Ultraviolet Radiation and Photooxidative Stress in Zooxanthellate Anthozoa: the Sea Anemone *Phyllodiscus semoni* and the Octocoral *Clavularia* sp.

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Abstract

Colonies of the stoloniferan octocoral *Clavularia* sp. and individuals of the aliciid sea anemone *Phyllodiscus semoni* were maintained for over five months in aquaria constructed of UV-Opaque and UV-Transparent acrylic, under incident sunlight reduced by 50% shadecloth. In *Clavularia*, the final concentrations of palythine and palythene (its principal UV-absorbing mycosporine-like amino acids, MAA) were significantly higher in UVT than in UVO acclimatized polyps. There were no significant differences between UVO and UVT polyps in the chlorophyll content of their zooxanthellae, or in the number of zooxanthellae per mg symbiosis protein, but net photosynthesis at maximum acclimation irradiance decreased by half in the UVT colonies. The greater activity of superoxide dismutase (SOD) in the animal fraction of the UVO polyps was consistent with the higher oxygen flux there, although no differences between the zooxanthellae were seen in SOD activity, which was rather low compared to that in the animal tissue. Depth-dependent photoadaptation in chlorophyll content occurred between 4 m and 17 m in zooxanthellae of *Clavularia* sp.; thus, similar oxygen fluxes may explain the similar activities of SOD and catalase (CAT) at these depths. There were no depth-related differences in the concentrations of palythine or palythene. Long-term acclimatization of *Phyllodiscus semoni* to UV exposure led to an increase in the concentration of palythine, and to a redistribution of mycosporine-glycine, between host and symbiont. Total concentration of MAAs increased two-fold in the zooxanthellae from UVT acclimatized

anemones, and was associated with a higher rate of photosynthesis and lack of photoinhibition by UV in freshly isolated zooxanthellae. Higher activities of SOD in the animal fraction, and of SOD, CAT, and ascorbate peroxidase in the zooxanthellae, are in keeping with this result. Together, the field and laboratory results suggest that the greater responsiveness of photooxidative defenses in the zooxanthellae of *Phyllodiscus semoni* than of *Clavularia* sp. is related to the former's occupation of shallow reef-flat habitats and the restriction of the later to greater depths. This study provides the first evidence of the regulation of the concentration of individual mycosporine-like amino acids by long-term UV exposure, and the first data showing that differences in the sensitivity of photosynthesis to UV are associated with different concentrations of MAAs in the zooxanthellae.

1. Introduction

There is a growing awareness of the separate and interacting effects of ultraviolet (UV) radiation and forms of active oxygen on coral reef organisms. Jokiel (1980) demonstrated that solar UV radiation causes outright mortality in "shade-loving" coral reef epifauna, and Jokiel and York (1982) showed the detrimental effect of such radiation on calcification in reef corals and on growth of their symbiotic dinoflagellates (zooxanthellae, *Symbiodinium* spp.) in culture. Shick and Dykens (1985) found a positive correlation between the activity of enzymes involved in detoxifying forms of active oxygen and chlorophyll content (an index of the potential for oxygen flux and photosensitization) in a taxonomic potpourri of symbiotic coral reef invertebrates. Subsequent experiments by Lesser et al. (1990) implicated UV radiation, elevated temperature, and increased levels of active oxygen species in the bleaching of a coral reef zoanthid.

Solar UV radiation has various detrimental effects on survival, growth, and physiology of invertebrates and algae, which may involve damage to DNA and proteins, oxidation of membrane lipids, and reduction of photosynthesis (via depression of chlorophyll content or inhibition of electron transport at the Q_B binding protein in photosystem II) (reviews in Caldwell, 1981; Worrest, 1982;

Renger et al., 1986; Kyle, 1987). Active oxygen is formed by the univalent reduction of molecular O_2 owing to spin restrictions on its valence electrons, yielding superoxide radical (O_2^-), and by further reduction of superoxide to hydrogen peroxide (H_2O_2) and hydroxyl radical $(HO\cdot)$; these reactions occur normally in many metabolic processes in the cytosol, mitochondria, and chloroplasts (reviews in Halliwell and Gutteridge, 1985; Asada and Takahashi, 1987; Cadenas, 1989). Transfer of energy to ground-state O_2 from an activated photosensitizer such as chlorophyll produces highly reactive singlet oxygen (Foote, 1976). Active forms of oxygen have multiple toxic effects on organisms, including damage to nucleic acids, inactivation of enzymes, disruption of membranes, and inhibition of photosynthesis (again, via damage to the Q_B binding protein) (reviews in Halliwell, 1982; Fridovich, 1986; Asada and Takahashi, 1987; Kyle, 1987; Cadenas, 1989). Formation of active oxygen species is enhanced by hyperoxia (Jamieson et al., 1986), and both their production and effects are exacerbated by the presence of sunlight, especially its energetic UV wavelengths (Clayton, 1977; Asada and Takahashi, 1987; Petasne and Zika, 1987; Valenzeno and Pooler, 1987).

Symbiosis with photosynthetic organisms requires that the invertebrate hosts incur the costs of exposing themselves to sunlight and to large fluxes of oxygen in their tissues, costs that include protection against UV and oxygen toxicity. Defenses against the former include a variety of UV-absorbing substances, e.g., "S-320" compounds (Shibata, 1969), known to comprise a family of mycosporine-like amino acids (MAAs) having absorption maxima ranging from 310 to 360 nm (Dunlap and Chalker, 1986; Dunlap et al., 1989). A defensive role of these compounds in zooxanthellate anthozoans has been inferred from their UV absorbing properties (e.g., Shibata, 1969; Dunlap et al., 1986), and their decrease in concentration with increasing depth (Dunlap et al., 1986; Scelfo, 1986) or after artificial screening from UV (Jokiel and York, 1982; Scelfo, 1986). Conversely, the concentrations of these compounds increase after transplantation of corals to shallower depths (Scelfo, 1986), and the greater resistance to UV in shallow-living than in deep-living corals (Scelfo, 1986; Siebeck, 1981, 1988) has been attributed in part to this relationship.

Not all anthozoans show such compensatory changes in MAA concentration. Scelfo (1985) found no differences in the concentrations of S-320 compounds in specimens of the zoanthid *Zoanthus pacificus* acclimatized to different irradiances in the presence and absence of UV for two months. Nor did the concentration of S-320 compounds in specimens of the scleractinian coral *Stylophora pistillata* change by one month after transplantation of colonies from 30 m to 5 m depth (Gattuso, 1987). Likewise, Stochaj (1988, 1989) found that concentrations of three MAAs in the actiniarian *Aiptasia pallida* did not change

during acclimation to the presence or absence of UV, and that two other compounds increased only slightly during two weeks of UV exposure. Finally, the concentrations of MAAs in the actiniarian *Anthopleura elegantissima* did not change during short- or long term acclimatization to natural or simulated solar UV (Scelfo, 1988; Stochaj, 1989). Further studies are needed to determine how widespread adaptive changes in MAA concentration may be.

