Marine Ecology. ISSN 0173-9565

ORIGINAL ARTICLE

Response of the soft coral *Heteroxenia fuscescens* to ultraviolet radiation regimes as reflected by mycosporine-like amino acid biosynthesis

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Keywords
Heteroxenia fuscescens; mycosporine-like amino acids; Red Sea; seasonal changes; soft corals; ultraviolet radiation resistance.

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Accepted: 18 August 2006

doi:10.1111/j.1439-0485.2006.00111.x

Abstract

The variation in mycosporine-like amino acid (MAA) concentration in the soft coral *Heteroxenia fuscescens* in relation to changes in ultraviolet radiation (UVR) regimes was investigated at the Gulf of Eilat, northern Red Sea. Solar radiation (300–700 nm) was measured for different depths and seasons. The UVR irradiance was measured to a depth of 25 m on the reef. The mean attenuation coefficient for UV-B measured in winter was twofold that of the summer value. Separation of *H. fuscescens* extracts by reverse-phase isocratic high-performance liquid chromatography revealed a single MAA compound, palythine ($\lambda_{max} = 320$ nm). Possible seasonal changes in MAAs in colonies of *H. fuscescens* along a depth gradient were examined on different dates. Palythine concentrations in the colonies were significantly higher in summer than in the other seasons particularly in shallow water. Possible changes in MAA content in colonies of *H. fuscescens* as a result of UVR protection, were determined by experiments conducted for periods of 1 week, 1 month and 3 months, at a depth of 5 m. In these experiments colonies were removed from the natural substrate and placed underwater, protected from UVR by a PVC filter. Significant differences between UV-exposed and protected colonies of *H. fuscescens* were found only in the 3-month experiment conducted during the summer. These findings demonstrate that UVR is an important environmental factor regulating MAA biosynthesis in the soft coral *H. fuscescens*.

Problem

In tropical regions, the flux of ultraviolet radiation (UVR) is at its global extreme and the coastal waters are characteristically clear and nutrient-poor (Kirk 1994). Oligotrophic waters are highly transparent to UVR (Dunne & Brown 1996) and, therefore, organisms there may experience harmful levels of radiation (Booth & Morrow 1997). Environmental UVB and the short wavelength of UVA can be detrimental to marine life (Shick *et al.* 1996; De Mora *et al.* 2000). Accordingly, shallow-water dwelling organisms exposed to high levels of solar UVR have evolved biochemical defences against such damage. This protection includes elaboration of natural UV-absorbing sunscreens, expression and regulation of antioxidant enzymes, accumulation or cycling of small-molecule antioxidants and molecular repair (Dunlap *et al.* 2000; Shick & Dunlap 2002).

Nearly ubiquitous among marine organisms is their ability to synthesize, or otherwise to acquire mycosporine-like amino acids (MAAs) that absorb radiation maximally in the range of 310–360 nm (Shick & Dunlap

2002). MAAs are particularly common in coral-reef algae and animals, especially among shallow-water organisms (Dunlap & Shick 1998; Karentz 2001). To date, there are 20 different known compounds of MAAs, all sharing the same basic molecular structure of a cyclohexenone or cyclohexenimine ring conjugated with the nitrogenous component of an amino acid (Carreto *et al.* 2005). Their concentration is greater in shallow than in deep-water stony corals (Shick *et al.* 1996; Dunlap *et al.* 2000; Gleason 2001) as an adaptive response to the exponential increase in the effects of UVR with decreasing depth.

Palythine ($\lambda_{max} = 320$ nm) seems to be the most prevalent MAA in soft corals (Shick *et al.* 1991; Michalek-Wagner 2001). The apparent multiplicity of interacting signals stimulating the biosynthesis of MAAs, as well as differences in the kinetics of their accumulation, result in temporal differences in the complement of MAAs and the combined UV-absorption spectra they present.

Annual patterns in content of MAAs may suggest response to a seasonal cue, which could be driven by solar radiation and temperature. Seasonal trends in concentrations of MAAs are probably determined by differences in solar UVR rather than by photosynthetic active radiation (PAR) or temperature (Drollet *et al.* 1997; Michalek-Wagner 2001), with the exception of very shallow-water coral populations in tropical regions (Kuffner 2005). Moreover, such trends are more clearly noticed in the photosynthetic organisms that produce the MAAs themselves than in others that obtain them via their food (Shick & Dunlap 2002).

Understanding the response of symbiotic systems on coral reefs to UVR requires an analysis of each component individually – the host and its complementary algal cells, as well as the intact symbiotic state. As part of an attempt to examine these complex interactions we have chosen to study the response of various stages in the life history of the soft coral *Heteroxenia fuscescens*, including its planulae, azoo- and zooxanthellate primary polyps and adult colonies (Zeevi Ben-Yosef 2003) because (i) it is a common inhabitant of Red Sea reefs along a wide depth gradient (Benayahu 1985) and (ii) its life history is well described (Ben-David-Zaslow & Benayahu 1996, 1998; Benayahu 1997). *H. fuscescens* thus provides a suitable model system in which all stages of the life cycle, including azoo- and zooxanthellate animals, can be studied conveniently in one species (see also Yacobovitch *et al.* 2003). The overall goal of this study was to determine the nature and extent of variation in MAA concentrations in mature colonies of *H. fuscescens* in Eilat in relation to changes in UVR regimes. For this purpose we studied the light regime of UVR adjacent to the colonies of *H. fuscescens*, the chemical nature and concentration of the MAA compounds along depths in different seasons, and the short- and long-term changes in MAAs in the coral host tissues.

