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Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates

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Abstract

Colonies of *Stylophora pistillata* maintained for four years in indoor aquaria in the near absence of ultraviolet radiation (UVR) contained only small amounts (<5 nmol mg^{-1} protein) of 10 identified mycosporine-like amino acids (MAAs, which act as UV sunscreens), the largest number reported in any organism. The concentrations of most MAAs increased linearly or exponentially when colonies were exposed to ultraviolet-A (UVA) and ultraviolet-B (UVB) for 8 h d^{-1} in the presence of photosynthetically active radiation (PAR). Total MAA concentration reached 174 nmol mg^{-1} protein after 30 d, with palythine and mycosporine-2 glycine constituting more than half of the final total. UVB specifically stimulated MAA accumulation: after 15 d, MAA levels in colonies exposed to PAR alone and to PAR and UVA did not differ (7 and 5 nmol MAA mg^{-1} protein, respectively), while those in colonies exposed to PAR and UVA + UVB were significantly higher (28 nmol mg^{-1} protein). Glyphosate, an inhibitor of the shikimate pathway, eliminated or reduced the UV-induced accumulation of most MAAs during 7 d of exposure, providing the first experimental evidence of their synthesis via this pathway in a coral symbiosis. Densities of zooxanthellae in colonies of *S. pistillata*, *Acropora* sp., and *Seriatopora hystrix* exposed to UVR for 15 d were only one-third of those in control colonies unexposed to UVR. This net decrease in the number of zooxanthellae in the corals (bleaching) occurred despite UV-stimulated increases in algal cytokinesis and in the host cell-specific density of zooxanthellae in hospite, increases that apparently destabilized the symbiosis and caused expulsion of the zooxanthellae.

The separate and interacting effects of solar ultraviolet radiation (UVR) and photosynthetically active radiation (PAR) on coral reef ecosystems are by now well documented and extensively reviewed (Chalker et al. 1988; Falkowski et al. 1990; Shick et al. 1996*b*). Considerable interest has centered on the mycosporine-like amino acids (MAAs), putative UV sunscreens that occur widely among freshwater (Sommaruga and Garcia-Pichel 1999; Xiong et al. 1999) and marine organisms, where the MAA concentration in corals is correlated with their exposure to solar radiation, usually over a depth gradient (reviewed by Dunlap and Shick 1998). Experiments involving differentially filtered natural sunlight and controlled water flow regimes have implicated ultraviolet-A (UVA, 320–400 nm), ultraviolet-B (UVB, 295–320 nm), and PAR (400–700 nm), as well as depth-dependent water movement, as determinants of this bathymetric relationship (Baker 1995; Jokiel et al. 1997; Dunlap and Shick 1998, for review of the earlier literature). Discussion of the foregoing factors has considered the direct stimulation by UVR of MAA synthesis, increased carbon flux into MAA biosynthetic pathways when photosynthesis is enhanced by higher levels of PAR and water movement, and increased capture of MAA-containing prey as water velocity increases at shallower depths.

Prior studies have been semicontrolled because total irradiance has depended on solar conditions during the experimental exposure in outdoor aquaria of corals freshly collected from the field, and because possible dietary sources of MAAs have not always been excluded. Largely because of the difficulty of the long-term maintenance of reef corals in indoor aquaria, controlled experiments using defined ra-

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diation sources to effect changes in MAA content and composition similar to those seen for cultured dinoflagellates (Carreto et al. 1990*a*,*b*) have not been undertaken heretofore. Nor have there been kinetic studies of the accumulation of individual MAAs in corals stimulated to do so. Studies of dinoflagellates (Carreto et al. 1990*a*,*b*), diatoms (Helbling et al. 1996), other microalgae (Xiong et al. 1997, 1999; Hannach and Sigleo 1998), and macrophytes (Rhodophyta) (Karsten et al. 1998) in culture indicate not only wavelength-specific effects on MAA accumulation but also reveal different kinetics for individual MAAs and a time-dependent change in algal MAA composition. Virtually all studies of MAAs in zooxanthellate corals and other phototrophic symbioses explicitly assume that most MAAs are synthesized de novo via the shikimate pathway (presumably in the algal endosymbionts because animals, or at least mammals, lack this pathway: Bentley 1990; Haslam 1993), although until now there has been no experimental test of the involvement of the shikimate pathway.

The role of increased solar UVR in coral bleaching has been controversial although its effects on cell division and photosynthesis in zooxanthellae and their loss from corals have been documented at high fluence rates in field-transplant and laboratory experiments (reviewed by Glynn 1996; Shick et al. 1996*b*; Brown 1997*a*). Natural bleaching events following prolonged calm sea conditions that allow greater penetration of UVR have occurred at intermediate depths where increases in UVR would be more modest (Goenaga et al. 1989; Gleason and Wellington 1993) and where UV fluences increase disproportionally to those of PAR (Gleason and Wellington 1994; Dunne and Brown 1996). Like other sublethal stressors such as temperature (Brown 1997*b*) and eutrophication (Muscatine et al. 1998), such increases in UVR might disrupt the coral symbiosis by affecting the proliferation of algae and their numbers in host cells, a possibility that we have tested.

The culturing of reef corals in indoor aquaria is becoming more tractable (Jaubert 1989) and allows assessment of the effects of environmental change under controlled conditions (Carlson 1999). The corals used in our experiments had been grown for several years under PAR provided by UV-shielded metal halide lamps and therefore had minimal prior exposure to UVR. Pieces of colonies were moved to an experimental aquarium and maintained under defined radiation sources in filtered, oligotrophic seawater at constant levels of temperature and water movement. The experimental variables were doses of UVA and UVB at constant fluences of PAR. Additional experiments examined the effect of chemical inhibition of the shikimate pathway on the accumulation of MAAs stimulated by UV exposure.

Materials and methods

Maintenance of corals—Experimental corals were obtained from the reserve in the Aquarium of the Musée Océanographique, Monaco, where they had been maintained for 4 yr since their collection from the Gulf of Aqaba, Jordan. Pieces 1.5–2.5 cm in length were cut from two parent colonies of *Stylophora pistillata* and suspended on nylon monofilaments in a 300-liter aquarium supplied with continuously flowing, oligotrophic Mediterranean seawater (salinity = 38) pumped from a depth of 55 m, heated to 27°C, and circulated past the corals by submersible pumps. Larger pieces (≈8–10 cm long) cut from terminal branches of single parent colonies of *Acropora* sp., *Seriatopora hystrix*, and *S. pistillata*, were suspended on nylon monofilaments in the same aquarium. All cut surfaces of skeleton were again covered by coral tissue within 1 month. Corals were fed weekly with freshly hatched *Artemia* nauplii; feeding was discontinued 5 d prior to the start of any experiment. Illumination (12 h light : 12 h dark) in the maintenance aquarium was provided by two metal halide lamps (Philips HQI-TS, 400 W), yielding PAR of 250 μmol photons m^{-2} s^{-1}, as measured with an LI-1000 DataLogger and 193SA spherical quantum sensor (LiCor) at the depth at which the coral pieces were suspended. Background levels of UVR emanating from the shielded halide lamps were measured using an IL1400A radiometer with SEL033 UVA and SEL240B UVB sensors (International Light) having peak sensitivities at 360 and 300 nm, respectively. Unweighted UVA and UVB fluences at the depth of the corals were 0.74–0.90 W m^{-2} and 0.001–0.005 W m^{-2}, respectively.

Experimental aquarium—In experiments involving controlled spectral irradiance, pieces of coral were suspended at a depth of 2 cm in a 50-liter aquarium divided vertically in half by UV-opaque Plexiglas perforated with baffles to allow circulation of seawater between the two halves of the aquarium but allowing no detectable leakage of UVR. The aquarium was supplied with flowing Mediterranean seawater heated to 27 ± 0.1°C, continuously circulated past the corals by a submersible pump in each half of the aquarium. Each pump drew its water from the other half of the aquarium. The velocity of turbulent flow across the corals was approximately 15 cm s^{-1}, as measured by timing the passage of neutrally buoyant beads. Experimental and control corals were exposed simultaneously in the same aquarium because it was infeasible to maintain water movement and quality and to assure even illumination for corals in individual chambers. Using a single aquarium for all treatments and controls prevented any tank effect of maintaining treated and control corals in two separate vessels.

Two 400-W Osram Powerstar HQI-T metal halide lamps above the aquarium provided PAR at 225 μmol photons m^{-2} s^{-1} at the depth of the corals. UVR was provided by two UVA-340 fluorescent lamps (Q-Panel Lab Products), one placed above each half of the aquarium, 4 cm from the surface of the water. The spectral power emission from such lamps is shown in Fig. 1. Colonies fully exposed to UVR in all experiments experienced a UVA fluence of 5.3 W m^{-2} and a UVB fluence of 0.065 W m^{-2} for 8 h in the middle of the 12-h PAR light cycle. Corals exposed to PAR but not to UVR were shielded by a UV-opaque (50% transmission at 400 nm) Lee 226 polycarbonate cutoff filter (Lee Filters) covering one half of the aquarium. Although this filter also attenuated PAR by about 8%, the halide lamps were adjusted to provide brighter incident illumination on the UV filter, so that PAR levels were the same beneath the UV filter as in the unshielded half of the aquarium. Unweighted UV flu-