Defenses against forms of active oxygen include various biochemical antioxidants, quenchers, and scavengers such as ascorbate, tocopherols, carotenoids, and urate, and enzymes such as superoxide dismutase (SOD, which removes O_2^- but in so doing produces H_2O_2), catalase (CAT, which removes H_2O_2) and ascorbate peroxidase (AsPX, which removes H_2O_2 and other peroxides) (for reviews, see Halliwell and Gutteridge, 1985; Asada and Takahashi, 1987; Di Giulio et al., 1989). By curbing the intracellular concentrations of O_2^- and H_2O_2, these enzymes also minimize the production of the highly reactive hydroxyl radical (Fridovich, 1986). Enzymic defenses in the host (SOD, CAT) occur in proportion to the potential for (photo)oxidative damage (Dykens and Shick, 1982, 1984; Shick and Dykens, 1985; Lesser et al., 1990), and likewise are robust in symbiotic dinoflagellates, where photoadaptive responses include increases in activities of SOD, CAT, and AsPX (Lesser and Shick, 1989a,b). Increases in SOD and CAT activities in response to UV exposure imply an involvement of UV in the production of active oxygen (Dykens and Shick, 1984; Lesser and Shick, 1989a; Lesser et al., 1990).

Although the concentration of total "S-320" compounds varies during experimental acclimatization of hermatypic corals to UV exposure (Jokiel and York, 1982; Scelfo, 1986), there are no published studies of UV-induced changes in identified mycosporine-like amino acids in these or other coral reef invertebrates. Neither the source nor the cellular localization of MAA is known. Although it has been assumed that UV-absorbing compounds in the host afford protection to the symbionts *in hospite* (e.g., Jokiel and York, 1982), the possibility that the compounds are synthesized by the zooxanthellae has been suggested (Dunlap and Chalker, 1986), but the concentration of MAAs in zooxanthellae has not been reported previously. The present study documents changes in the concentrations of individual MAAs in host and symbionts, together with variations in the activities of antioxidant enzymes in the partners, and the photosynthetic performance of whole polyps and isolated zooxanthellae. The study provides insight concerning the presumed protective role of UV-absorbing MAAs, and an integrated look at the defenses against photooxidative stress, in zooxanthellate representatives of two groups of Anthozoa other than scleractinian corals under controlled conditions of long-term UV exposure. It forms part of a larger, long-term program investigating coral reef

photobiology (Shick and Dykens, 1985; Chalker et al., 1988; Shick et al., 1989, and unpublished results).

2. Materials and Methods

Collection and maintenance of specimens

Multiple colonies of the stoloniferan octocoral *Clavularia* sp. (the species having dove-gray polyps common on dead coral and coral rubble at depths of 5–12 m over much of the Great Barrier Reef; Fig. 1) were collected from a depth
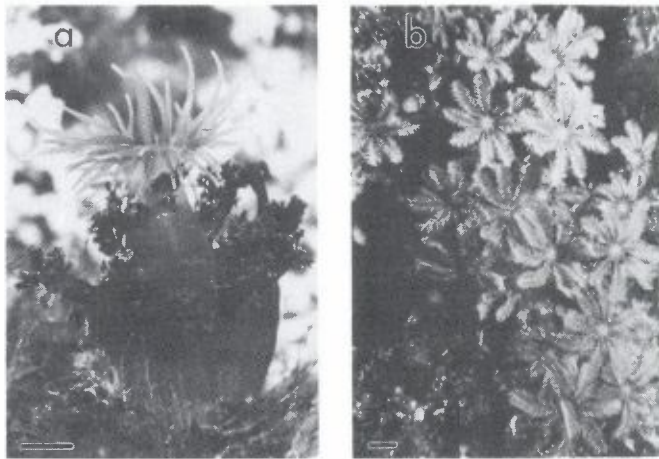

Figure 1. (a) *Phyllodiscus semoni* Kwietniewski. Expanded specimen showing feeding tentacles (which lack zooxanthellae) surrounding the oral disc at the apex of the column, which is extended only at night. Branched "pseudotentacles" radially arranged on the mid-column remain extended day and night. Zooxanthellae are concentrated in the pseudotentacles, which also bear vesicles where large amastigophore nematocysts used in defense are located. Scale bar = 1 cm.
(b) *Clavularia* sp. Small colony on coral rubble at a depth of 8 m on Grub Reef (central Great Barrier Reef). Scale bar = 0.5 cm. Both photographs by J.M. Shick.

of 5 m at Grub Reef (central GBR) in August 1988. Colonies were maintained in the system of flowing seawater at the Australian Institute of Marine Science (AIMS), Townsville, Queensland at ambient temperature and salinity. For the first 36 days, all colonies were held in tanks shielded by glass opaque to ultraviolet (UV) light below $\approx$ 400 nm, at irradiances of photosynthetically active radiation (PAR) < 10% of incident sunlight. Some colonies were then transferred to acrylic aquaria (200 dm^3; with covers) that differed in their UV transmission characteristics. UV-opaque (UVO) aquaria excluded wavelengths

below 385 nm, whereas UV-transparent (UVT) aquaria admitted wavelengths
down to 290 nm, as indicated by scanning in a UV-visible spectrophotometer
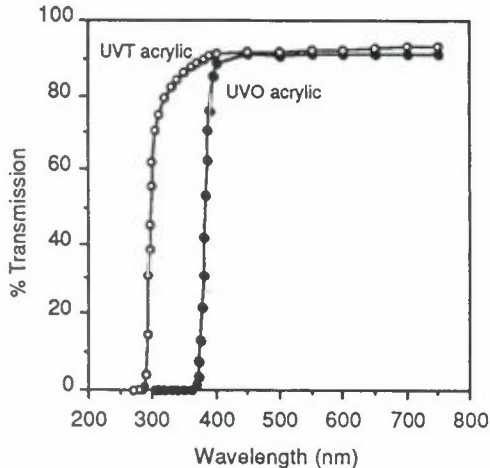(Fig. 2). Incident solar irradiance was reduced using 50% neutral density

Figure 2. Transmission spectra of UV-opaque and UV-transparent acrylic sheets used in
constructing the UVO and UVT aquaria. UVT acrylic was also used for the cover
to the chamber in which photosynthesis by *Clavularia* sp. colonies was measured.

shadecloth, which produced a photic regime approximating that at 4–5 m
(B.E. Chalker, personal communication), the shallowest depth at which *Clavularia* sp. was found. Seawater flowed through the aquaria at ≤ 3 dm^{3}min^{-1}.

Subsamples of 10 polyps were taken from freshly-collected colonies of *Clavularia* sp., and additional subsamples of three or five polyps were removed
from colonies in the holding tank after 36 and 77 days of maintenance there,
and from the UVO and UVT aquaria following a further 76 and 172 days of
acclimatization to the different UV regimes. Individual polyps were processed
for high performance liquid chromatographic (HPLC) analysis of UV-absorbing
compounds. At the end of 170–175 days of acclimatization in the UVO and
UVT aquaria, additional polyps were removed for measurements of their rates
of photosynthesis, density of zooxanthellae, chlorophyll content, and activities
of antioxidant enzymes. Fresh specimens were collected at depths of 4 m and
17 m at Bowl Reef (central GBR) in March 1989 and analyzed immediately
for UV-absorbing compounds, chlorophyll, and enzyme activities.

Specimens of the aliciid sea anemone *Phyllodiscus semoni* (Fig. 1) ranging
from 1 to 3 cm basal diameter were taken from the fouling community in the
seawater system at AIMS in October 1988. Individual were placed in UVO
and UVT aquaria for 163 days and subsequently their pseudotentacles were

removed and analyzed for UV absorbers, density of zooxanthellae, chlorophyll content, and activities of oxygen detoxification enzymes. Rates of photosynthesis also were measured in zooxanthellae freshly isolated from pseudotentacles of UVO and UVT acclimatized anemones.

