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Sex and microhabitat influence the uptake and allocation of mycosporine-like amino acids to tissues in the purple sea urchin, *Strongylocentrotus purpuratus*

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Abstract UVR-absorbing mycosporine-like amino acids (MAAs) were detected in tissues of *Strongylocentrotus purpuratus* and in ten species of Rhodophyte macroalgae (eight previously untested) collected from intertidal microhabitats in November and January 2006–2007 in Central California (35°09′N, 120°45′W). In sea urchins, MAA concentrations were higher in ovaries than testes, while epidermal concentrations were similar between sexes. Ovaries and epidermal tissues had similar MAA signatures and broadband UVA/UVB absorbance, while testes had a narrower absorption ranges shifted toward higher energy wavelengths. Sea urchins occupying pits in the substrate exhibited lower MAA concentrations than those outside pits, suggesting adult microhabitat may impact UV protection. Light levels did not influence gonadal MAA concentrations, but correlated with elevated epidermal MAA concentrations for males in the sunniest microhabitat. This study suggests sex and habitat strongly influence MAA concentrations among individual *S. purpuratus* and that allocation of MAA sunscreens to tissues in response to UVR is sex-dependent.

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Introduction

Despite the efforts to reduce emissions of ozone-depleting substances, the penetration of harmful solar ultraviolet radiation (UVR) through the thinned ozone layer, is predicted to continue above pre-1970s levels for several decades (Madronich et al. 1998; Forster et al. 2007) and may increase with global climate change (McKenzie et al. 2007; Zepp et al. 2011). UVR is detrimental to many marine organisms, including macroalgae and marine invertebrates (Franklin and Forster 1997; Lesser et al. 2003; Campanale et al. 2011; Häder et al. 2011; Lamare et al. 2011). In temperate coastal waters, UVB (280–320 nm) and UVA (320–400 nm) penetrate to several meters depth (Tedetti and Sempere 2006). Therefore, marine organisms inhabiting shallow waters or exposed during low tides are at risk for UVR damage. Many organisms have defenses that mitigate UVR damage including photorepair and antioxidants (van de Poll et al. 2002; Lamare et al. 2006, 2011; Lesser 2010). However, it is likely advantageous to prevent UVR-induced damage by utilizing UVR-absorbing sunscreens.

One preventative defense against UVR in marine organisms is the presence of mycosporine-like amino acids (MAAs). MAAs are a suite of approximately 21 water-soluble compounds (Rastogi et al. 2010) that maximally absorb light in the UVR range (309–360 nm, Dunlap and Shick 1998). They are characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol (Sinha et al. 1998) and are stable over long periods of time in vivo (Adams and Shick 2001; Adams et al. 2001). MAAs are synthesized by macroalgae (especially Rhodophytes) and microorganisms including microalgae and cyanobacteria (Sinha et al. 1998; Oren and Gunde-Cimerman 2007). MAAs are also present in many marine invertebrates and

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fishes (Shick and Dunlap 2002; Sinha et al. 2007; Rastogi et al. 2010), and are acquired through diet or symbioses (Carroll and Shick 1996; Shick et al. 1999; Carefoot et al. 2000; Newman et al. 2000). Exposure to UVR can stimulate MAA production in algae and uptake in other invertebrates (Franklin et al. 1999; Karsten et al. 1999; Shick et al. 1999; Hoyer et al. 2002). Further, MAAs protect sea urchin embryos from UVR-induced damage (Adams and Shick 1996, 2001) and phytoplankton against photoinhibition caused by UVR (Neale et al. 1998; Karsten et al. 1999), implicating MAAs as protective UVR sunscreens.

Sea urchins, especially their embryos, have served as model organisms for examining the negative effects of UVR on development and the protective role of MAAs (Adams and Shick 1996, 2001; Lesser et al. 2004, 2006; Lamare et al. 2011). Though much of this work has been conducted using the green sea urchin, *Strongylocentrotus droebachiensis*, little work has been performed on the California purple sea urchin *Strongylocentrotus purpuratus*, which is widely studied and was the first invertebrate deuterostome to have its genome sequenced and annotated (Sea Urchin Genome Sequencing Consortium et al. 2006). There is only one published account of MAAs in a single *S. purpuratus* (Lamare and Hoffman 2004), which reported extremely low concentrations. Nevertheless, the ecology of purple sea urchins in California indicates they are likely to contain MAAs. Adults eat MAA-producing Rhodophyte macroalgae (Ebert 1968; Dayton 1975) and are frequently exposed to UVR when inhabiting the intertidal and shallow subtidal. Also, developing *S. purpuratus* larvae are vulnerable to UVR (Adams and Shick 2001; Campanale et al. 2011) during the weeks to months they develop in the water column (Strathmann 1987).

The traits of individual sea urchins may strongly affect MAA concentrations in their tissues, with potentially far-reaching fitness effects. Intertidal *S. purpuratus* are relatively sedentary (Grupe 2006; personal observation), so it is likely that their microhabitat will determine both their exposure to the sun (e.g. shady or sunny microhabitat) and their intake of algae, which may affect MAA uptake or allocation. Further, sexes may differ in their uptake or allocation of MAAs, especially to gonadal tissues.

To further understand how MAA concentrations in intertidal *S. purpuratus* tissues are affected by sex, season, microhabitat, and algal availability, we sampled sea urchin tissues and ten common species of red macroalgae for MAAs. Sea urchins and algae were collected from four tidepool microhabitats with differing sunlight exposure. We hypothesized that sea urchin ovaries would have higher concentrations of MAAs than testes and that both tissues would increase in MAAs as the spawning season approached, but that epidermal concentrations of MAAs would be similar between sexes and collection months. We hypothesized that algal tissue and sea urchin epidermal tissue would show higher concentrations of MAAs in high light microhabitats, but that gonadal tissues would not, as has been found for ovaries of *S. droebachiensis* exposed to UVR in the laboratory (Adams et al. 2001).

Materials and methods

Ten common species of Rhodophyte macroalgae and 119 adult *Strongylocentrotus purpuratus* were collected in October 2006 (algae) and in November 2006 and January 2007 (sea urchins) from four rocky intertidal microhabitats on the jetty at Port San Luis, California (35°09′N, 120°45′W). The microhabitats included horizontally oriented surfaces in tide pools where sea urchins were (1) not burrowed into pits ("Non-pit" microhabitat), (2) burrowed into pits ("Pit" microhabitat) which sea urchins excavate from the substratum, and vertical walls in tide pools facing either (3) south or (4) north with sea urchins outside of pits ("South-facing" and "North-facing" microhabitats, respectively). For each microhabitat, three representative tide pool stations were chosen; all stations were in the mid-intertidal zone within 50 meters distance of each other, were between 0.91 and 6.51 m^2 in area, and had similar sea urchin densities. We hypothesized that sun exposure would be highest for sea urchins in horizontally oriented Non-pit microhabitats, followed by South facing then North-facing microhabitats, and lowest in the Pit microhabitat.

