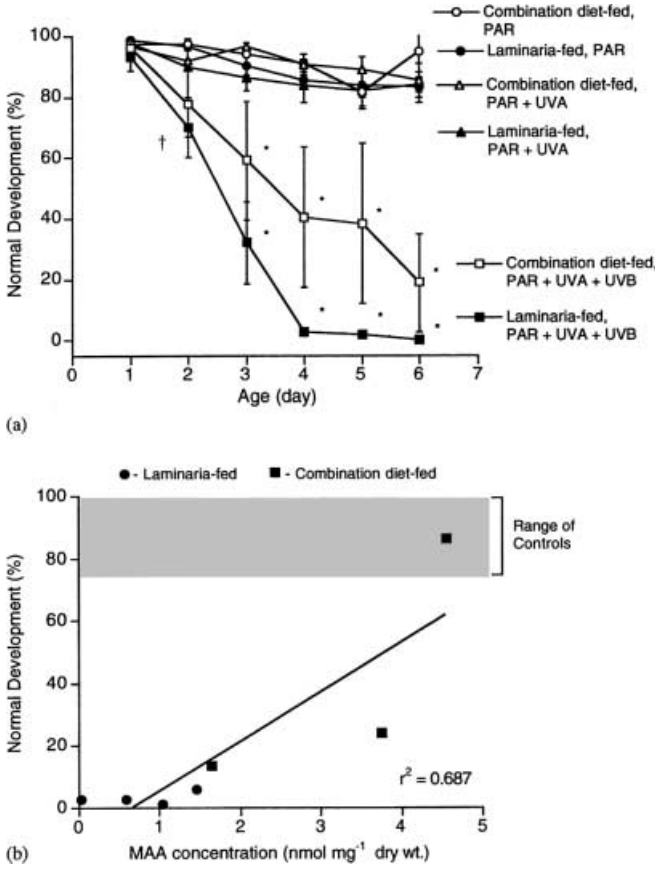

Fig. 7a, b *Strongylocentrotus droebachiensis*. Effects of UV radiation on development. **a** Percentage of normal development (mean ± SE) by day and UV-treatment of embryos from adults fed either *Laminaria saccharina* (no MAAs) or a combination diet of *L. saccharina* and *Chondrus crispus* (high MAAs) from October 1998 to June 1999. A dagger (†) indicates a significant difference in the percentage of UVB-irradiated embryos from *L. saccharina* compared with all control and UVA-irradiated treatments ($P < 0.05$, $n = 4$). Asterisks indicate a significant difference in the percentage of embryos developing normally compared with all other treatments on that day ($P < 0.01$, $n = 4$). **b** The percentage of individuals from each batch of eggs developing normally on day 4 in embryos exposed to PAR + UVA + UVB correlates linearly with total MAA concentration ($r^2 = 0.687$, $P = 0.008$). *Shaded area* denotes range of percentage of normal development in PAR-irradiated (control) embryos from the same batches on day 4

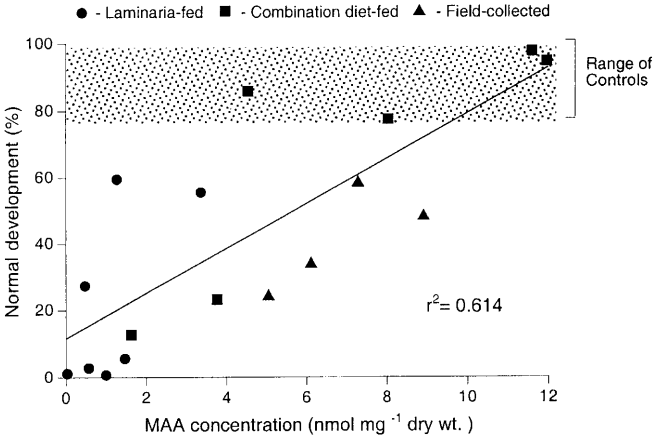

Fig. 8 *Strongylocentrotus droebachiensis*. When exposed to PAR + full UVR, the percentage of embryos developing normally correlates linearly with the total MAA concentration in eggs ($r^2 = 0.614$, $P = 0.001$) among all experiments on embryos from adults fed *Laminaria saccharina*, a combination diet of *L. saccharina* and *Chondrus crispus*, and field diet. *Shaded area* denotes range of percentage of normal development in PAR-irradiated (control) embryos from the same batches on day 4