Analysis of UV-absorbing compounds and chlorophyll

Extraction and analysis of UV-absorbing compounds (mycosporine-like amino acids, MAA) in minced whole polyps of *Clavularia* sp., pseutotentacles of *Phyllodiscus semoni*, and in freshly isolated zooxanthellae pooled from several specimens of *P. semoni* under each UV regime were performed according to the procedures in Dunlap and Chalker (1986), Dunlap et al. (1986), and Dunlap et al. (1989). Extraction efficiency in 100% methanol was determined by four sequential 30 min extractions, as described by Dunlap and Chalker (1986). Individual MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column protected with an RP-8 guard column, in an aqueous mobile phase including 0.1% acetic acid and 25% methanol. Detection of peaks was by UV absorbance at 313 and 340 nm. Identities of peaks were confirmed by co-chromatography with standards of mycosporine-glycine, palythine, and asterina-330 isolated from the zoanthid *Palythoa tuberculosa*, and of palythene from lens tissues of the red coral trout *Plectropomus leopardus*. Peaks were integrated on Hewlett-Packard or Spectra-Physics integrators, and quantification of individual MAAs (corrected for extraction efficiency) was as described in Dunlap and Chalker (1986) and Dunlap et al. (1989) using published molar extinction coefficients summarized by those authors.

Chlorophylls a and c_2 were quantified in $MgCO_3$ buffered 90% acetone extracts as described in Lesser and Shick (1989a), both for whole polyps and for freshly isolated zooxanthellae (FIZ) from *Phyllodiscus semoni*. Zooxanthellae (cleaned of animal debris as in Lesser and Shick, 1989a) were counted in triplicate using an AO bright-line hemacytometer. Protein was determined by the method of Bradford (1976) using Coomassie Brilliant Blue (G-250) (Bio-Rad Laboratories) and bovine gamma globulin standards. It should be noted that the Bradford method routinely underestimates the amount of protein in cnidarian tissues (Zamer et al., 1989).

Enzyme assays

Activities of superoxide dismutase (SOD, including both the Cu-Zn and the CN^--insensitive forms of the enzyme), catalase (CAT), and ascorbate peroxidase (AsPX, zooxanthellae only) in the animal tissues and in FIZ

were measured spectrophotometrically at 23–25°C as described in Lesser and Shick (1989a). Biochemicals and SOD standards were purchased from Sigma Chemical Co. Soluble protein was determined by the Bradford (1976) method.

Measurement of photosynthesis

UVO and UVT acclimatized colonies of *Clavularia* sp. (consisting of five and six polyps, respectively) were placed on a perforated platform in a 0.48 dm^3 closed respirometer chamber in which the seawater (Millipore-filtered, 0.45 μm pore size; 27.5°C; 35‰ S) was continuously stirred (200 rpm) by a magnetic spinbar located beneath the platform. The lid to the respirometer was of the same UVT acrylic used in constructing the aquaria, and was fitted with a port for a Radiometer E5046 oxygen sensor. Duplicate measurements of photosynthesis lasting 35–60 min were made at an irradiance of 975 μmol photons m^{-2}s^{-1} (PAR, 400–700 nm, as measured with a LI-CCR 190SB cosine-corrected quantum sensor), which approximates the peak daily irradiance experienced by the colonies in the shaded aquaria. Light was provided by a Thorn 500 W quartz halogen lamp situated above the respirometer, which was immersed in a constant-temperature water bath. Following measurement of photosynthesis, dark respiration was measured in seawater equilibrated with 50% O_2 : 50% N_2 (see Shick, 1990a). After a dark recovery period of > 1 hr, duplicate measurements of photosynthesis were repeated on UVO and UVT acclimatized colonies at the same irradiance of PAR with the addition of UV-B and UV-A radiation. This was provided by a Westinghouse FS20 fluorescent lamp (312 nm peak emission) and an NEC T10, 20 W fluorescent blacklight (358 nm peak emission) at a distance of 15 cm above the colonies. Immediately following these experiments, individual polyps were extracted for MAA analysis and determination of total protein content.

Zooxanthellae freshly isolated from *Phyllodiscus semoni*, cleaned and suspended in Millipore-filtered (0.22 μm pore size) seawater (35‰ S), were placed in a temperature-controlled (27.5°C) chamber (3.52 cm^3) similar to that described by Dubinsky et al. (1987). The chamber, which has quartz windows transparent to UV-A and UV-B, was fitted with the Radiometer E5046 oxygen sensor connected to a Radiometer PHM72 Mk2 Acid-Base Analyzer and strip-chart recorder. Illumination was provided by an Osram tungsten halogen lamp in a slide projector. Photosynthesis was measured for 15–25 min at several irradiances with constant stirring of the chamber's contents by a small spinbar. The highest irradiance (1, 000 μmol photons m^{-2}s^{-1}) corresponds to the peak daily irradiance experienced by the anemones during the acclimatization period, although zooxanthellae *in hospite* would have experienced lower

photon fluxes. Dark respiration was measured before and after each determination of photosynthesis. Measurements at each level of PAR were repeated in the presence of UV light from the PS20 and T10 lamps situated 12 cm from the chamber window. These lamps were operated continuously during all photosynthesis measurements, but for those involving PAR only, a filter of UVO acrylic was placed across the quartz window. This filter was replaced by one of UVT acrylic for measurements involving UV light. Fresh aliquots of the UVO and UVT zooxanthella suspensions (5.9 and 6.2×10^5 cells cm^{-3}, respectively) were used for measurements at every irradiance. Measurements were discontinued when gross photosynthesis in the stock suspensions had declined by 10% from the rate measured immediately after isolation of the zooxanthellae. Zooxanthellae isolated from *Clavularia* sp. were not photosynthetically competent.