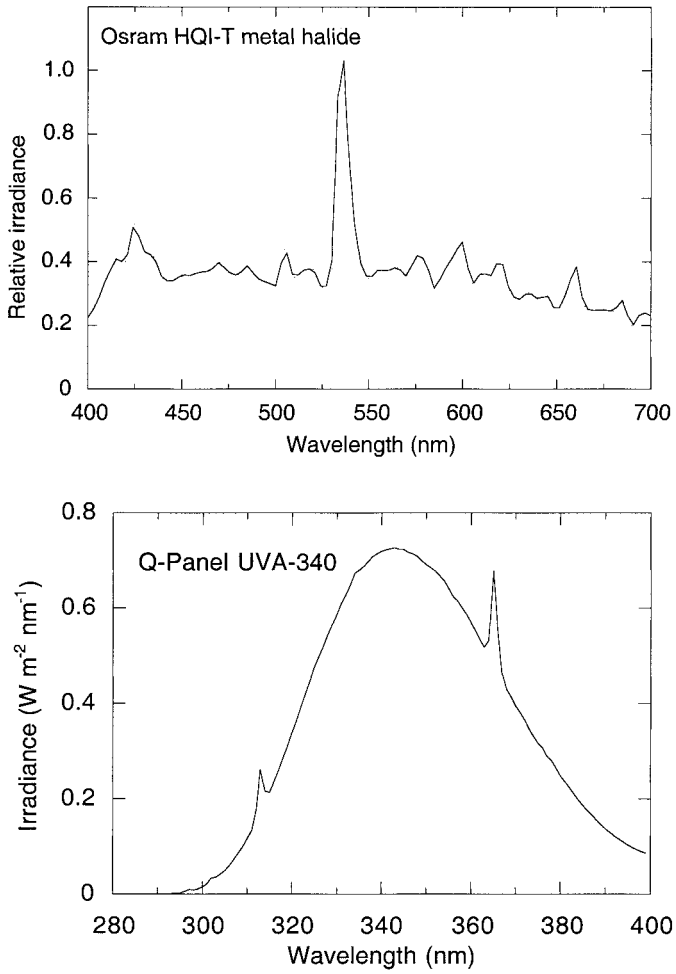


Fig. 1. Spectral power emission from lamps illuminating the experimental aquarium. Upper panel: Osram Powerstar 400 W HQI-T metal halide (in shielded housing); lower panel: Q-Panel 40 W UVA-340 fluorescent. Data provided by the manufacturers.

ences measured beneath the UV filter were 0.045 W m^{-2} for UVA and 0.003 W m^{-2} for UVB, at or below UV levels in the Musée's reserve and the maintenance aquarium.

Kinetics of accumulation of MAAs—Individual pieces of one colony of *S. pistillata* in the experimental aquarium were exposed to PAR plus full UVR (see above). Three pieces were analyzed on day 0, and three additional pieces were removed on each of days 3, 7, 15, and 30. Corals were extracted in three changes of 3 ml of high-performance liquid chromatography (HPLC)-grade methanol for 1 h each in the dark at 4°C, and the pooled extracts for each piece were analyzed for MAAs as described below. The methanol-extracted skeletons were stored at −20°C and subsequently analyzed for total protein content as described below. Additional pieces were simultaneously exposed to the same experimental conditions and analyzed for chlorophylls (Chls) *a* and c_2 and total protein (see below). To control for any effect of maintenance in the experimental aquarium unrelated to UV exposure, further pieces were held simultaneously in the UV-shielded half of the aquarium and analyzed for MAAs, chlorophyll, and protein after 16 d. (Control colonies intended for analysis of MAAs and chlorophyll, after 30 d, were lost owing to mishap.) Data for days 0, 3, 7, and 15 of UV exposure were analyzed by separate one-way analysis of variance (ANOVA) for Chl *a*, Chl c_2, and protein, followed by Student-Newman–Keuls multiple comparison tests (StatView, SAS Institute). We were unable to sample both the experimental and control colonies on the same day but used the chlorophyll and protein data for day 16 (no UV exposure) as a separate control for any effects of maintenance in the experimental aquarium for a similar duration (within 7%) as the corals exposed to UVR for 15 d, comparing the data for days 15 and 16 using unpaired *t*-tests. One-way ANOVA for individual and total MAA concentrations were performed using data for days 0, 3, 7, 15, and 30. MAA data for day 16 (control) were compared with those for day 15 of UV exposure, and with day 0 MAA data, using unpaired *t*-tests. Critical values of the *t*-distribution were obtained from Sidák's multiplicative inequality for two comparisons (Rohlf and Sokal 1995). Changes in MAA concentrations from day 0 to day 30 of UV exposure were analyzed by linear (StatView) and nonlinear regression (SYSTAT for Macintosh). In each nonlinear regression, the mean MAA concentration on day 0 was the starting datum.

Differential effects of UVA and UVB radiation on MAA accumulation—In a separate experiment of 15 d duration, three groups of pieces of another colony were simultaneously exposed to the following conditions in the experimental aquarium: PAR (225 μmol photons m^{-2} s^{-1}), UVA (5.3 W m^{-2}), and UVB (0.065 W m^{-2}); PAR and UVA only (5.3 W m^{-2}, UVB being reduced to undetectable levels by a Mylar D filter having 50% transmission at 324 nm, above which wavelength the UVB sensor does not respond); or PAR only (UVA and UVB being attenuated by the Lee 226 filter to levels given above). To correct for the slightly greater transmission of PAR by the Lee filter than by the Mylar filter, incident illumination from the metal halide lamps was adjusted accordingly, so that PAR exposures were constant among treatments. MAAs were extracted as described above. Data for individual and total MAAs were analyzed by ANOVA followed by Student–Newman–Keuls tests.

Effect of glyphosate on MAA accumulation—*N*-(phosphonomethyl)-glycine (glyphosate), an inhibitor of several enzymes in the shikimate pathway (Bentley 1990), was purchased from ICN Biomedicals. Pieces of the same colony of *S. pistillata* as used in the kinetics experiment were held in individual 150-ml beakers on nylon mesh above magnetic stirrers. Beakers were placed in a constant temperature bath at 27°C beneath Osram metal halide and Q-Panel UVA-340 lamps adjusted to provide fluences of PAR and UVR to within 10% of levels stated previously. Three pieces of coral were exposed to each of the following conditions for 7 d: full UVR (UVA + UVB) and PAR; full UVR and PAR, plus 1 mM glyphosate; no UVR (PAR only); and no UVR (PAR only) plus 1 mM glyphosate. All glyphosate-treated corals were initially exposed to the inhibitor for 24 h prior to the start of the experiment to allow tissues to absorb the

inhibitor before exposure to the various photic treatments. Seawater (100 ml, with or without fresh glyphosate) in each beaker was changed daily. All colonies were subsequently analyzed for MAAs. Effects of UVR and of glyphosate on MAA accumulation were analyzed by ANOVA followed by Student–Newman–Keuls tests.

Effects of UVR on zooxanthella density and numbers of zooxanthellae in host cells—Six branches each of *Acropora* sp., *S. hystrix,* and *S. pistillata* were exposed to full UVR in one half of the experimental aquarium for 15 d, while another six branches of each species served as controls shielded from UVR in the other half of the aquarium. Fluences of UVR and PAR were as stated for the kinetics experiment with *S. pistillata.* Three branches of each species from each treatment were used for the determination of number of zooxanthellae per host cell. Corals were crushed, placed in a 50-ml Ehrlenmeyer flask, and macerated by agitation on a wrist-action shaker as described by Muscatine and Cernichiari (1969). An aliquot of the suspension was taken for analysis of total protein (see below). Intact host cells were then processed and counted as described in Muscatine et al. (1998). Maceration, sampling, and counting were carried out between 1000 and 1600 h, and cells were observed within 1 h of maceration. Host cells containing one or more endosymbiotic algae were recognized as in Muscatine et al. (1998) by the presence of host cytoplasm (fluorescein diacetate staining) and a host cell nucleus (4′,6-diamidino-2-phenylindole (DAPI) staining). Approximately 500 host cells from each colony were observed and ranked according to the number of zooxanthellae (from one to eight) that each contained. The cell-specific density was estimated as: CSD $= \Sigma(f_i \times r_i)/\Sigma f_i$, where f_i is the frequency of occurrence of host cells in each rank (r_i). The frequency of algal cells undergoing cytokinesis was also recorded.

The other three colonies from each treatment were used for analysis of total number of zooxanthellae and total colony protein. Tissues were removed using an air-pick, homogenized, and two aliquots taken for measurement of protein and numbers of zooxanthellae. Zooxanthellae were counted in a Neubauer cell.

The likelihood chi-square test (*G*-test; SYSTAT) was used to compare the zooxanthella-frequency distributions between UV-exposed and control colonies. Because of the low frequency of host cells containing more than two zooxanthellae, the frequencies of cells containing from three to eight algae were summed in the foregoing analyses. Percentages of zooxanthellae dividing in UV-exposed and control colonies were compared using unpaired *t*-tests of arcsin-transformed data. Effects of UVR on total numbers of zooxanthellae per mg colony protein and on CSD were compared using unpaired *t*-tests.

Analysis of chlorophylls and protein—Chls *a* and c_2 were extracted from colonies of *S. pistillata* and analyzed as described in Romaine et al. (1997). Total protein was solubilized and measured as in Romaine et al. (1997).

Analysis of MAAs—Fresh methanolic extracts (9 ml for each piece) were scanned spectrophotometrically from 200 to 750 nm. Each extract was then passed through two C-18 Sep-Pak Plus cartridges (Waters Corp.) in series to remove chromatographically intractable materials; care was taken to collect the void volume remaining in the cartridges to minimize loss of material (cf. Teai et al. 1997). Finally, all extracts were evaporated to dryness under a stream of N_2 gas at room temperature, lyophilized to remove residual water, and stored at −20°C before analysis in Maine. Extraction efficiency for MAAs was conservatively assumed to be 95% (Shick et al. 1995).

Briefly, dried extracts were redissolved in deionized water and subjected to reverse-phase HPLC on a Phenosphere C-8 column (4.6 mm inner diameter × 250 mm) (Phenomenex) protected by a Brownlee Spheri-5 RP-8 guard (4.6 mm inner diameter × 30 mm) (Applied Biosystems) in a mobile phase of 0.1% acetic acid in 55% aqueous methanol. The eluate was monitored at 310, 320, and 334 nm with a Waters 490E detector and peak areas measured with a Waters 746 integrator. Common MAAs were identified by their retention time and wavelength ratio and by cochromatography with authenticated standards provided by W. C. Dunlap (Australian Institute of Marine Science, Townsville).

