

SEX AND MICROHABITAT INFLUENCE THE ALLOCATION OF MYCOSPORINE  
-LIKE AMINO ACIDS TO TISSUES IN THE PURPLE SEA URCHIN,  
*STRONGYLOCENTROTUS PURPURATUS*

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Master of Science in Biological Sciences

by  
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COMMITTEE MEMBERSHIP

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

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## ABSTRACT

Sex And Microhabitat Influence The Allocation Of Mycosporine

-Like Amino Acids To Tissues In The Purple Sea Urchin,

*Strongylocentrotus Purpuratus*

Sarah A. Gravem

Field surveys of *Strongylocentrotus purpuratus* demonstrated that concentrations of natural sunscreens, mycosporine-like amino acids (MAAs), were higher in females than males for both gonadal and epidermal tissues, increased in ovaries as spawning season approached, and were influenced by the sea urchins' microhabitat. Sea urchins occupying burrows, or "pits", had lower concentrations of MAAs than those outside pits, suggesting a trade-off between physical and UV protection. Overall, UV irradiance did not influence MAA accumulation in gonadal tissues. However, males increased their allocation of MAAs to epidermal tissues in the microhabitat with the highest irradiance. Relative concentrations of individual MAAs were similar for epidermal tissues from both sexes and ovaries, providing broadband UVA/UVB absorbance, but testes contained principally one MAA, palythine. This is the first study to demonstrate that *S. purpuratus* and eight species of macroalgae in California have MAAs, and that the concentrations can be influenced by microhabitat.

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### CHAPTER 1. INTRODUCTION TO THE THESIS

Despite efforts to reduce emissions of ozone-depleting substances, the penetration of harmful ultraviolet radiation (UVR), specifically UVB (280- 320 nm) through the thinned ozone layer is predicted to continue above pre-1970's levels for several decades (McKenzie et al. 2007). UVR levels reaching Earth's surface may also be increased by



global climate change through alteration of cloudiness and albedo, and through continued polar stratospheric cooling, which reduces ozone and may hinder ozone-recovery at mid-latitudes (McKenzie et al. 2007). In the ocean, UVB penetrates to several meters depth in temperate coastal waters, with UVA (320-400 nm) penetrating meters deeper than UVB (Franklin and Forster 1997; Banaszak et al. 1998; Tedetti and Sempere 2006). Increasing UVR levels can also cause photobleaching in chromophoric dissolved organic matter (CDOM), further increasing UVR penetration (Anderson et al. 2001). Therefore, marine organisms inhabiting shallow waters, such as macroalgae and invertebrates, are at particular risk. Further, intertidal organisms that are emersed during daytime low tides are exposed to unfiltered UVR.

UVR, particularly UVB, is detrimental to many marine organisms, including benthic macroalgae, pelagic invertebrate larvae (Adams and Shick 1996; 2001), and adult invertebrates (Dey et al. 1988; Gleason 1993). The direct effects of UVB include damage to DNA and RNA through formation of pyrimidine dimers (Buma et al. 1995; van de Poll et al. 2002), and protein damage (Bischof et al. 2000; Sinha et al. 2005). UVA can indirectly damage cells by creating reactive oxygen species (ROS), which can damage DNA, proteins, lipids and cause apoptosis (Tyrrell 1991; Pourzand and Tyrrell 1999; Lesser 2006). In benthic macroalgae, exposure to UVR reduces growth and photosynthesis and cause decreased offspring survival (Wood 1987; Wiencke et al. 2000). In sea urchins, which are classic model organisms for studies of UV-effects on marine invertebrates, solar UVR can cause cyclobutane pyrimidine dimers, developmental delays, abnormalities and death in larvae (Adams and Shick 1996; 2001; Lesser et al. 2004; Lamare et al. 2007) and is behaviorally avoided by adults (Sharp and

Gray 1962; Adams 2001). At the community level, UVR can reduce biomass, productivity and diversity and alter marine community composition (Worrest et al. 1978; Bothwell et al. 1994; Lotze et al. 2002).

Many organisms contain defenses against UVR. For example, many organisms mitigate DNA damage using the enzyme photolyase, which monomerizes pyrimidine dimers (Eker et al. 1990; van de Poll et al. 2002). In addition, algae and invertebrates contain many molecules with antioxidant capabilities, some of which are upregulated in response to UVR (Lesser 1996; Lesser et al. 2003). While important, repair mechanisms are likely energetically costly; energy may be better spent in preventing, rather than repairing, damage using UVR-absorbing sunscreens.

A preventative defense against UVR-induced damage that is ubiquitous in marine organisms, including macroalgae and invertebrates, is the presence of mycosporine-like amino acids (MAAs). MAAs are a suite of water-soluble compounds that absorb light in the UVA and UVB range (310-360 nm) and may dissipate its energy harmlessly (Conde et al. 2000; Shick et al. 2000). They are stable over long periods of time *in vivo* (Adams and Shick 2001; Adams et al. 2001) and have overlapping absorption ranges, which in concert cover much of the harmful natural UVR spectrum (Dunlap and Shick 1998). Synthesis of MAAs occurs through the shikimic acid pathway (Favre-Bonvin et al. 1987), which appears to be absent in animals (Shick et al. 1999). MAAs are produced by shallow-water red macroalgae from the phylum Rhodophyta, and also by some species of brown and green macroalgae, various phytoplankters, and fungi (Shick and Dunlap 2002). They have also been found in many marine invertebrate species and some fishes (Shick and Dunlap 2002), and experimental evidence shows that these organisms obtain

MAAs through their diet or symbioses with MAA-producing organisms (Carroll and Shick 1996; Shick et al. 1999; Newman et al. 2000).

Multiple studies have shown a correlation between exposure to UV and the concentration of MAAs within organisms. Natural and artificial UVR can stimulate MAA production in algae and MAA accumulation in invertebrates with photosynthetic symbionts (Dunlap et al. 1986; Franklin et al. 1999; Shick et al. 1999; Hoyer et al. 2002; Shick and Dunlap 2002). The concentration of MAAs decreases with increasing depth in macroalgae and sea urchins (Karentz et al. 1997; Karsten et al. 1999; Karsten and Wiencke 1999) and upregulation of MAAs often occurs when transplanting macroalgae to shallower waters, especially in treatments using unfiltered sunlight (i.e. with UVR; Wood 1989). Increased concentrations of MAAs in macroalgae have also been found in lower latitude compared to higher latitude species (Karsten et al. 1998a; 1998b), during the summer months (Wood 1987; Karsten et al. 1999; Aguilera et al. 2002) and in conspecifics in sun-exposed compared to shaded microhabitats (Karsten et al. 1999; Figueroa et al. 2003). Both algae and invertebrates show increased concentrations of MAAs in reproductive tissues and gametes (Adams and Shick 1996; Carroll and Shick 1996; Carefoot et al. 2000; Karsten et al. 2000) as well as in epidermal tissues in invertebrates and growing tissues in algae (Shick et al. 1992; Carroll and Shick 1996; Bandaranayake and Des Rocher 1999; Karsten et al. 1999; Karsten et al. 2000). The correlation of MAA concentration with these factors further implicates MAAs as protective UVR sunscreens.

Other studies have demonstrated that MAAs serve at least in part as sunscreens against UV- induced damage. MAAs protect sea urchin embryos from UVR-induced

delay of the first cell division and developmental abnormalities (Adams and Shick 1996; 2001). The concentration of MAAs also correlates positively with the ability of algae to resist inhibition of photosynthesis in the presence of UVR (Neale et al. 1998; Karsten et al. 1999).

Sea urchins, especially their larvae, have served as good model organisms for testing model of accumulation and the protective role of MAAs (Adams and Shick 1996; 2001; Lesser et al. 2004; 2006). However, at present there is only one published account of the widely studied California purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson), being tested for MAAs (Lamare and Hoffman 2004). The concentration of MAAs found in the individual *S. purpuratus* was extremely low (0.004 and 0.022 nmol mg<sup>-1</sup> dry wt. for ovaries and eggs, respectively), and spectrophotometric scans showed little light absorbance in the UV range (Lamare and Hoffman 2004; Sinha et al. 2007). *S. purpuratus* was the first invertebrate deuterostome to have its genome sequenced (Sodergren et al. 2006), and is important for structuring ecologically and economically valuable kelp forest and rocky intertidal ecosystems (Dayton 1975; Pearse 2006). It has long been a model organism for developmental biology (Jasny and Purnell 2006), including numerous studies on the effects of UV radiation on development (Wells and Giese 1950; Giese 1964; Zeitz et al. 1968; Lamare and Hoffman 2004). Thus, an understanding of the presence, distribution and functions of MAAs in adult and larval *S. purpuratus* is imperative to understand the physiology of this model organism.

The ecology of *S. purpuratus* makes it likely that they contain MAAs. Larvae of *S. purpuratus*, like other pelagic larvae, seem especially vulnerable to UVR because of their small size, lack of protective covering, and rapid rates of cell division. Though

echinoderm larvae have been found to sink in response to solar UVR and are negatively geotropic (Pennington and Emllet 1986), they are likely advected into shallow parts of the water column (Denny and Shibata 1989), where they would be exposed to UV irradiance. Adult intertidal *S. purpuratus* are also frequently exposed to UVR, and the majority of their diet consists of macroalgae, including MAA-rich Rhodophytes. Purple sea urchins exhibit two feeding modes. When grazing, they scrape benthic algae from the substrate. In addition, they use their tube feet to catch floating drift algae for consumption (Ebert 1968; Dayton 1975). A similar species, *S. droebachiensis*, has the ability to acquire dietary MAAs in various body tissues (Carroll and Shick 1996) and their embryos are protected from UVR by MAAs (Adams and Shick 1996; 2001). The exposure of purple sea urchins to UVR as larvae and as adults in the intertidal, the fact that they consume a diet potentially rich in MAAs, and that tissues of congeners contain MAAs, makes it likely that purple sea urchins can acquire MAAs from their diets.

A preliminary study on *S. purpuratus* collected on California's Central Coast, confirmed the presence of MAAs in methanolic extracts of mature gonadal tissues (Chang and Adams, unpublished data). In the study, adult *S. purpuratus* were collected from each of four sites on the Californian Central Coast. Sites included three rocky intertidal locations: Hazards Beach at Montaña de Oro State Park, the jetty at Port San Luis in Avila Beach, and the University of California Kenneth S. Norris Rancho Marino Reserve in Cambria; the fourth site was a subtidal rocky reef in Goleta, California. The results of this study showed some differences between sites, with intertidal sea urchins from Avila having higher concentrations of MAAs than subtidal sea urchins from Santa Barbara ( $P = 0.031$ ), but in general the variation within the sites was extremely high

(Hazards:  $0.12 \pm 0.28$ , Avila:  $0.54 \pm 0.62$ , Rancho Marino:  $1.64 \pm 3.05$ , Santa Barbara:  $0.02 \pm 0.03$  mean nmol mg<sup>-1</sup> dry wt.  $\pm$  S.D.). Further, there was no significant difference in concentrations of MAAs between the sexes ( $P = 0.349$ ), as has been found in other *Strongylocentrotus* species (Carroll and Shick 1996). This was because the concentrations of MAAs were extremely variable (ovaries:  $0.58 \pm 1.6$ , testes:  $0.19 \pm 0.14$  mean nmol mg<sup>-1</sup> dry wt.  $\pm$  S.D.), especially for ovaries, which ranged in MAAs from 0.00 nmol mg<sup>-1</sup> dry wt. to 7.09 nmol mg<sup>-1</sup> dry wt. Because the sea urchins used in this study were collected without regard for microhabitat conditions (e.g. tidal height, degree of shading or burrowing behavior), the large variation observed in this study may have been due to fine-scale microhabitat variation affecting the availability or uptake of MAAs in purple sea urchins.

Because intertidal purple sea urchins are fairly sedentary (Grupe 2006, personal observation), it is likely that their local environmental conditions, their microhabitat, will affect their exposure to UVR and their algal intake. Microhabitat factors, including exposure to UVR and diet, could affect MAA uptake and allocation to sea urchin tissues, which should affect the health of adult urchins, as well as offspring survival (Adams and Shick 1996; 2001) and ultimately fitness. While exposure to UVR triggers increased uptake of MAAs in algae and symbiotic invertebrates (Franklin et al. 1999; Shick et al. 1999; Hoyer et al. 2002), it has been shown to be ineffective in increasing the concentration of MAAs in the green sea urchin *S. droebachiensis* (Adams et al. 2001). However, Adams et al. (2001) only sampled ovarian tissue, which is shielded from UVR by the test. Further, *Strongylocentrotus* species are broadcast spawners with pelagic larval phases lasting weeks to months (Strathmann 1987), so it would likely be

maladaptive for an adult sea urchin to allocate MAAs to its offspring based on its own immediate irradiance condition. Conversely, microhabitat variation in UVR exposure may cause changes in the uptake or allocation of MAAs to other tissues, such as the epidermis, or cause changes in the relative concentrations of individual MAAs among various tissues, as has been suggested for tropical sea cucumbers (Shick et al. 1992).

For this study, we identified microhabitat conditions that may affect intertidal purple sea urchin exposure to UVR and MAA accumulation from algal diets. The first is rock surface orientation to the sun and the other is the occurrence of sea urchins in “pits”. Intertidal *S. purpuratus* exhibit burrowing behavior, where they slowly excavate pits in the substratum (Morris et al. 1980), which may serve to protect them from predation (Grupe 2006) and from waves easily capable of dislodging sea urchins (Denny and Gaylord 1996). Sea urchins living inside pits (hereafter “Pit urchins”) may also experience decreased desiccation, temperature stress, and exposure to UVR, all of which would likely be advantageous. However, the potential advantages of burrowing behavior may also reduce the ability of *S. purpuratus* to graze and to catch drift algae, and Pit urchins have been shown to experience lower growth rates, likely due to reduced feeding, compared to nearby sea urchins living outside of pits (hereafter “Non-pit urchins” after Grupe 2006). This probable reduction in feeding in Pit urchins compared to Non-pit urchins could affect not only MAA intake, but also total reproductive output, because gonad size and the abundance of nutritive cells is associated with increased nutrition (Minor and Scheibling 1997).