3. Results

UV-absorbing compounds and chlorophyll in Clavularia *sp.*

An HPLC chromatogram of the methanolic extract of a UVT-acclimatized specimen of *Clavularia* sp. is shown in Fig. 3. Polyps of *Clavularia* sp. collected in winter (August 1988) at Grub Reef contained palythine ($\lambda_{max} = 320$ nm) as their principal mycosporine-like amino acid (Fig. 4), and only trace amounts of palythene ($\lambda_{max} = 360$ nm). In some chromatograms, small unidentified peaks occurred as shoulders on the palythine and palythene peaks (Fig. 3); these unknowns were not included in the integration of the known peaks. There was no change in the concentration of palythine during the first 36 days of maintenance under $< 10\%$ of ambient irradiance and absence of UV (Fig. 4), but a significant decline occurred by day 77 of maintenance (Student's $t = 3.599$, df $= 8$, $P = 0.007$). After 74 days of acclimatization in UVO and UVT aquria, polyps in the latter had significantly higher concentrations of palythine than those in the former ($t = 4.167$, df $= 4$, $P = 0.014$). Palythine concentration in UVT polyps was greater than that in UVO polyps at the end of 172 days of acclimatization (208 days after the start of the experiment; Fig. 3) ($t = 3.341$, df $= 8$, $P = 0.010$). Asterina-330 ($\lambda_{max} = 330$ nm) and palythene were quantifiable in all UVT polyps at day 172 of UV acclimatization; the former compound was above the level of detection in three of five UVO polyps tested, and the latter in two of the five (Fig. 5). There was no significant difference between UVO and UVT polyps in the concentration of asterina-330, but palythene was significantly more concentrated in UVT polyps ($t = 5.416$, df $= 8$, $P = 0.001$). Mortality of polyps in both groups was estimated to be $> 75\%$ during the 172 days of acclimatization in the aquaria.
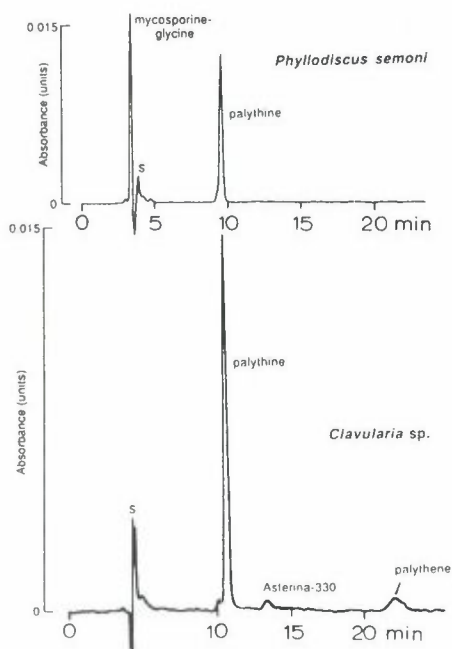
Figure 3. HPLC chromatograms of methanolic extracts of *Phyllodiscus semoni* (top) and *Clavularia* sp. (bottom) acclimatized to the presence of solar UV. In the former, peak detection was at 313 nm and quantification was on a Hewlett-Packard integrator; in the latter, detection was at 340 nm and quantification was on a Spectra-Physics integrator. S = salts (refractive).

There was no significant difference between UVO and UVT polyps of *Clavularia* in number of zooxanthellae per mg total polyp protein, values being $5.56 \times 10^6 \pm 1.21$ cells (mg protein)$^{-1}$ and $5.30 \times 10^6 \pm 0.50$ cells (mg protein)$^{-1}$, respectively ($t = 0.199$, df $= 8$, $P = 0.847$). Likewise, zooxanthellae from UVO and UVT polyps did not differ in total chlorophyll, chlorophyll a, or chlorophyll c_2 per cell (df $= 8$ and $P > 0.42$ in all cases; Fig. 6). The ratio of chlorophyll a to c_2 calculated for individual polyps was 1.80 ± 0.22 (S.E.) in UVO polyps and 1.90 ± 0.22 in UVT polyps, which was not a significant difference ($t = 0.333$, df $= 8$, $P = 0.748$).

Specimens of *Clavularia* sp. collected from Bowl Reef in late austral summer (March 1989) generally had higher concentrations of MAA than did winter specimens from Grub Reef (cf. Fig. 7 and Fig. 4). There were no significant differences between polyps collected at 4 m and 17 m in their concentrations of palythine or palythene (df $= 10$ and $P > 0.35$ in both cases). Asterina-330 was not detected in these polyps. Zooxanthellae from polyps living at 17 m depth had significantly more total chlorophyll, chlorophyll a, and chlorophyll c_2 than
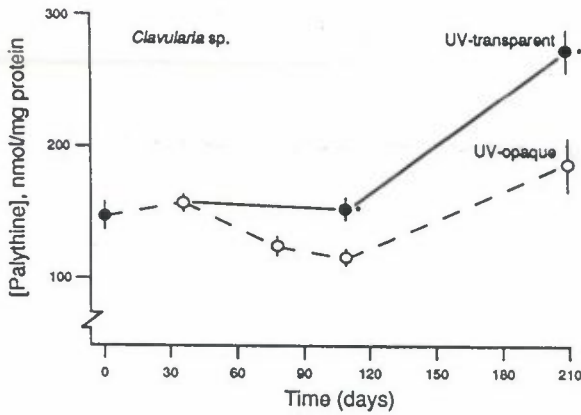
Figure 4. Time course of change in the concentration of palythine in polyps of *Clavularia* sp. during maintenance under controlled conditions of UV exposure. The point at day 0 is for freshly-collected polyps from 5 m depth (N = 10). For the first 36 days, colonies were held in the AIMS seawater system under UV-opaque glass at < 10% of incident solar irradiance. Some colonies were then transferred to UVO and UVT aquaria exposed to 50% of incident solar irradiance for the duration of the experiment (total time: 208 days = 0.71 sabbatical). Vertical bars indicate ±S.E. Asterisks indicate significant differences between UVO and UVT acclimatized polyps.
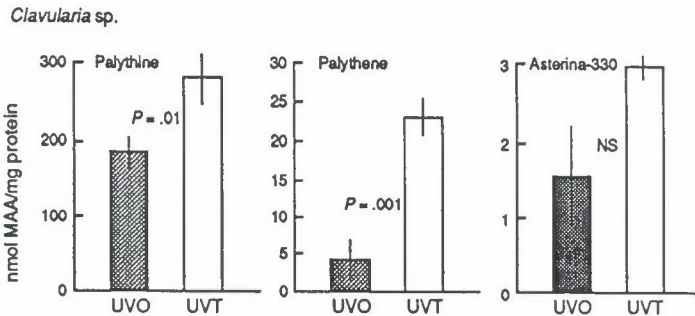

Figure 5. Concentrations of individual mycosporine-like amino acids in polyps of *Clavularia* sp. after 172 days of acclimatization in UVO and UVT aquaria. Vertical bars indicate ±S.E. N = 5 polyps in each group. NS = not statistically significant at $P = 0.05$.
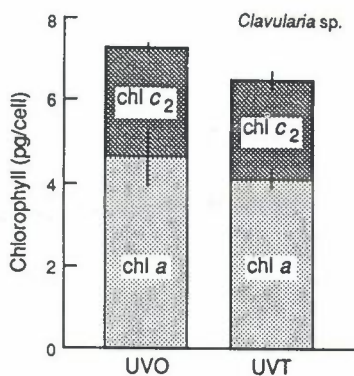
Figure 6. Concentrations of chlorophylls *a* and c_2 in polyps of *Clavularia* sp. after 172 days of acclimatization in UVO and UVT aquaria. Vertical bars indicate ±S.E. N = 5 polyps in each group.
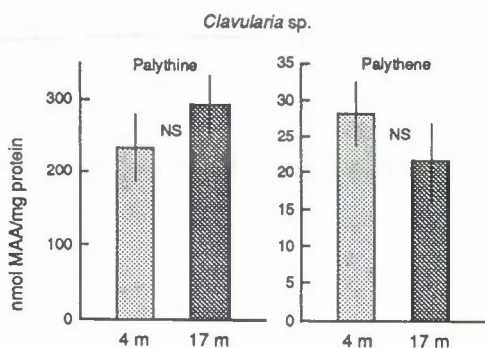

Figure 7. Concentrations of individual mycosporine-like amino acids in polyps of *Clavularia* sp. freshly collected from depths of 4 m and 17 m at Bowl Reef. Vertical bars indicate ±S.E. N = 6 polyps in each group. NS = not statistically significant at $P = 0.05$.
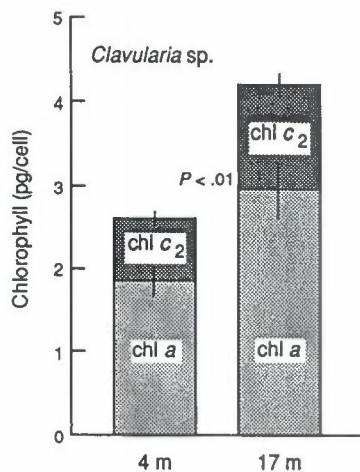

Figure 8. Concentrations of chlorophylls *a* and c_2 in polyps of *Clavularia* sp. freshly collected from depths of 4 m and 17 m at Bowl Reef. Vertical bars indicate ±S.E. N = 5 polyps in each group.

those at 4 m depth (df $= 8$, $P \leq 0.01$ in all cases; Fig. 8). The ratio of chlorophyll a to c_2 did not differ significantly with depth ($t = 1.814$, df $= 8$, $P = 0.054$), being 2.49 ± 0.04 at 4 m and 2.38 ± 0.11 at 17 m.