Material and Methods

Underwater light regime

The study was conducted from August 1997 to September 2001 in Eilat (Gulf of Eilat, northern Red Sea) on the reef across from the Interuniversity Institute for marine sciences in Eliat (IUI). Visible radiation and UVR (300–700 nm) were measured at different depths (see below) during August 1997 (summer) and February 1998 (winter). Spectral irradiance data at each depth were obtained using a Li-Cor 1800 UW scanning spectroradiometer (Li-Cor, Lincoln, NE, USA). The cosine-corrected collector and sensors were programmed to scan from 300 to 700 nm at 1-nm intervals. At each depth (see below), separate scans (scan time approximately 45 s) were collected and reported in units of $W \cdot m^{-2} \cdot nm^{-1}$. Vertical attenuation coefficients (K_d m^{-1}) for both visible radiation and UVR were calculated following Kirk (1994). In addition, a three-channel dosimeter (Eldonet) for continuous monitoring of solar radiation with the filter functions PAR (280–315), UVA (315–400) and UVB (400–700), was placed on the IUI reef on 5 September 1999 and on 22 September 2002, adjacent to colonies of *Heteroxenia fuscescens* at a depth of 3 m. These measurements were taken between 4:30 am and 17:30 pm. The measurements taken on 5 September 1999 at 3 m were stopped between 12:30 am and 13:50 pm in order to conduct measurements also at 0, 5, 7 and 10 m. At each depth, readings consisting of three separate scans (total scan time approximately 1 min) were collected and the average (±SD) is reported in units of $W \cdot m^{-2}$.

Analysis of MAAs

In June 1999, 10 colonies of *H. fuscescens* were collected from the IUI reef at 1–2 m depth, frozen to −20 °C and transported to Tel-Aviv. They were lyophilized and extracted three times in 80% methanol. The crude extracts were examined by spectrophotometer and individual MAAs were separated by reverse-phase isocratic high-performance liquid chromatography (HPLC) following a protocol modified from Dunlap & Chalker (1986) using a RP-8 preparative column (Supelco, 25 cm × 10 mm, 5 μm). The mobile phase consisted of 0.1% acetic acid in water and was run at a flow rate of $0.7 \ ml \cdot min^{-1}$. Peaks were detected by UV absorbance at 320 nm and then collected, evaporated and analysed by ^{1}H-NMR and ^{13}C-NMR (Bruker ARX-500 MHz). The results of the NMR were compared with previously published data of known MAA compounds (see Dunlap *et al.*

1986, 1989). The material was diluted to appropriate concentration for running on an HPLC analytical column (Merck, Lichrosphere 25 cm × 4 mm, 5 μm). After defining the appropriate conditions for separation on the HPLC, a sample of known concentration was set as standard. The standard was used to calculate the concentrations of MAAs in all subsequent studied samples (Michalek-Wagner & Willis 2001).

UVR and MAAs along a depth gradient

In order to examine possible seasonal changes in MAAs in coral tissues along a depth gradient, colonies of *H. fuscescens* were sampled randomly at the IUI reef in December (winter) 2000, March and April (spring) and September 2001 (summer), at 3, 7 and 20 m (n = 5 colonies each depth, 3 polyps each). Samples (each polyp separately) were stored in 1 ml 100% MeOh at 4 °C for at least overnight prior to further HPLC analysis following the protocol of Dunlap & Chalker (1986) and Lesser (2000) with modifications. Quantification of an individual MAA compound was achieved by comparing the integrated HPLC peak to a quantified standard. MAA concentrations were normalized to protein concentration for each sample (*i.e.* one polyp), using the micro-Lowry spectrophotometric technique, with bovine serum albumin (Sigma) as a standard (Lowry *et al.* 1951).

Effect of UV shielding on *Heteroxenia fuscescens* colonies

To determine possible changes in MAA contents in colonies of *H. fuscescens* as a result of UVR protection, experiments were conducted for various periods of time. In these experiments colonies were removed from the reef substrate and attached to the bottom of PVC boxes (50 × 50 cm) with 10-cm high perforated sides (holes 5 × 5 cm) and placed on the reef. Colonies were protected from UVR by a PVC filter (50 × 50 cm, 0.5 mm width) placed 10 cm above them. The control colonies were placed as described above but in an uncovered box. The experiments were conducted as follows: (i) 1-week experiment conducted in January 2000 at 5 m, comprised one UV-protected and one control group; all colonies originated from 5 m (n = 6 colonies each); (ii) 1-month experiment conducted in May 2000 at 5 m, comprised a UV-protected group and two control groups, all originating from 5 m, where one of the controls was translocated adjacent to the UV-protected group and the second was kept at the original site (n = 3 colonies each) and (iii) 3-month experiment (April–June 2001) conducted at 3 m, comprised two UV-protected and two control groups, originating from 3 and 20 m (n = 5 colonies each, 4 groups in total).