To further understand how microhabitat, algal availability, season and sex affect MAA concentrations in intertidal purple sea urchin tissues, *S. purpuratus* and ten

common species of red macroalgae were collected from a rocky intertidal zone and the concentrations of MAAs were measured. Algae and sea urchins were taken from tide pools representing four microhabitats. To determine the effect of sea urchin burrowing behavior on MAA content in sea urchins, the first two microhabitats contained sea urchins inside and outside of pits on horizontally oriented rock surfaces (Pit and Non-pit urchins, respectively). To determine the effects of differential UV irradiance on MAA content in sea urchins and algae, the other two microhabitats were vertical surfaces oriented Southward (increased irradiance) or Northward (decreased irradiance), and sea urchins were not in pits. The concentration of MAAs in sea urchins was measured for both gonadal and epidermal tissues to determine whether microhabitat affected preferential allocation of MAAs to different tissues. Both male and female urchins were collected and analyzed to investigate whether any patterns in uptake and allocation were sex-specific. Algal and sea urchins collections were done during the season when sea urchins were accumulating gonadal tissue and gametes were developing, and sea urchins were collected again just before spawning to track changes in the concentration of MAAs as gametes approached maturity.

Based on previous studies, we predicted that intertidal *S. purpuratus* gonads and epidermis would contain MAAs. We hypothesized that all the species of red macroalgae sampled would contain MAAs, and their concentrations of MAAs would be increased in higher irradiance microhabitats. We also expected Pit urchins to have lower total concentrations of MAAs than Non-pit urchins, and that epidermal, but not gonadal, concentrations of MAAs would be increased in higher irradiance microhabitats. In addition, we predicted that epidermal concentrations of MAAs should be similar among



the sexes, but that concentrations of MAAs in ovaries would be higher than those in testes, as was found in *S. droebachiensis* (Carroll and Shick 1996).

## CHAPTER 2. MANUSCRIPT

### **Introduction to the Manuscript**

Despite efforts to reduce emissions of ozone-depleting substances, the penetration of harmful ultraviolet radiation (UVR), specifically UVB (280-320 nm) through the thinned ozone layer is predicted to continue above pre-1970's levels for several decades (Madronich et al. 1998; McKenzie et al. 2007). Levels of UVR reaching Earth's surface may also be increased by global climate change through continued polar stratospheric cooling, which may hinder ozone-recovery at mid-latitudes (McKenzie et al. 2007). In temperate coastal waters, UVB penetrates to several meters depth, with UVA (320-400 nm) penetrating meters deeper than UVB (Franklin and Forster 1997; Banaszak et al. 1998; Tedetti and Sempere 2006). Therefore, marine organisms inhabiting shallow waters or exposed during low tides, such as macroalgae and invertebrates, are at particular risk for UVR damage.

UVR is detrimental to many marine organisms, including macroalgae and adult and larval invertebrates (Dey et al. 1988; Gleason 1993; Franklin and Forster 1997; Adams and Shick 2001). UVR can directly damage DNA, RNA and proteins as well as promote the formation of reactive oxygen species, which can also damage cellular components (Tyrrell 1991; Buma et al. 1995; Pourzand and Tyrrell 1999; van de Poll et al. 2002; Lesser 2006). Many organisms have defenses that mitigate UVR damage including photorepair and antioxidative enzymes (Eker et al. 1990; Lesser 1996; van de Poll et al. 2002; Lesser and Barry 2003). While important, repair mechanisms are likely energetically costly; energy may be better spent in preventing, rather than repairing, damage using UVR-absorbing sunscreens.

One such preventative defense against UVR-induced damage that is ubiquitous in marine organisms is the presence of mycosporine-like amino acids (MAAs). MAAs are a suite of water-soluble compounds that absorb UVR in the UVA and UVB range (309-360 nm) (reviewed by Shick et al. 2000). MAAs are stable over long periods of time *in vivo* (Adams and Shick 2001; Adams et al. 2001) and their overlapping absorption ranges cover much of the UVR spectrum (Dunlap and Shick 1998). MAAs are synthesized using the shikimic pathway (Favre-Bonvin et al. 1987) by red macroalgae (Phylum Rhodophyta), some species of brown and green macroalgae, various phytoplankters, symbiotic zooxanthellae and fungi (reviewed by Shick and Dunlap 2002). In addition, many marine invertebrate species and some fishes contain MAAs (reviewed by Shick and Dunlap 2002; Sinha et al. 2007), and appear to obtain the MAAs through their diet or symbioses (Carroll and Shick 1996; Shick et al. 1999; Carefoot et al. 2000; Newman et al. 2000). MAAs have been shown to protect *Strongylocentrotus droebachiensis* 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). The findings of these studies implicate 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). Although much work has been performed on the role of MAAs in the green sea urchin, *Strongylocentrotus droebachiensis*, little work has been done on the California purple sea urchin *Strongylocentrotus purpuratus* (Stimson). The purple sea urchin stands out as a widely-studied model organism in molecular biology, and was the first invertebrate deuterostome

to have its genome sequenced (Sodergren et al. 2006). However, to our knowledge there is only one published account of MAAs in the widely studied California purple sea urchin, *S. purpuratus* (Lamare and Hoffman 2004). In their study, Lamare and Hoffman (2004) found extremely low concentrations of MAAs in gonadal extracts of a single *S. purpuratus* individual from Washington State.

The above result seems unusual because the ecology of purple sea urchins makes it likely that they can contain MAAs. Transparent, developing larvae of *S. purpuratus* are especially vulnerable to UVR and they are likely advected into shallow waters (Denny and Shibata 1989) during the weeks to months they develop in the water column (Strathmann 1987). Adult intertidal *S. purpuratus* are also frequently exposed to UVR, and a large proportion of their diet consists of MAA-producing Rhodophyte macroalgae, which they either graze from the substrate or catch with their tube feet from the water column (Ebert 1968; Dayton 1975).

Moreover, a preliminary study in our laboratory on *S. purpuratus* collected from four sites along California's Central Coast confirmed the presence of MAAs in mature gonadal tissues (Chang and Adams, unpublished data). Some ovary samples of *S. purpuratus* collected in this study had higher concentrations of MAAs (0.08 – 7.09 nmol mg<sup>-1</sup> dry wt.) than testes (0.02 – 0.44 mg<sup>-1</sup> dry wt.), but in general the variation was extremely high within sexes and sites. Because the sea urchins used in this study were collected without regard for microhabitat conditions (e.g. tidal height, degree of shading or burrowing behavior), the large variation observed may have been due to fine-scale variation in microhabitat, affecting the availability or uptake of MAAs by sea urchins.

Intertidal purple sea urchins are fairly sedentary (Grupe 2006; personal observation), so it is likely that their local environmental conditions, their microhabitat, will affect their exposure to UVR and their algal intake over time. These could affect MAA uptake and allocation to tissues, which should affect the health of adult urchins as well as offspring survival (Adams and Shick 1996; 2001) and ultimately fitness. While exposure to UVR triggers increased uptake of MAAs in algae and symbiotic invertebrates (Franklin et al. 1999; Karsten et al. 1999; Shick et al. 1999; Hoyer et al. 2002), it was ineffective in increasing the concentration of MAAs in the gonads of adult green sea urchins (*S. droebachiensis*) (Adams et al. 2001). However, Adams et al. (2001) only examined ovaries, and increased UVR exposure may cause an increase in the uptake or allocation of MAAs to other tissues, such as the epidermis or testes.

To further understand how microhabitat, algal availability, season and sex affect MAA concentrations in intertidal purple sea urchin tissues, concentrations of MAAs were measured in adult *S. purpuratus* and ten common species of red macroalgae collected from the intertidal zone. Sea urchins and algae were collected from microhabitats which may affect intertidal purple sea urchins' exposure to UVR or MAA acquisition from algal diets, including those with sea urchins on rock surfaces facing Northward (more shaded) and Southward (less shaded) as well as those with sea urchins in and out of "pits," which they excavate and inhabit in the substratum (Morris et al. 1980). The concentrations of MAAs in sea urchins were measured for both gonadal and epidermal tissues, and collections were performed two times, before and at the start of peak reproductive season for sea urchins; algal collections were performed in the fall before sea urchin reproductive season.

We predicted that intertidal *S. purpuratus* gonads and epidermis would contain MAAs and that all the species of red macroalgae sampled would contain MAAs. We also expected sea urchins in pits to have lower total concentrations of MAAs than those out of pits because of less access to algal resources, and that epidermal, but not gonadal, concentrations of MAAs would be increased in higher irradiance microhabitats. We also predicted that epidermal concentrations of MAAs should be similar among the sexes, but that concentrations of MAAs in ovaries would be higher than those in testes, similar to patterns observed in *S. droebachiensis* (Carroll and Shick 1996).

## Methods

Adult *S. purpuratus* and ten species of macroalgae from the phylum Rhodophyta were collected from a rocky intertidal bench on the jetty at Port San Luis in Avila Beach, California (35°09'N, 120°45'W). Sea urchins and macroalgae were collected from four microhabitats including: 1) horizontally-oriented surfaces in tide pools where sea urchins were not burrowed into pits (hereafter the “Non-pit” microhabitat), 2) horizontally-oriented surfaces in tide pools where sea urchins were burrowed into pits (hereafter the “Pit” microhabitat), 3) South facing vertical surfaces in tide pools with sea urchins outside of pits (hereafter the “South facing” microhabitat) and 4) North facing surfaces walls in tide pools with sea urchins outside of pits (hereafter the “North facing” microhabitat). Inhabiting a pit may decrease *S. purpuratus*' algal intake because of reduced mobility as well as decrease irradiance levels experienced, and inhabiting a vertical wall should decrease the irradiance levels experienced by sea urchins, more so in the North facing than South facing microhabitat. For each microhabitat, three representative tide pool stations were chosen, and all stations were within 50 meters of one another.

### *Station Measurements*

In order to ensure that the microhabitats' orientation to the sun (i.e. South facing vs. flat vs. North facing) was related to actual irradiance level, a Solar Pathfinder (Perusion) was used to obtain monthly average irradiance measurements for each station based on the angle of the sun and the amount of shading from nearby rocks. August through January measurements were used in analyses to coincide with algal and sea

urchin collections as well as the gametogenic cycle of *S. purpuratus* (Pearse et al. 1970; Giese et al. 1991).

Because temperature may affect algal and sea urchin physiology, the average temperature for each station was determined from November 2006 to January 2007 using HOBO pendant temperature recorders. Tidal height was also measured for each station, and all stations were between 0.8 and 1.4m above mean lower low water (MLLW). This small (< 1m) range in intertidal height helped validate the comparison of the irradiance measurements above, because variation among stations in attenuation of solar irradiance by water was likely low. Prior to collections, sea urchin density for each station was measured to enable testing for any effects of herbivory on algae or competition in sea urchins on the concentration of MAAs.

In order to characterize the availability of MAAs to sea urchins among the microhabitats, both attached and drift algal availability were sampled monthly from October 2006 to May 2007 in each station. Attached algal availability was sampled by measuring attached algal cover for each species within 0.50 m<sup>2</sup> random quadrats in each station over time. Drift algal availability to sea urchins was measured monthly at each station by collecting and weighing macroalgae stuck to the tube feet of fifty random sea urchins.

#### *Collection and Preparation of Specimens*

Algae. Twelve specimens of each of ten common algal species were collected in the early afternoon on October 7, 2006 and included *Calliarthron tuberculosum* (Postels and Ruprecht) Dawson, *Corallina chilensis* (Decaisne), *Corallina vancouveriensis*



(Yendo), *Endocladia muricata*, *Mastocarpus jardinii* (J. Agardh) J.A. West, *Mastocarpus papillatus*, *Mazzaella flaccida* (Setchell and N.L. Gardner) Fredericq, *Osmundea spectabilis* (Postels and Ruprecht) Nam, *Prionitis lanceolata* and *Pterocladia capillacea* (Gmelin) Santelices and Hommersand. In order to characterize MAAs in the species, one healthy, whole algal specimen from each species was collected from each station when available. Because each station did not contain every algal species, some specimens were collected within the same station to bring the total to twelve specimens. Algae were transported to Cal Poly within a few hours of collection and frozen at -80°C. Algae were identified using guides and dichotomous keys (Abbott and Hollenberg 1993; Mondragon and Mondragon 2003) as well as the assistance of Dr. Kathy Ann Miller from the University of California, Berkeley. Whole algal specimens were thawed and cleaned of epibiota using forceps, then frozen in liquid nitrogen and pulverized. Samples were then re-frozen, lyophilized and stored at -80°C for later extraction and analysis of MAAs. Drift algal samples were separated into phyla, and Rhodophytes were treated in the same way as attached samples detailed above. Samples from the November 2006 collection served as representative drift algal specimens for MAA analysis. These samples were frozen in liquid nitrogen and pulverized before extraction and analysis of MAAs.

Sea Urchins. Adult *S. purpuratus* were collected in November 2006 before sea urchins were fully gravid, and again in January 2007 just before the spawning season when sea urchins were gravid. Five female and five male sea urchins, at least 30mm in diameter to ensure sexual maturity (Giese et al. 1991), were collected from each station in both November and January for analyses. Sea urchins were kept in seawater-filled

coolers for an hour during transport before being transferred to enclosed seawater aquaria for no more than four days before dissection.