UV-absorbing compounds and chlorophyll in Phyllodiscus semoni

Very high mortality of anemones during the 163 days of acclimatization in UVO and UVT aquaria meant that few specimens were available for analysis at the termination of the experiment. Priority was placed on the MAA analyses. Mycosporine-glycine($\lambda_{max} = 310$ nm) and palythine were the only MAAs detected in *Phyllodiscus semoni* (Fig. 3). UVO and UVT acclimatized anemones did not differ in their concentrations of mycosporine-glycine ($t = 0.290$, df $= 4$, $P = 0.786$), while the concentration of palythine was significantly higher in UVT specimens ($t = 2.809$, df $= 4$, $P = 0.048$) (Fig. 9).

Freshly isolated zooxanthellae from about half of the pseudotentacles of three anemones from each group were pooled and extracted from MAA analysis. By calculating the ratio of zooxanthella protein to total symbiosis (zooxanthellae plus animal) protein, and knowing the concentration of MAAs in the whole symbioses and in the zooxanthellae, the partitioning of MAAs between zooxanthellae and host animal could be estimated. Zooxanthella protein biomass comprised 47.6% of the total protein biomass in pseudotentacles of UVO anemones and 48.7% of that in UVT anemones. Mycosporine-glycine present in the zooxanthellae accounted for 40.1% of the amount of this compound in the symbiosis in UVO acclimatized anemones (Fig. 9), approximately in proportion to the biomass of the zooxanthellae. In UVT acclimatized anemones, virtually all (94.9%) of the mycosporine-glycine present was in the zooxanthellae. Palythine was undetectable in zooxanthellae from UVO acclimatized anemones, but comprised 8.3% of this compound present in UVT acclimatized anemones, and accounted for about one-third of the increase in palythine seen in the UVT symbiosis (Fig. 9).

Data for zooxanthellae pooled from several specimens each of UVO and UVT acclimatized anemones indicate no difference between these groups in the number of zooxanthellae per mg symbiosis protein (Fig. 10), but those isolated from UVT anemones tend to have a greater protein content (43 pg protein cell^{-1} vs. 36 pg protein cell^{-1}; Fig. 10). Zooxanthellae from anemones exposed to solar UV during the acclimatization period showed a 50% reduction in their content of chlorophyll a and an 8.4% decline in chlorophyll c_2 content (Fig. 10), resulting in a decline in the chlorophyll $a : c_2$ ratio from 2.02 to 1.10.
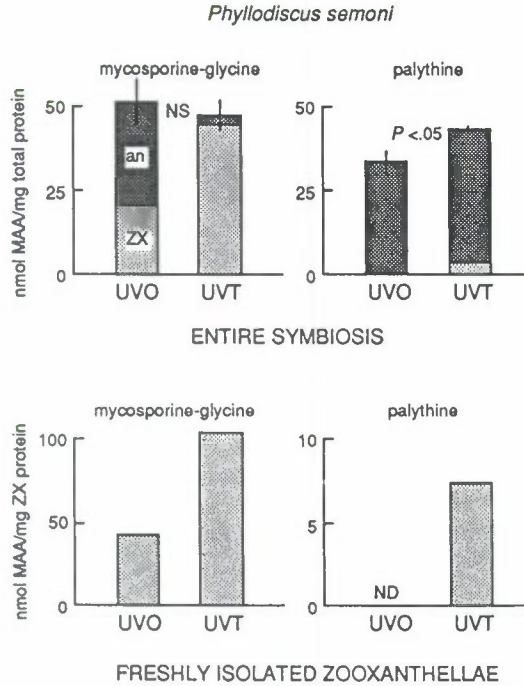
Figure 9. Concentrations of individual mycosporine-like amino acids in pseudotentacles of *Phyllodiscus semoni* after 163 days of acclimatization in UVO and UVT aquaria. Vertical bars indicate ±S.E. for mean concentrations in entire symbiosis (animal tissue plus zooxanthellae), where N = 3 individuals in each treatment. Concentrations in the zooxanthellae (ZX) were determined for algae pooled from 3 specimens in each treatment; concentrations in the animal tissue (an) were calculated as the difference between symbiosis and zooxanthellae concentrations, and the relative amounts of protein biomass in the two fractions. NS = not statistically significant at $P = 0.05$. ND = not detected. See text for discussion.

Enzyme activities in Clavularia *sp.*

The activity of SOD in the host animal tissue of specimens shielded from UV during the acclimatization period was significantly greater than that in UV acclimatized specimens ($t = 2.053$, df = 8, $P = 0.037$; Fig. 11). The activity of cyanide-insensitive SOD (probably the manganese-containing form of the enzyme localized in mitochondria) increased to the same extent as total SOD activity in the animal tissues (data not shown). There was no significant difference between UVO and UVT acclimatized polyps in total SOD activity in their zooxanthellae, no significant difference between the groups in CAT activity in animal tissue or zooxanthellae, and no significant difference in AsPX activity in the zooxanthellae (df = 8, $P > 0.05$ in all cases; Fig. 11), although
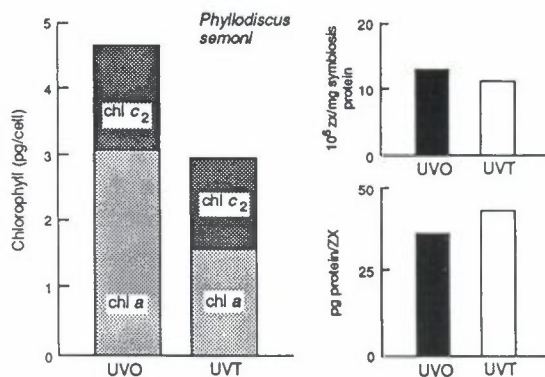
Figure 10. Concentrations of chlorophylls a and c_2, and zooxanthella biomass parameters, in pseudotentacles of *Phyllodiscus semoni* after 163 days of acclimatization in UVO and UVT aquaria. Note decline in ratio of chlorophyll a to c_2 in UVT acclimatized specimen.
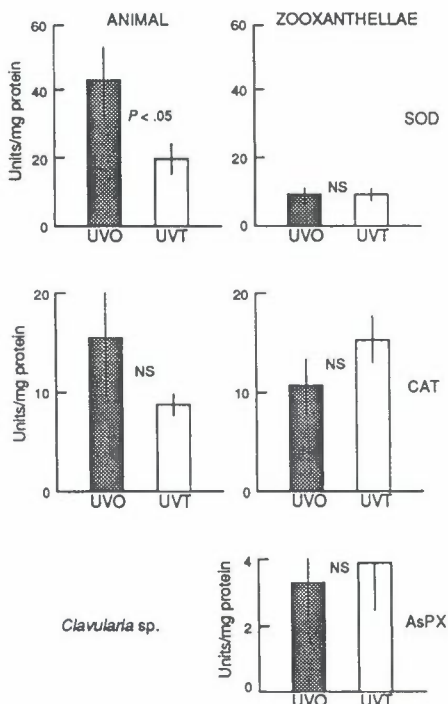