In each experiment, groups of three polyps from the upper portions of each colony were removed prior to placing the filters. The first experiment was sampled on day 0 and day 7, the second was sampled once a week and the third after 60 and 90 days (see above). MAA concentrations per polyp wet weight in the first experiment were calculated from both the absorbance value and the extinction coefficient of Palythine ($\varepsilon = 36200\ M^{-1} \cdot cm^{-1}$), while in the other experiments MAA concentrations were normalized to protein concentration per polyp as described above.

Statistical analyses

Statistical analyses were performed using STATISTICA (data analysis software system), version 7.0 (StatSoft, Inc. 2004). Homogeneity of variance was tested using Leven's test. Significance between the MAAs concentrations of colonies at different depths and dates and in the 1- and 3-month experiments and the depth gradient, was determined by the Friedmans and Kruskal–Wallis non-parametric ANOVA, followed by *post hoc* multiple comparisons. The data of the 1-week experiment were analysed using two-way ANOVA, followed by Tukey's *post hoc* multiple comparisons.

Results

Underwater light regime on the IUI reef

Ultraviolet radiation measurements on the IUI reef are presented in Fig. 1. In August 1997 (summer) at a depth of 1 m, 90–100% water surface radiation was recorded, and at 5 m a steep decline occurred, ranging between 13% and 41% in the UV range (300–360 nm respectively). At 20 m only 1.8–18.4% water surface irradiance was recorded (Fig. 1a). In February 1998 (winter) the average UV (300–360 nm) surface radiation was 14–67% of summer values and at 20 m the values for the same wavelength resembled those of summer for this depth (Fig. 1b). The PAR/UV ratio at the water surface in winter was higher than in summer, but the 20 m values were almost the same for both seasons. The coefficient values K_d [m^{-1}] recorded at the UV portion in summer (Fig. 2, 0.1–0.2 at 300–400 nm) were lower than those in winter (0.2–0.5).

Maximal values (noon-time) of PAR, UV-A and UV-B recorded on 5 September 1999 at 3 m were 290, 36 and 1 $W \cdot m^{-2}$ respectively (Fig. 3). The values of PAR, UVA and UVB from sunrise to sunset at 3 m in September 2001 are presented in Fig. 4; this specific day was cloudy and, therefore the values are lower by *c.* 50% than those presented for the same depth in September 1999. Daily maximal values of PAR, UVA and UVB recorded on 5

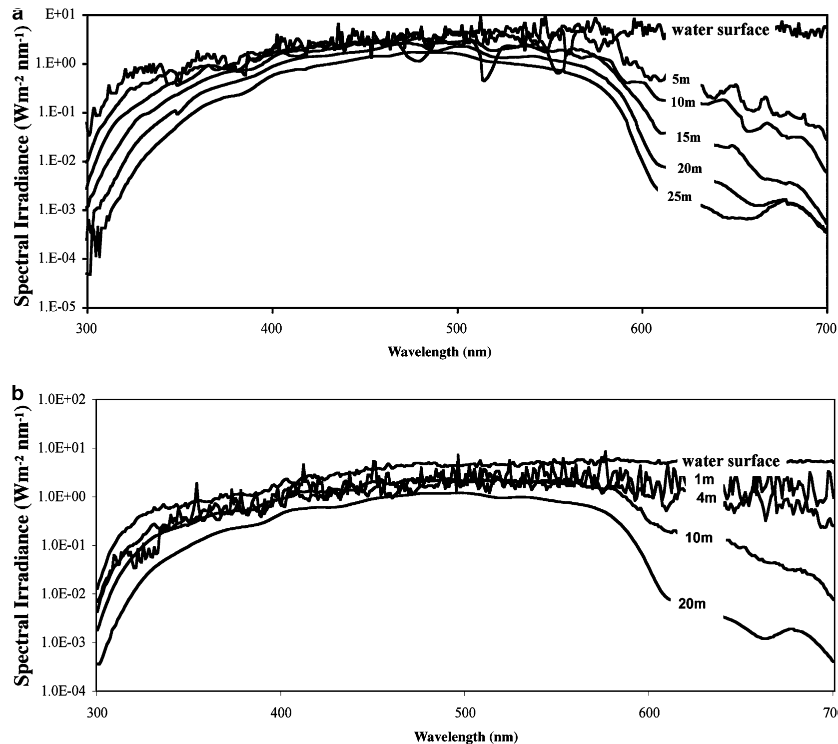

Fig. 1. Depth profile of spectral data at a range of 300–700 nm recorded on the reef across from the Interuniversity Institute of Eilat in August 1997 (a) and February 1998 (b) using Li-cor LI-1800 UW scanning spectroradiometer placed at various depths.