There was little effect of UVA on the development of sea urchin embryos. The availability of small-molecule antioxidants may decrease the effect of UVA on these embryos. Ascorbic acid (vitamin C), carotenoids, and ovothiols may moderate oxidative damage (Shapiro and Hopkins 1991; Dunlap and Yamamoto 1995; Shick et al. 1996; Dunlap et al. 2000). Sea urchin eggs contain ovothiols at concentrations of ~4.3 mM, which prevent damage arising from the release of hydrogen peroxide during fertilization (Shapiro and Hopkins 1991). Carotenoids are present in eggs of sea urchins at concentrations ranging from at least 40–90 pg per embryo and decrease with development, possibly as a result of interaction with ROS (Vershinin and Lukyanova 1993). However, more study is needed to determine whether carotenoids provide protection in these embryos. Ascorbic acid has a very low one-electron reduction potential and, thus, is easily oxidized, i.e. it is a good antioxidant (Buettner 1993), potentially a stronger one than ovothiols and carotenoids. Ascorbic acid in eggs of *S. droebachiensis* ranged from 0.2 to 1.8 mM (2- to 20-fold lower than ovothiols), but its concentration did not correlate with diet or with the incidence of developmental abnormalities caused by UVB in eggs.

In electron paramagnetic studies, UV-irradiated ovaries of *S. droebachiensis* produced ascorbate radicals (an endogenous indicator of oxidative stress) inversely related to their MAA concentration, indicating a protective role of MAAs against UVA-induced production of free radicals in ovaries in vitro (Shick et al. 2000). Thus, because cellular damage, including that caused by ROS, can be accumulated despite the amelioration by photorepairing enzymes and antioxidants, it seems important for embryos to contain compounds, such as MAAs, that both protect against direct (primarily UVB) damage and against indirect, photoxidative damage from UVA by absorbing UV-energy and dissipating it.

Application of Garcia-Pichel's (1994) model for self-shading (Adams and Shick 1996) suggests that fortification of sea urchin eggs with the MAAs shinorine and porphyra-334 provides effective protection at 334 nm, yet the present experiments were performed using broad-band UVR (295–400 nm, with peaks at 311 and 365 nm). Our results show that these MAAs provide protection against broad-band UVR.

Eggs of *S. droebachiensis* contain mostly shinorine and porphyra-334, which absorb maximally at 334 nm, and no great amounts of specific UVB-absorbers (Fig. 1b). A partially purified shinorine extract having a minor amount (4.4%) of porphyra-334 absorbed across a range of 275–400 nm (Fig. 1a). The high extinction

coefficients for shinorine in this extract at 334 nm (44,568 M^{-1} cm^{-1}), 320 nm (31,670 M^{-1} cm^{-1}), and 310 nm (16,178 M^{-1} cm^{-1}) show that it is a strong absorber of both UVA and UVB. The extinction coefficient of shinorine at 320 nm is 87% of that of palythine at its λ_{max} of 320 nm (36,200 M^{-1} cm^{-1}) and 60% of that of mycosporine-glycine at its λ_{max} 310 nm (28,100 M^{-1} cm^{-1}), indicating that shinorine alone can absorb not only UVA wavelengths, as commonly assumed, but also nearly as much UVB as the most common UVB absorbers in marine organisms. This may explain how shinorine and porphyra-334, traditionally considered UVA-absorbers, protect against UVB-induced damage; these results are also in agreement with the predictions of Garcia-Pichel (1996). Tropical marine organisms that accumulate more UVB-absorbing mycosporine-glycine and palythine may be protected against the much higher fluences of UVB experienced at those latitudes (Gleason 1993; Shick et al. 1996; Banaszak et al. 1998), and may also benefit from the antioxidant properties of mycosporine-glycine, particularly in phototrophic symbioses experiencing high levels of molecular oxygen (Dunlap and Shick 1998).