Extracts of a colony containing several additional, unknown MAAs were lyophilized and sent to T. Teai (Institut Malardé, Papeete, French Polynesia), who identified and quantified mycosporine-methylamine:serine and mycosporine-methylamine:threonine, and to J. Wu Won (AIMS), who investigated the possible presence of sulfated MAAs using the sulfate isolates prepared by Wu Won et al. (1997). Although the original nuclear magnetic resonance spectra indicated the presence of only one compound in the palythine-serine sulfate isolate, newer chromatography revealed a second peak in that isolate. An unknown peak in our extract that absorbed maximally at 320–321 nm cochromatographed with one of the peaks in the isolate (J. Wu Won pers. comm.). Thus, our unknown may be palythine-serine sulfate or an isomer or decomposition product of this MAA. Additional aliquots of our extract were retained at the University of Maine and used as secondary standards for quantifying the foregoing MAAs on our HPLC. Palythine-serine was identified at the Institut Malardé and was quantified using the calibration factor for palythine (Teai 1996 and pers. comm.). Similarly, the calibration factor for palythine was used to quantify the putative palythine-serine sulfate, no quantitative standard for this MAA being available. It is unlikely that either the addition of the $-CH_2OH$ group to the amino acid moiety in palythine-serine, or the sulfation of this molecule would substantially affect its extinction coefficient at 320 nm (W. C. Dunlap pers. comm.), justifying our method of tentative quantification. The independent quantitative analyses at the University of Maine and Institut Malardé of mycosporine-glycine, shinorine, porphyra-334, mycosporine-2 glycine, palythine, and palythinol agreed to within an average of 10%.

Results

Kinetics of accumulation of MAAs—Spectrophotometry revealed a large absorption peak between 325 and 329 nm

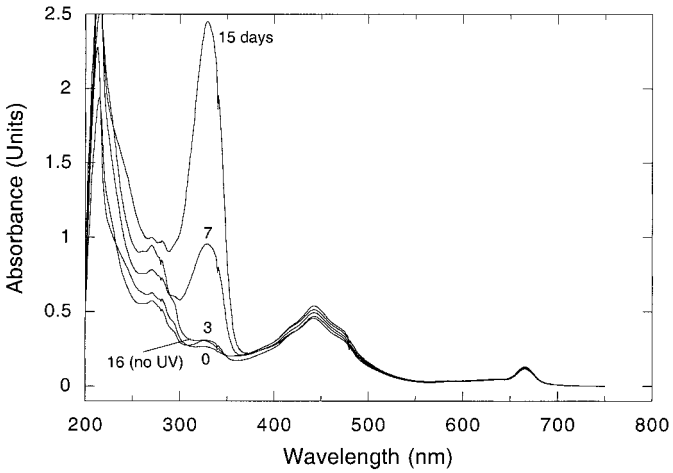


Fig. 2. Spectrophotometric scans of methanolic extracts of *S. pistillata* exposed to UVR for 0, 3, 7, and 15 d and of control colonies held simultaneously in the same aquarium for 16 d but shielded from UVR.

in extracts of colonies of *S. pistillata* exposed to UVR, indicating the presence of MAAs (Fig. 2). This peak (normalized to those for algal chlorophyll and accessory pigments) was all but absent from colonies unexposed to UVR (days 0 and 16) and from colonies exposed to UVR for only 3 d, and increased in magnitude with the duration of UV exposure.

HPLC analyses of these extracts confirmed the presence of at least 10 MAAs (Fig. 3). The concentrations of individual (Fig. 4) and total (Fig. 5) MAAs increased with cumulative exposure to UVR, the increase being linear for porphyra-334 and mycosporine-2 glycine, best described by an exponential increase in five others and more complexly in the remaining three (Fig. 4). A lag period of 3–7 d occurred in all MAAs except for mycosporine-glycine that showed no lag and palythine-serine sulfate that did not increase in concentration until after 15 d of UV exposure. The rate constant, *K*, ranged from 0.117 to 0.173 (mean = 0.153) in all of the exponentially increasing MAAs, implying similar kinetics for their accumulation. The exponential regression gave a marginally better fit than the linear for total MAA accumulation (Fig. 5). Seven of 10 MAAs (and total MAAs) showed significantly higher levels in colonies after 15 d of UV exposure than in controls shielded from UVR for 16 d (Figs. 4, 5). Only one MAA, shinorine, changed significantly in concentration between day 0 (0.23 nmol mg^{-1} protein ± 0.06 SE) and day 16 (0.02 ± 0.01 nmol mg^{-1} protein) in the UV-shielded colonies (Fig. 4). Total MAA concentration (Fig. 5) also declined in the absence of UVR from day 0 (3.42 ± 0.14 nmol mg^{-1} protein) to day 16 (2.17 ± 0.24 nmol mg^{-1} protein), although these decreases are small compared with MAA increases under UVR in the unshielded half of the aquarium.

UVR apparently decreased the concentrations of Chls *a* and c_2, but not total protein, in corals during 15 d of exposure (Fig. 6). Concentrations of both Chls *a* and c_2 at day 15 were significantly lower than on all other days, which did not differ among themselves. Some of this decline may have been owing to confinement in the experimental aquarium or

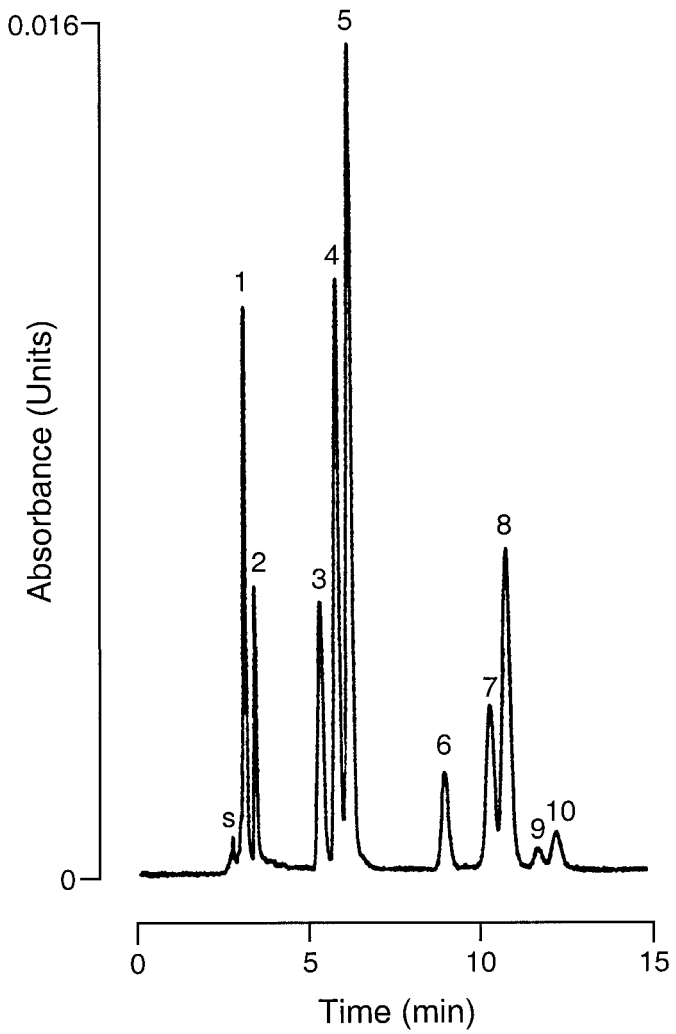
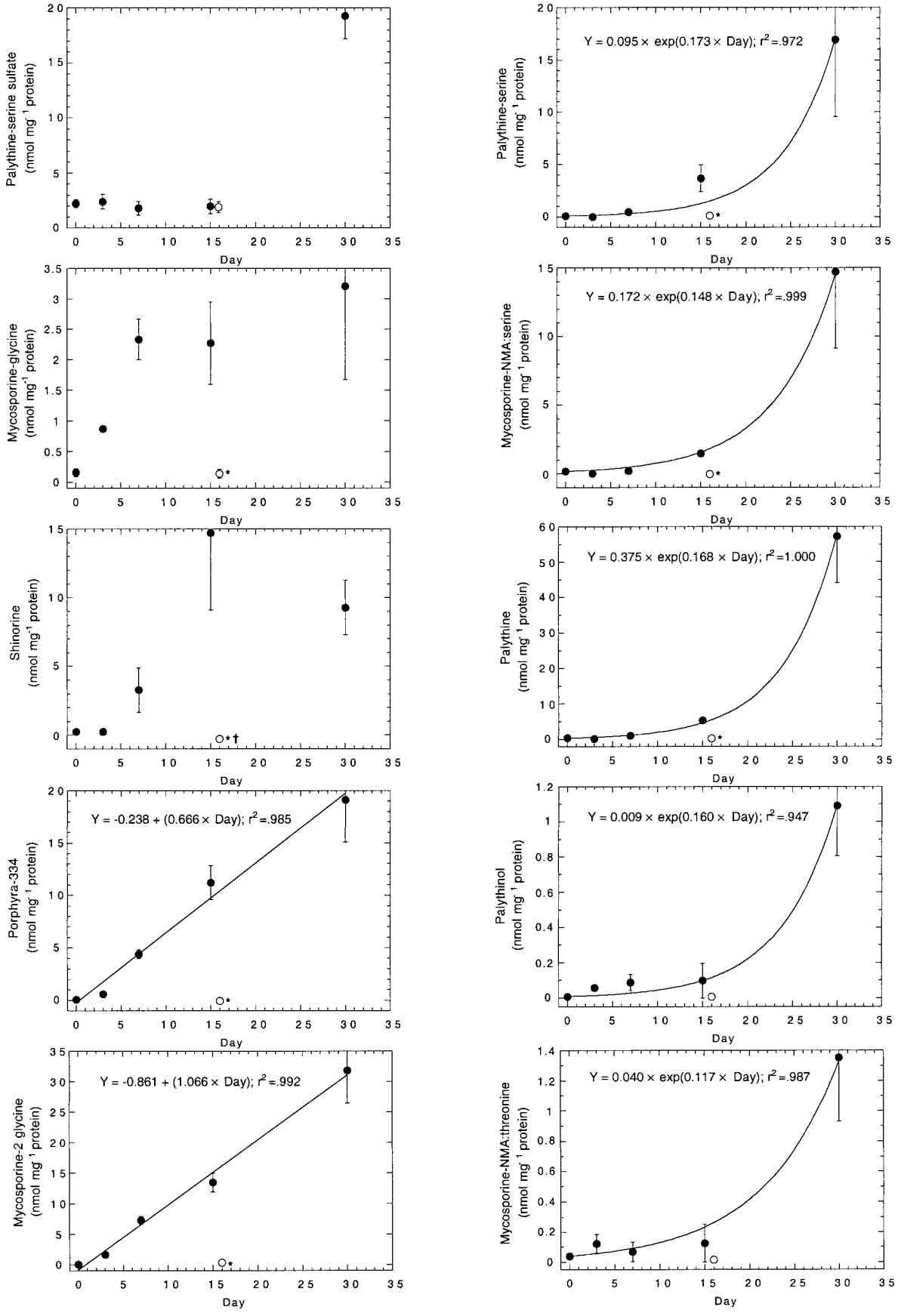