Station measurements

To compare the relative solar exposure of sea urchins inhabiting the four microhabitats, we used three complementary approaches. First, in November 2006 three Solar Pathfinder (Perusion) measurements were taken and averaged for each station, except for the three-Pit microhabitat stations because the instrument did not fit inside the pits. The Solar Pathfinder is commonly used in terrestrial and stream ecology to measure sun exposure (Li et al. 1994; Naumburg and DeWald 1999; Arkle and Pilliod 2010) and consists of a spherical dome that reflects a panorama of the location, including any shade-casting objects. Solar Pathfinder Assistant (Perusion) imaging software was used calculate daily solar energy from a photograph for each month of the year based on shading as the sun angle changes predictably daily and seasonally. We compared August through January light measurements because solar exposure during the months prior to collections potentially influenced MAA uptake; sea urchins can retain MAAs in their tissues for months (Adams and Shick 1996), and *S. purpuratus* gametes were likely developing during this period (Giese et al. 1991). Second, to obtain sunlight

measurements inside pits and to supplement data from the Solar Pathfinder, HOBO Pendant® light loggers (Onset) were deployed in each station for 16 days in December 2011. Though these data were taken years after sea urchin collections, the angle of the sun follows a fixed path through the sky each year, so these measurements provide good approximations of the relative sun exposure of sea urchins collected during the same season in past years. Although these loggers measure illuminance (lux), which is not easily converted to a standard irradiance metric, they were helpful for this study because they are small enough to fit inside pits and measure relative differences in sunlight among microhabitats. Finally, to further validate the Solar Pathfinder data and to specifically compare UVA and UVB levels among microhabitats (except the Pit microhabitat, because the instruments were too large), we used an IL 1400A radiometer (International Light, Newburyport, MA, USA) coupled with a UVA (model SEL033) or UVB sensor (model SEL240) with maximal peak sensitivities at 350 nm and 295 nm, respectively, on two sunny afternoons in December 2011. We averaged measures from two or three locations in each station held at $\sim$15 cm below the water's surface. Admittedly, the Solar Pathfinder, HOBO light logger and UV sensor measurements have limitations (e.g., too large for pits, mismatch in timing, units in lux), but these three strategies complement one another and when combined obtain our objective of statistically testing differences in relative sunlight exposure among microhabitats. Shore level (vertical height above mean lower low water, MLLW) was also measured for each station, because it potentially affected sun exposure and algal availability to sea urchins.

To compare differences in sea urchin dietary MAA availability among microhabitats, both attached and drift algal availability were surveyed four times. Two surveys were performed simultaneous with sea urchin collections in November 2006 and January 2007, and two surveys were performed in December 2006 to assess algae available to grazing sea urchins prior to the January sampling period. Multiple survey dates allowed us to test whether trends were consistent despite inherent variation in algal abundance over time. Because it was not possible to quantify algal intake by sea urchins, our goal was to estimate relative algal availability in the different microhabitats and contrast this with concentrations of MAAs in sea urchins. Algal cover ($cm^2\ m^{-2}$) of each species attached to the substrate was sampled using a 0.25 m^2 quadrat in each station. Because MAAs vary among algal species, we converted algal cover values to "attached algal MAA availability" using the following equation:

$$\sum_{\text{algal species}} \left(\frac{\text{species cover } (\text{cm}^2)}{\text{m}^2} * \frac{\text{species meandryweight } (\text{mg})}{\text{cm}^2} * \frac{\text{species meantotal[MAA] } (\text{nmol})}{\text{dry weight } (\text{mg})} \right) = \frac{\text{MAAs available to sea urchins } (\text{nmol})}{\text{m}^2}$$

To determine the mean dry wt cm^{-2} for each species, we scraped algae from five plots (25 or 6.25 cm^2 depending on the species) near the stations for each of 10 common species and lyophilized, weighed and averaged the scrapes. The mean concentration of MAAs (nmol mg^{-1} dry wt) for each species was calculated using 12 algal specimens collected and analyzed for MAAs (Table 1, methods described below). Rarely occurring species were not included in the calculations. Drift algal availability was measured in each station by collecting and weighing Rhodophyte macroalgae held by 50 random sea urchins. A conversion to MAA availability was not performed for drift algae because samples were not consistently identifiable to species.

Collection and preparation of specimens

Twelve specimens of each of ten common algae species were collected in October 2006 and included *Calliarthron tuberculosum, Corallina officianalis* var. *chilensis, Corallina vancouveriensis, Endocladia muricata, Mastocarpus jardinii, Mastocarpus papillatus, Mazzaella flaccida, Osmundea spectabilis, Prionitis lanceolata*, and *Pterocladia capillacea*. One healthy, whole algal specimen from each species was collected from each station within 30 cm of sea urchins when available (algae in the Pit microhabitat were collected adjacent to pits). They were immediately transported to Cal Poly, cleaned of epibiota, frozen in liquid nitrogen and pulverized. Samples were then lyophilized and stored at -80 °C for later MAA extraction and analysis. Rhodophytes from the November 5, 2006, drift algal samples in each station were also prepared for MAAs analysis as described above.

Adult *S. purpuratus* were collected on November 5, 2006, before they were fully gravid and on January 15, 2007, just before the spawning season. Sea urchins were at

Table 1 Maximal UV absorption wavelengths (λ_{max}) and mean concentrations of individual and total MAAs (nmol mg^{-1} dry wt ± SD) for the ten species of red algae collected among the stations. Multiplying concentrations of individual MAAs below by their molecular weights yields mg MAA g^{-1} dry wt. Molecular wts (g mol^{-1}) are Shin: 332, PT: 224, P-334: 346, A-330: 288, PL: 302, Usu: 284

Species	*N*	λ_{max} (nm)	Concentration of MAAs (nmol mg^{-1} dry weight)						
			Shin	PT	P-334	A-330	PL	Usu	Total
Calliarthron tuberculosum[a]	12	329	0.50 ± 0.30	0.24 ± 0.18	tr.	0.02 ± 0.02	n.d.	0.03 ± 0.03	0.79 ± 0.52
Corallina officianalis var. *chilensis*[a]	12	320	0.28 ± 0.12	0.24 ± 0.21	tr.	0.02 ± 0.03	n.d.	0.03 ± 0.02	0.57 ± 0.31
Corallina vancouveriensis[a]	12	321	0.35 ± 0.18	0.19 ± 0.10	tr.	0.02 ± 0.01	n.d.	0.01 ± 0.01	0.57 ± 0.30
Endocladia muricata	12	335	8.16 ± 2.17	0.24 ± 0.83	0.01 ± 0.02	n.d.	n.d.	n.d.	8.41 ± 2.63
Mastocarpus jardinii	12	335	9.60 ± 3.01	n.d.	0.09 ± 0.08	n.d.	n.d.	n.d.	9.69 ± 3.01
Mastocarpus papillatus	12	335	11.41 ± 3.32	n.d.	0.05 ± 0.05	n.d.	n.d.	n.d.	11.46 ± 3.34
Mazzaella flaccida	12	327	1.38 ± 0.63	3.63 ± 1.30	tr.	0.32 ± 0.14	n.d.	0.47 ± 0.26	5.81 ± 2.16
Osmundea spectabilis	11	327	0.12 ± 0.06	0.60 ± 0.17.	0.38 ± 0.13	1.87 ± 0.63	0.23 ± 0.07	n.d.	3.20 ± 0.99
Prionitis lanceolata	12	326	4.78 ± 1.84	6.21 ± 2.56	0.01 ± 0.02	0.41 ± 0.18	n.d.	0.28 ± 0.18	11.69 ± 4.34
Pterocladia capillacea	12	330	3.09 ± 1.85	1.77 ± 0.99	0.02 ± 0.02	0.25 ± 0.16	n.d.	0.22 ± 0.12	5.34 ± 3.02

Shin shinorine, *PT* palythine, *P-334* porphyra 334, *A-330* asterina 330, *PL* palythinol, *Usu* usujirene, *tr.* trace MAAs detected, *n.d.* MAAs not detected

[a] Coralline algae species with calcium carbonate skeletons

least 30 mm in diameter to ensure sexual maturity (Giese et al. 1991), and were held in enclosed seawater aquaria for less than 4 days. Whole wet wt, test diameter and epidermis and gonad wet wt were recorded for five female and male sea urchins from each station. Sea urchins were rinsed, trimmed of their spines, gonads tissues were dissected, and epidermal tissues from the five ambulacral sections of the test (those including tube feet) were sampled (~15–20 % of whole wet wt, between 4.3 and 20.8 g). Gonadal and epidermal tissues were frozen, lyophilized, and stored at −80 °C for later MAA extraction and analysis. Gonadal index (GI), a measure of fecundity, was calculated for each sea urchin as: (wet wt of gonad/wet wt of whole urchin) × 100.