Figure 11. Specific activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (AsPX, zooxanthellae only) in tissues of host and symbiont following 172 days of acclimatization of polyps of *Clavularia* sp. in UVO and UVT aquaria. Vertical bars indicate $\pm$S.E. $N = 5$ determinations in each group. NS = not statistically significant at $P = 0.05$.

the activities of CAT and AsPX were highly variable. However, the activity of cyanide-insensitive SOD was greater in zooxanthellae from UVT acclimatized polyps [5.37 ± 0.34 Units (mg protein)$^{-1}$] than in those from UVO polyps [3.64 ± 0.49 Units (mg protein)$^{-1}$] ($t = 2.896$, df $= 8$, $P = 0.01$). Total SOD activity in the zooxanthellae was only one-half to one-fourth that in the animal tissue. Comparing polyps freshly collected from 4 m and 17 m depth, there was no significant difference in total SOD or CAT activity in the animal tissues (df $= 8$, $P > 0.08$ in both cases; Fig. 12); enzyme activities were not measured in their zooxanthellae.
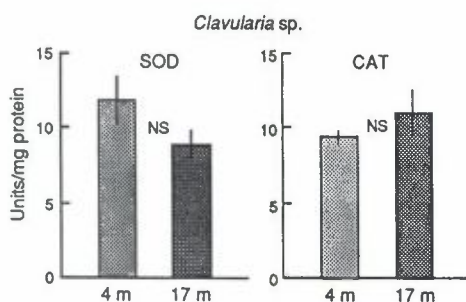

Figure 12. Specific activities of superoxide dismutase (SOD) and catalase (CAT) in animal tissues of polyps of *Clavularia* sp. freshly collected from 4 m and 17 m depth on Bowl Reef. Vertical bars indicate $\pm$S.E. N $= 5$ polyps in each group. NS $=$ not statistically significant at $P = 0.05$.

Enzyme activities in Phyllodiscus semoni

Only single specimens of UVO and UVT acclimatized *P. semoni* were available for analysis of enzymes (and as sources of zooxanthellae used in photosynthesis experiments). With the exception of CAT in the animal tissue, the activities of all enzymes tended to be higher in the host and in the zooxanthellae when the anemone had been exposed to UV during the acclimatization period (Fig. 13). SOD activity in the zooxanthellae was approximately three times that in the animal tissue.

Photosynthesis

Net photosynthesis (expressed per mg total polyp protein) measured at the peak irradiance that the colonies experienced during the acclimatization period was twice as great in UVO acclimatized as in UVT acclimatized colonies of *Clavularia* sp. (Fig. 14). There was no striking short-term effect of UV on photosynthesis, but the rate in UVO acclimatized polyps was reduced by
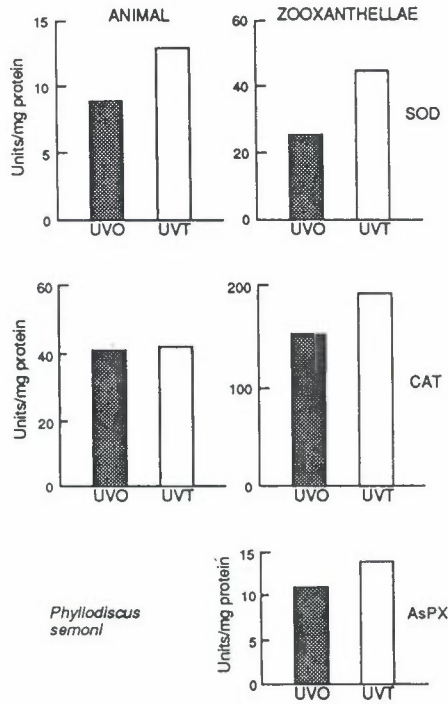
Figure 13. Specific activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (AsPX, zooxanthellae only) in tissues of host and symbiont following 163 days of acclimatization of *Phyllodiscus semoni* in UVO and UVT aquaria.
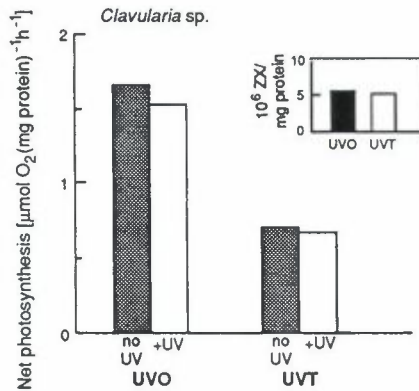
Figure 14. Net photosynthetic oxygen flux at 975 μmol photons $m^{-2}s^{-1}$ (PAR) in colonies of *Clavularia* sp. after 170–175 days of acclimatization in UVO and UVT aquaria. Duplicate measurements on each colony were repeated in the presence of UV-A + UV-B irradiation (see text for description of light sources). Inset shows that both UVO and UVT polyps had the same density of zooxanthellae, which themselves had the same chlorophyll content (see Fig. 6).

7.5% during their acute exposure to UV-A and UV-B. Owing to the small number of polyps surviving the acclimatization period, those used in the photosynthesis measurements were subsequently extracted for MAA analysis; this precluded quantification of their chlorophyll content, and necessitated expressing photosynthetic oxygen flux on the basis of polyp protein. This facilitated interpretation of the data on antioxidant enzyme activities in the host tissues (which are also expressed on a protein basis) relative to the oxygen regime they experienced. For similar reasons, photosynthesis was expressed as net oxygen flux, as antioxidant enzyme activity in the host should be determined more by this than by gross photosynthesis.

Gross photosynthesis (expressed per 10^6 zooxanthellae) was greater at all irradiances in zooxanthellae isolated from the UVT acclimatized specimen of *Phyllodiscus semoni* than in those from the UVO acclimatized anemone (Fig. 15), despite the greater chlorophyll content in the latter (Fig. 10). Because photosynthesis declined with increasing time after isolation of the zooxanthellae, and the experiments were terminated accordingly, too few data points are available to allow meaningful calculations of photosynthetic parameters for the photosynthesis – irradiance curves shown in Fig. 15. However, the data seem sufficiently consistent that a $\approx 25\%$ depression of $P_{\max}$ by UV-A and UV-B during measurement of photosynthesis is evident in zooxanthellae from the anemone shielded from solar UV during the acclimatization period, but not in zooxanthellae from the anemone acclimatized to UV exposure. There is no indication of a decline in photosynthesis in either group at the highest irradiance, which approximates the maximum irradiance experienced by the host anemones during the acclimatization period.