Fig. 3. Chromatogram showing 10 MAAs present in a specimen of *S. pistillata* exposed to UVR for 15 d. Detection at 310 nm. s = salt (refractive); 1 = palythine-serine sulfate (λ_{max} = 320 nm; tentative identification: see Materials and methods); 2 = mycosporine-glycine (λ_{max} = 310 nm); 3 = shinorine (λ_{max} = 334 nm); 4 = porphyra-334 (λ_{max} = 334 nm); 5 = mycosporine-2 glycine (λ_{max} = 331 nm); 6 = palythine-serine (λ_{max} = 320 nm); 7 = mycosporine-NMA:serine (λ_{max} = 325 nm); 8 = palythine (λ_{max} = 320 nm; 9 = palythinol (λ_{max} = 332 nm); 10 = mycosporine-NMA: threonine (λ_{max} = 328 nm).

cessation of feeding (Titlyanov et al. 1999) and not just to UV exposure, although the data are equivocal. The concentration of Chl c_2 in colonies after 15 d of UV exposure was less than that in day 16 control colonies unexposed to UV in the same tank (indicating a specific effect of UVR on Chl c_2 content), but Chl *a* did not differ between UV-exposed and UV-shielded colonies (days 15 and 16, respectively), amid much variation in the former. The Chl $a:c_2$ ratio was little changed, ranging from 1.42 to 1.70 among all treatments.

Differential effects of UVA and UVB on MAA accumulation—Spectrophotometric scans of extracts of corals normalized to absorption by algal pigments suggested a primary


Palythine-serine sulfate (nmol mg⁻¹ protein)
Day
Palythine-serine (nmol mg⁻¹ protein)
Y = 0.095 × exp(0.173 × Day); r² = .972
Mycosporine-glycine (nmol mg⁻¹ protein)
Mycosporine-NMA:serine (nmol mg⁻¹ protein)
Y = 0.172 × exp(0.148 × Day); r² = .999
Shinorine (nmol mg⁻¹ protein)
Palythine (nmol mg⁻¹ protein)
Y = 0.375 × exp(0.168 × Day); r² = 1.000
Porphyra-334 (nmol mg⁻¹ protein)
Y = -0.238 + (0.666 × Day); r² = .985
Palythinol (nmol mg⁻¹ protein)
Y = 0.009 × exp(0.160 × Day); r² = .947
Mycosporine-2 glycine (nmol mg⁻¹ protein)
Y = -0.861 + (1.066 × Day); r² = .992
Mycosporine-NMA:threonine (nmol mg⁻¹ protein)
Y = 0.040 × exp(0.117 × Day); r² = .987

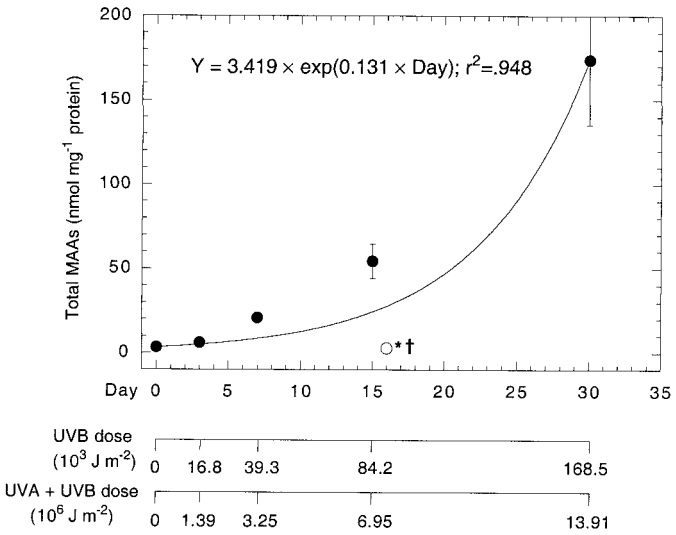


Fig. 5. Accumulation of total MAA concentration in *S. pistillata* during exposure to UVR (filled circles) shown as days of exposure and cumulative, unweighted total (UVA + UVB) and UVB doses. Also shown are data for control colonies unexposed to UVR (open circles) in the same aquarium for 16 d. Values are means ± SE; where error bars are not visible, they are smaller than the size of the symbol. $n = 3$ in all cases. ANOVA indicates a significant effect of duration of UV exposure on total MAA concentration ($F = 27.833$; $P = 0.0001$). Equations relating total MAA concentration to unweighted total (UVA + UVB) and UVB doses, respectively, are: $Y = 3.419 \times \exp(0.283 \times \text{total UV})$ and $Y = 3.419 \times \exp(0.023 \times \text{UVB})$. Asterisk (*) indicates that MAA concentration in control colonies differs significantly from that in UV-exposed corals at day 15 (*t*-test, $P < 0.05$). Dagger (†) indicates a significant difference in MAA concentration between control colonies on day 16 and colonies on day 0 (*t*-test, $P < 0.05$).

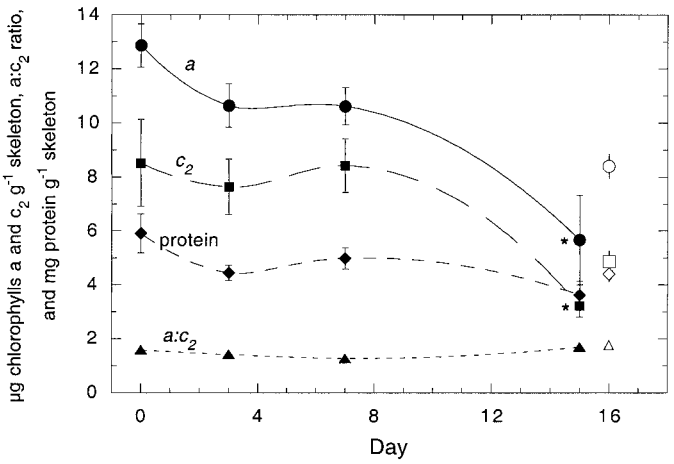


Fig. 6. Concentrations of Chls *a* (filled circles) and c_2 (filled squares) and protein (filled diamonds) in *S. pistillata* exposed to UVR for 15 d and in control colonies (open symbols) unexposed to UVR in the same aquarium for 16 d. Values are means ± SE; $n = 3$ in all cases. Also shown is the ratio of Chl $a : c_2$ (filled triangles, experimental; open triangle, control). ANOVA indicates significant effects of UV exposure on concentrations of Chls *a* and c_2 ($P < 0.05$ in both cases) but no effect on protein ($P > 0.05$). Asterisks (*) indicate that concentrations of Chl *a* and c_2 on day 15 are significantly different from all other days of UV exposure, which do not difer among themselves (SNK tests). Chl c_2 differs between UV-exposed colonies on day 15 and controls on day 16 (*t*-test, $P < 0.05$), but Chl *a* does not.

effect of combined UVA + UVB, and possibly a lesser effect of UVA alone, on MAA concentration (Fig. 7A). ANOVA indicated that total MAA concentration varied significantly among colonies exposed to PAR & UVA + UVB, PAR & UVA, or PAR only for 15 d (Fig. 7B), as did the concentrations of 6 of 10 individual MAAs (Table 1). Among these, five showed no separate effect of UVA on MAA concentration (i.e., PAR & UVA + UVB > PAR & UVA = PAR only) and only one (the least concentrated MAA, palythinol) showed a significant effect of UVA alone (i.e., PAR & UVA + UVB = PAR & UVA > PAR only). Likewise, total MAA concentration was significantly affected by UVA + UVB but not by UVA alone (Fig. 7B).

Effect of glyphosate on MAA accumulation—Spectrophotometric scans of extracts of corals normalized to absorption by algal pigments indicated both an enhancement of MAA concentration by UVR and an inhibition of this by glyphosate (Fig. 8A). Only nine MAAs were detected in pieces of coral used in this experiment; palythine-serine was not detected in any specimen. Concentrations of eight of these nine MAAs (Table 2), and total MAA concentration (Fig. 8B), showed a significant treatment effect among corals exposed or unexposed to UVR, with or without the addition of glyphosate. The most common pattern of response, seen in total MAA concentration and in five of the eight MAAs responding to the treatments, was for corals receiving UVR and no glyphosate to have the highest MAA concentrations, with no significant differences among the other treatments. Thus, in most cases the elevation of MAA concentration by UVR was abolished by exposure to glyphosate, and glyphosate did not affect the MAA concentration in colonies unexposed to UVR. The level of palythine-serine sulfate (the second most-concentrated MAA in these specimens) was unaffected by exposure to UVR during the 7 d of this experiment (as in the kinetics experiment: Fig. 4), but there was an inexplicable increase in this MAA in glyphosate-treated colonies

←

Fig. 4. Accumulation of individual MAAs in *S. pistillata* during exposure to UVR (filled circles). Also shown are data for control colonies unexposed to UVR (open circles) in the same aquarium for 16 d. Values are means ± SE; where error bars are not visible, they are smaller than the size of the symbol. $n = 3$ in all cases. ANOVA indicates a significant effect of duration of UV exposure on concentration of MAAs ($P < 0.05$ in all cases). Asterisks (*) indicate significant differences in MAA concentration between control colonies and those exposed to UVR at day 15 (*t*-test, $P < 0.05$). Dagger (†) indicates a significant difference between the shinorine concentration in control colonies on day 16 and colonies on day 0 (*t*-test, $P < 0.05$). Equations for the regressions describing the relationship between MAA concentration and days of UV exposure are given for MAAs that increase linearly or exponentially.