Mycosporine-like amino acid analysis

A fixed dry weight of each tissue type (200 mg gonads, 1.000 g epidermis, and 200 mg algae) was extracted for MAAs using three serial 60-min. extractions in 80 % HPLC-grade methanol at room temperature. At the start of the first extraction, the samples were sonicated (Branson Sonifier 250) for 20 s to lyse cells. The three extracts were pooled, and algae extracts were filtered through a Waters Sep Pack Plus C-18 column to remove pigments as per Adams et al. (2001).

Extracts were analyzed for MAAs using a Hewlett Packard 1100 Series reverse-phase high performance liquid chromatography (HPLC) with a Phenomenex Phenosphere C-8 column at a flow rate of 0.8 mL min^{-1} (as per Adams et al. 2001). MAAs were identified by comparing peak absorbance and retention times with known MAAs, and identification of representative peaks was confirmed by co-chromatography using standards provided by Dr. W. C. Dunlap. The peak areas of the MAAs mycosporine glycine, shinorine, porphyra-334, and mycosporine 2-glycine were calculated using a 55 % methanol and 0.1 % acetic acid mobile phase, and the MAAs palythine, asterina-330, palythinol and usujirene were calculated using a 25 % methanol and 0.1 % acetic acid mobile phase. MAA concentrations in nmol mg^{-1} dry wt were calculated from HPLC peak areas using peak area integration of MAA standards calibrated in this system, then adjusted for extraction efficiency for each tissue as per Dunlap and Chalker (1986). No standard was available for the MAA usujirene, so its identity was inferred by elution time, peak absorption at 357 nm, and co-chromatography with a methanolic extract of *Palmaria palmata*, an algal species known to contain usujirene (Sekikawa et al. 1986). The concentration and extraction efficiency of usujirene was calculated using the standard for palythene, its *trans* isomer. Unidentified MAAs were rare and not quantified. Absorption spectra (290–700 nm) were measured using a Jasco Model V550 UV/Vis spectrophotometer for one representative of each of the four tissue types (ovaries, testes, female, and males epidermis) containing equal concentrations of total MAAs.

Statistical analyses

All data were analyzed on JMP software using Fit Model and sequential sums of squares. Models including random variables were tested using Restricted Maximum Likelihood (REML) tests and those without were analyzed using ANOVA. Significant terms ($P < 0.05$) in all models were

further analyzed using Student Newman–Keuls post hoc analyses. Transformations were applied where appropriate to best achieve linearity, homogeneity of variance, and normal distribution of residuals. Select model outputs and supplemental figures with statistical results are provided in Online Resource 1.

To analyze trends among microhabitats in sun exposure (Solar Pathfinder), drift and attached algal MAA availability, and wet wt of sea urchins, we used mixed REML models with collection date, microhabitat and their interactions as categorical predictors, and station nested within microhabitat as a random variable to control for among-station variation within microhabitats. Maximum and mean daily illuminance (HOBO light loggers) and average solar UVA and UVB irradiance (IL UV sensors) in each station were compared among microhabitats with date included as a categorical random predictor variable. Variation in shore level and sea urchin density among microhabitats was analyzed using a one-way ANOVA.

Microhabitat variation in total concentration of MAAs and relative concentration of shinorine in algae was analyzed using REML analyses with species and station (nested within microhabitat) included as random variables to control for species or stations that may have masked any microhabitat trends.

Models analyzing concentrations of MAAs in sea urchin gonadal and epidermal tissues were performed separately because epidermal samples contained spine and test tissues, so standardizing concentrations by dry wt rendered the two tissue measurements incomparable. Nevertheless, we were able to examine allocation of MAAs to epidermal versus gonadal tissues (concentration of epidermal MAAs/[concentration of epidermal + gonadal MAAs]). For the three response variables outlined above (gonadal and epidermal concentrations of MAAs and the proportion of MAAs in the epidermis) and for gonadal index, we ran models using microhabitat, month, sex, and their interactions as predictors and included station nested within microhabitat as a random variable. We also compared relative concentrations of individual MAAs (concentration of individual MAA/concentration of total MAAs) among microhabitats, sexes and tissues (month was removed because no terms were significant) and sea urchin number and station (nested within microhabitat) were included as random variables.

Results

Microhabitat characteristics

All three methods of light measurement document differences in solar exposure among microhabitats. First, the Solar Pathfinder analysis performed in 2006 showed higher solar exposure levels in the Non-pit microhabitat compared to the South-facing microhabitat (though this was not significant), and lower levels in the North-facing microhabitat (Fig. 1a, REML, $F_{3,186} = 10.3$, $P = 0.004$). As expected, solar exposure decreased approaching winter months, but the pattern among microhabitats was consistent each month (REML, Month x Microhabitat: $F_{15,186} = 1.13$, $P = 0.335$). Second, measurements from HOBO light loggers taken in December 2011 corroborate this pattern and highlight low solar exposure in Pits, with average daily maximum illuminance highest in the Non-pit (57,920 ± 4,470 lux) followed by the South-facing microhabitat (33,450 ± 3,040 lux), with North-facing and Pit microhabitats (3,110 ± 250 and 7,450 ± 3,270 lux, respectively) having similar low solar exposure (Fig. 1b; REML, $F_{3,149} = 128.24$, $P < 0.001$). Average daily mean illuminance from HOBO light loggers followed a similar trend, being highest in the Non-pit (6,760 ± 540 lux), followed by the South-facing and North-facing pit microhabitats (2,960 ± 300, 490 ± 140, 420 ± 40 lux; Fig. 1b, REML, $F_{3,149} = 118.4$, $P < 0.001$). The difference between Non-pit and North-facing microhabitats was about 55,000 lux at maximum each day (Fig. 1b), which is approximately equivalent to direct versus indirect sunlight. Finally, instantaneous UV measures further supported this trend, showing higher UVA in the Non-pit compared to North-facing microhabitats, with South facing as intermediate (REML, $F_{3,17} = 5.14$, $P = 0.010$; $X \pm SE = 360.3 \pm 49.5$, 239.2 ± 40.1, and 143.3 ± 16.3 μW UVA cm^{-2} and $N = 6$ for Non-pit, South facing and North facing, respectively). For UVB measurements, North-facing microhabitats showed low levels, but no significant differences were detected, possibly due to small sample size and low UVB levels during winter afternoon measurements ($X \pm SE = 1.0 \pm 0.2$, 1.1 ± 0.4 and 0.5 ± 0.1 μW UVB cm^{-2} and $N = 6$ for Non-pit, South facing and North facing, respectively). Together these three approaches test differences in relative sun exposure of sea urchins and algae in the separate microhabitats.