Dark respiration (measured before exposure to light in the photosynthesis chamber) was 51.0 ± 9.9 nmol O_2 (10^6 zooxanthellae)$^{-1}$h^{-1} in 12 replicates of zooxanthellae isolated from the UVO acclimatized anemone and 95.5 ± 8.1 nmol O_2 (10^6 zooxanthellae)$^{-1}$h^{-1} in 8 replicates of those taken from the UVT acclimatized specimen. Such pseudoreplication does not permit statistical analysis, but the nearly twofold difference was consistent, and greater than can be accounted for by the slightly higher protein content of UVT zooxanthellae (cf. Fig. 10). Dark respiration measured after photosynthesis was consistently greater than that measured before photosynthesis, and generally increased with irradiance (data not shown), perhaps due to an increase in substrate supply from photosynthesis (see Weger et al., 1989). Gross photosynthesis (i.e., net photosynthesis + respiration) in each case was therefore calculated using the higher, post-photosynthesis value for dark respiration; this is because it was
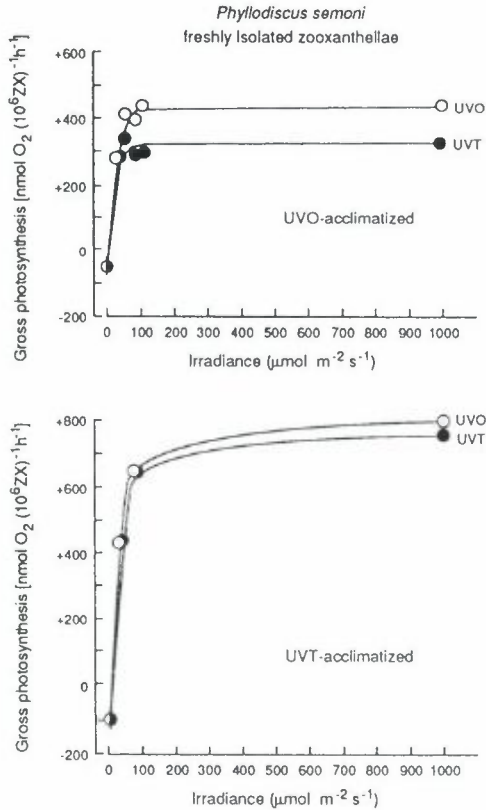
Figure 15. Photosynthesis–irradiance curves for zooxanthellae freshly isolated from UVO and UVT acclimatized (> 150 days specimens of *Phyllodiscus semoni*. Measurements were made in the absence and in the presence of UV-A + UV-B light, achieved by placing either a UVO or a UVT acrylic filter between the "Dubinsky" chamber and the FS20 and T10 fluorescent lamps that provided UV-B and UV-A irradiation (see text for further description). Dark respiration (measured before photosynthesis) was consistently about twice as great in zooxanthellae from UVT anemone as in those from UVO anemone.

assumed that respiration during illumination and photosynthesis would approximate that value more than it would the pre-photosynthesis rate of dark respiration prior to exposure to light.

4. Discussion

When shielded from solar UV radiation and held at an irradiance of visible light < 10% of ambient sunlight, the stoloniferan *Clavularia* sp. maintained the concentration of palythine (its principal UV-B absorbing, mycosporine-like amino acid) at the level found in freshly-collected polyps, for at least one

month. A $\approx 15\%$ decline in the concentration of palythine was evident only after two months under such photobiologically benign conditions, which suggests a very low turnover of this compound in this species. The higher concentration of palythine in polyps acclimatized to 50% of ambient solar irradiation in the presence of UV than in those shielded from UV for ≥ 2 months was not due to an increase of palythine in the former, but rather to its further decline in the latter (Fig. 4). After ≥ 5 months under UVT conditions the concentration of palythine did increase, but its concentration in the UVO acclimatized individuals shielded from UV was still nearly 70% of that in UVT acclimatized polyps (Figs. 4 and 5). Such a response is decidedly unlike the rapid and pronounced changes in the concentrations of S-320 compounds reported in some hermatypic corals during exposure to increased levels of UV (e.g. Jokiel and York, 1982; Scelfo, 1986).

Likewise, the lack of bathymetric differences in the concentrations of palythine and palythene in polyps collected from 4 m and 17 m at Bowl Reef deviates from the scleractinian paradigm. The concentrations of palythine in polyps of *Clavularia* sp. from both sites are higher than those reported for scleractinian *Acropora* spp. (Dunlap and Chalker, 1986; Dunlap et al., 1986), which together with the lack of bathymetric effect and relatively small change during long-term acclimatization to the presence and absence of UV, suggests that the levels of palythine in *Clavularia* sp. may be constitutive. This is especially so because a classic depth-dependent photoadaptation is seen in the elevated chlorophyll content in the deeper polyps (Fig. 8), indicative of their exposure to a lower flux of photons at the greater depth.

Conversely, the intraspecific difference in palythine concentration at two collection sites (cf. Figs. 4 and 7) may indicate an adaptive response. That is, the higher concentration in the Bowl Reef specimens might be related to a greater UV flux that would be expected (1) in summer (when they were collected) than in winter (when the Grub Reef colonies were collected), or (2) in the clearer seawater at Bowl (an outer-shelf reef) than at Grub (a mid-shelf reef).

The absence of asterina-330 from freshly-collected colonies of *Clavularia* sp. at the two reef sites, and its appearance during long-term maintenance of Grub Reef specimens, is unexplained. Its appearance even in some UVO acclimatized polyps indicates that it is not specifically induced by increased exposure to UV; its synthesis during different seasons or reproductive states (colonies were maintained for 208 days), or its origin in the diet (unfiltered coastal seawater flowed through the maintenance aquaria), are two possibilities. Its synthesis as a generalized response to stress (elevated temperature, and dilution of seawater

by rain and coastal run-off, occasionally occurred in the flow-through aquaria) is a third possibility.

Net photosynthesis measured at the peak daily irradiance prevailing in the aquaria was reduced by half in the UVT polyps compared to the UVO polyps (Fig. 14) of *Clavularia* sp. This was not due to UV-induced "bleaching" through the loss of zooxanthellae or a depression of their chlorophyll content, as there were no significant differences between UVO and UVT polyps in the number of zooxanthellae (Fig. 14), or in the contents of chlorophyll a and c_2 in the zooxanthellae (Fig. 6). UV-visible scans of methanolic extracts of UVO and UVT polyps showed no obvious difference in the ratio of carotenoids and accessory pigments to chlorophyll (data not shown). Collectively, these results imply that the reduction in photosynthetic capacity in this symbiosis during chronic exposure to UV fluxes characteristic of those occurring at its shallowest depth of occurrence is due to UV effects other than destruction of photosynthetic pigments. Renger et al. (1986) have shown an inhibition of photosynthesis by UV effects on the plastoquinone A-B apoprotein in photosystem II in spinach chloroplasts, but this has not been studied in zooxanthellae.

This reduction in photosynthesis in UVT acclimatized polyps of *Clavularia* sp. was seen whether or not UV-A and UV-B were present during the measurement (Fig. 14), suggesting a direct effect of long-term exposure to UV on photosynthetic capacity. A slight reduction in photosynthesis was seen in the UVO acclimatized colony when exposed to UV-A + UV-B in duplicated measurements. This may indicate an additional, acute effect of UV on photosynthesis in these polyps, but the experiment must be replicated, which was precluded by the high mortality during the acclimatization period.