Whole wet weight, test diameter and gonad wet weight were recorded for each sea urchin and gonadal index (GI), a measure of fecundity (Giese et al. 1991), was calculated for each sea urchin as:  $100 \times (\text{wet weight of gonad} / \text{wet weight of whole urchin})$ .

Epidermal tissue was collected by trimming off the spines and sampling the ambulacral sections of the test. Gonadal and epidermal tissues were frozen, lyophilized, and stored at  $-80^{\circ}\text{C}$  for later extraction and analysis of MAAs.

#### *Mycosporine-Like Amino Acid (MAA) analysis*

A fixed dry weight of each tissue type (0.200g gonads, 1.000g epidermis and 0.200g algae) was extracted for MAAs using three serial 60-minute extractions in HPLC-grade methanol at room temperature, and the three extracts were pooled. During the first extraction the samples were sonicated for approximately 20 seconds using a Branson Sonifier 250. The pooled extracts for algal specimens were filtered through a Waters Sep Pack Plus C-18 column to remove chlorophyll and other pigments as per Adams et al. (2001). Presented absorption spectra (290-700 nm) were measured for representative tissue samples with equal total MAA concentrations using a Jasco Model V550 UV/Vis spectrophotometer. Extracts were analyzed for MAAs using Hewlett Packard 1100 Series reverse-phase high performance liquid chromatography (HPLC) with a Phenomenex Phenosphere C-8 column at a flow rate of  $0.8 \text{ ml min}^{-1}$ .

MAAs were identified by comparing peak absorbance and retention times with known MAAs, and identification of representative peaks were confirmed by

cochromatography using quantified standards provided by Dr. W.C. Dunlap of the Australian Institute of Marine Science in Townsville, Australia. 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 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, palythanol and usujirene were calculated using a 25% methanol and 0.1% acetic acid mobile phase. MAA concentrations in nmol mg<sup>-1</sup> dry weight 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). The MAA concentration of usujirene was calculated using a calibrated standard for palythene, its *trans* isomer.

#### *Unknown MAAs*

HPLC chromatograms of the sea urchin tissue MAA extracts repeatedly showed four unknown peaks absorbing in the UV range. Two of the peaks were occasionally present in ovary tissue and absorbed at 322 and 324 nm. They eluted before mycosporine-glycine, in the range where we have never previously observed MAAs. Another peak eluted directly after mycosporine-2-glycine and was common in epidermal but not gonadal tissue, but the maximal absorbance could not be determined. The final peak eluted before palythine, had a maximum absorption of 338 nm and was common in ovarian and epidermal tissue. Karsten et al. (2000) and Karsten et al. (2005) documented

novel UV-absorbing compounds using HPLC that absorbed at 334 nm from red algae and 324 nm from green algae, respectively. Both these compounds eluted early in the HPLC chromatogram and could be the same as the 332 nm and 324 nm found here, though standards were not available for these possible MAAs and more testing is needed. In this study, the occurrences of these unknown peaks were rare, and when present, were drastically smaller (and hence less concentrated) than other MAAs peaks (such as shinorine and palythine) in the chromatogram. No standards were available for these peaks, so none of them are included in calculations of total concentrations of MAAs for any sample. While their presence is intriguing, they did not likely contribute drastically to the UV-absorbance of the extracts or the total MAA concentrations of the tissues.

#### *Attached Algal MAA Availability to Sea Urchins*

In October 2006, when the algae were collected for MAA analysis, ten 5 cm<sup>2</sup> or 2.5 cm<sup>2</sup> plots for each of the ten algal species identified above were scraped from the substrate, lyophilized and weighed to determine the approximate dry weight per unit area for each species. For each algal species, this measurement of biomass per unit area was multiplied by the average MAA concentration per unit biomass and the measurement of average monthly cover for the species per station, giving the average monthly MAA availability to sea urchins in each station per unit area using the equation below. We then summed this value for all the species found at each station each month, and were able to compare the MAA availability to sea urchins among stations and microhabitats. This metric is a better indication of attached algal MAA availability to sea urchins in each station than simple algal percent cover measurements, because it incorporates MAA

concentrations of individual algal species as well as the biomass per unit area of cover for each algal species. Species other than those analyzed for MAAs were not included in these conversions, but were only occasionally identified in attached algal cover measurements, so MAA availability to sea urchins from those species was likely negligible.

$$\frac{\text{mean mg dry wt.}}{\text{cm}^2 \text{ cover}} \times \frac{\text{mean nmol total MAAs}}{\text{mg dry wt.}} \times \frac{\text{mean monthly cm}^2 \text{ cover}}{\text{m}^2 * \text{station}} = \frac{\text{monthly nmol total MAAs}}{\text{station}}$$

### *Statistical Analyses*

Graphical results are presented using untransformed data and are given as means  $\pm$  one standard error unless otherwise indicated. All data were analyzed using General Linear Models (similar to multi-factor ANOVA) on Minitab software using sequential sums of squares. Significant terms ( $P < 0.05$ ) in all models were further analyzed using Tukey's post-hoc analyses. Square root, natural log, negative inverse or arcsine transformations were applied to the data to best achieve linearity, homogeneity of variance and normal distribution of residuals for statistical analyses.

To analyze microhabitat variation in concentrations of total MAAs in algal specimens, we used species and microhabitat as predictor variables and included station nested within microhabitat to control for among-station variation within each microhabitat in concentration of MAAs. Because wet to dry weight ratios varied greatly among algal species, species comparisons of total concentration of total MAAs per unit dry weight were not accurate. However, we were able to compare the species in the relative concentration of shinorine (concentration of shinorine / concentration of total MAAs) found in algal tissues because this metric was a relative rather than an absolute

concentration of MAAs. In this model we also used species and microhabitat as predictor variables included station nested within microhabitat to control for among-station variation within the microhabitats. Other individual MAAs were similarly tested, but were either not significant or did not meet general linear model assumptions. For drift algal analyses, we tested microhabitat variation in MAAs using microhabitat as a predictor and concentration of MAAs in homogenized drift algal collections as the response variable for the November 2006 collection of drift algae.

For analyses on sea urchins, models analyzing concentrations of MAAs in gonadal and epidermal tissues were performed separately because epidermal samples contained spine and test tissues, so standardizing measurements by dry weight rendered the two tissue measurements incomparable. In order to examine variation in the allocation of MAAs within each sea urchin to the separate tissues, the proportion of total MAAs per sea urchin that was detected in the epidermal tissue (versus the gonadal tissue, i.e. concentration of epidermal MAAs / [concentration of epidermal + gonadal MAAs]) was used as a response variable for the analysis. For the three response variables outlined above (gonadal and epidermal concentrations of MAAs and the proportion of MAAs in the epidermis) as well as for gonadal index, we ran models using microhabitat, month, sex and their interactions as predictors, and again included station nested within microhabitat to control for among-station variation within microhabitats.

In order to compare variation among sea urchins in the relative concentrations of individual MAAs, the relative concentrations of the two most concentrated MAAs, shinorine and palythine (i.e. concentration of shinorine or palythine / concentration of total MAAs), were used as response variables in separate models, each with microhabitat,

sex, tissue and their interactions as predictors; month was removed because no terms including month were significant. For each model, sea urchin number (nested within microhabitat and sex) was included to control for the non-independence of tissue measurements within sea urchins. Because shinorine and palythine were calculated as relative proportions of gonadal and epidermal MAA concentrations rather than absolute concentrations, these metrics were comparable between gonadal and epidermal tissues, and tissue type was included in these models rather than analyzing trends in epidermis and gonad tissues separately.

Having included the “microhabitat” predictor term in all above the models (but after removing the station nested within microhabitat term) we included the various environmental parameters (irradiance, tidal height, temperature, sea urchin density, attached algal cover, drift algal availability and attached algal MAA availability) and sea urchin attributes (size, gonadal index) in each model, but none proved significant. However, after removing the “microhabitat” term from the above models, and re-running the models including environmental variables and sea urchins attributes as predictors in each model, the environmental parameters and sea urchin attributes often became significant. Because we were especially interested in retaining the microhabitat term in these models, we performed separate analyses for these environmental and sea urchin attribute variables to examine among-microhabitat patterns. For each of these “microhabitat characterization” analyses, date and algal species (where applicable) and microhabitat were treated as predictor variables, with station nested within microhabitat was included to control for among-station variation within microhabitats (except when testing tidal height and sea urchin density, where there was only one measurement per station).

## Results

### *Microhabitat Characterizations*

Irradiance. Mean irradiance levels varied significantly among the microhabitats (Fig. 1a;  $P < 0.000$ ,  $R^2 = 0.72$ ) with the North facing microhabitat ( $2.54 \pm 0.22 \text{ kW hr}^{-1} \text{ m}^{-2}$ ) having lower irradiance than the other microhabitats, as expected ( $P < 0.001$ ). Because of afternoon shading, the South facing microhabitat had a moderately lower mean irradiance ( $3.29 \pm 0.19 \text{ kW hr}^{-1} \text{ m}^{-2}$ ) than both the horizontally oriented Pit ( $3.67 \pm 0.01 \text{ kW hr}^{-1} \text{ m}^{-2}$ ) and Non-pit ( $3.66 \pm 0.02 \text{ kW hr}^{-1} \text{ m}^{-2}$ ) microhabitats, ( $P = 0.031$  and  $P = 0.049$ , respectively). Though the mean irradiance levels for the Pit and Non-pit microhabitats were not different ( $P = 0.996$ ), the irradiance measurements for the Pit microhabitat were taken on the rock surfaces and not inside the pits; therefore it can be assumed that portions of the epidermis of Pit urchins are substantially more shaded by the walls of their pits than irradiance measurements indicate.

Height above MLLW. Even within the small range in vertical height ( $< 1\text{m}$ ) among the stations, there were significant differences in height above MLLW among the microhabitats (Fig. 1b;  $P = 0.007$ ,  $R^2 = 0.77$ ). On average, stations in the Non-pit microhabitat were at a higher tidal height level than the other three microhabitats ( $P < 0.031$ ;  $1.34 \pm 0.04 \text{ m}$ ), which did not differ from one another ( $P > 0.688$ ;  $0.91 \pm 0.11$ ,  $0.96 \pm 0.03$  and  $0.84 \pm 0.09 \text{ m}$  above MLLW for South facing, North facing and Pit microhabitats, respectively).



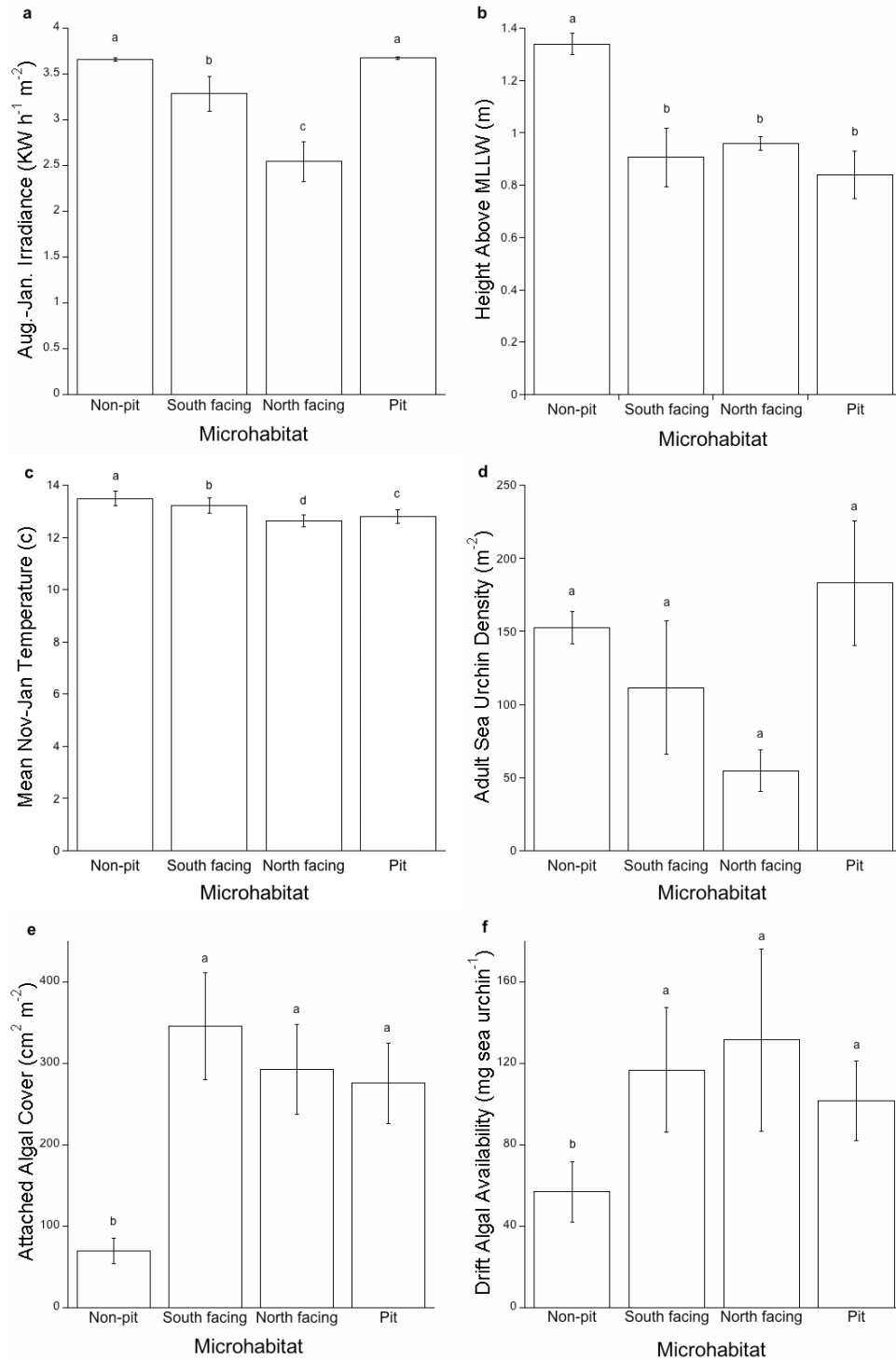


Figure 1. Measurements of mean ( $\pm$  S.E.) a) irradiance (n=9), b) intertidal height above MLLW (n=3), c) temperature (n=3), d) adult *S. purpuratus* density (n=3), e) attached algal cover (n=15) and f) drift algal availability (n=15) among the microhabitats. Matching letters above bars represent means that were not significantly different from one another. Irradiance in Pits (1a) may have been lower than the figure indicates because irradiance measurements were taken outside of pits.