Though there was a small range in shore level (0.8–1.4 m above MLLW) among the 12 stations, MLLW in the Non-pit microhabitat station was higher ($X \pm SE = 1.34 \pm 0.04$ m, $N = 3$) than those in the other three microhabitats, which did not differ from one another (ANOVA, $F_{3,8} = 8.77$, $P = 0.007$; $X \pm SE = 0.91 \pm 0.11$, 0.96 ± 0.03, and 0.84 ± 0.09 m above MLLW and $N = 3$ for South-facing, North-facing and Pit microhabitats, respectively). Any effect this shore level difference may have had on sunlight levels was captured by the HOBO light loggers (Fig. 1b), and likely only increased exposure in the already sunny Non-pit microhabitat.

Algal availability

The availability of MAAs to *Strongylocentrotus purpuratus* from algae growing nearby (see calculation in methods)

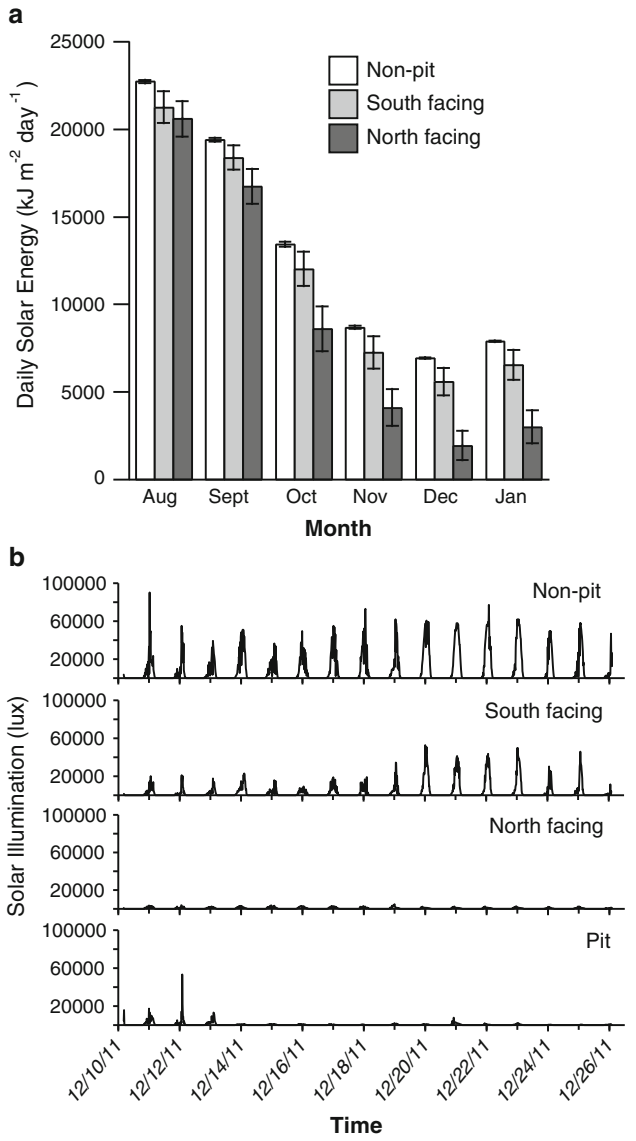

Fig. 1 Solar exposure among the four microhabitats. **a** Average daily solar energy (kJ m^{-2} day^{-1} ± SE) each month in the microhabitats as estimated by Solar Pathfinder measurements in each station ($N = 3$) in November 2006. Measurements inside the Pit microhabitat are not included, because the instrument did not fit inside the pits. **b** Time series over 16 days in December 2011 of average solar illumination in stations representing the four microhabitats recorded by HOBO light loggers. These data were acquired as a comparison to supplement Solar Pathfinder data and assess low light levels inside pits. ($N = 3$, except for the pit microhabitat where $N = 2$). Tick marks indicate noon on each day

was generally lower in the Non-pit microhabitat than in the other three microhabitats, (Fig. 2a; REML, $F_{3,30} = 5.41$, $P = 0.025$). This varied among surveys but 3 of the 4 surveys exhibited this trend (Fig. 2a; REML, Date x Microhabitat: $F_{9,30} = 4.06$, $P = 0.003$). The amount of Rhodophyte drift algae available to sea urchins was similar among microhabitats on most sampling dates, except in November, when there was low availability in the Non-pit microhabitat (Fig. 2b; REML, Date x Microhabitat: $F_{9,32} = 4.03$, $P = 0.003$).

Concentration of MAAs in algae

MAAs were present in every algal specimen tested, and species varied in concentration of total and individual MAAs (Table 1). Having statistically controlled for inter-specific differences, algal specimens in the Non-pit microhabitat showed higher mean concentrations of MAAs than algae from the Pit microhabitat, with the South-facing and North-facing microhabitats being intermediate (REML, $F_{3,105} = 13.20$, $P = 0.001$). The relative concentration of the MAA shinorine tended to be highest in algae from Non-pit microhabitats and lowest in those from Pit microhabitats, but this was not significant (Fig. S1).

MAAs were detected in all samples of Rhododphyte drift algae. Drift algal samples were composed primarily of fleshy algae, but the mean concentration of MAAs in drift algal samples ($X \pm SE = 1.54 \pm 0.37$ nmol mg^{-1} dry wt, $N = 12$) was much lower than those of the fresh fleshy attached algal species tested ($X \pm SE = 7.34 \pm 0.50$ nmol mg^{-1} dry wt, $N = 71$).

Gonadal indices

Gonadal indices (GIs) of adult *S. purpuratus* in the North-facing microhabitat were significantly greater than those in the South-facing and Pit microhabitats, with Non-pit urchins having intermediate GIs (REML, $F_{3,221} = 3.62$, $P = 0.032$; $X \pm SE = 11.76 \pm 0.4$, 10.0 ± 0.5, 12.8 ± 0.6 and 11.9 ± 0.5 and $N = 20$ for Non-pit, South-facing, North-facing and Pit microhabitats, respectively). These results were consistent among sexes and months (microhabitat × sex: $F_{3,221} = 0.72$, $P = 0.544$, microhabitat × month: $F_{3,221} = 0.36$, $P = 0.780$). GIs decreased from November to January for females but not males, (REML, sex × month: $F_{1,221} = 7.83$, $P = 0.006$), suggesting females may have begun spawning before the January collection, though ovaries were still ripe upon dissection. No correlation between GI and concentration of MAAs was found for the 119 individual *S. purpuratus* sampled at this study site (Linear correlation: $r^2 < 0.01$, $F_{1,235} = 1.97$, $P = 0.160$).