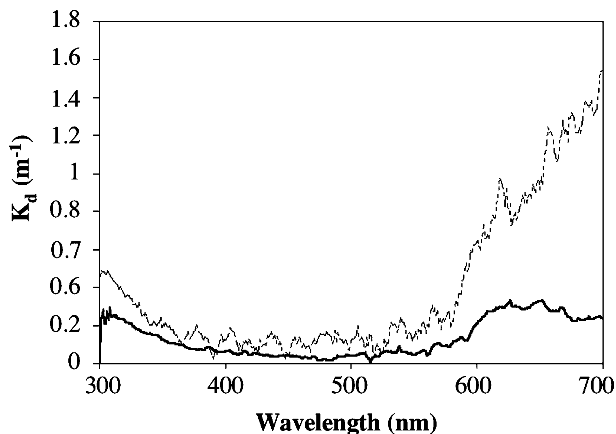

Fig. 2. Attenuation coefficient (K_d [m^{-1}]) using the spectral data (300–700 nm) recorded on the reef across from the Interuniversity Institute of Eilat, in August 1997 (bold line) and February 1998 (dashed line).

and 6 September 2001 were 360, 47, 1.5 $W \cdot m^{-2}$ and 330, 43 and 1.3 $W \cdot m^{-2}$, respectively (data not presented). These values resemble those recorded on 5 September 1999 (Fig. 3).

Analysis of MAAs in colonies of *Heteroxenia fuscescens*

The crude methanol extracts obtained from 10 colonies of *Heteroxenia fuscescens* showed strong absorbance at the UV range with $\lambda_{max} = 320$ nm (Fig. 5). The extracts were separated on the preparative column and the chromatogram revealed a single peak at 320 nm (Fig. 6), which was collected and evaporated. Ten milligrams of this material was analysed by NMR; the results were compared with published data and revealed that the main compound isolated from *H. fuscescens* was palythine (Dunlap *et al.* 1986, see also Discussion ahead).

UVR and MAAs along a depth gradient

There was no clear pattern in concentration of palythine in colonies of *H. fuscescens* along a depth gradient in December (winter) or March (early spring) 2001, but in April (late spring) and September (summer) 2001 shallow colonies appeared to have more palythine (Fig. 7). There were no significant differences among concentrations of MAAs of all depths at different dates (Friedman's ANOVA for repeated measures, $P < 0.001$) while at 3- and 20-m depths there were significant differences between the different dates attributed mainly to differences between September (summer) and the other dates (Kruskal–Wallis ANOVA and *post hoc* multiple comparisons, $P < 0.001$).

Effect of UV protection on *Heteroxenia fuscescens* colonies

There was no significant difference between the MAAs in the experimental and control colonies of *H. fuscescens* in

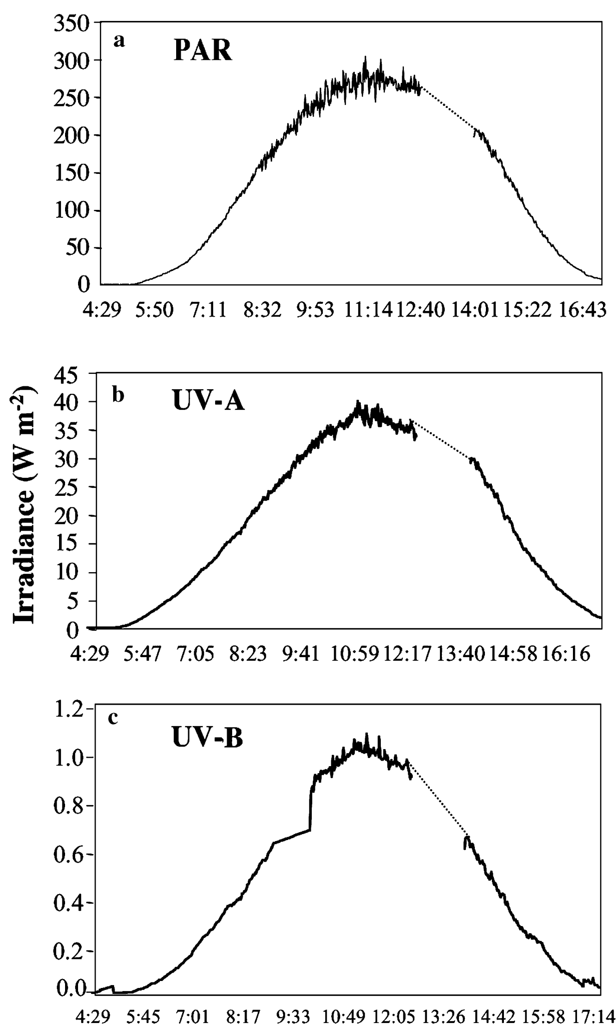

Fig. 3. Photosynthetic active radiation (a) UV-A (b) and UV-B (c) measurements from sunrise to sunset taken on 5 September 1999 at 3 m on the reef across from the Interuniversity Institute of Eilat. Dotted line indicates no data.