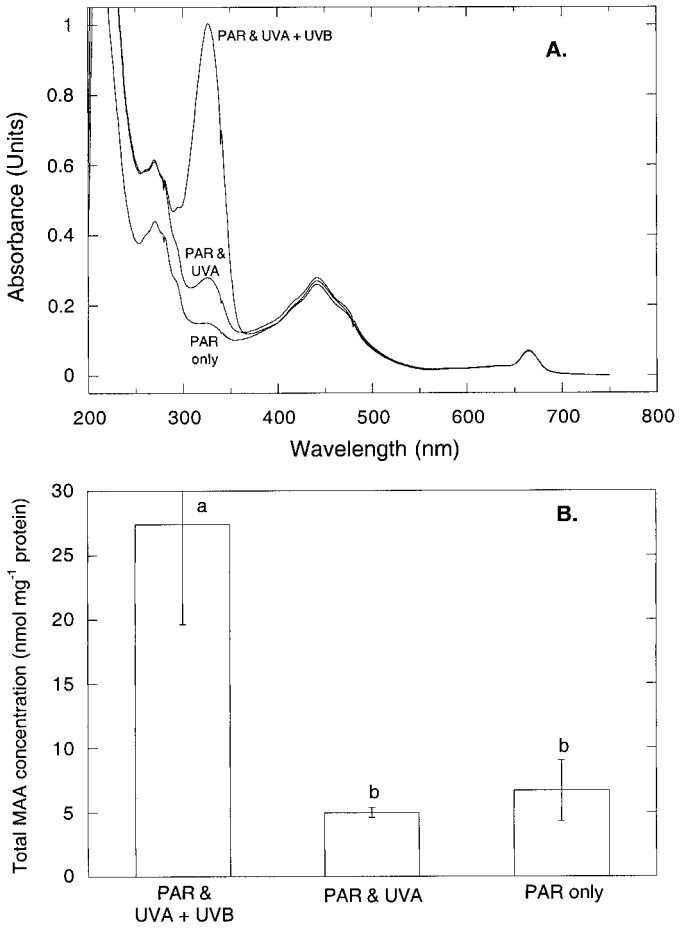


Fig. 7. (A) Spectrophotometric scans of methanolic extracts of *S. pistillata* exposed to PAR & UVA + UVB, PAR & UVA, or PAR only for 15 d. All specimens were exposed to the same level of PAR. (B) Total MAA concentration in *S. pistillata* exposed to PAR & UVA + UVB, PAR & UVA, or PAR only for 15 d. Values are means ± SE; $n = 3$ in all cases. ANOVA indicates a significant treatment effect ($F = 7.089$; $P = 0.026$). Treatment means that do not differ significantly from each other at $P < 0.05$ in SNK tests share superscripts.

unexposed to UVR (Table 2). Mycosporine-methylamine: threonine varied only between UV-exposed colonies exposed and unexposed to glyphosate but not among any other treatments. Glyphosate did not completely inhibit the UV-induced increase in shinorine, with the concentration of this MAA in colonies exposed to UV + glyphosate being intermediate to UV-exposed, uninhibited colonies and the groups unexposed to UVR. Glyphosate significantly inhibited the UV-induced accumulation of total MAAs (Fig. 8B).

Effects of UVR on zooxanthella density and numbers of zooxanthellae in host cells—Consistent with the decline in Chls *a* and c_2 in the other colonies of *S. pistillata*, the number of zooxanthellae per mg colony protein (Fig. 9) declined significantly in this species and in *Acropora* sp. following 15 d of exposure to UVR (in the presence of PAR). An apparent decline in zooxanthella density in *S. hystrix* was not significant in these highly variable samples.

In all control colonies, host cells containing a single zooxanthella (singlet) predominated, the frequency of singlets being 85% in *Acropora* sp., 60% in *S. hystrix*, and 79% in *S. pistillata* (Fig. 10). *G*-tests indicated that frequency distributions of zooxanthellae per host cell within treatments were homogeneous in all species but that UV exposure significantly affected this distribution (Fig. 10; Table 3). In the *S. pistillata* colonies, for example, singlets decreased from 79% in the control to 64% under UVR, while doublets increased from 18% in the control to 28% under UVR. The effect of UVR in decreasing the frequency of singlets and increasing the frequencies of, especially, doublets, triplets, and quadruplets (Fig. 10) resulted in a significant increase in the cell-specific density of zooxanthellae in all species (Fig. 11). Finally, the frequency of zooxanthellae undergoing cytokinesis (Fig. 12) was significantly higher in UV-exposed than in control colonies of *Acropora* sp. and *S. pistillata*, but not in *S. hystrix* (again because of high variability among colonies of the last species).

Discussion

The use of defined radiation sources confirms a primary role of UVR in stimulating the accumulation of 10 MAAs

Table 1. Results of analysis of variance for effects of exposure to PAR & UVA + UVB, to PAR & UVA, and to PAR only on concentration of individual MAAs (nmol mg^{-1} protein) in colonies of *S. pistillata*. All corals were exposed to the same level of PAR. Values are means (±SE), where $n = 3$. Treatment means for each MAA that are not significantly different from each other at $P < 0.05$ in Student–Newman–Keuls tests share superscripts.

MAA	PAR & UVA + UVB	PAR & UVA	PAR only	*F* value	*P* value
Palythine-serine sulfate	3.95 (0.91)	3.81 (0.29)	5.86 (2.18)	0.695	0.535
Mycosporine-glycine	0.73^a (0.01)	0.09^b (0.02)	0.13^b (0.01)	37.629	0.0004
Shinorine	1.99^a (0.82)	0.01^b (0.01)	0.00^b (0.00)	5.836	0.039
Porphyra-334	5.02^a (1.76)	0.09^b (0.03)	0.02^b (0.02)	7.932	0.021
Mycosporine-2 glycine	7.61^a (1.41)	0.46^b (0.20)	0.11^b (0.03)	26.354	0.001
Palythine-serine	1.20 (0.77)	0.00 (0.00)	0.00 (0.00)	2.455	0.166
Mycosporine-NMA : serine	1.54 (0.69)	0.04 (0.01)	0.08 (0.08)	4.504	0.064
Palythine	5.05^a (1.31)	0.15^b (0.03)	0.24^b (0.14)	13.671	0.006
Palythinol	0.14^a (0.03)	0.09^a (0.02)	0.01^b (0.01)	8.138	0.020
Mycosporine-NMA : threonine	0.16 (0.06)	0.24 (0.01)	0.25 (0.17)	0.235	0.798

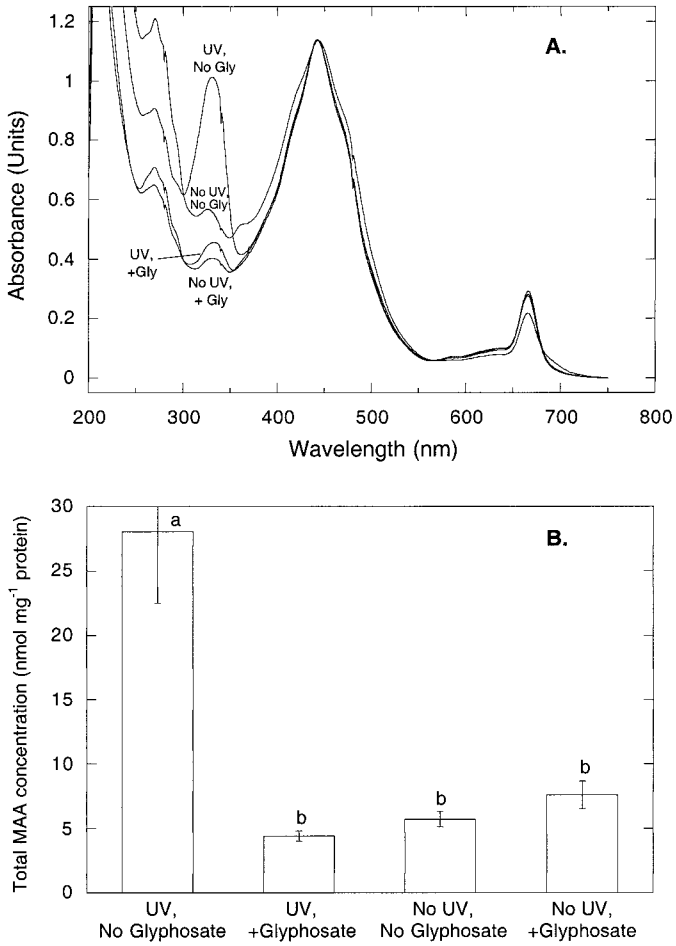


Fig. 8. (A) Spectrophotometric scans of methanolic extracts of *S. pistillata* exposed to UV but not to glyphosate (UV, no Gly), UV + 1 mM Gly, no UV and no Gly, and no UV + 1 mM Gly, for 7 d. All colonies were exposed to the same level of PAR. (B) Total MAA concentration in *S. pistillata* exposed to UV but not to glyphosate (UV, no Gly), UV + 1 mM Gly, no UV and no Gly, and no UV + 1 mM Gly, for 7 d. All colonies were exposed to the same level of PAR. Values are means ± SE; $n = 3$ in all cases. ANOVA indicates a significant treatment effect ($F = 15.429$; $P = 0.0011$). Treatment means that do not differ significantly from each other at $P < 0.05$ in SNK tests share superscripts.