Concentration of total MAAs in sea urchins

Overall, concentrations of MAAs in sea urchin gonads increased as spawning season approached (Fig. 3a, b; Table S1, $P = 0.005$), while the concentrations of MAAs in epidermal tissues did not (Fig. 3c, d; Table S2, $P = 0.569$). All results outlined below were consistent between months unless otherwise noted (Tables S1 and S2,

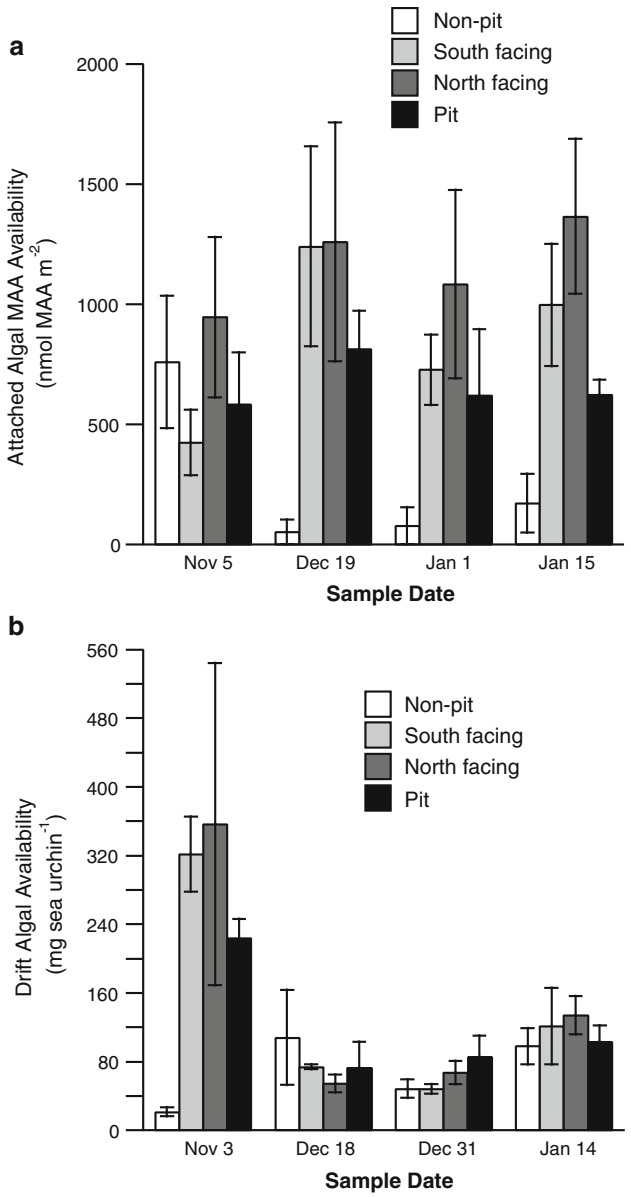

Fig. 2 Measurements among four microhabitats of mean monthly (±SE) **a** attached algal MAA availability to sea urchins (see calculation in methods, $N = 3$) and **b** drift algal availability to sea urchins ($N = 3$) in winter 2006–2007. Sea urchins were collected for MAA analysis on November 5, 2006, and January 15, 2007

month interaction terms) so the November and January data are presented together below, but are separate in figures to illustrate consistency in trends over time (Fig. 3).

Females had generally higher concentrations of MAAs in their ovaries than males had in their testes (Fig. 3a, b; Table 2; Table S1, $P = 0.006$) though this difference was only significant in the Non-pit and South-facing microhabitats (Fig. 3a, b; Table S1, $P = 0.024$). Surprisingly, females had overall higher epidermal MAA concentrations than males (Fig. 3c, d; Table 2; Table S2, $P = 0.01$). However, this relationship was variable with microhabitat and month (Fig. 3c, d; Table S2, $P = 0.005$ and $P = 0.026$) and the difference in concentrations of MAAs was extremely small (Table 2), so this trend is not likely ecologically relevant. Variation in concentration of total MAAs (nmol mg^{-1} dry wt) in all tissues was high and ranged from 0.02 to 9.5 in ovaries, 0.05–1.65 in testes, <0.01–0.66 in female epidermis, and <0.01–1.28 in male epidermis.

MAA concentrations tended to be lower in sea urchins from Pit microhabitats compared to those from other microhabitats, though this varied among the sexes and tissues considered (Fig. 3; Tables S1 and S2: $P = 0.024$ and $P = 0.005$ for gonads and epidermis, respectively). The lower concentration in Pit urchins was found for ovaries (Fig. 3a, b; Student's, $P < 0.02$ for all comparisons), for female epidermal tissues (Fig. 3c, d; Student's, $P < 0.02$ for all comparisons) and for male epidermal tissues (Fig. 3c, d) though only when compared to the Non-pit and North-facing microhabitats (Student's, $t_{57} = -4.17$, $P < 0.01$ and $t_{58} = -2.53$, $P = 0.02$, respectively). Conversely, concentrations of MAAs in testes showed little variation among microhabitats, perhaps because concentrations were low (Fig. 3a, b; Student's, $P > 0.07$ for all comparisons).

Males collected from the sunniest Non-pit microhabitat (Fig. 1) in January had much higher concentrations of epidermal MAAs than males from the other microhabitats (Fig. 3d; Table S2: REML, $P = 0.026$; Student's, $P < 0.01$ for all pairwise comparisons). We then compared the proportion of MAAs in the epidermal versus gonadal tissues from both months for each sea urchin and found that males allocated nearly twice as many MAAs to their epidermal tissues in the sunny Non-pit microhabitat compared to males in the other microhabitats (Fig. 4; Table S3: $P = 0.034$; Student's, $P < 0.01$ for all pairwise comparisons), which did not differ significantly from one another (Student's, $P > 0.29$ for all comparisons). Conversely, female sea urchins showed no differences in the mean proportion of MAAs in epidermal tissues among the microhabitats, (Fig. 4; Student's, $P > 0.59$ for all comparisons).

Concentrations of individual MAAs in sea urchins

We identified seven MAAs in *S. purpuratus* tissues including shinorine, palythine, porphyra-334, mycosporine glycine, asterina-330, mycosporine 2-glycine and usujirene (Table 2) which was similar to the results of Adams et al. (2001) and Carroll and Shick (1996) for ovaries of *S. droebachiensis*. Relative concentrations of individual MAAs (concentration of individual MAA/concentration of total MAAs) showed typical "MAA signatures," which were similar in ovaries and epidermis of both sexes and