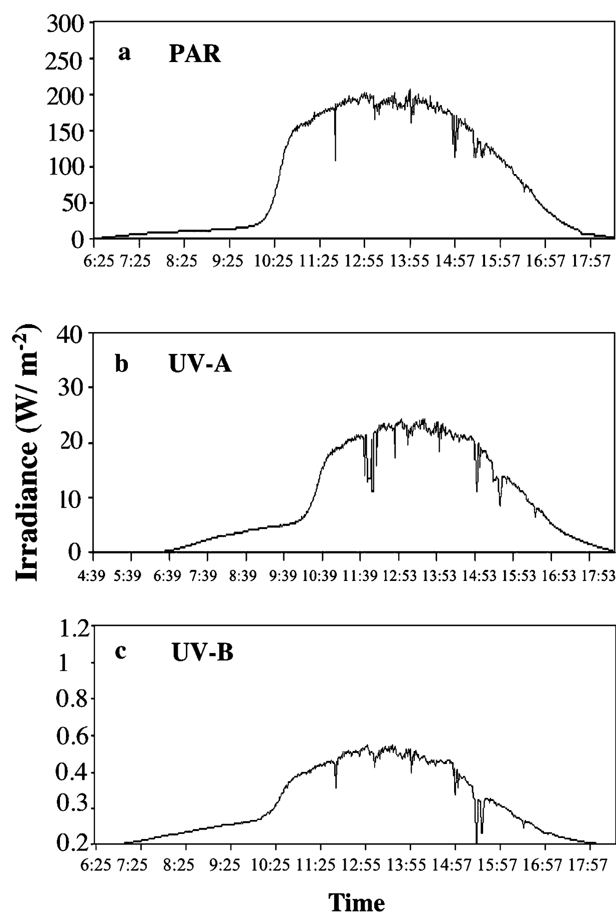

Fig. 4. Photosynthetic active radiation (a), UV-A (b) and UV-B (c) measurements from sunrise to sunset taken on 21 September 2001 at 3 m, on the reef across from the Interuniversity Institute of Eilat.

the 1-week experiment (Fig. 8, *t*-test, $P > 0.05$). Similarly, there were no significant differences in palythine concentrations between the two groups of colonies in the 1-month experiment among sampling dates (Fig. 9, two-way ANOVA, $P > 0.05$). In the 3-month experiment there were significant differences in MAA concentrations among dates and depths (Fig. 10, Friedman's ANOVA for repeated measures, $P < 0.001$). The palythine concentration of UV-protected colonies originating from 3 and 20 m declined to similar values on day 92 of the experiment (6.8 ± 1 and 4.8 ± 1 nmol palythine mg^{-1} protein, $n = 5$ colonies each; Kruskal–Wallis ANOVA and *post hoc* multiple comparisons, $P < 0.001$). Similar comparisons showed significant differences between the UV-protected and the exposed colonies of both depths and for all sampling dates ($P < 0.05$). The shallow-water control colonies did not reveal significant changes in concentration of palythine between sampling dates (*post hoc* comparisons, $P > 0.05$), while in the deep-water control colonies the concentration was significantly higher after 92 days than those obtained for all other dates and in the other experimental and control colonies (Kruskal–Wallis ANOVA and multiple *post hoc* comparisons, $P < 0.001$).

Discussion

Underwater light regime on the IUI reef

Ultraviolet radiation irradiance, including UVB, was measured to a depth of 25 m on an Eilat coral reef. The mean attenuation coefficient for UVB wavelength in summer (0.24) resembles the mean attenuation coefficient measured in the Conch reef, Key Largo, Florida (0.22, Lesser 2000). Therefore, the maximum depth for downwelling irradiances of UVB at 1% and 10% of surface irradiance for the oceanographic conditions encountered

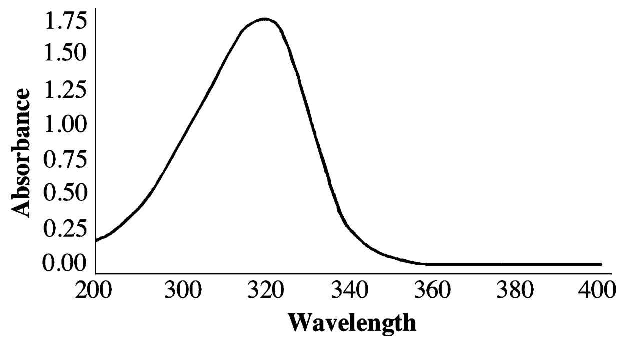

Fig. 5. *Heteroxenia fuscescens*: absorption spectrum of 100% methanol extracts of mature colonies.