Temperature. Average monthly temperature was highest the Non-pit microhabitat ( $13.49 \pm 0.28$  °C) followed by the South facing, Pit and North facing microhabitats ( $13.21 \pm 0.30$ ,  $12.63 \pm 0.23$  and  $12.81 \pm 0.26$  °C, respectively), all of which differed significantly from one another (Fig. 1c;  $P < 0.003$  for all comparisons,  $R^2 = 0.99$ ). The mean monthly temperature was coldest in January compared to November and December ( $P < 0.001$  for both comparisons), but November and December did not differ from one another ( $P = 0.402$ ). The above trend in mean monthly temperature among microhabitats was affected by the month (Month x Microhabitat:  $P = 0.002$ ); however, the above order among microhabitats in mean monthly temperature was consistent each month, though the microhabitats were not always significantly different from one another each month.

Adult Sea Urchin Density. There was no significant variation in starting adult sea urchin density among the microhabitats (Fig. 1d;  $P = 0.100$ ,  $R^2 = 0.52$ ). The Pit microhabitat was most densely populated with sea urchins ( $183.1 \pm 42.8$  sea urchins  $m^{-2}$ ), followed by the Non-pit ( $152.7 \pm 11.1$  sea urchins  $m^{-2}$ ), South facing ( $111.5 \pm 45.5$  sea urchins  $m^{-2}$ ) and North facing ( $54.7 \pm 14.1$  sea urchins  $m^{-2}$ ) microhabitats.

Attached Algal Cover. Attached algal cover is used here as a proxy for the amount of algae available to grazing sea urchins in each microhabitat (Fig. 1e). Algal cover was lowest in the Non-pit microhabitat ( $70.0 \pm 15.5$   $cm^2 m^{-2}$ ) compared to the other microhabitats ( $P < 0.007$  for all comparisons,  $R^2 = 0.24$ ), which did not significantly differ from one another ( $P > 0.711$  for all comparisons,  $345.8 \pm 66.0$ ,  $292.6 \pm 55.4$  and  $275.6 \pm 49.0$   $cm^2 m^{-2}$ , for South facing, North facing and Pit microhabitats, respectively). Attached algal cover did not vary among the sampling dates ( $P = 0.380$ ).

Drift Algal Availability The dry weight of Rhodophyte drift algae that was held on the tube feet of sea urchins was used as an estimate of drift algal availability to sea urchins (Fig. 1f). In general, the mean abundance of drift algae was lowest in the Non-pit microhabitat ( $P < 0.008$ ,  $R^2 = 0.81$ ;  $57.0 \pm 14.7$  mg dry wt. sea urchin<sup>-1</sup>) than the other three microhabitats, which did not differ from one another ( $P > 0.844$ ;  $116.8 \pm 30.6$ ,  $131.5 \pm 44.7$  and  $101.7 \pm 19.6$  mg dry wt. sea urchin<sup>-1</sup> for South facing, North facing and Pit microhabitats, respectively). The average dry weight of drift algae also varied among sample dates ( $P < 0.001$ ), and this trend was dependent upon microhabitat (Microhabitat x Date,  $P = 0.009$ ). The abundance of drift algae was highest in the fall collection (November 2006), intermediate in the three winter collections (December 2006 and January 2007) and was lowest in the spring collection (March 2007). Among microhabitats, the trend of low drift algal abundance in the Non-pit microhabitat was consistent among months, though this was not always significant. Sea urchins at this collection site were observed to eat a variety of the attached fleshy Rhodophyte species examined as well as fleshy drift algae.

Sea Urchin Size and Gonadal Index. Sea urchin size (diameter and whole wet weight) showed no significant correlation with MAA concentration in sea urchin gonadal or epidermal tissues, the proportion of MAAs detected in the epidermal tissue, or with the relative concentrations of shinorine and palythine. Similarly, gonadal index showed no correlation with MAA concentration in sea urchin gonadal and epidermal tissues, the proportion of MAAs detected in the epidermal tissue or the relative concentrations of shinorine and palythine in the tissues.

In general, males had a higher mean GI than females ( $P = 0.012$ ,  $R^2 = 0.33$ ) and mean GI decreased from November to January ( $P < 0.000$ ). However, the observed decrease in mean GI over time was dependent upon the sex of the sea urchins (Sex x Month:  $P = 0.004$ ). From November to January, mean GI for females decreased significantly ( $P < 0.000$ ;  $12.86 \pm 0.45$  and  $9.33 \pm 0.49$  for November and January, respectively) while mean GI for males did not change ( $P = 0.371$ ;  $12.69 \pm 0.42$  and  $11.68 \pm 0.54$  for November and January, respectively). Non-pit, North facing and Pit urchins had significantly higher mean GIs ( $11.76 \pm 0.44$ ,  $12.83 \pm 0.59$  and  $12.02 \pm 0.47$ , respectively) than the South facing urchins ( $9.96 \pm 0.47$ ;  $P = 0.012$ ,  $P < 0.001$  and  $P = 0.004$ , respectively), but were not significantly different from one another ( $P > 0.314$  for all comparisons). Gonad weight was positively correlated with test diameter ( $P < 0.000$ ,  $R^2 = 0.295$ , Gonad wet wt. (g) =  $- 7.81 + 0.278 * \text{Test Diameter (mm)}$ ).

In summary, sea urchins in the Non-pit microhabitat were at higher intertidal heights and probably experienced higher irradiance levels than sea urchins in the other three microhabitats. They also had less attached and drift algal food available compared to sea urchins in the other microhabitats, assuming that all urchins eat algae at the same rate. Sea urchins and algae in the South facing microhabitat were likely exposed to higher irradiances than those in the North facing microhabitat, but slightly lower levels than those in the Non-pit microhabitat. Sea urchins in pits appeared to experience lower irradiance levels than Non-pit urchins, at least on portions of their test, due to constant shading by the pit walls, while algae in the Pit microhabitat likely experienced similar irradiance levels to algae in the Non-pit microhabitat (Fig. 1a), because they were not located inside pits. Mean monthly temperature (Fig. 1c) was different among

microhabitats, but the range in mean temperatures among microhabitats was small (< 0.9 °C among microhabitats).

Regression analyses among the stations of all the above variables against one another revealed that attached algal availability decreased significantly with tidal height ( $P = 0.002$ ,  $R^2 = 0.640$ , attached algal cover =  $1239 \text{ cm}^2 \text{ m}^{-2} - 936 \text{ cm}^2 \text{ m}^{-2} * \text{m}$  above MLLW). No other significant correlations among the other microhabitat characteristics were observed.

#### *Concentration of Total MAAs in Algae*

Attached Algae. At least one MAA was present in every algal species and specimen tested (Table 1). Species varied in their mean concentration of MAAs ( $P < 0.000$ ,  $R^2 = 0.89$ ), though the magnitudes and significance tests of the differences are likely inaccurate due to standardization by dry weight. Having controlled for species differences, there was variation among microhabitats in the concentration of MAAs in algae ( $P < 0.001$ ), with algae in the Non-pit microhabitat containing a higher mean concentration of MAAs than algae from the Pit microhabitat ( $P = 0.003$ ). The mean concentration of MAAs in algae from the South facing and North facing microhabitats was not significantly different from the other two microhabitats or from one another ( $P > 0.067$  for all comparisons).

Attached Algal MAA Availability. Because the MAA content of algae in the stations as well as algal cover should affect the amount of MAAs consumed by sea urchins, we translated the measurements for attached algal cover per station to a measurement of the attached algal MAA availability to sea urchins in each station (see

equation in methods). There was significant variation in attached algal MAA availability among microhabitats (Fig. 2;  $P < 0.001$ ,  $R^2 = 0.77$ ), with the Non-pit microhabitat having lower mean attached algal MAA availability ( $16 \pm 5 \text{ nmol m}^{-2}$ ), compared to the other three microhabitats ( $P < 0.001$  for all comparisons), which did not differ from one another ( $P < 0.514$  for all comparisons;  $93 \pm 18$ ,  $103 \pm 17$ ,  $90 \pm 18 \text{ nmol m}^{-2}$  for South facing, North facing, and Pit microhabitats respectively).

Table 1. Maximal UV absorption wavelengths ( $\lambda_{\text{max}}$ ) and mean concentrations of individual and total MAAs ( $\text{nmol mg}^{-1}$  dry weight  $\pm$  S.D.) for the ten species of red algae collected among the stations. Asterisks indicate coralline species with calcium carbonate skeletons. Trace amounts of mycosporine-2-glycine were detected in *O. spectabilis*, but none in other species. Shin: shinorine, P-334: porphyra 334, PT: palythine, A-330: asterina 330, PL: palythanol, Usu: usujirene, tr.: trace MAAs detected, n.d.: MAAs not detected.

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

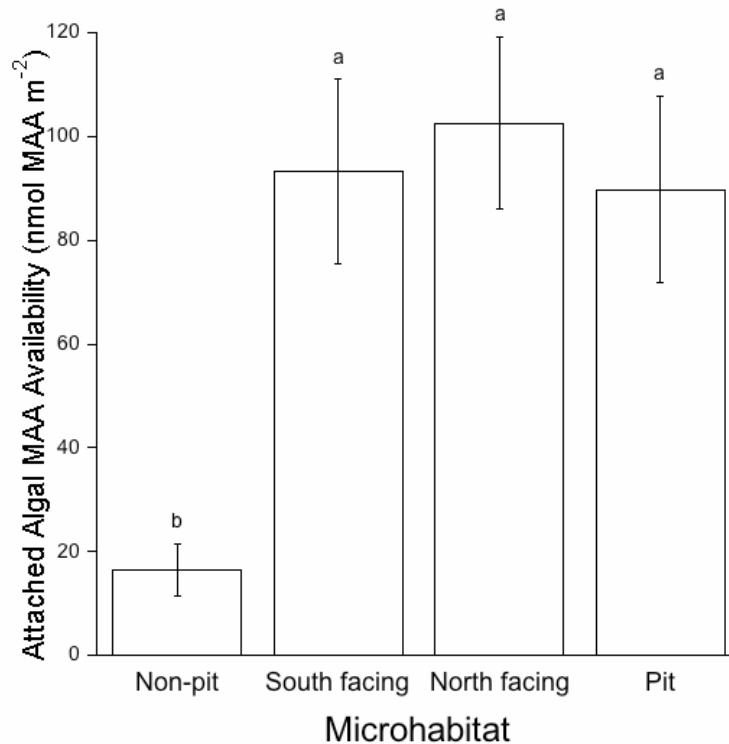


Figure 2. The availability of MAAs to *S. purpuratus* from all species of attached algae by microhabitat (nmols of MAAs/m<sup>-2</sup> ( $\pm$ S.E.), n = 18 for each microhabitat). See methods for calculation of attached algal availability.

*Drift Algae.* MAAs were detected in all samples from the November 2006 collection of drift algae. Drift algal samples were primarily composed of fleshy algae, and the mean concentration of MAAs in the fleshy attached algal species was much higher ( $7.34 \pm 0.50$  nmol mg<sup>-1</sup> dry wt.) than that of drift algal samples ( $1.54 \pm 0.37$  nmol mg<sup>-1</sup> dry wt.), suggesting drift algae lost MAAs after detachment and may not provide sea urchins with as many MAAs as attached algae per unit biomass, though sample size was small (N = 12). MAA concentration in our small subset of drift algae (N = 3 samples per microhabitat) did not differ among microhabitats (P = 0.221).

#### *Concentration of Shinorine in Algae*

Shinorine was the most abundant specific MAA detected in most algal species, followed by palythine (Table 1). As expected, the relative concentration of shinorine to total MAAs was different among algal species (Table 1,  $P < 0.001$ ). When having considered the variation among species, there was a difference among microhabitats in the relative concentration shinorine in algae, which seemed to decrease with decreasing irradiance levels, though this trend was not significant (Fig. 3;  $P = 0.067$ ,  $R^2 = 0.98$ ). The relative concentration of shinorine in all algal specimens was highest in the South facing ( $70.6 \pm 4.9\%$ ) and Non-pit microhabitats ( $69.9 \pm 5.1\%$ ), followed by the North facing ( $59.7 \pm 4.5\%$ ), and Pit ( $45.7 \pm 6.5\%$ ) microhabitats.