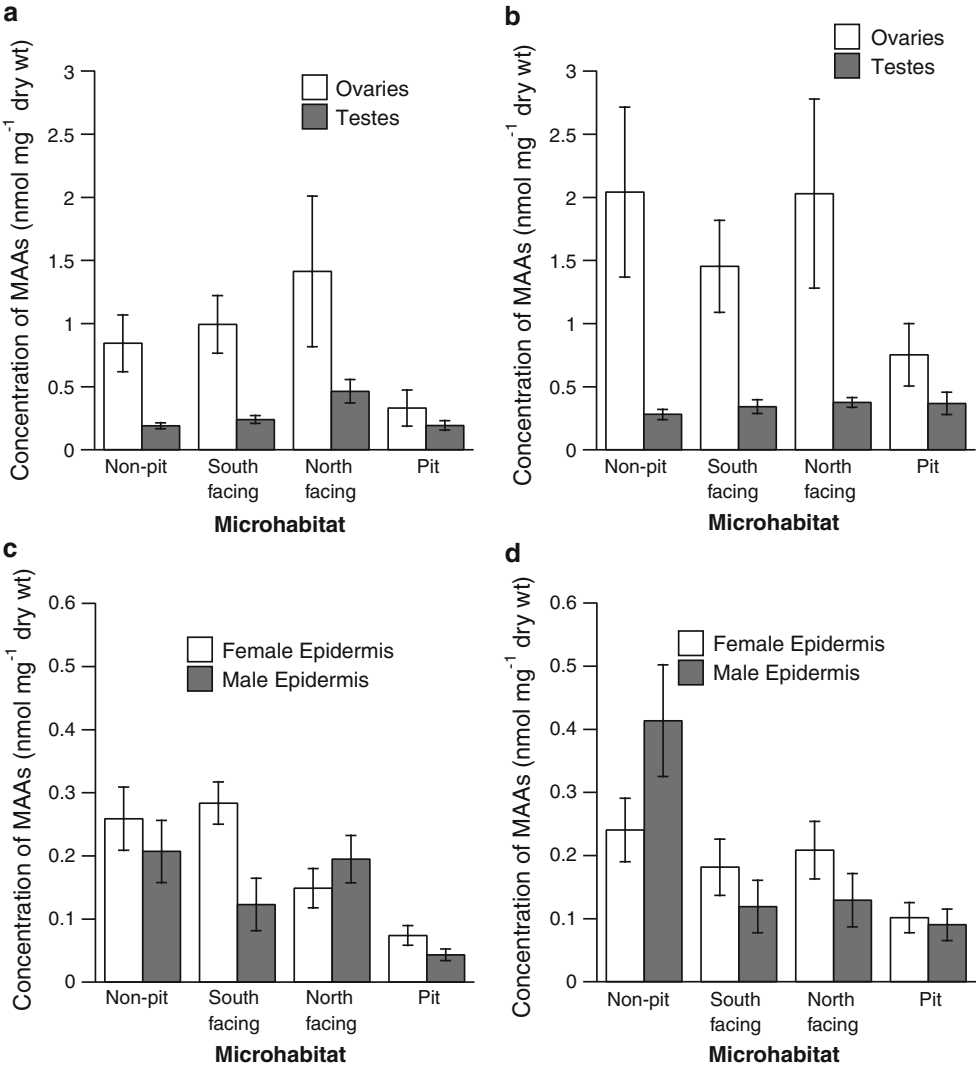

Fig. 3 The mean concentration of MAAs (nmol mg^{-1} dry wt. ± SE) in *S. purpuratus* tissues among microhabitats for gonadal tissues in **a** November and **b** January and for epidermal tissues in **c** November and **d** January. White bars represent female tissues and gray bars represent male tissues ($N = 15$ except for females from the South-facing microhabitat in November where $N = 14$)

Table 2 UV absorption maxima (λ_{max}) and mean concentrations of individual and total MAAs (nmol mg^{-1} dry wt ± SE) for each sea urchin tissue type averaged among microhabitats for the two collection months. The total concentrations of MAAs by dry wt in gonadal and epidermal tissues are not directly comparable, due to inclusion of pieces of test in epidermal samples. An asterisk (*) indicates the individual MAA with the highest relative concentration compared to other MAAs in the tissue type ([individual MAA]/[total MAAs]). Multiplying concentrations of individual MAAs below by their molecular weights yields mg MAA g^{-1} dry weight. Molecular wts (g mol^{-1}) are Shin: 332, PT: 224, P-334: 346, Myc-gly: 245, A-330: 288, Myc-2-gly: 288, Usu: 284

Tissue type	N	λ_{max} (nm)	Concentration of MAAs (nmol mg^{-1} dry wt)							
			Shin	PT	P-334	Myc-gly	A-330	Myc-2-gly	Usu	Total
Ovaries	118	331	*0.82 ± 0.11	0.21 ± 0.06	0.05 ± 0.01	0.05 ± 0.02	0.02 ± <0.01	0.07 ± 0.07	tr.	1.22 ± 0.17
Testes	119	322	0.02 ± <0.01	*0.28 ± 0.02	tr.	tr.	tr.	n.d.	tr.	0.31 ± 0.02
Female epidermis	119	330	*0.16 ± 0.01	0.02 ± <0.01	0.01 ± <0.01	tr.	tr.	tr.	n.d.	0.19 ± 0.06
Male epidermis	116	331	*0.13 ± 0.02	0.02 ± <0.01	0.01 ± 0.001	tr.	tr.	tr.	n.d.	0.16 ± 0.02

Shin shinorine, *PT* palythine, *P-334* porphyra 334, *myc-gly* mycosporine glycine, *A-330* asterina 330, *myc-2-gly* mycosporine 2-glycine, *Usu* usujirene, *tr*. trace MAAs detected, *n.d.* MAAs not detected

contained mostly shinorine, (X ± SE: 53.4 ± 2.7, 72.8 ± 2.0 and 62.3 ± 2.7 % and $N = 118$, 119 and 116 for ovaries, female and male epidermis, respectively) some palythine (X ± SE: 38.6 ± 2.9, 19.8 ± 2.1 and 30.3 ± 2.7 % and $N = 118$, 119, and 116 for ovaries, female and male epidermis, respectively), and small

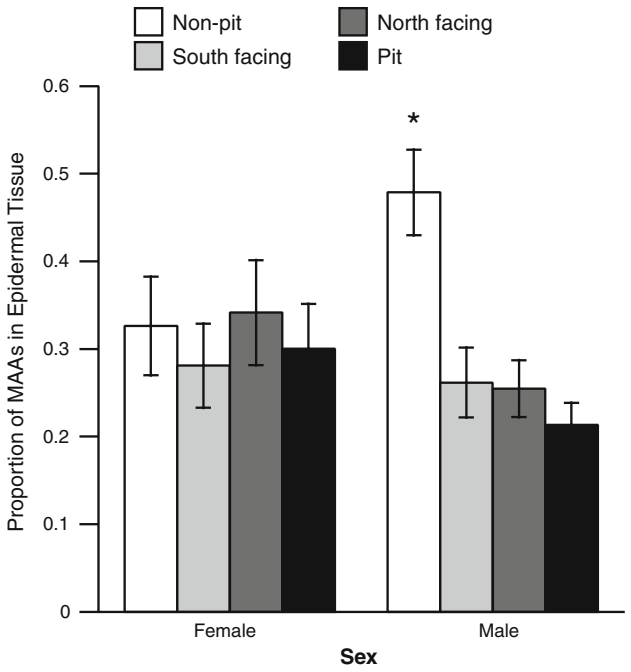

Fig. 4 The mean (±SE) proportion of the total concentration of MAAs detected in *S. purpuratus* epidermal tissues ([epidermal MAAs]/[gonadal + epidermal MAAs]) in the microhabitats for each sex for both November and January ($N = 30$ except for females in the South-facing microhabitat where $N = 29$). The *asterisk* (*) denotes a significantly higher mean proportion of MAAs in the epidermis for the males from the Non-pit microhabitat compared to males from the other three microhabitats

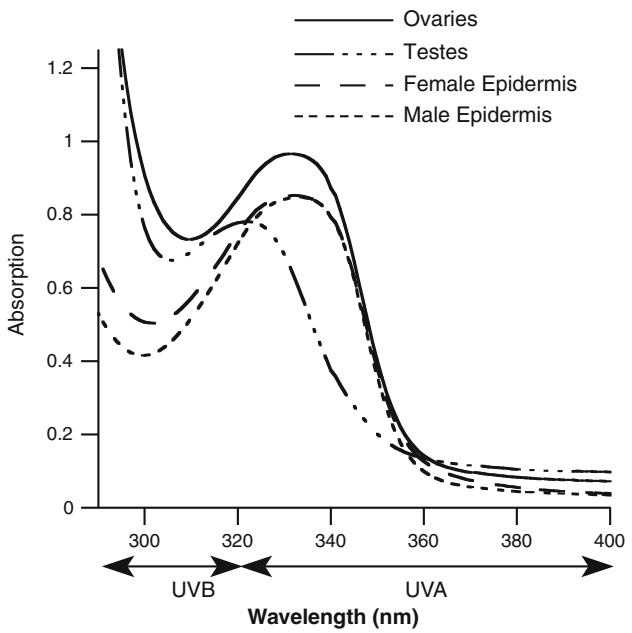

Fig. 5 Typical absorption spectra of MAA extracts for gonadal and epidermal tissues for male and female *S. purpuratus*. Each *spectrum* shown is from a single representative tissue extract, each with the same concentration of total MAAs