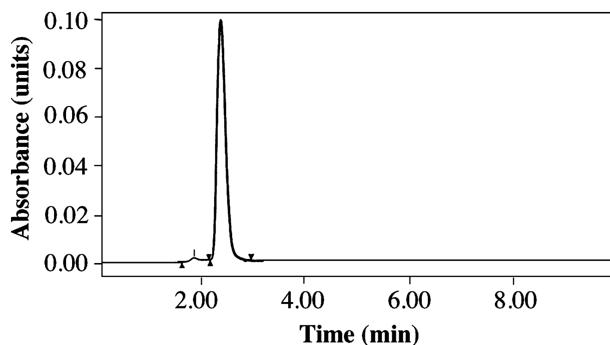

Fig. 6. *Heteroxenia fuscescens*: separation of methanol extracts on a high-performance liquid chromatography RP-8 preparative column (Supelco, 25 cm × 10 mm, 5 μm): mobile phase, 0.1% acetic acid in water; flow rate 0.7 ml · min^{-1}, detector 320 nm.

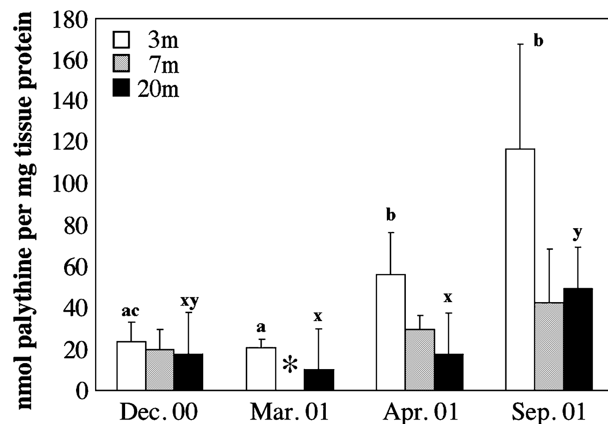

Fig. 7. *Heteroxenia fuscescens*: changes in concentrations of palythine per milligram tissue protein in colonies (+ 1 SD, n = 3 colonies, 3 polyps each) at 3, 7 and 20 m at different dates. *No data. Letters on top of bars indicate statistical groups.

during this study are 22 and 11 m respectively, following Kirk (1994). The mean attenuation coefficient for UV-B that was measured in winter in the present study (0.44)

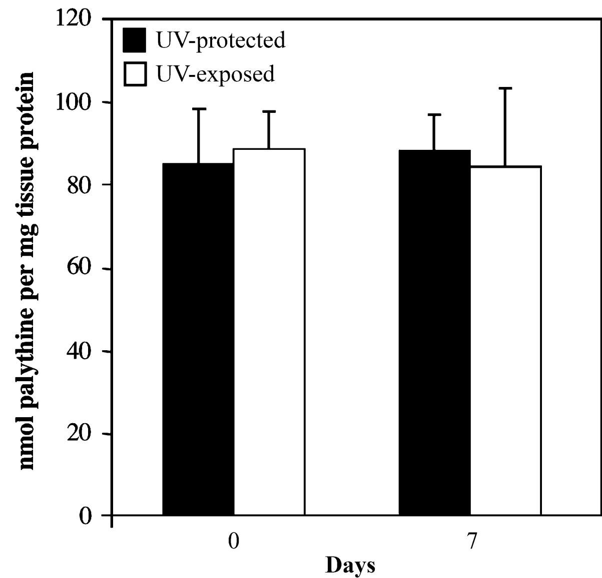

Fig. 8. *Heteroxenia fuscescens*: changes in concentration of palythine (+SD, n = 6 colonies, 3 polyps each) in UV-protected and UV-exposed colonies at day 0 and 7 of the experiment (5 m depth).

was twofold that of the summer mean. Similar differences between seasons were recorded in a previous study conducted in Eilat between 1993 and 1996 (Iluz 1997). The latter study revealed the highest values between January and March and the lowest between May and December, which are the upwelling and the thermocline periods, respectively, in the Gulf of Eilat (Genin *et al.* 1995). These results show that UV penetration is higher during the thermocline periods (summer) than during the upwelling periods characterized by algal blooms, which might affect UV penetration.

Analysis of MAAs in colonies of *Heteroxenia fuscescens*

Stony corals collectively contain at least 13–20 different MAAs (Shick & Dunlap 2002; Carreto *et al.* 2005). The presence of a combination of MAAs with slightly different absorption maxima within marine organisms has been interpreted as a 'broad-band' filter against UVR damage (Dunlap *et al.* 1986). In *Heteroxenia fuscescens* we found one MAA compound, palythine. Palythine has been described as a typical MAA component in other corals (reviewed in Shick & Dunlap 2002; Yakovleva & Hidaka 2004), and is predominant in the soft corals *Lobophytum compactum* and *Sinularia flexibilis* (Michalek-Wagner 2001). Palythine has an absorption peak at 320 nm, and is thus effective close to the UV-B region, which has been shown to be the major biological effective component in the solar spectrum (Chalker-Scott *et al.* 1992; Berghahn