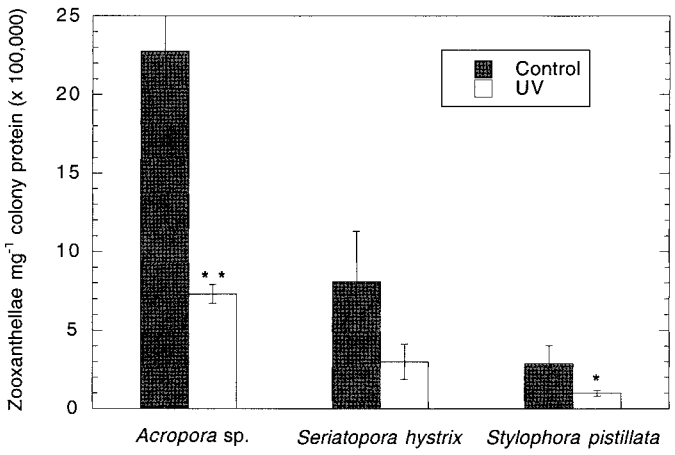
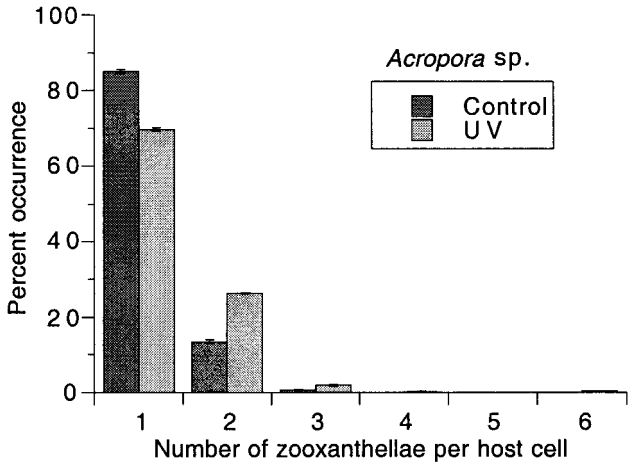


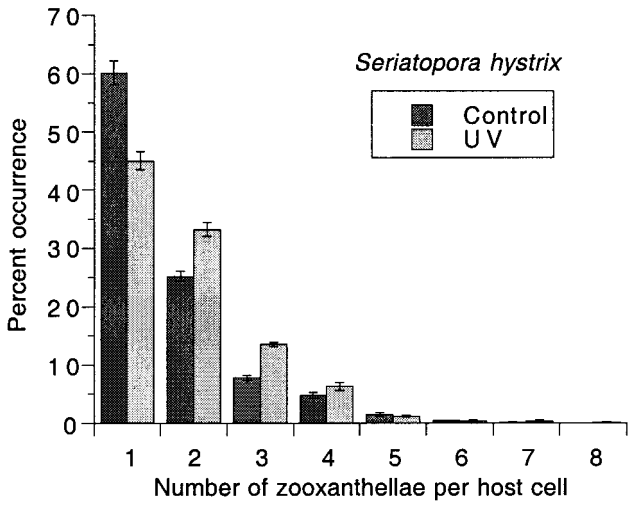
Fig. 9. Number of zooxanthellae per mg colony protein in three species of coral after 15 d of exposure to UVR in the presence of PAR and in control colonies exposed to PAR only. Values are means ± SE; $n = 3$ in all cases. Unpaired *t*-tests indicate that UV-exposed specimens differ from controls at $P < 0.05$ (*) or $P < 0.01$ (**).

in unfed specimens of *S. pistillata* under constant levels of PAR, water movement, and temperature. Experimental colonies were irradiated with an artificial UVR source producing combined UVA and UVB fluences equivalent to what corals would experience at 6–9 m depth in clear reef water (see, e.g., table 1 in Shick et al. 1996*b*), and after 30 d of exposure, the total MAA concentration (174 nmol MAA mg^{-1} protein) was the same as that in freshly collected *Acropora microphthalma* living at those depths (≈165 nmol mg^{-1} protein; Shick et al. 1995) and similar to the concentrations in *Acropora* spp. (≈100 nmol mg^{-1} protein; Dunlap et al. 1986) and *Porites astreoides* (≈250 nmol mg^{-1} protein; Gleason 1993) at similar depths. The MAA concentrations in our experimental corals are also similar to those reported in a diversity of Caribbean and Hawaiian corals in several genera freshly collected at 5–10 m, generally 150–200 nmol mg^{-1} protein (Kuffner et al. 1995; Banaszak et al. 1998). At an average molecular weight of 300 Da for MAAs, and assuming conservatively that protein constitutes 60% of the organic mass of coral tissues (cf. Zamer et al. 1989; Achituv et al. 1994), the foregoing MAA concentrations are equivalent to ≈1.8–4.5% of the total organic mass of the

Table 2. Results of analysis of variance for effects of exposure to UVR and glyphosate (Gly) on accumulation of individual MAAs (nmol mg^{-1} protein) in colonies of *S. pistillata*. All corals were exposed to the same level of PAR. Values are means (±SE), where $n = 3$. Treatment means for each MAA that are not significantly different from each other at $P < 0.05$ in Student–Newman–Keuls tests share superscripts.

MAA	UV, no Gly	UV, + Gly	No UV, + Gly	No UV, no Gly	*F* value	*P* value
Palythine-serine sulfate	$3.98^{a,b}$ (0.67)	2.22^{b} (0.62)	6.39^{a} (1.03)	$4.66^{a,b}$ (0.52)	5.51	0.024
Mycosporine-glycine	1.66^{a} (0.47)	0.24^{b} (0.04)	0.47^{b} (0.02)	0.20^{b} (0.03)	8.62	0.007
Shinorine	0.89^{a} (0.02)	0.13^{b} (0.05)	0.00^{c} (0.00)	0.02^{c} (0.01)	248.04	0.0001
Porphyra-334	3.27^{a} (0.09)	0.11^{b} (0.06)	0.07^{b} (0.04)	0.06^{b} (0.02)	797.62	0.0001
Mycosporine-2 glycine	15.98^{a} (4.35)	2.15^{b} (0.25)	0.25^{b} (0.06)	0.20^{b} (0.03)	12.18	0.002
Mycosporine-NMA : serine	0.07 (0.04)	0.02 (0.02)	0.02 (0.02)	0.05 (0.02)	0.87	0.497
Palythine	1.62^{a} (0.30)	0.24^{b} (0.08)	0.29^{b} (0.11)	0.35^{b} (0.05)	15.88	0.001
Palythinol	0.41^{a} (0.03)	0.11^{b} (0.04)	0.02^{b} (0.01)	0.03^{b} (0.01)	48.36	0.001
Mycosporine-NMA : threonine	0.18^{a} (0.04)	0.05^{b} (0.02)	$0.08^{a,b}$ (0.01)	$0.14^{a,b}$ (0.03)	4.56	0.038





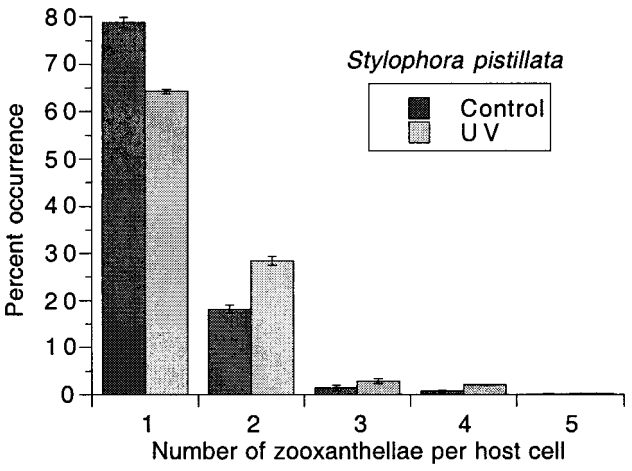


Fig. 10. Frequency distribution of number of zooxanthellae per host cell in three species of coral after 15 d of exposure to UVR in the presence of PAR, and in control colonies exposed to PAR only. Values are means ± SE; $n = 3$ in all cases. All species show a significant effect of UVR on the distribution (see Table 3).

Table 3. Results of likelihood chi-square test (G test) for differences in the frequency distribution of number of zooxanthellae per host cell in control and in UV-exposed colonies of three species of coral. Data are shown in Fig. 10. Host cells containing from three to eight zooxanthellae were pooled for statistical analysis. Intra control refers to differences in the zooxanthella distribution among replicate control colonies; intra UV refers to differences in the zooxanthella distribution among replicate UV-exposed colonies; and inter control × UV refers to the comparison of the zooxanthella distribution between control and UV-exposed colonies.

Source	Value	df	Probability
Acropora sp.			
Intra control	0.313	4	0.989
Intra UV	0.692	4	0.952
Inter control × UV	77.289	2	0.999×10^{-15}
Total	78.294	10	0.108×10^{-11}
S. hystrix			
Intra control	1.723	4	0.787
Intra UV	1.361	4	0.851
Inter control × UV	66.218	2	0.422×10^{-14}
Total	69.302	10	0.605×10^{-10}
S. pistillata			
Intra control	2.235	4	0.693
Intra UV	0.546	4	0.969
Inter control × UV	57.283	2	0.364×10^{-12}
Total	60.064	10	4.0×10^{-9}

tissues. This is well below the extraordinary values in other Caribbean field specimens of *Montastraea* spp. and *Diploria strigosa* (Banaszak et al. 1998), where a concentration of 1,832 nmol total MAAs mg^{-1} protein (in *Montastraea cavernosa* at 7.6 m depth) would be equivalent to 0.53 mg of the identified MAAs per mg protein or >30% of the total organic mass of the tissues. On balance, the MAA concentrations in our experimental colonies are consistent with

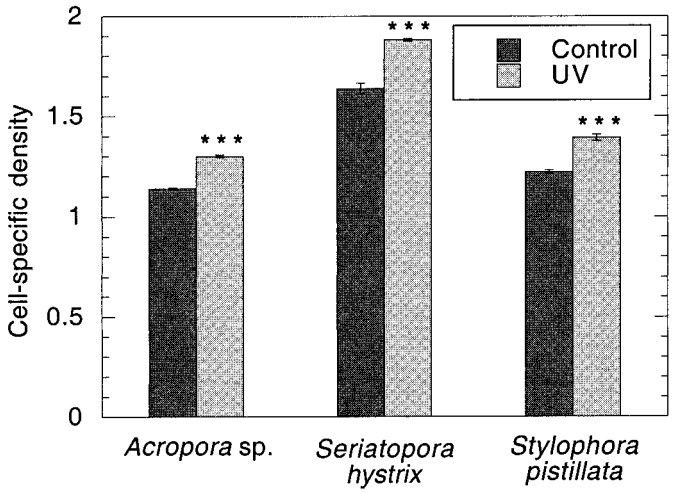


Fig. 11. Cell-specific density of zooxanthellae in host cells of three species of corals after 15 d of exposure to UVR in the presence of PAR and in control colonies exposed to PAR only. Values are means ± SE; $n = 3$ in all cases. Unpaired t-tests indicate that UV-exposed specimens differ from controls at $P < 0.001$ (***).