amounts of other MAAs. Conversely, testes had low relative concentrations of shinorine compared to the other tissues ($X \pm$ SE: 10.7 ± 1.2 %, $N = 119$; Student's, $P < 0.01$ for all comparisons) but over twice the relative concentration of palythine ($X \pm$ SE: 88.5 ± 1.3 %, $N = 119$; Student's, $P < 0.001$ for all comparisons). Ovaries also had higher relative concentrations of mycosporine glycine than the other three tissue types ($X \pm$ SE: 3.3 ± 0.9 % $N = 118$; Student's, $P < 0.01$ for all comparisons), which only contained trace amounts (Table 2). The relative concentration of shinorine also tended to be higher in sea urchins from Non-pit microhabitats and lower in those from Pit microhabitats (Fig. S2a), while palythine showed the opposite trend (Fig. S2b).

MAA absorption spectra in sea urchins

Absorption maxima of ovaries and female and male epidermal tissues were similar and in the low UVA range (Fig. 5; Table 2; $\lambda_{max} = 331$, 330 and 331 nm, respectively), while the absorption maximum for testes was at a shorter, higher energy wavelength near the UVA/UVB cusp (Fig. 5; Table 2: $\lambda_{max} = 322$ nm). Ovaries had the broadest absorption spectrum, spanning the UVA and some of the UVB range, while testes showed a constricted spectrum. Within the UVB range (280–320 nm), ovaries and testes showed similar absorbencies, while both epidermal tissues absorbed less UVB (Fig. 5).

Discussion

Our study establishes the presence of MAAs in the tissues of the widely studied purple sea urchin *Strongylocentrotus purpuratus*, as well as ten species of Rhodophyte macroalgae on California's Central Coast. Multiple patterns in concentrations of MAAs were identified that may affect fitness or survival of sea urchins. Most importantly, we found that ovaries exhibit higher MAA concentrations than testes, that different MAA signatures among sexes and tissues influence UVR absorption, that microhabitat affects concentrations of MAAs in sea urchins, and that males appear to more readily allocate MAAs to epidermal tissues in response to UVR than females. These findings are important for understanding how MAAs compare among different species of sea urchins, how sea urchins allocate MAAs among their tissues, and how sea urchins may respond increased UVR stress with climate change (McKenzie et al. 2007; Zepp et al. 2011).

Total MAAs in algae

This study is the first to demonstrate the presence of MAAs in eight species of red algae including *Calliarthron*

tuberculosum, *Corallina vancouveriensis, Endocladia muricata, Mastocarpus jardinii*, *Mastocarpus papillatus, Mazzaella flaccida, Osmundea spectabilis,* and *Prionitis lanceolata* (reviewed by Sinha et al. 2007). MAAs have been previously detected in only two species in our study; extracts from *Corallina officianalis* from the North Sea (Karsten et al. 1998) contained somewhat higher concentrations of shinorine ($X \pm$ SD: 0.88 ± 0.27 nmol mg^{-1} dry wt) than our samples (Table 1, $X \pm$ SD: 0.28 ± 0.12 nmol mg^{-1} dry wt, $N = 12$), but contained no palythine, asterina 330, or usujirene. Specimens of *Pterocladia (Ptercladiella) capillacea* from Japan (Lee and Shiu 2009) showed similar concentrations of total MAAs (maximum $\sim$9 nmol mg^{-1} dry wt) to our samples (Table 1, $X \pm$ SD: 5.34 ± 3.02 nmol mg^{-1} dry wt, $N = 12$), but while they found porphyra-334 to be dominant and shinorine and palythinol to be present, we found very little porphyra-334 and no palythinol (Table 1). Our results demonstrate the ubiquity of MAAs in Rhodophyte macroalgae and establish that MAAs are available to consumers, such as purple sea urchins, in Central California's coastal marine environment.

Sex- and tissue-specific differences in concentrations of MAAs in sea urchins

The existence of higher concentrations of MAAs in ovaries compared to testes is not surprising (Fig. 3; Table 2) and is consistent with other studies in corals (Michalek-Wagner 2001), holothuroids (Karentz et al. 1991) and sea urchins (Bosch et al. 1994; Carroll and Shick 1996; Karentz et al. 1997; McClintock and Karentz 1997). MAAs confer protection from cleavage delay and fatal developmental abnormalities in irradiated *S. droebachiensis* embryos (Adams and Shick 1996, 2001), so MAAs may protect *S. purpuratus* embryos similarly. In comparison, MAAs can only protect sperm during the $\sim$20 min (Pennington 1985) fertilization period. Further, MAA uptake in sperm is limited by sperm's small size, while eggs, which are 34,000 times larger (Levitan 1993), can more easily utilize MAAs. Adams and Shick (1996) calculated that MAAs in *S. droebachiensis* sperm may not protect the nucleus due to sperm's small optical radius. However, it is possible that MAAs present the fluid surrounding the sperm could provide protection, similar to MAA-containing cells surrounding tunicate embryos (Epel et al. 1999).

Concentrations of individual MAAs and absorption spectra of sea urchin tissues

The differences in MAA signatures and absorption spectra of testes versus the other tissues (Table 2; Fig. 5) appear to represent a strategy by males where the breadth of UVR absorption by MAAs is decreased, while the relative absorption at higher energy UVR wavelengths is increased. The small size of sperm limit the number of MAA molecules they can contain (Adams and Shick 1996), but the over twofold increase in the relative concentration of UVB-absorbing palythine in testes ($\lambda_{max} = 320$ nm, $X \pm$ SE: 88.5 ± 1.3 %, $N = 119$) may protect sperm DNA by maximally absorbing UVR in the shorter, higher energy wavelengths (Fig. 5, $\lambda_{max} = 322$ nm). The concentration of palythine in the ovaries was comparable to that in testes (Table 2), which correlates with elevated absorption of these tissues in the UVB range compared to epidermal tissues (Fig. 5). Shinorine ($\lambda_{max} = 334$ nm) dominated ovary and epidermal tissues and likely caused the maximal absorption of UVR to occur at longer wavelengths compared to testes (Table 2; Fig. 5). Finally, the combination of MAAs found in ovaries and epidermal tissues (Table 2) caused UV absorption spanning a broad range of wavelengths (Fig. 5), which could protect the more diverse cellular components of developing larvae and epidermis.

Some MAAs have variable antioxidant capabilities, which may mitigate negative effects of oxidation in sea urchin tissues. For example, mycosporine glycine effectively scavenges free radicals (Dunlap and Yamamoto 1995; de la Coba et al. 2009) and was primarily found in ovaries (Table 2) so may provide further protection to developing embryos. Elevated relative concentrations of palythine in testes compared to other MAAs (Table 2) may also confer increased scavenging of superoxide radicals and inhibition of lipid peroxidation as found for asterina-330 plus palythine in algal extracts (de la Coba et al. 2009).