The concentration of MAAs in sea urchin embryos is constant from the egg, through the blastula, to the gastrula, the most vulnerable stages of development. MAA concentrations in pluteus larvae were lower than in earlier embryonic stages, as observed previously (Adams and Shick 1996). The present experiments extend these results and confirm that long-term UV-irradiation of embryos does not alter their MAA concentrations, so the MAAs are photochemically stable in vivo. This is consistent with the failure of partially purified shinorine to form radical intermediates when irradiated by UVR in vitro (Shick et al. 2000). Although there is a decrease in the MAA concentration in plutei developing from eggs having high levels of MAAs, the optical model for sunscreening factors (Garcia-Pichel 1994) indicates that MAA concentrations remain high enough to confer up to 78% protection during the life of the larva.

The decline in the concentrations of MAAs in embryos coincides with the point at which larvae will start to feed at ~8 days or need supplemental energy from dissolved organic sources (Stephens 1972; Adams and Shick 1996), but it is not known whether the feeding larvae accumulate MAAs from their microalgal diets in nature. Embryos and larvae of sea urchins acquire free amino acids (FAAs) in seawater by active transport and thereby supplement their energetic stores (Jaeckle and Manahan 1992). Transmembrane net entry of FAAs into echinoderm larvae occurs against concentration gradients in excess of 10^5:1 from external amino acid concentrations below 125 nM (Davis and Stephens 1984; Davis et al. 1985). Similar active transport of MAAs had not previously been tested in sea urchin larvae.

MAAs have been measured in the medium used to culture dinoflagellates (Banaszak et al. 2000), and UV-absorbing compounds have been detected dissolved in seawater during plankton blooms (Vernet and Whitehead 1996), and might be available to pluteus larvae. We tested whether such larvae can accumulate MAAs from the surrounding medium at concentrations similar to FAAs and found that larvae of sea urchins and sand dollars do not accumulate shinorine available in seawater in concentrations up to 100 μM and across a transcellular gradient of up to 12:1, a mild gradient compared with that against which FAAs are absorbed.

Their capacity to take up FAAs but not shinorine suggests that there may be no transport mechanism for shinorine in larvae. Neutral amino acids are transported more readily by larvae of sea urchins than are acidic or basic amino acids (Davis et al. 1985), and shinorine and porphyra-334, the MAAs most commonly found in sea urchin eggs, are acidic. Mason et al. (1998) suggested that differences in bioaccumulation of MAAs from similar diets indicate that acidic-MAA transporters may exist in the intestines of sea urchins, while neutral-MAA transporters may occur in the intestines of fish that accumulate neutral MAAs (Dunlap et al. 1989). It is possible that the nutritive phagocytes in ovaries transport shinorine and porphyra-334 into eggs, but the later larvae themselves have no ability to absorb dissolved MAAs. Once the larvae are fully developed and have functional digestive tracts, they may further accumulate MAAs from their diet of microalgae.

In conclusion, this is the first demonstration that MAAs in eggs, embryos, and larvae of *S. droebachiensis* protect against UVB-induced damage during development from fertilization to the pluteus larva. Embryos retain the MAAs contained in their eggs up to at least the gastrula stage, and the MAAs are photostable during this exposure to UVR. Although the concentrations of MAAs are lower in the plutei, and plutei do not acquire additional MAAs from the surrounding seawater, the concentrations are high enough to afford protection to the four-armed pluteus stage. Ascorbic acid did not provide protection against UVB-induced damage, although absorption of UVR by MAAs may lessen the formation of ROS (Shick et al. 2000), possibly expanding their protective role. MAAs are part of a suite of defenses against UV-induced damage, especially during development. Such protection against the damaging UVB wavelengths during development of sea urchins may increase the number of larvae that successfully metamorphose into juvenile urchins and ultimately influence adult urchin populations.

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