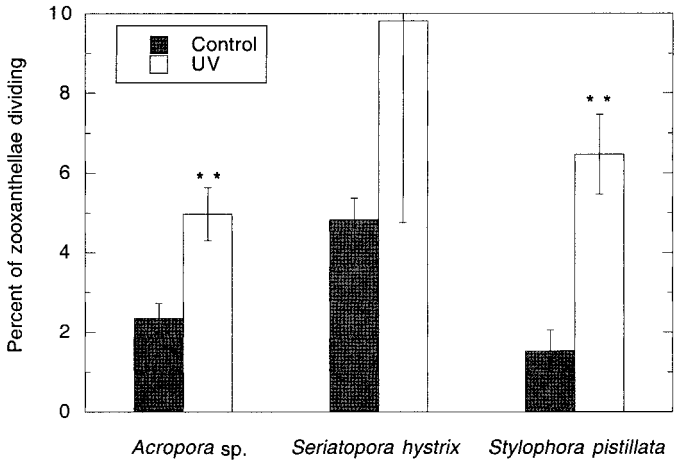


Fig. 12. Percentage of zooxanthellae dividing in three species of coral on day 15 of exposure to UVR in the presence of PAR and in control colonies unexposed to UVR. Values are means ± SE; $n = 3$ in all cases. Unpaired *t*-tests indicate that UV-exposed specimens differ from controls at $P < 0.01$ (**).

most of those reported for corals collected in the field and indicate that laboratory experiments can be used to evaluate quantitatively the factors controlling MAA synthesis with application to conditions in nature.

Our studies uniquely examined the effects of UVR on corals that had been maintained in an indoor mesocosm under minimal levels of UVR for 4 yr since their collection. These background UVA and UVB fluences are comparable to those prevailing in clear seawater at depths of >20–30 m on coral reefs in nature (cf. Gleason and Wellington 1995; Lesser 1995; Dunne and Brown 1996; Shick et al. 1996*b*), a depth range over which there is no apparent effect of ambient irradiation on MAA concentration (Dunlap et al. 1986; Shick et al. 1995). Still, the presence of measurable amounts of all 10 MAAs in our colonies prior to experimental UV irradiation and of MAAs in colonies living deeper than 30 m in nature indicate that the biochemical pathway leading to their synthesis is operating and is either extraordinarily sensitive to UVR or is constitutive and operates at a low rate without a UV cue. Such results are consistent with the reports by Banaszak and Trench (1995*a*) and Lesser (1996) that MAA synthesis in *Symbiodinium* spp. proceeds in the absence of UVR.

Experimental evidence for wavelength-specific effects in stimulating MAA biosynthesis (or more generally, accumulation) in corals is fragmentary. The pioneering experiments by Jokiel and York (1982) reveal that exposing colonies of *Pocillopora damicornis* to full solar UVR results in higher concentrations of S-320 compounds than in colonies exposed to the same fluence of PAR but shielded from UVR. Scelfo (1986) reports fluctuations in S-320 concentrations attributable to increases in both solar PAR and UVR following transplantation of *Montipora verrucosa* from moderate depth to shallow aquaria under full sunlight.

Few studies have both manipulated the spectral irradiance and measured individual MAAs. Transplantation of colonies of *P. astreoides* from moderate to shallow depths led (after 104 d) to increases in their concentrations of shinorine and asterina-330 (but not mycosporine-glycine or palythine) when UVR was present but not when it was eliminated by a UV-cutoff filter (Gleason 1993). His results thus demonstrate a dose-effect of UVR but not of PAR, on the accumulation of particular MAAs in this coral. Jokiel et al. (1997) report separate and interacting effects of PAR and UVR (the latter having the greater effect) on concentrations of palythine and palythinol in *P. damicornis* but no effect of irradiance on mycosporine-glycine during 7 d of exposure.

Our results with colonies under controlled conditions of PAR and water movement, and using defined radiation sources, clearly implicate UVB (but not UVA) as the principal stimulant for accumulation of MAAs in *S. pistillata.* In the only other study of separate effects of UVA and UVB on MAA accumulation in a coral, Baker (1995) reports higher concentrations of mycosporine-glycine in planulae from colonies of *P. damicornis* receiving full solar irradiance for 1 month than in planulae from conspecific colonies shielded from UVB, indicating an effect of UVB similar to our results. Conversely, UVA and PAR increase the concentrations of asterina-330 and mycosporine-glycine : valine above the levels obtained when UVB is also included (UVA + UVB and PAR). There is no significant effect of UVA or UVB on the concentrations of shinorine or porphyra-334.

There are few studies of the effects of UVR on MAA synthesis by zooxanthellae in culture. In some cases zooxanthellae in culture produce no MAAs whether or not UVR is present (Banaszak and Trench 1995*a*), whereas in others certain MAAs are synthesized under PAR alone, and in still others MAA synthesis is greatest under combined UVA + UVB and PAR (Banaszak and Trench 1995*a*; Lesser 1996).

Considering interspecific differences and wavelength-specific effects on accumulating MAAs separately from any adaptive advantage of doing so (cf. Baker 1995; Riegger and Robinson 1997), the foregoing results on invertebrate–zooxanthella symbioses seem as mixed as those on cyanobacteria, phytoplankton, and macroalgae, where separate and interacting effects of PAR, UVA, and UVB are documented (Carreto et al. 1990*b*; Garcia-Pichel et al. 1993; Riegger and Robinson 1997; Xiong et al. 1997; Hannach and Sigleo 1998; Karsten et al. 1998). The relative importance (indeed, the actual operation) of postulated increases in photosynthetic carbon flow into MAA biosynthetic pathways caused by higher PAR (Jokiel et al. 1997), wavelength-specific photoregulatory systems (Riegger and Robinson 1997), and separate molecular mechanisms for UVR- and PAR-induced synthesis of MAAs (Karsten et al. 1998) remains to be validated.

Any examination of the mechanisms and regulation of MAA synthesis must consider the shikimate pathway. Generalizing from experiments on the biosynthesis of fungal mycosporines (Favre-Bonvin et al. 1987), workers on corals and other phototrophic symbioses have generally assumed that MAAs are produced via the shikimate pathway (for review, see Bandaranayake 1998; Dunlap and Shick 1998), although this has not heretofore been verified experimentally. Moreover, lack of the shikimate pathway in animals and its presumed presence in zooxanthellae as in other photoautotrophic algae that synthesize essential, aromatic amino acids

has led to the related widespread assumption that MAAs in symbiotic cnidarians are produced by the algal partner (e.g., Dunlap and Chalker 1986 and most authors since then).

Our experiments using glyphosate, a specific inhibitor of the shikimate pathway, provide evidence that MAAs in *S. pistillata* are indeed synthesized via this route. Glyphosate at micromolar concentrations is a competitive inhibitor of substrate (phosphoenol pyruvate) binding by 5-enolpyruvyl-shikimate 3-phosphate (EPSP) synthase (Haslam 1993), which occurs late in the pathway, just prior to the chorismate branchpoint leading to essential aromatic amino acids, inter alia. At millimolar concentrations it also inhibits the first two steps in the pathway—3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase and 3-dehydroquinate (DHQ) synthase (Bode et al. 1984). This is important because in the biosynthetic scheme proposed by Favre-Bonvin et al. (1987), the carbon ring structure of mycosporines is derived from DHQ produced early in the pathway, well before the EPSP synthase-catalyzed step. Exposing *S. pistillata* to 1 mM glyphosate all but completely inhibits UV-stimulated MAA synthesis, consistent with the evidence that the mycosporine base structure originates in the early shikimate pathway.

Our data also suggest that MAAs in *S. pistillata* are produced via a cytosolic DAHP synthase-Co^{2+} isozyme (which is glyphosate-sensitive) and not the glyphosate-resistant DAHP synthase-Mn^{2+} isozyme in the chloroplast (Rubin et al. 1982). However, the presence of the latter isozyme might also account for the production of the observed small but statistically significant increases in shinorine despite the use of glyphosate. Alternatively, this increase in shinorine in corals exposed both to UV and to glyphosate compared with unexposed colonies may be owing to shinorine synthesis from a precursor such as DHQ or deoxygadusol (see Dunlap and Shick 1998) formed prior to the application of glyphosate, or originating in the diet.

Our finding that MAAs in *S. pistillata* are synthesized via the early shikimate pathway further implicates the zooxanthellae as the proximal source of these MAAs and complements the data of Banaszak and Trench (1995*a*), who directly show the biosynthesis and translocation of MAAs by zooxanthellae from the scyphozoan *Cassiopeia xamachana.* There is considerable interspecific diversity among corals in their MAA complement (Dunlap and Chalker 1986; Dunlap et al. 1986; Gleason 1993; Baker 1995; Gleason and Wellington 1995; Kuffner et al. 1995; Shick et al. 1995; Drollet et al. 1997; Jokiel et al. 1997; Teai et al. 1997, 1998; Wu Won et al. 1997; Banaszak et al. 1998; Muszynski et al. 1998). Although this might reflect a corresponding metabolic diversity among their zooxanthellae, the situation is more complicated because zooxanthellae cultured in the presence of UVR produce a more restricted suite of MAAs (from 0 to 3: A. T. Banaszak and R. K. Trench pers. comm.) than is found in the corals (up to 10 in the present study of *S. pistillata*). The zooxanthellae isolated from the corals *P. damicornis* (which contains five to seven MAAs: Baker 1995; Kuffner et al. 1995; Jokiel et al. 1997; Teai et al. 1997) and *M. verrucosa* (containing five or six MAAs: Kuffner et al. 1995; Teai et al. 1997) themselves produce no MAAs in culture (Banaszak and Trench pers. comm.), and those from *S. pistillata* produce only one MAA, shinorine (Banaszak, Shick, and Trench unpubl. results).