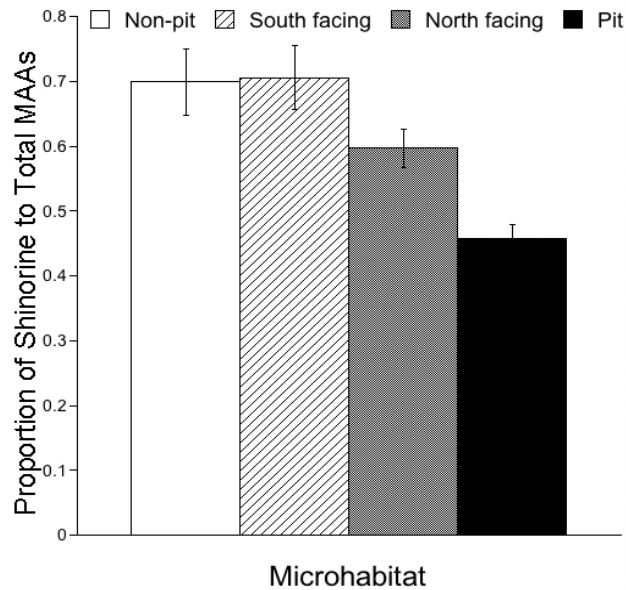


Figure 3. The mean ( $\pm$  S.E.) relative concentration of shinorine to total MAAs in algal samples ( $[\text{shinorine}]/[\text{total MAAs}]$ ). The relative concentration of shinorine was not significantly different among the microhabitats, but there was a trend of decreasing relative concentration of shinorine with decreasing irradiance in the microhabitats ( $n = 35$  for the Non-pit,  $n = 25$  for the South and North facing, and  $n = 33$  for the Pit microhabitats).

#### *Concentration of Total MAAs in Sea Urchins*



Microhabitat. The mean concentration of MAAs for both gonadal and epidermal sea urchin tissues was dependent on microhabitat (Figs. 4 and 5;  $P = 0.001$ ,  $R^2 = 0.22$  and  $P < 0.001$ ,  $R^2 = 0.26$  for gonadal and epidermal tissues, respectively). Pit urchins had lower mean concentrations of MAAs in both gonadal (Fig. 4;  $0.41 \pm 0.08$  nmol mg<sup>-1</sup> dry wt.) and epidermal (Fig. 5;  $0.08 \pm 0.01$  nmol mg<sup>-1</sup> dry wt.) tissues than sea urchins in the other three microhabitats ( $P < 0.045$  and  $P < 0.005$  for all comparisons for gonads and epidermis, respectively), and this was found in both the November and January collections (Figs. 4 and 5; Microhabitat x Month:  $P = 0.281$  and  $P = 0.302$ , for gonadal and epidermal tissues, respectively). Gonadal concentrations of MAAs were not significantly different in sea urchins from the South facing, North facing or Non-pit microhabitats ( $P > 0.721$  for all comparisons;  $0.75 \pm 0.12$ ,  $1.07 \pm 0.25$  and  $0.84 \pm 0.20$  nmol mg<sup>-1</sup> dry wt., respectively). Epidermal concentrations of MAAs were nearly significantly higher in the Non-pit microhabitat (compared to the South facing microhabitat ( $P = 0.051$ ), but no other differences were observed among the Non-pit, South facing and North facing microhabitats for epidermal tissues ( $P > 0.331$  for all comparisons;  $0.28 \pm 0.03$ ,  $0.18 \pm 0.02$  and  $0.17 \pm 0.02$  nmol mg<sup>-1</sup> dry wt., respectively).

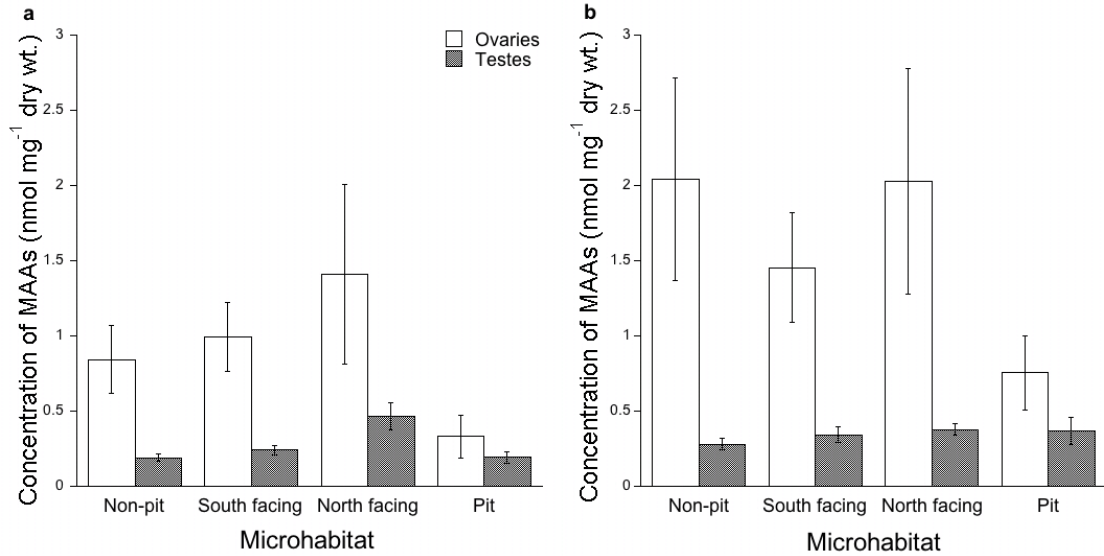


Figure 4. Variation in the mean ( $\pm$  S.E.) concentration of MAAs ( $\text{nmol mg}^{-1}$  dry wt.) in *S. purpuratus* gonadal tissues among microhabitats for each sex for a) November 2006 and b) January 2007 ( $n = 15$  for each bar except for South facing females in January, where  $n = 14$ ).

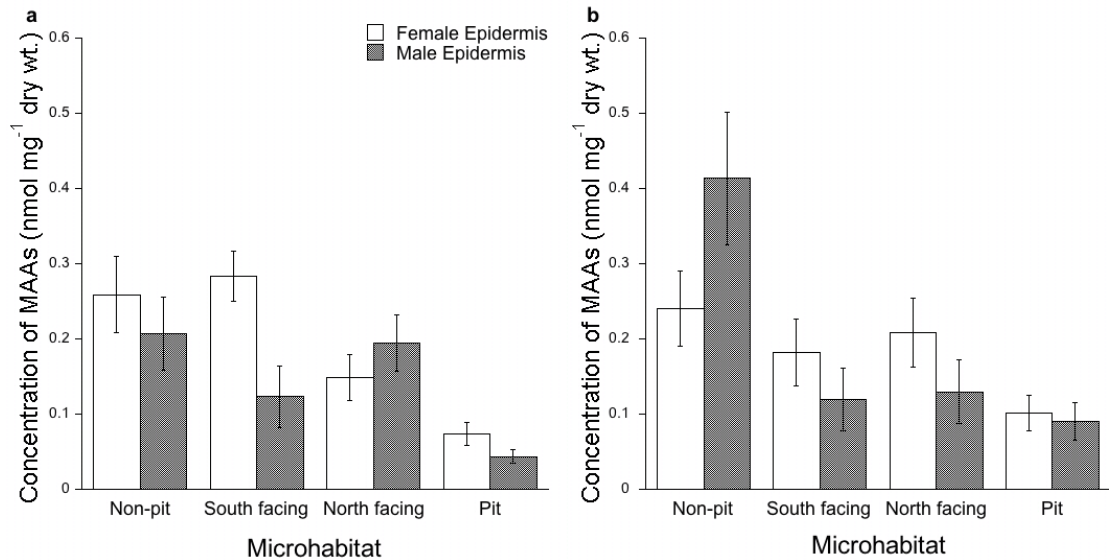


Figure 5. Variation in the mean ( $\pm$  S.E.) concentration of MAAs ( $\text{nmol mg}^{-1}$  dry wt.) in *S. purpuratus* epidermal tissues among microhabitats for each sex for a) November 2006 and b) January 2007 ( $n = 15$  for each bar except for South facing females in January, where  $n = 14$ ).

In gonadal tissues, the trend of low concentrations of gonadal MAAs in Pit urchins was dependent upon the sex of the sea urchins, though this trend was not

significant (Fig. 4; Microhabitat x Sex:  $P = 0.051$ ). In male sea urchins, concentration of MAAs in testes did not vary among microhabitats (Fig. 4;  $P > 0.50$ ;  $0.42 \pm 0.05$ ,  $0.29 \pm 0.03$ ,  $0.28 \pm 0.05$  and  $0.24 \pm 0.02$  nmol  $\text{mg}^{-1}$  dry wt. for North facing, South facing, Pit and Non-pit urchins, respectively). However, female Pit urchins had lower concentrations of MAAs in their ovaries ( $0.54 \pm 0.15$  nmol  $\text{mg}^{-1}$  dry wt.) compared to females in the other microhabitats (Fig. 4;  $P < 0.031$  for all comparisons;  $1.44 \pm 0.37$ ,  $1.22 \pm 0.21$   $1.72 \pm 0.47$  and nmol  $\text{mg}^{-1}$  dry wt. for Non-pit, South facing and North facing urchins, respectively). These sex-dependent trends among microhabitats were not affected by collection month (Microhabitat x Sex x Month:  $P = 0.887$ ). For epidermal tissues, low concentrations of MAAs in Pit urchins were not significantly affected by the sex of the sea urchins (Fig. 5; Microhabitat x Sex:  $P = 0.071$ ), or by the collection month (Fig. 5; Microhabitat x Month:  $P = 0.302$ ).

The mean proportion of total MAAs allocated to the epidermal tissue for each sea urchin (i.e. epidermal MAAs/[epidermal + gonadal MAAs]) was influenced by sea urchins' microhabitat ( $P = 0.006$ ,  $R^2 = 0.184$ ), and this was variable between the sexes (Fig. 6; Microhabitat x Sex:  $P = 0.031$ ). Female sea urchins showed no differences in the mean proportion of MAAs in epidermal tissue among the four microhabitats, (Fig. 6;  $P > 0.998$ ;  $0.33 \pm 0.06$ ,  $0.28 \pm 0.05$ ,  $0.34 \pm 0.06$  and  $0.30 \pm 0.05$  for Non-pit, South facing, North facing and Pit urchins, respectively), while male Non-pit urchins had a significantly higher proportion of MAAs in their epidermis ( $0.47 \pm 0.05$ ) than male sea urchins from the other three microhabitats (Fig. 6;  $P < 0.012$ ;  $0.26 \pm 0.04$ ,  $0.25 \pm 0.03$  and  $0.21 \pm 0.03$  for South facing, North facing and Pit urchins, respectively), which did not differ significantly from one another ( $P > 0.998$ ). These trends were not affected by collection

month (Microhabitat x Month:  $P = 0.528$ ; Microhabitat x Sex x Month:  $P = 0.078$ ).

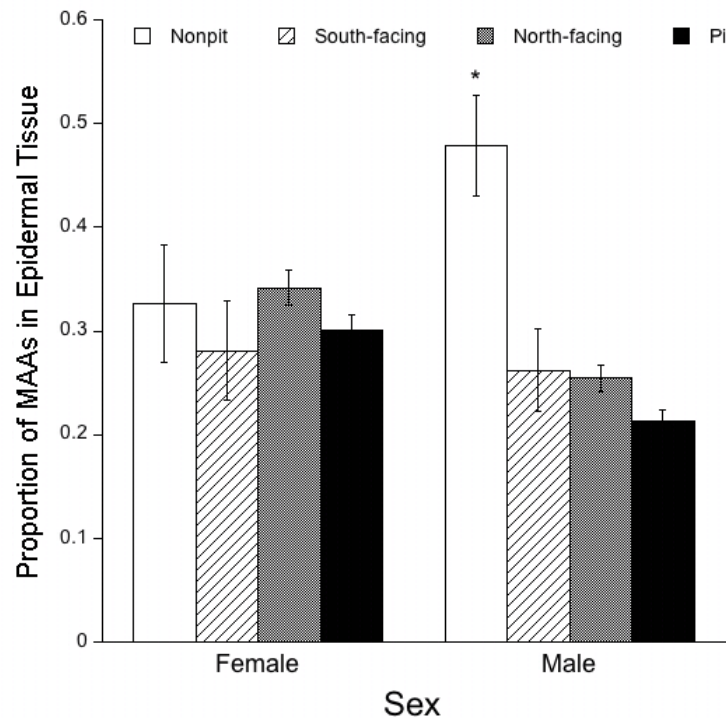


Figure 6. The mean ( $\pm$  S.E.) proportion of MAAs detected in *S. purpuratus* epidermal tissues ([epidermal MAAs]/[gonadal + epidermal MAAs]) in the microhabitats for each sex ( $n = 30$ , except South facing female sea urchins, where  $n = 29$ ). The asterisk (\*) indicates there was a significantly higher mean proportion of MAAs in the epidermis for the males in the Non-pits microhabitat compared to the other three microhabitats.

Collection Month. The concentration of MAAs in sea urchin gonadal tissues increased significantly between the November 2006 and the January 2007 collections (Fig. 4;  $P = 0.009$ ). The increase in the concentration of MAAs from November to January was much stronger in females (from  $0.74 \pm 0.10$  to  $1.57 \pm 0.28$  nmol  $\text{mg}^{-1}$  dry wt. from November to January) than in males (from  $0.27 \pm 0.03$  to  $0.34 \pm 0.03$  nmol  $\text{mg}^{-1}$  dry wt. from November to January), though neither sex increased significantly when considered separately ( $P = 0.054$  and  $P = 0.6129$  for females and males, respectively). There was no difference in MAA concentrations in epidermal tissues between months

(Fig. 5;  $P = 0.734$ ;  $0.17 \pm 0.01$  and  $0.19 \pm 0.02$  nmol mg<sup>-1</sup> dry wt. for November and January, respectively), for either sex (Month x Sex:  $P = 0.127$ ). The mean proportion of MAAs found in epidermal tissues also did not vary between the collection months ( $P = 0.165$ ), for either sex (Month x Sex:  $P = 0.069$ ).