These sex- and tissue-specific differences in MAA signatures may be due to sex-specific dietary preferences, to physiological differences in MAA uptake or transport to different tissues, or to interconversion of MAAs by sea urchins or by bacteria. No studies have shown sex-specific feeding preferences for any sea urchin species, probably because sex is difficult to determine externally. Alternatively, uptake of MAAs may vary between sexes with differing rates of interconversion of shinorine to other MAAs by the bacteria *Vibrio harveyii,* which is found in the guts of other sea urchins and holothuroids (Dunlap and Shick 1998; Bandaranayake and Des Rocher 1999; Shick and Dunlap 2002). Tissue-specific differences in concentrations of individual MAAs may also be caused by interconversion of MAAs within tissues (Whitehead et al. 2001; Conde et al. 2003; Shick 2004; reveiwed by Carreto and Carignan 2011) or by differences in the transfer of MAAs from the gut to tissues (Mason et al. 1998; Carefoot et al. 2000; Shick and Dunlap 2002). For instance, increased palythine in *S. purpuratus* testes may be a result of conversion of dietary shinorine or porphyra-334 to mycosporine glycine then palythine in testes (Whitehead et al. 2001;

Shick 2004). Alternatively, different carrier proteins may transfer palythine to testes (Dunlap et al. 1989; Mason et al. 1998), or differences in chemical properties, such as the more non-polar character of palythine (Furusaki et al. 1980) or the acidic and polar nature of shinorine, may allow them to be more easily transferred to or interconverted within different tissues.

Microhabitat variation in concentrations of MAAs in sea urchins

Our data suggest that microhabitat has long-term fitness or survival consequences for intertidal *S. purpuratus*. Specifically, intertidal *S. purpuratus* living in pits may produce larvae with lower survival due to decreased MAAs (Fig. 3a, b; Adams and Shick 1996, 2001). This potential disadvantage of inhabiting a pit may be offset by higher adult survival due to reduced predation (Grupe 2006), desiccation, or dislodgement by waves (Denny and Gaylord 1996). Thus, inhabiting a pit likely increases adult survival but reduces offspring viability.

Though we do not have data on the specific UV exposure nor the actual dietary MAA intake of each sea urchin studied, we used microhabitat as a proxy for both solar exposure (Fig. 1) and dietary MAA availability (Fig. 2). Thus, our inferences regarding these factors on concentrations of MAAs in sea urchins are speculative. However, many studies have detected effects of UV exposure and dietary MAAs on concentrations of MAAs in animal tissues (Karentz et al. 1997; Newman et al. 2000; Adams et al. 2001; Lamare et al. 2004).

Males appear to respond more strongly to UVR than females; a nearly twofold increase in the proportional concentrations of MAAs in epidermal tissues of males (Fig. 4) in the sunniest (Fig. 1) and highest Non-pit microhabitat was observed. This could mitigate negative effects of UVR, potentially maintaining growth or survival in these high stress environments. Interestingly, this response did not occur in females (Fig. 4). MAAs are important for embryo and larval health and survival (Adams and Shick 1996, 2001), so females may allocate a higher proportion of MAAs to their ovaries regardless of their own exposure to UVR. These findings suggest the sexes have differing abilities to protect themselves from increased UVR with climate change (McKenzie et al. 2007; Zepp et al. 2011).

We observed no clear trend of increased concentrations of MAAs in gonadal tissue (Fig. 3a, b) with sunlight exposure across microhabitats (Fig. 1), consistent with the results for *S. droebachiensis* under different UVR treatments (Adams et al. 2001). This is not surprising because very little ambient light penetrates the test to expose the gonads to UV (Walker et al. 2007; Adams, unpublished data for *S. purpuratus*). Thus, it would not be advantageous for females to alter concentrations of MAAs in their ovaries based on their immediate UVR conditions before releasing embryos into the plankton.

Surveys of attached algal MAA and drift algal availability show no association with the concentrations of MAAs in sea urchin tissues (Figs. 2, 3). These results are surprising because strong effects of dietary MAAs has been documented in other sea urchins (Adams et al. 2001; Lamare et al. 2004), seahares (Carefoot et al. 2000), krill (Newman et al. 2000), and marine snails (Przeslawski et al. 2005). Unfortunately, it was impossible to sample actual intake of algae in the field, and the lack of correlation between these factors suggest algal availability does not necessarily translate to algal consumption. For example, Pit urchins had low concentrations of MAAs (Fig. 3) but had moderate levels of attached and drift algal availability (Fig. 2). Conversely, Non-pit urchins had moderate to high concentrations of MAAs (Fig. 3) but low attached and perhaps drift algal availability (Fig. 2).

The lack of correlation among the microhabitats between gonadal index (an indicator of dietary biomass, Vadas 1968; Himmelman 1978) and concentrations of MAAs (an indicator of dietary MAAs, Adams et al. 2001; Lamare et al. 2004) suggests sea urchins in different microhabitats had different diets, and may explain the lack of association between algal availability and concentrations of MAAs in sea urchins. For example, both Pit and Non-pit urchins had moderate to high gonadal indices, but Pit urchins had low concentrations of MAAs and Non-pit urchins had moderate to high concentrations (Fig. 3). Pit urchins likely stayed in their pits (Grupe 2006) and relied mostly on caught drift algae, which showed lower concentrations of MAAs by weight than attached fleshy algae. Further, brown algae (Phaeophyceae) were common in drift but do not contain high amounts of MAAs (Shick and Dunlap 2002). Non-pit urchins may have been obtaining MAAs by consuming algae faster, supplementing their diets with microalgae, or consuming available algae high in MAAs. Individual algae from the Non-pit microhabitat contained higher concentrations of MAAs than conspecifics in other microhabitats (see results), possibly due to both high sunlight levels (Fig. 1) and high shore levels. Further, high relative concentrations of shinorine, the primary MAA accumulated by sea urchin tissues (Table 2), were found in both sea urchins and algae in the Non-pit microhabitat (Figs. S1 and S2) suggesting a potential transfer from algae to sea urchins, as has been noted between trophic levels for MAAs in several other organisms (Carroll and Shick 1996; Newman et al. 2000; Carefoot et al. 2000).

Future research

Our study highlights correlations of UV exposure and MAAs from algal diets with concentrations of MAAs in

S. purpuratus tissues. However, specific relationships between these factors remain unclear and further research is necessary and underway in our laboratory. Our current research examines synergistic effects of natural UV exposure and controlled MAA diets on the concentrations of MAAs in gonads and epidermis tissues of *S. purpuratus* over time. These experiments will explicitly test the correlative patterns in concentrations of MAAs in sea urchins with UV exposure and algal availability noted in this field survey.

Conclusion

This study is the first to identify multiple MAAs in the purple sea urchin, *S. purpuratus*, and in eight species of Rhodophyte macroalgae. High concentrations of MAAs in ovaries and broadband UV protection from many types of MAAs suggest they are important for protecting sea urchin embryos from developmental delay and abnormalities (Adams and Shick 1996, 2001). The narrower absorption spectrum of testes peaked at higher energy UV wavelengths and suggests males were maximizing the concentration the MAA palythine to protect sperm DNA. Inhabiting a pit reduced overall concentrations of MAAs, with potentially far-reaching fitness consequences, especially for offspring survival. Our data suggest that male *S. purpuratus* increase concentrations of epidermal MAAs in sunny environments, but that females do not, perhaps because allocation of MAAs to ovaries by females is prioritized and crucial for embryo survival. However, the ability to phenotypically adjust concentrations of MAAs depending on sunlight levels may be important for *S. purpuratus* populations in the near future, as the penetration of UVR into the atmosphere is predicted to continue to be above natural levels due to ozone thinning and ozone interactions with climate change (McKenzie et al. 2007; Zepp et al. 2011).

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