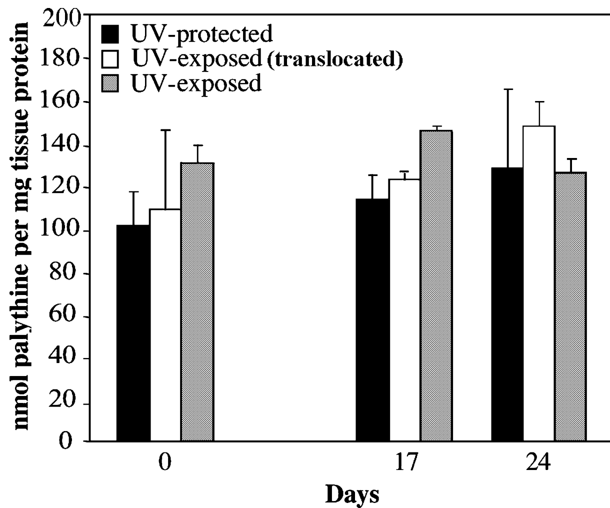

Fig. 9. *Heteroxenia fuscescens*: changes in concentration of palythine (+SD, n = 5 colonies, 3 polyps each) in UV-protected, translocated UV-exposed and ambient UV-exposed colonies at day 0, 17 and 24 of the experiment (5 m depth).

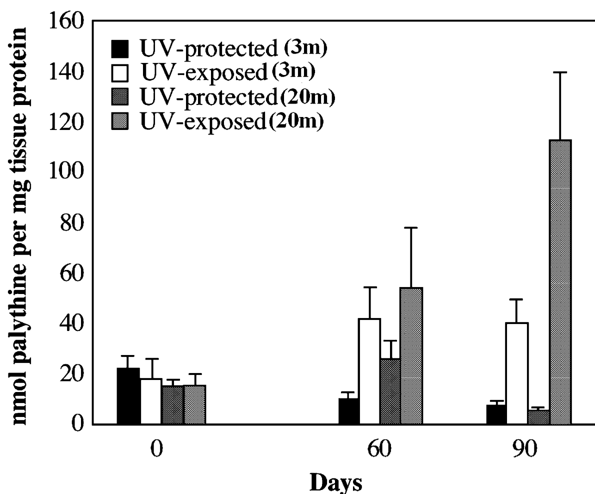

Fig. 10. *Heteroxenia fuscescens*: changes in concentration of palythine (+SD, n = 3 colonies, 3 polyps each) in shallow UV-protected and UV-exposed colonies, deep translocated (20 to 5 m) UV-protected and UV-exposed colonies at day 0, 60 and 90 of the experiment (5 m depth).

et al. 1993). UV-B radiation is particularly detrimental because its wavelength of 290–320 nm overlaps the upper absorption range of DNA and proteins. Therefore, lower-end absorption of UV-B by MAAs, such as palythine, probably provides certain direct protection for DNA and proteins (Carroll & Shick 1996). MAAs in microalgal-invertebrate symbiosis presumably originates in the phototrophic partner via the shikimate pathway (Shick & Dunlap 2002). In extreme cases, however, the host inhabited by zooxanthellae that produce no MAAs *in vitro*, may have up to seven MAAs (Shick *et al.* 1999, 2000). Thus, in these corals the compounds originate from other sources than the corals' algae (Shick *et al.* 2002). In *H. fuscescens* adult colonies there was only a single MAA compound found, in contrast to other corals with six different MAAs and more (see above). Thus, *H. fuscescens* resembles the low MAA diversity predominated by palythine, found in other soft corals (*Clavularia* sp. three MAAs: Shick *et al.* 1991, and *L. compactum* two MAAs: Michalek-Wagner 2001). In these studies the MAA compound mycosporine-glycine was also absent from the coral tissues. This finding is puzzling because mycosporine-glycine is the parent MAA from which all others are derived (Bandaranayake 1998; Shick & Dunlap 2002; Portwich & Garcia-Pichel 2003). While heterotrophy plays a major role in many corals (Sebens *et al.* 1996), *H. fuscescens* has a unique autotrophic nature. However, studies have also shown an uptake of dissolved organic matter in the form of FAAs from seawater (Schlichter 1982). Indeed, MAAs have been measured in the medium of dinoflagellate cultures (Banaszak *et al.* 2000) and UV-absorbing compounds have been detected dissolved in seawater during plankton blooms (Vernet & Whitehead 1996). Therefore, the source of palythine might be exogenous, thus explaining the absence of the parent MAA mycosporine-glycine. A nutritional source of MAAs cannot be excluded in symbiotic organisms, which could explain the existence of a broader band of MAAs in some heterotrophic corals (Shick & Dunlap 2002) than that found in the autotrophic *H. fuscescens* (this study).