Sex. Female sea urchins had a higher concentration of MAAs than male sea urchins in both gonadal (Fig. 4;  $P = 0.005$ ) and epidermal (Fig. 5;  $P = 0.020$ ) tissues ( $1.15 \pm 0.15$ ,  $0.31 \pm 0.02$ ,  $0.19 \pm 0.02$ , and  $0.17 \pm 0.02$  nmol mg<sup>-1</sup> dry wt. for ovaries, testes and female and males epidermal tissues, respectively). These trends were consistent among months (Sex x Month:  $P = 0.345$  and  $P = 0.127$ , for gonadal and epidermal tissues, respectively). There was no difference in the proportion of MAAs in the epidermal tissues between the sexes (Fig. 6;  $P = 0.896$ ). However, there were interactive effects of sex and microhabitat on the gonadal MAA concentration (Fig. 4;  $P = 0.051$ ) and the proportion of MAAs detected in the epidermis (Fig. 6;  $P = 0.031$ ) as discussed above.

#### *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 7;  $\lambda_{\max} = 331$  nm, 330 nm and 331 nm, respectively), while the absorption maximum for testes was at a shorter, higher energy wavelength ( $\lambda_{\max} = 322$  nm), near the UVA/UVB cusp. Ovaries showed the widest range of absorption, spanning the UVA and some of the UVB ranges. Within the UVB range (280-320 nm), ovaries and testes showed similar absorbances, while both epidermal tissues absorbed less UVB (Fig. 7).

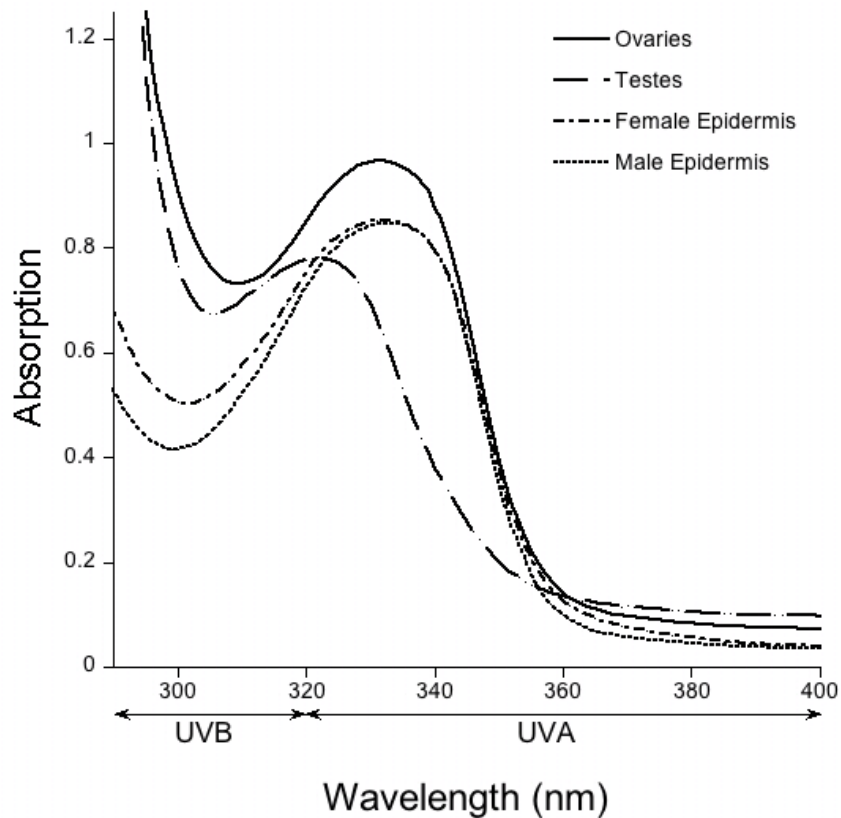


Figure 7. Absorption spectra of representative MAA extracts of gonadal and epidermal tissues of each sex. All extracts had the same concentration of total MAAs. The maximum absorbances of ovaries, female and male epidermal tissues were similar and in the UVA range ( $\lambda_{\max}$ = 331 nm, 330 nm and 331 nm, respectively). The maximum absorbance for testes was at a shorter, higher energy wavelength ( $\lambda_{\max}$ = 322 nm) near the UVA/UVB cusp.

#### *Concentrations of Individual MAAs in Sea Urchins*

Seven MAAs were identified in the gonadal and epidermal tissues of *S. purpuratus* and included shinorine, palythine, porphyra-334, asterina-330, mycosporine glycine, mycosporine 2-glycine and usujirene (Fig. 8). Most MAAs were identified in all tissue types, except no mycosporine 2-glycine was detected in the testes and no usujirene was detected in epidermal tissues. In general, the relative concentrations of MAAs in the sea urchin tissue types were very consistent, with the standard error about the mean

percentages of each MAA at  $\pm 2.8\%$  for shinorine and palythine and  $\pm 0.7\%$  for all other MAAs. Sea urchin testes showed a very different MAA signature than ovaries and epidermal tissues, and contained principally palythine, and some shinorine (Fig. 8).

Testes had lower mean relative concentrations of many MAAs when compared to ovaries and epidermal tissues (shinorine:  $P < 0.000$  mycosporine-glycine:  $P < 0.032$ , porphyra-334:  $P < 0.000$  and asterina-330:  $P < 0.000$ ), but had a higher relative concentration of palythine ( $P < 0.000$ ). The relative concentrations of individual MAAs in the ovaries and epidermal tissues for both sexes were similar, but ovaries had a higher concentration of mycosporine-glycine than the epidermal tissues ( $P < 0.000$ ).

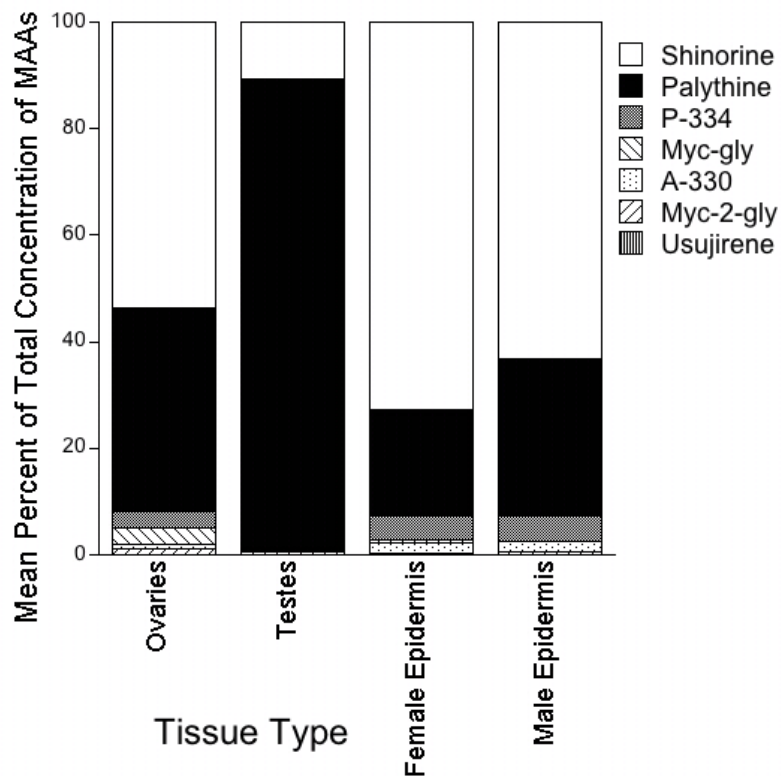


Figure 8. The relative concentrations of individual MAAs in the gonadal and epidermal tissues for each sex of *S. purpuratus* (n = 119 for female tissues and n = 120 for male tissue). Error bars are not shown, but were  $\pm 2.8\%$  for shinorine and palythine and  $\pm 0.7\%$  for all other MAAs.

Shinorine and palythine were the dominant MAAs detected in all sea urchin tissues (Fig. 8) and other individual MAAs tended to follow a similar pattern to either shinorine or palythine. For example, porphyra-334 and mycosporine-2-glycine were similar in variation among sexes and tissues to shinorine, and asterina-330 was similar in variation to palythine. Nevertheless, the absence of the five MAAs besides shinorine and palythine in many samples prevented their inclusion in statistical analyses. The relative concentrations of shinorine and palythine were not affected by collection month ( $P = 0.586$  and  $P = 0.184$  for mean relative concentrations of shinorine and palythine, respectively) nor were any of the trends in the other variables different between the months ( $P > 0.05$  for all interactions of month with microhabitat, sex and/or tissue for both relative concentrations of shinorine and palythine analyses).

*Microhabitat.* In general, the relative concentration of shinorine ( $[\text{shinorine}]/[\text{total MAAs}]$ ) was highest in sea urchins in the Non-pit microhabitat, followed by those in the South facing, North facing and Pit microhabitats, all of which differed significantly from one another (Fig. 9;  $P < 0.003$  for all comparisons,  $R^2 = 0.87$ ). This pattern was affected by the sex of the sea urchins, (Microhabitat x Sex:  $P = 0.002$ ). However, the above order among microhabitats in relative concentration of shinorine was consistent for both sexes, though the relative concentration of shinorine were not always significantly different among microhabitats.

Among microhabitats, the trend for the relative concentration of palythine ( $[\text{palythine}]/[\text{total MAAs}]$ ) in sea urchin tissues was opposite that of shinorine (above): the highest relative concentration of palythine was in sea urchins from the Pit microhabitat, followed by those in the North facing, South facing then Non-pit



microhabitats, all of which differed significantly from one another (data not displayed;  $P < 0.008$  for all comparisons,  $R^2 = 0.87$ ). This trend varied between the sexes (Microhabitat x Sex:  $P = 0.004$ ). Nevertheless, the relative order of relative concentrations of palythine for females was consistent with the trend described above, but were not always significantly different among microhabitats.

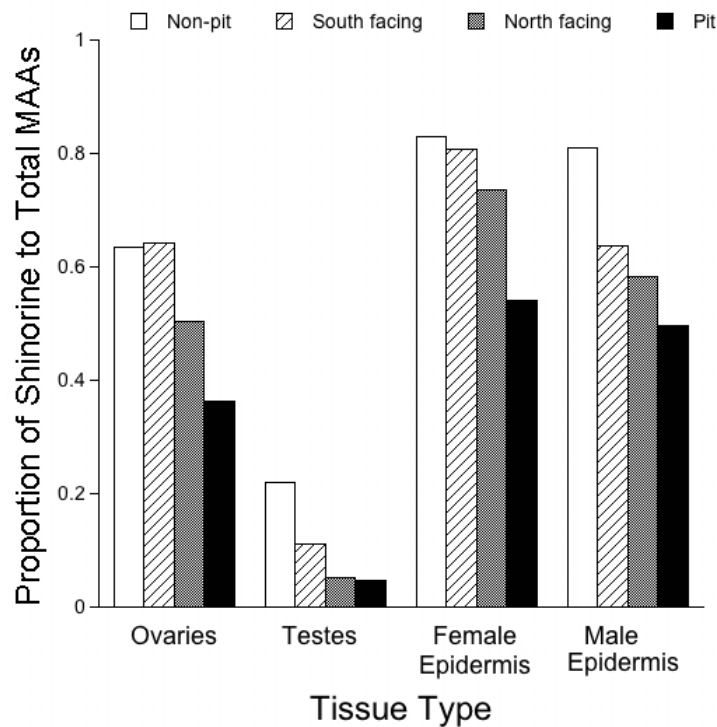


Figure 9. The mean ( $\pm$  S.E.) relative concentration of shinorine compared to the total concentration of MAAs ( $[\text{shinorine}]/[\text{total MAAs}]$ ) among the microhabitats for the separate tissue types of *S. purpuratus* ( $n = 29$  for females tissues and  $n = 30$  for male tissues for each microhabitat).

Among microhabitats, the trends in both relative concentrations of shinorine and palythine were similar in the gonadal and epidermal tissues (Tissue x Microhabitat:  $P = 0.371$  and  $P = 0.902$  for relative concentrations of shinorine and palythine, respectively). These trends were also consistent between sexes (Tissue x Microhabitat x Sex:  $P = 0.842$  and  $P = 0.516$  for relative concentrations of shinorine and palythine, respectively).

Therefore, although tissue (gonads and epidermis) was included in statistical models, microhabitat trends in relative concentrations of shinorine and palythine are presented above without distinction between gonadal and epidermal tissues.

The relative concentrations of shinorine and palythine in sea urchins were dependent on the sex of the sea urchins and the tissue examined (Tissue x Sex:  $P < 0.001$  for both relative concentrations of shinorine and palythine). The relative concentration of shinorine was highest in female epidermal tissue, followed by male epidermal tissue, ovaries then testes, all of which were significantly different from one another (Fig. 9;  $P < 0.001$  for all comparisons). Palythine showed the opposite trend, with the highest relative concentration of palythine in the testes, followed by the ovaries, female epidermis then male epidermis, all of which were significantly different from one another ( $P < 0.003$  for all comparisons). These trends were not affected by microhabitat (Tissue x Sex x Microhabitat:  $P = 0.842$  and  $P = 0.516$  for relative concentrations of shinorine and palythine, respectively).