The foregoing discrepancies might be reconciled if zooxanthellae living in hospite produce more diverse MAAs as compared with the same zooxanthellae cultured in vitro. There have been no experiments to test this possibility directly, and there is evidence from other zooxanthellate anthozoans against qualitative differences in algal photosynthate (glucose, glycerol, and amino acids) produced in hospite versus in vitro (Trench 1971). Greater diversity of MAAs in the holobiont may therefore also arise from yet-undescribed enzymes catalyzing condensation reactions that add or substitute various amino acids and amino alcohols to the central carbon ring, probably in the zooxanthellae but possibly also in the animal host (see Dunlap and Shick 1998).

It remains a fair assumption that the animal moieties of MAA-containing corals, other cnidarians, and diverse invertebrates that harbor zooxanthellae lacking the capacity to produce MAAs do not have the shikimate pathway themselves. This has not been tested directly using enzyme assays or molecular probes of host tissues, but the lack of an MAA branch of the early shikimate pathway is at least suggested by the failure of aposymbiotic hosts to synthesize MAAs when stimulated by UVR (Stochaj et al. 1994; Banaszak and Trench 1995*a*). There is, however, evidence that non-zooxanthellate corals may synthesize essential amino acids (Fitzgerald and Szmant 1997), some of which would necessarily involve the shikimate pathway.

It is also possible that some MAAs in our corals were obtained from the diet, as in the case of heterotrophic animals (Shick et al. 1992; Adams and Shick 1996; Carroll and Shick 1996; Carefoot et al. 1998; Mason et al. 1998). Trophic accumulation is also likely in sea anemones in the genus *Anthopleura,* where both symbiotic and naturally aposymbiotic specimens of *Anthopleura elegantissima* contain MAAs (Stochaj et al. 1994; Banaszak and Trench 1995*a*), the zooxanthellae from this anemone do not synthesize MAAs (Banaszak and Trench 1995*a*), and the nonsymbiotic congener *Anthopleura artemisia* contains MAAs (Shick et al. 1996*a*). The *Artemia* nauplii fed to corals prior to the start of the present experiments contained low levels of shinorine (<0.1 nmol mg^{-1} dry weight; data not shown) and presumably of gadusol (Grant et al. 1985), which via deoxygadusol may be a precursor of MAAs (Dunlap and Shick 1998). However, endogenous concentrations of shinorine in control colonies unexposed to UVR (at most 0.2 nmol mg^{-1} protein) are insufficient to be converted to the total concentration of MAAs that accumulates during exposure to UVR (maximally 174 nmol mg^{-1} protein). It also seems unlikely that dietary gadusol would accumulate to levels sufficient to be converted metabolically into the full complement of MAAs, especially in the absence of an exogenous cue such as UVR. The combined 0.45-μm and 0.22-μm filtrates of 15 liters of seawater supplying the experimental aquarium did not contain detectable MAAs (data not shown), which eliminates this as a source of the compounds. Thus, the bulk of the MAAs that accumulate in *S. pistillata* is synthesized de novo.

The taxonomic diversity within and among species of

Symbiodinium is manifested in differences in their tolerance and physiological performance related to irradiance (Iglesias-Prieto and Trench 1994, 1997). A natural bleaching event following a prolonged period of unusually high water clarity selectively decreased a *Symbiodinium* clade associated with low-irradiance habitats in *Montastraea annularis* and *Montastraea faveolata* (Rowan et al. 1997), which implicates increased solar irradiance at depth as a cause, although an additional effect of temperature cannot be excluded nor are the relative effects of solar PAR and UVR known. Experiments by Gleason and Wellington (1993) support the hypothesis that UVR is involved in such bleaching events at intermediate depths. The former authors opine that MAA concentrations in the coral do not increase rapidly enough to offset short-term increases in UVR during periods of increased water clarity, an observation consistent with the kinetics of MAA accumulation that we document, although there are no studies showing that corals or zooxanthellae differing in their MAA concentration or metabolism vary systematically in their susceptibility to bleaching.

Our experiments were not designed to investigate the phenomenon of coral bleaching, but the results do indicate a bleaching effect in corals acclimatized for years to low fluences of UVR (comparable to those at depths >20 m) upon acute exposure to moderate fluences of UVR (equivalent to those at 6–9 m depth in clear reef water) at constant, environmentally relevant, nonstressful levels of temperature and PAR. In all species tested (*Acropora* sp., *S. hystrix,* and *S. pistillata*) the total density of zooxanthellae in colonies exposed to UVR and PAR for 15 d is about one-third of that in colonies experiencing PAR only. The results are consistent with the foregoing field studies in which increases in UVR during periods of high water clarity are manifested in coral bleaching at intermediate depths.

The net loss of zooxanthellae from our colonies under UVR occurs despite increases in algal cell division and their specific density in host cells. Although studies of zooxanthella division in hospite and in vitro using high fluences of UVR at high PAR reveal either no effect (Banaszak and Trench 1995*b*) or an inhibition by UVR (Jokiel and York 1982; Read 1986; Lesser and Shick 1989; Gleason 1993), some in vitro experiments under light-limited conditions show a stimulation of cell division by UVR (Jokiel and York 1984; Santos 1995). Karentz et al. (1991) attribute such an enhancement of algal growth alternatively to stimulation of photosynthesis by UVR under limiting PAR (Halldal 1968) or to increases in DNA-replicating enzymes necessary for excision repair of UV-induced damage to nucleotides. Because parallel experiments on *S. pistillata* using the same fluences of PAR and UVR as in the study where bleaching occurred revealed no UV-dependent changes in colony photosynthesis (Romaine-Lioud and Shick unpubl.), the latter explanation seems more likely, although experiments designed specifically to examine these hypotheses are needed.

Our experiments apparently are the first to show a stimulation by moderate fluences of UVR on zooxanthella cytokinesis in hospite under environmentally realistic conditions, and also to demonstrate an effect of UVR on zooxanthella density in host cells. If, as Muscatine et al. (1998) argue, there is an optimum CSD of endosymbionts in hospite, then increases in CSD during exposure to UVR are an indication of stress, which when prolonged leads to a destabilization of the symbiosis and eventually to the observed net loss of algae. It is unknown whether the effects of UVR on increased CSD and a higher frequency of host cells having two or more zooxanthellae are owing more to the enhancement of algal cell cytokinesis or to a delay of host cell cytokinesis (which when uninhibited reduces the number of endosymbionts per cell). Exposure to UVR from the same UVA-340 lamp used in our experiments causes a delay in cytokinesis in sea urchin eggs (N. L. Adams pers. comm.), an effect that is offset by high intracellular levels of MAAs (Adams and Shick 1996). The targets of UVR affecting cytokinesis remain unknown, but might include, inter alia, the cytoskeleton (Godar et al. 1993). If the host cytoskeleton is damaged, this might lead not only to an inhibition of cytokinesis in the cell but also to alterations in cytoskeletal interactions with cell-surface integrins. If so, both the host cell and its contained algae might be lost, accounting for the decline in the algal population. It is interesting that the UV-induced loss of zooxanthellae (bleaching) occurs during the 15-d period when UV-enhanced concentrations of protective MAAs have reached only ≈15–30% of their eventual level at 30 d. There are delays of similar duration in MAA accumulation following increases in solar UVR, as well as differences among MAAs in their rates of accumulation, in nature (Drollet et al. 1997).

Interpreting the kinetics of MAA synthesis and accumulation is complicated by the decline in zooxanthella standing crop at the same time that most MAAs are increasing. If indeed shinorine is the only MAA synthesized by the zooxanthellae within *S. pistillata,* as is the case for these zooxanthellae in culture, then the linear increase in concentration of this MAA during the acute exposure of colonies to UVR indicates not only an immediate activation of pre-existing synthetic machinery but also a further increase in specific synthetic activity, because the number of sites (zooxanthellae) of shinorine synthesis are decreasing. Moreover, if shinorine translocated from the zooxanthellae to the host is metabolically converted there to form the additional nine MAAs that accumulate in the coral, this implies a still greater increase in synthesis and export of this substrate under UVR, together with an upregulation of host enzymes involved in bioconversions among MAAs. A similar argument applies if most or all MAAs are synthesized by the zooxanthellae in hospite, where the kinetics might indicate different sensitivities of individual MAA biosynthetic mechanisms to UVR or differences in the time required for enzymic upregulation in the endosymbionts.

Other than shinorine, the most rapidly increasing MAAs in *S. pistillata* are porphyra-334 and mycosporine-2 glycine, algal-type MAAs that differ from shinorine only by the amino acid substituent at C1 in the central ring (see Dunlap and Shick [1998] for structures). Most of the remaining MAAs are structurally more divergent and increase exponentially with similar rate constants, suggesting a time- or UVB dose-dependent upregulation of the enzymes necessary for the biosynthesis of these MAAs or the bioconversion of shinorine (which, suggestively, is the only MAA to decrease in concentration between 15 and 30 d of UV-exposure, when

most other MAAs are decreasing exponentially) or other precursors. Although the zooxanthellae probably provide MAAs or their precursors in the present study, future investigations must also consider exogenous sources such as dietary plankton and seston, as well as bacteria in the surface mucus (Lyons et al. 1998) or coelenteron (Fitzgerald and Szmant 1997) of corals, especially should the zooxanthellae in some MAA-containing corals (like those in *A. elegantissima*) prove not to synthesize MAAs in hospite.

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