UVR and MAAs along a depth gradient

Previous studies have shown a depth-related decline in MAA concentrations in corals (Gleason & Wellington 1993; Corredor *et al.* 2000; Lesser 2000). In those studies the significance of differences between depths varied among MAA compounds and sites. In the stony coral *Acropora* spp. on the Great Barrier Reef (GBR, Australia), the ratio of surface concentration of palythine, the main compound of the coral, to that found at 20 m, was 16:1 (Dunlap *et al.* 1986). In the Caribbean stony coral *Montastraea faveolata*, mycosporine-glycine, the main compound of this coral decreased significantly with depth, while no significant decline occurred in palythine in the same study (Lesser 2000). Such differences among species and their MAA compounds in the different studies could be related to the UV spectra at the coral's specific habitat on the reef. Bathymetric adaptations in different MAAs of *Acropora* sp. were studied in the GBR by Dunlap *et al.* (1986), and it has been shown for three Caribbean stony coral species that they may be able to adjust their MAA

contents not only in terms of increasing total UV absorption capacity, but also in adjustment of UV-absorbing compound suites to spectral quality of UV irradiation (Corredor *et al.* 2000). In the present study, palythine concentrations in *H. fuscescens* colonies in different seasons showed significant differences mainly in shallow water. These differences suggest that they respond to a seasonal cue, which could result from changes in solar radiation or seawater temperature, which in Eilat occur mainly in shallow water (Zeevi Ben-Yosef 2003). Annual fluctuations in MAAs were found in the soft corals *L. compactum* and *S. flexibilis* on the GBR and were significantly correlated to annual cycles in the above-mentioned factors (Michalek-Wagner 2001). Patterns of high MAA tissue concentrations in summer, when incident solar radiation is the highest, and low concentrations in winter, when radiation is the lowest, are consistent with MAAs functioning for photo-protection (Gleason & Wellington 1995; Shick *et al.* 1995). Comparable changes in MAA levels in the mucus of the stony coral *Fungia repanada* were found to be positively correlated with annual cycles of solar radiation (Drollet *et al.* 1997). Thus, it may be that significant differences in MAA concentrations occur when solar radiation is the highest. Production of MAAs involves a substantial investment of nitrogen, which is considered to be highly limited in the nutrient-poor waters of coral reefs (Cook 1983; Gleason 2001; Shick *et al.* 2005). Induction of energetically costly MAAs only in response to environmental stress minimizes metabolic cost until the ecological benefit of their production can be realized (Michalek-Wagner 2001). This could be the reason for the higher concentrations of palythine in shallow water corals in summer found in the present study.

Effect of UV shielding on *Heteroxenia fuscescens* colonies

In our experiments conducted for 1 week in January 2000 and 1 month in May 2000, no significant differences in the concentration of palythine between UV-protected and UV-exposed colonies were found. In the 3-month experiment conducted between April and July 2001, there were significant differences in the UV-exposed groups but no significant differences in the UV-protected ones. Similarly a *c.* 15% decline in the concentration of palythine in the soft coral *Clavularia* sp. was evident only after 2 months when shielded from UV radiation, and it has been suggested that this was because of a very low turnover of this compound in the species (Shick *et al.* 1991). UV-native stony corals synthesize detectable MAAs within days of exposure to UV (Shick *et al.* 1999, 2005; Shick 2004). Our UV-shielding experiments reflected a relatively slow accumulation of MAAs and may thus imply that the source of MAAs in *H. fuscescens* is exogenous rather than endogenous. This may also explain the finding of significant differences in the species' palythine concentrations only for the 3-month experiment. In order to verify this assumption, future experiments should include continuous measurements of daily UVR fluxes. However, it is also possible that significant changes in MAA concentrations occur mainly in the summer when UV radiation increases. In the 3-month experiment, the colonies translocated from deep to shallow water and the UV-exposed groups showed a very sharp increase in palythine concentration, reaching concentrations that were four times higher than those in the shallow UV-exposed colonies. Other studies have shown that water motion is important for the maintenance of MAAs in corals (Jokiel *et al.* 1997; Kuffner 2002). It is possible that the deep colonies, which normally encounter lower water energy, are capable of producing higher levels of MAAs than the ambient colonies when exposed to higher water motion combined with exposure to higher UVR. To verify this assumption, further investigation concerning the synergistic effects of UVR and water motion on MAA production is required. In addition, it is possible that the exposed colonies were slightly more subject to water motion and sedimentation. In the 1-week and 1-month experiments we did not find any significant differences in MAA concentration between covered and uncovered colonies, thus indicating that UVR radiation is predominant.

Conclusions

The finding that palythine concentration declined in UV-protected colonies of *Heteroxenia fuscescens*, mainly in shallow water and in summer, provides evidence for the role of UVR as an important environmental factor regulating MAA biosynthesis. This soft coral incorporates the MAA compound palythine. Palythine is effective close to the UV-B region, which penetrates in significant amounts along its depth distribution. This, together with the ability to alter the concentration of palythine as a response to environmental changes, provides evidence that colonies of *H. fuscescens* are well-adapted for protection against UVR.

Acknowledgements

The authors thank Prof. A. Oren, Prof. Z. Dubinski, Prof. S. Beer and Prof. M. Ilan for their useful advice and help. Prof. H. Schubert is acknowledged for kind hospitality to D. Z. B.-Y. during her visit to Griefswald University (Germany) and for his insightful advice. We are also indebted to the staff of the Interuniversity Institute of Marine Biology at Eilat for their hospitality and assistance. N. Paz is

acknowledged for her skillful editorial assistance. We wish to acknowledge M. J. Shick and an anonymous reviewer for useful comments on the manuscript.

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