## Discussion

To our knowledge, our study is the first to identify ecologically-relevant concentrations of MAAs in the tissues of the widely studied purple sea urchin, *Strongylocentrotus purpuratus*, as well as nine species of Rhodophyte macroalgae on California's central coast. The concentrations of these MAAs in sea urchins appears to be influenced primarily by the tissue type examined and the sex of the sea urchin (Figs. 4, 5 and 8), as well as the specific microhabitat inhabited by the sea urchin (Figs. 4 and 5). Microhabitat variation in concentrations of MAAs in total MAAs in sea urchins appears to be influenced by diet, while the allocation of MAAs to the epidermis and gonads may be influenced by irradiance levels in males, but not in females. Multiple trade-offs concerning the uptake and allocation of MAAs to sea urchins tissues were detected. The first was detected in sea urchins inhabiting pits, which may confer more physical protection, but was associated with low concentrations of MAAs. The second trade-off was found in males only, and it appeared that they allocated more MAAs to their epidermis in the sunny Non-pit microhabitat compared to the other microhabitats. Lastly, many types of MAAs were detected in ovaries and epidermis of both sexes, but males had primarily one MAA in their testes. This seemed to be related to shift in absorbance of testes to higher energy wavelengths of UV and suggests a sex and tissue-dependent trade off in breadth of absorbance versus the wavelength of peak absorbance. These findings are important for understanding how organisms adapt to changing environments.

### *Microhabitat Variation in Total MAAs in Sea Urchins*

The variation in total concentration of MAAs in sea urchin tissues among microhabitats suggests that both the ability to acquire MAAs and physiological trade-offs

between adult and larval protection may be dictated by the adult's microhabitat. Sea urchins inhabiting pits showed lower total concentrations of epidermal MAAs in both sexes compared to sea urchins from the other three microhabitats, regardless of the month collected (Fig. 5). This trend of low concentrations of MAAs Pit urchins was also detected in female sea urchins' ovaries, but not in male sea urchins' testes; testes showed low concentrations of MAA in all microhabitats (Fig. 4).

The generally reduced concentration of MAAs epidermal tissues of both sexes and in ovary tissues in Pit urchins may have been caused by reduced food intake, lower uptake of dietary MAAs, or lower MAA concentrations in algal diets compared to sea urchins in the Non-pit, South facing and North facing microhabitats. Increased gonad index is associated with increased food intake (Vadas 1968; Himmelman 1978) and the gonadal index of Pit urchins was not reduced compared to the other microhabitats. Therefore, it appears that Pit urchins did not have a lower food intake than sea urchins in other microhabitats, and decreased nutrition is not likely responsible for low concentrations of MAAs in Pit urchins.

In contrast, it is possible that Pit urchins did not absorb the MAAs from their food as readily as sea urchins in other microhabitats. Residing in pits may have reduced their overall exposure to UVR and potentially reduced the need for sunscreens, which may have caused the uptake of MAAs to be downregulated accordingly. However, this is unlikely, and although two studies indicate there is a decrease in the concentration of MAAs in sea urchin gonads with depth (Karentz et al. 1997; Lamare et al. 2004), they indicate it is due to algal MAA concentrations. Moreover, previous results with a congeneric sea urchin, *S. droebachiensis*, indicate that moderate UV levels do not affect

the accumulation of MAAs in sea urchin ovary tissues (Adams et al. 2001). In addition, a recent long-term study examining whether natural solar UV-irradiation of *S. purpuratus* adults affects MAA accumulation in ovaries by Adams et al. (unpublished data) indicates that *S. purpuratus* do not alter their MAA accumulation in eggs in response to UVR. It is unclear whether it is ecologically advantageous for adult female sea urchins to increase their accumulation of MAAs to their ovaries depending on their own irradiance condition, because the larvae do not develop in the same habitat as the adults.

The aforementioned study only examined the effect of UVR on ovary tissues (Adams et al. 2001), and it is possible that UV stimulates the uptake of MAAs into epidermal tissues. This could explain the reduction of MAAs in the epidermal tissues of the shaded Pit urchins of both sexes. However, when comparing the concentrations of MAAs in epidermal tissues in this study among the Non-pit, South facing and North facing microhabitats we did not see any differences (Fig 5), even though those three microhabitats had differing irradiance levels (Fig 1a). Because the microhabitat trend existed in both gonads and epidermis, our data suggest that the same mechanism was responsible for the lower MAA concentration in both tissues for Pit urchins. A reduced concentration of MAAs in the algae eaten by Pit urchins is the most likely explanation for the low concentration of MAAs in Pit urchins.

The concentration of MAAs in algae, and not the biomass of algae, has previously been shown to affect the concentration of MAAs in sea urchin ovaries (Adams et al. 2001), and we believe that a reduced concentration of MAAs in the algae eaten by Pit urchins is the reason for the reduced concentration of MAAs in their tissues. Though our measurements of attached algal cover, attached algal MAA availability and drift algal

availability do not suggest that Pit urchins had lower food availability than the other three microhabitats (Figs. 1e, 1f, and 2), we suspect that Pit urchins had different constraints on their diets than sea urchins living in the other microhabitats, and that this may have created the pattern seen here. Sea urchins in pits probably have limited access to algae growing near them because they seem to stay within the pits (Grupe 2006), so the attached MAA availability to Pit urchins was probably lower than our measurements indicate (Fig. 2). We often observed macroalgae growing very near sea urchins in pits, without evidence of herbivory. Moreover, our analysis of both attached algae and sea urchins among the microhabitats indicated there are lower relative concentrations of shinorine, the primary MAA accumulated by sea urchin tissues (Fig. 8), in both algae and sea urchins in the Pit microhabitat (Figs. 3 and 9). This suggests a dietary transfer from algae to sea urchins of the relative concentrations of shinorine, and that the trend of lowered relative concentrations of shinorine in Pit urchins was due to lowered concentrations in their diets.

Conversely, inhabiting pits did not seem to lower sea urchins' abilities to catch drift algae (Fig. 1f). The lower concentration of MAAs in the tissues of Pit urchins may be caused by Pit urchins consuming a higher proportion of drift algae, which (in our study) had a lower average concentration of MAAs per unit dry weight than all of the species of attached algae collected (excluding coralline algae). Further, brown algae, which do not contain high amounts of MAAs (Shick and Dunlap 2002), were much more common in drift samples than in attached algal samples, and may constitute a large portion of Pit urchins' diets.

Regardless of the mechanism creating the reduced concentration of MAAs in Pit urchins, there seems to be a trade-off involved for sea urchins living in versus out of pits. Pit urchins are probably more protected from predators (Grupe 2006), desiccation, and dislodgement by waves (Denny and Gaylord 1996). However, Pit urchins may have decreased offspring survival due to the lower concentrations of MAAs in their ovaries (Adams and Shick 1996, 2001), and adult survival may be reduced due to lower concentrations of MAAs in the epidermis. A study on *S. purpuratus* in Oregon found that Pit urchins have slower growth rates and smaller test diameters than Non-pit urchins (Grupe 2006). These factors combine to suggest there is a trade-off between adult sea urchin survival, which is likely higher inside pits, and fitness and growth, which is likely higher outside of pits.

Initially, the importance of food availability in controlling MAA concentrations in sea urchin tissues seems to conflict with the data for Non-pit urchins; Non-pit urchins had concentrations of MAAs in both gonadal and epidermal tissues that were similar to sea urchins in the South and North facing microhabitats (Figs. 4 and 5), but attached algal MAA availability, algal cover and drift algal availability (Figs. 2, 1e, 1f) were lowest in the Non-pit microhabitat, suggesting they did not have high food availability. However, gonadal indices for Non-pit urchins were not smaller than sea urchins in the other microhabitats, suggesting that their algal intake was not actually low. How are Non-pit sea urchins getting food and consequently MAAs when there was little attached or drift algae observed to be available to them? Non-pit urchins may eat available attached and drift algae faster than sea urchins in other microhabitats, or they may supplement their diets with microalgae, including benthic diatoms and periphyton. Further studies on

dietary differences in sea urchins among these microhabitats may clarify the patterns in concentrations of MAAs seen here.

An alternative explanation is that the concentrations of MAAs are higher in algae consumed by sea urchins in the Non-pit microhabitat. Even when having considered species-specific differences in algal specimens in concentrations of total MAAs, we detected high concentrations of total MAAs as well as high relative concentrations of the important MAA shinorine (Fig. 3) in algal specimens from the Non-pit microhabitat, though these differences were not always significant compared to the other microhabitats. Further, algal species that were common along the edges of Non-pit tidepools included *P. capillacea*, *E. muricata*, and *M. papillatus*, which had medium to high concentrations of MAAs (Table 1), while coralline algae tended to be common in pools in the other microhabitats, and are low in concentration of MAAs per unit dry weight (Table 1). It is possible that high concentrations of shinorine in algae from the Non-pit microhabitat was transferred up the food chain to sea urchins, as discussed above for Pit urchins and as has been noted between trophic levels for MAAs in several other studies (Carroll and Shick 1996; Carefoot et al. 2000; Newman et al. 2000).

#### *Microhabitat Variation in Allocation of MAAs Between Tissues*

Males had a higher proportion of MAAs in their epidermal tissues in the Non-pit microhabitat compared to those in the other three microhabitats, while the proportion of MAAs in females' epidermal tissues was the same in all of the microhabitats (Fig. 6). The Non-pit microhabitat had higher irradiance levels than the other microhabitats (Fig. 1a), and was also higher in the intertidal zone (Fig. 1b) meaning there was less shade and



that less water filtered the incoming UVR. These data suggest that males trade-off allocation of MAA resources to epidermal versus gonadal tissue depending on abiotic conditions. Though the survival of offspring and thus fitness of the adult males may be decreased by having reduced MAAs in the gametes, this preferential allocation of sunscreens to epidermal tissues could indirectly positively affect fitness by increasing the growth or survival of adult male sea urchins, allowing increased reproductive output over time. In contrast, our data suggest females did not change the percentage of MAA resources allocated to adult versus reproductive tissue depending on the microhabitats and the environmental factors associated with them (Figs. 1 and 2). A females' fitness may not benefit from reducing resources to eggs in favor of transferring them to her epidermis, because the survival of her offspring and consequently her own fitness may be reduced (Adams and Shick 1996; 2001).

#### *Microhabitat Variation in Gonadal Indices*

Lowered food availability did not seem to be limiting to sea urchins in the South facing microhabitat (Figs. 1e, 1f and 2), yet they exhibited a lowered mean gonadal index compared to sea urchins in the other three microhabitats. It could be that sea urchins in the South facing microhabitat had spawned previous to our collections. However, the above pattern was detected in November, before the spawning season had commenced, as well as in January when spawning season had begun (Giese et al. 1991), suggesting that early spawning was not the source of this pattern. Further investigation will need to be performed concerning the lowered gonadal indices in sea urchins in South facing microhabitat compared to sea urchins in the other microhabitats at this and other sites.

The pattern of low gonadal indices in sea urchins in the South facing microhabitat was not accompanied by a concurrent reduction of gonadal concentration of MAAs, suggesting that concentrations of MAAs are not tightly linked with gonad maturation. Indeed, when including data from all microhabitats, neither gonadal index nor sea urchin size showed a correlation with concentrations of MAAs, which suggests that neither the number of gametes nor the size of a sea urchin affects its ability to sequester MAAs.

#### *Sex and Tissue-Specific Differences in Concentrations of MAAs*

The different trends in concentrations of MAAs in the gonadal and epidermal tissues between the sexes suggest that female *S. purpuratus* utilize MAAs differently than males, which is consistent with other studies that observed sex-specific differences in concentration of MAAs 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 were more highly concentrated in ovaries than in testes, and in female epidermis than in male epidermis (Figs. 4 and 5). Because males and females live interspersed, it is likely that these differences are not caused by sex-specific differences in diet or abiotic conditions, but are physiological differences between the sexes.

The higher concentration of MAAs in ovaries compared to testes may simply be a physical limitation set by gamete size. The volume of an *S. purpuratus* egg is over 34,000 times larger than a sperm (Levitan 1993) so eggs can acquire a higher amount of MAAs in the cytoplasm and adult female sea urchins may allocate a greater percentage of MAA resources to their gonads simply because that resource is more easily utilized by the

gametes. Adams and Shick (1996) calculated the optical radius for *S. droebachiensis* eggs and sperm and hypothesized that eggs can use MAAs to absorb up to 86% of UVR reaching the nucleus, but that it is physiologically impossible for sperm (due to their small size) to sequester enough MAAs to obtain protection against UVR. Indeed, the male with the highest concentration of MAA in its testes (1.646 nmol mg<sup>-1</sup> dry wt.) had a much lower concentration of MAAs than the female with the highest (9.465 nmol mg<sup>-1</sup> dry wt.), suggesting that the physical maximum MAA concentration for testes is much lower than that of ovaries. Further, the lowered concentration of MAAs in testes compared to ovaries was not associated with a concurrent increase in males' (compared to females') epidermal MAAs (Figs. 4 and 5). This suggests that MAA concentrations in males were not limited by the amount of MAAs in their diets, but that testes were limited in their ability to accumulate MAAs physiologically. However, upon examining the rank-order concentrations of MAAs in testes, our data do not indicate that concentrations of MAA in the testes reached some threshold where no further MAAs could be allocated to the testes.

Regardless, it is possible that MAAs could still function to protect sperm even if they are not highly concentrated within sperm cytoplasm. During spawning, MAAs could be present in the fluid surrounding the sperm, which could shade sperm from UV radiation. Further, high densities of sperm would result in some sperm cells being protected by MAAs present in other sperm, even at low concentrations of MAAs. A similar phenomenon, where MAA-containing cells surround gonadal tissue and protect embryos, has been demonstrated in tunicates (Epel et al. 1999).

The presence of MAAs in eggs may have larger implications for the fitness of females than the presence of MAAs in sperm could have for the fitness of males. In female green sea urchins (*S. droebachiensis*) MAAs are transferred from the diet into the eggs, are retained in the cytoplasm at least through the early pluteus stages, and confer protection from cleavage delay and fatal developmental abnormalities when embryos are exposed to UVR (Adams and Shick 1996, 2001). However, any MAAs in sperm would not be transferred to the offspring and could serve only to protect the sperm for the short ~20 minute period (Pennington 1985) that the sperm is viable. Therefore, any MAAs contained in the testes may confer a small increase the male sea urchin's individual fitness through increased sperm survival, but are not likely to affect offspring survival.

Similar to the pattern seen in gonadal tissues, females had a higher mean concentration of MAAs in epidermal tissues than males. We expected a similar concentration of MAAs in epidermal tissues between the sexes, because UV-exposure and diet probably does not differ. It is possible that the increased concentration of MAAs in female epidermal tissues is a by-product of the higher uptake and allocation of MAAs to gonadal tissues in females; an increased uptake of MAAs from female's diets may result in the increased concentrations in the epidermal tissues. Though the mean differences between the sexes were significant, the magnitude of the differences was small, ( $0.19 \pm 0.01$  and  $0.017 \pm 0.02$  nmol mg<sup>-1</sup> dry wt. for mean female and male epidermal concentrations, respectively) and not likely to be ecologically relevant.

The ranges in concentrations of MAAs detected in this study encompassed the ranges found in intertidal field-collected intertidal green sea urchins, *S. droebachiensis*, from Maine (Carroll and Shick 1996). We observed much higher and much lower

concentrations for each tissue than those found in *S. droebachiensis*, and this was probably due to the larger number of sea urchins tested, though it may indicate that *S. purpuratus* have more variability in concentrations of MAA than *S. droebachiensis* (for *S. purpuratus* and *S. droebachiensis*, respectively: Ovaries: 0.02-9.47 and 1.630-3.101 nmol mg<sup>-1</sup> dry wt., Testes: 0.05-1.65 and 0.167-1.173 nmol mg<sup>-1</sup> dry wt., Combined Epidermis: 0.00-1.28 and 0.135-0.183 nmol mg<sup>-1</sup> dry wt.). However, the mean ovary concentration in females collected in January from our study ( $1.57 \pm 0.28$  nmol mg<sup>-1</sup> dry wt.) was lower than the mean concentrations of MAAs found in five *S. droebachiensis* ( $7.57 \pm 0.66$  nmol mg<sup>-1</sup> dry wt.) also collected intertidally during the spawning season in Maine (Adams et al. 2001). This suggests that *S. purpuratus* may have lower average concentrations of MAAs in their ripe ovaries and eggs compared to their congeneric *S. droebachiensis*, but more studies are needed to conclude whether this is consistent over the ranges of both species and whether it is consistent for other tissues.

#### *Concentrations of Individual MAAs and Absorption Spectra of Tissues*

The similarities in the absorption spectra for extracts of ovaries and epidermal tissues from both sexes (Fig. 7) are consistent with the similarities seen in the relative concentrations of individual MAAs in these tissues (Fig. 8). The various MAAs detected absorb maximally at different wavelengths in the UV spectrum, and the spectral analysis suggests that the MAAs together in these tissues cover a broad portion of the UV spectrum (Fig. 7). Adult epidermis and embryos produced from the ovaries are fairly long-lived tissues that may receive prolonged exposure to sunlight, and therefore may benefit from broadband absorbance of UV (Fig. 7). Mycosporine-glycine was more

concentrated in ovaries than in epidermal tissues (Fig. 8). Though the actual mean concentration of mycosporine-glycine was low, its maximal absorbance is at a shorter, higher energy UVB wavelength ( $\lambda_{\text{max}} = 310 \text{ nm}$ ) and it may be responsible for the higher absorbance seen in ovaries versus epidermal tissues in the UVB range (Fig. 7). Because lower wavelengths are more damaging than higher wavelengths, especially to DNA, protection in the UVB range may be extremely beneficial to small, transparent, developing pelagic larvae. Further, it has been speculated that mycosporine-glycine has antioxidant capabilities (Dunlap and Yamamoto 1995), which could further protect eggs from the oxidative effects of UVA.

The shift of the testes absorption signature toward the shorter, higher energy wavelengths ( $\lambda_{\text{max}} = 322 \text{ nm}$ ) (Fig. 7) may have been due to the high relative concentration of palythine found in testes (Fig. 8), which absorbs maximally at the UVA/UVB transition ( $\lambda_{\text{max}} = 320 \text{ nm}$ ). If MAAs in the testes actually protect sperm, a shift in absorption toward the UVB part of the spectrum may better protect the valuable DNA contained within the sperm, because DNA absorbs UV at higher energy short wavelengths ( $\lambda_{\text{max}} = 260 \text{ nm}$ ).

The preferential allocation of palythine to the testes could represent a trade-off between the breadth of MAA absorption and the wavelength of peak MAA absorbance depending on sex, and may occur because the small size of sperm limits the number of MAA molecules that can be stored in the cytoplasm. The larvae that develop from eggs live for months in the water column (Strathmann 1987), so they may benefit from broadband protection from both UVA and UVB.

Our results concerning the individual MAAs found in *S. purpuratus* sea urchins tissues were very similar to those seen by Adams et al. (2001) and Carroll and Shick (1996) for ovary tissues of *S. droebachiensis*: *S. purpuratus* also accumulated mostly shinorine and small amounts of porphyra-334, mycosporine-2-glycine, palythine, asterina-330 to their ovaries. However, we detected the MAA usujirene in the ovaries and testes of *S. purpuratus* (Fig. 8), whereas Adams et. al (2001) did not detect usujirene in *S. droebachiensis* ovaries, even when sea urchins were maintained on a usujirene-rich diet of *Chondrus crispus*. This suggests that there is a difference in uptake of dietary MAAs between these two *Strongylocentrotus* species. Alternatively it may be that that the ovaries of *S. droebachiensis* in their study did contain usujirene, but that the HPLC used in the study was not able to detect it because it was less sensitive than the one used here, or that usujirene was lost in the algae fed to urchins prior to consumption because algae was frozen.

The mechanism of the higher concentrations of total MAAs in females as well as sex and tissue-specific differences may be physiological differences between the sexes, especially for gonadal tissue, in the numbers and types of cross-membrane proteins that may transfer water-soluble MAAs between cells. It has been suggested that MAAs can be structurally altered internally by corals and carnivorous pteropods (Whitehead et al. 2001; Shick 2004) as well as by the bacteria *Vibrio harveyi* in the guts of sea urchins and holothuroids (Dunlap and Shick 1998). Further, a diversity of carrier-mediated MAA-transport mechanisms may exist in the gut and other tissues of organisms (Mason et al. 1998; Shick et al. 2000), allowing selective uptake of MAAs as well as tissue-specific control of MAA transport within a sea urchin. This information suggests that individual

MAAs may be selectively acquired or modified by *S. purpuratus*, and that this accumulation or modification is different between the sexes and is tissue-specific.

#### *Concentrations of MAAs Over Time*

The mean concentration of MAAs in ovaries increased in from November to January (Fig. 4) suggesting that female sea urchins were “packing” the MAA resource into the gonads, and presumably eggs, as the spawning season approached. Conversely, the males did not show an increase in the concentration of MAAs in testes over time (Fig. 5), suggesting that either the testes are constitutively low in MAAs throughout the year, or that they had reached a low but maximum concentration early in gonadal ripening (i.e. before November). The increase in MAA concentration in ovaries as spawning season neared indicates some functional role of MAAs in ovaries, and it been suggested that MAAs may play a role in gametogenesis (Bandaranayake et al. 1997; Bandaranayake and Des Rocher 1999), though this is subject to debate (Adams et al. 2001). Our data did not show a positive correlation between gonadal index and MAAs, and many sea urchins of both sexes had extremely low concentrations of MAAs, so it is not clear that MAAs necessarily regulate reproduction. It is not surprising that the epidermal MAA concentrations did not change in either sex from November to January (Fig. 5), as the solar irradiance levels do not differ drastically over that time.

The decrease in gonadal index for female sea urchins, but not males, from November to January indicates that the females may have spawned during that time, while the males did not. Though spawning before January is early in the reproductive season, the winter of 2006/2007 was exceptionally warm, and it has been documented



that sea urchins can spawn in response to increased temperatures (Farmanfarmaian and Giese 1963). A high fertilization percentage was observed under the microscope between gametes of the dissected sea urchins in January, so egg maturity was likely. Nevertheless, ovaries were ripe with eggs and concentrations of MAAs increased in ovaries from November to January, so females were still reproducing in January.

#### *Total MAAs in Algae*

To our knowledge, our study is the first to demonstrate the presence of MAAs in eight red algae species from California including *Calliarthron tuberculosum*, *Corallina vancouveriensis*, *Endocladia muricata*, *Mastocarpus jardinii*, *Mastocarpus papillatus*, *Mazzaella flaccida*, *Osmundea spectabilis*, and *Prionitis lanceolata* (Sinha et al. 2007). Other studies have previously demonstrated the presence of MAAs in *Corallina officianalis* from Patagonia (Karsten et al. 1998a; Häder et al. 2003) and *Pterocladia capillacea* from Japan (as cited in Sinha et al. 2007). We detected MAAs in every one of the 120 algal specimens collected (Table 1); this further established the ubiquity of MAAs in Rhodophytes algae around the world. These data are especially important for establishing that MAAs are available to consumers, such as purple sea urchins, in central California's coastal marine ecosystem.

#### *Mechanisms for Variability in Total MAAs*

The large number of samples we analyzed allowed us to detect patterns among sea urchins in the concentrations of MAAs in their tissues, but we still observed extremely high variability in the mean amount of MAAs detected for all tissue types

( $1.15 \pm 1.66$ ,  $0.31 \pm 0.23$ ,  $0.19 \pm 0.16$  and  $0.17 \pm 0.21$  nmol mg<sup>-1</sup> dry wt. ( $\pm$  S.D.) for ovaries, testes and female and male epidermis, respectively). There are a few mechanisms that could have created this high variability: individual sea urchins could differ in their ability to sequester MAAs, there could be short retention times of MAAs in tissues and only recently-ingested MAAs are detected, or there could be high variability in the diets of individual *S. purpuratus*. It is improbable that there are short retention times for MAAs in sea urchin tissues, because *S. droebachiensis* held on a MAA-poor diet still contained similar concentrations of MAAs months into the experiment (Adams et al. 2001). A follow-up feeding experiment in this laboratory will examine whether sequestration of MAAs in tissues of *S. purpuratus* is affected by diet and/or by exposure to natural UVR, and examine the level of variability among individuals kept on controlled diets.

## Conclusion

This study is the first to identify multiple mycosporine-like amino acids in the purple sea urchin, *Strongylocentrotus purpuratus*, and in eight new species of Rhodophyte macroalgae. Further, MAA concentrations in sea urchins were shown to vary with the sex, tissue type, time of year and the microhabitat that sea urchins occupied. *S. purpuratus* seems to have similar amounts of MAAs in its tissues as its congeneric *S. droebaciensis*, though with perhaps more variability. Multiple trade-offs concerning MAAs in sea urchins tissues were noted, and because MAAs protect sea urchin embryos from developmental delay and abnormalities (Adams and Shick 1996; 2001), these trade-offs have potentially far-reaching fitness consequences. This study is unique because it considered fine (microhabitat scale) variation in the field as well as investigated differences between sexes and differences in allocation of MAAs between tissues.

The first apparent trade-off was noted in sea urchins inhabiting pits; pits likely provide protection from abiotic and biotic stressors, while living outside of a pit is associated with increased size, fecundity and growth (Grupe 2006) and in our study was associated with an increased concentration of MAAs in both gonadal and epidermal tissues, at least for female sea urchins. This trade-off illustrates the importance that microhabitat choice may have for long-term fitness and survival of individual *S. purpuratus*. A second trade-off was detected in males only: in the Non-pit microhabitat, which was highest in irradiance as well as highest in the intertidal, male sea urchins allocated relatively higher concentrations of MAAs to their epidermal tissues (rather than their testes) than did males in the other three microhabitats. This suggests that males trade-off resources between somatic and reproductive tissues depending on the abiotic

environment. This trend was not observed in females, suggesting that females invest a consistently high proportion of their MAA resources to their offspring, regardless of abiotic conditions. The trade-off may only be advantageous for males because their short-lived sperm are not as likely to be affected by UV, so MAA resources may be better utilized in the epidermis when irradiance levels are high. Conversely, adjusting MAA allocation to eggs depending on a female's abiotic environment may not be advantageous, because UV likely negatively affects embryos and larvae regardless of their mothers' microhabitat.

Males also principally allocated only two MAAs to their testes, while multiple MAAs were detected in ovaries and epidermal tissues for both sexes, suggesting another trade-off in MAA allocation that is dependent upon sex. The different concentrations of individual MAAs found in testes compared to ovaries and epidermis seemed to be related to the wavelengths of absorption of UVR (Fig. 7), which could have implications for the level of protection that adult tissues and gametes have against UVR.

The ability to phenotypically adjust concentrations of sunscreens MAAs depending on irradiance levels may be important for *S. purpuratus* populations in the near future, as the penetration of UV 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). Our study suggests that male, but not female, *S. purpuratus* have some ability to adjust epidermal sunscreen levels depending on UV levels, and that microhabitat selection may have an impact on sea urchins' ability to obtain sunscreens. Current and future research in our laboratory will address the ability of *S. purpuratus* to adjust the MAA concentrations in their gonads and epidermis in response to UVR.

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