The significantly higher activity of SOD, and tendency for CAT activit to be elevated, in the animal fraction of UVO acclimatized specimens of *Cl* - *ularia* sp. (Fig. 11) is in keeping with the greater net oxygen flux in their tis (Fig. 14), as the production of superoxide radical should increase with oxyge concentration (Jamieson et al., 1986). However, no differences in activities of total SOD, CAT, or AsPX were seen in zooxanthellae from UVO and UVT acclimatized polyps (Fig. 11). Moreover, specific activity of total SOD was much lower in the zooxanthellae than in the host animal, which is unlike the case in *Phyllodiscus semoni* (see below), *Anthopleura elegantissima* (Dy e 1984), and *Aiptasia pallida* (cf. Lesser and Shick, 1989a, and Tapley, 1 8 .

There were no significant differences in SOD or CAT activities in the ani tissues of *Clavularia* sp. from 4 m and 17 m depth (Fig. 12) This might be expected from the photoadaptation evident in the chlorophyll contents of their zooxanthellae (Fig. 8). That is, if the higher concentration of chlorophyll in the 17 m zooxanthellae compensated for the lower light availability and

raised their total photosynthesis, net oxygen flux in the host's tissues would not be as different from that at 4 m as predicted simply from the difference in depth/irradiance. Thus, the similar activities of SOD and CAT at 4 m and 17 m may reflect similar tissue oxygen regimes at these depths. Still, there was a tendency for SOD activity to be greater at the shallower depth ($P = 0.085$); this might reflect less than perfect photoadaptation in the deeper polyps (due to self-shading as total chlorophyll concentration increased), and perhaps an added photochemical generation of oxygen radicals necessitating greater defenses against them under the higher photon fluxes (including UV: see Dykens and Shick, 1984; Lesser and Shick, 1989a; Lesser et al., 1990) at the shallower depth. SOD and CAT activities in host tissue from freshly-collected polyps were similar to those in UVT acclimatized specimens (cf. Figs. 11 and 12).

Like other sea anemones examined in this light (Scelfo, 1988; Stochaj, 1988, 1989), *Phyllodiscus semoni* showed little elevation in the concentration of its mycosporine-like amino acids during prolonged acclimatization to UV exposure (Fig. 9). Also, when expressed per mg total protein, the concentrations of individual MAAs in *P. semoni* were lower than those in *Clavularia* sp., but similar to those in the anemone's closer relatives among the scleractinians (Dunlap and Chalker, 1986; Dunlap et al., 1986). There was, however, a marked redistribution of MAAs between host and endosymbionts during acclimatization of *P. semoni* to UV: the concentration of mycosporine-glycine declined in the animal but increased two-fold in the zooxanthellae, whereas palythine both increased in the host and appeared in the zooxanthellae, from which it had previously been absent. It is not known whether this redistribution of mycosporine-glycine was due to a decrease in its translocation from symbiont to host, whether the increase in palythine was due to greater rates of synthesis and translocation from the zooxanthellae, or indeed whether the animal can synthesize MAAs *de novo* or modify precursors provided by the algae and transfer the product back to its endosymbionts.

Regarding total UV protection in the host, its seeming decline due to the decrease in mycosporine-glycine was partially offset by the increase in palythine, especially as the latter has a $\approx$ 20–25% greater molar absorptivity for UV (see Dunlap and Chalker, 1986). Total UV protection more than doubled in the zooxanthellae during acclimatization of the anemones to solar UV (Fig. 9).

Unlike the case in *Clavularia* sp., prolonged exposure of *P. semoni* to solar UV reduced the chlorophyll content in zooxanthellae compared to those shielded from UV (Fig. 10). Therefore, the role of UV-absorbing MAAs in the zooxanthellae seems not to lie in absolute protection of the photosynthetic apparatus. A disproportional decrease in chlorophyll *a* relative to chlorophyll

c_2 (i.e., decrease in the $a : c_2$ ratio) was noted in UV-exposed zooxanthellae in *Aiptasia pallida* (Lesser and Shick, 1989a) and the effect was pronounced in *P. semoni* (Fig. 10). The slightly greater protein content of zooxanthellae and their marginally lower density in the tissues of the UVT acclimatized anemones (Fig. 10) is consistent with a UV-related decrease in cell division seen in zooxanthellae from *Aiptasia* spp. exposed in culture (Jokiel and York, 1982; Lesser and Shick, 1989a) and their smaller size *in hospite* (Lesser and Shick, 1989a). The relatively small UV effect on cell size of zooxanthellae exposed *in hospite* compared to those exposed in culture suggests that some UV protection is afforded by the host's tissues (see also Lesser and Shick, 1989a, 1990), which may be the case in *P. semoni* as well.

The effect of UV acclimation on photosynthesis in zooxanthellae freshly isolated from *Phyllodiscus semoni* was quite unlike that in *Aiptasia pallida* (cf. Fig. 15 and Lesser and Shick, 1989a), where UV acclimation led to a reduction in photosynthetic capacity. In *P. semoni*, gross photosynthesis per zooxanthella was at least 75% greater in those isolated from the UVT acclimatized anemone than in those from the UVO anemone, whether or not UV was present during the measurement of photosynthesis. This result occurred despite the lower chlorophyll content in the zooxanthellae from the UVT anemone (Fig. 10), so that the difference would be even greater if expressed per unit chlorophyll a. The basis for this difference is unknown, but might be related to the rather high concentration of chlorophyll in zooxanthellae from the UVO anemone, resulting in greater self-shading in these than in the less-pigmented zooxanthellae from the UVT anemone when tested at the same cell density in the photosynthesis chamber. Such an effect would be accentuated *in hospite*, where the density of zooxanthellae in the pseudotentacles of *P. semoni* (Fig. 10) is greater than that reported in other anemones (reviewed by Shick, in press), and the pseudotentacles appear optically black (Fig. 1a).

Theoretically, our results could be due to an increase in the number of photosynthetic units (PSU) and an accompanying decrease in PSU size (to account for the decrease in chlorophyll during UV acclimatization), but we have no data germane to this possibility. Also, Paerl (1984) showed that long-term exposure of cyanobacteria to UV irradiation increased the synthesis of carotenoids, which both provided photoprotection and enhanced photosynthesis. UV-visible scans (data not shown) of methanolic extracts of zooxanthellae from UVO and UVT acclimatized anemones showed no obvious differences in carotenoid content that would account for the enhanced photosynthesis in the UVT zooxanthellae.

Photosynthesis in zooxanthellae freshly isolated from the UVT acclimatized anemone showed no indication of inhibition by UV-A + UV-B, whereas such

inhibition occurred in zooxanthellae from the UVO acclimatized specimen (Fig. 15). This result seems related to the much higher concentration of MAAs in the former zooxanthellae, but which components of the photosynthetic process may be protected by the UV-absorbing compounds is unknown. Also, respiration in zooxanthellae from the UVT acclimatized anemone was twice as great as respiration in those from the UVO specimen. The difference may indicate the ongoing energetic cost of defending against and repairing damage from UV exposure. It is unknown whether the higher respiratory rate is associated with differences in mitochondrial volume density or ultrastructure (see Lesser and Shick, 1990).

Assuming that the rates of photosynthesis measured in freshly isolated zooxanthellae also occurred *in hospite* (see above), the activities of SOD in the host, and those of SOD, CAT, and AsPX in the zooxanthellae, are interpretable in an adaptive context: higher fluxes of oxygen and UV radiation in the UVT acclimatized anemones necessitated higher activities of antioxidant enzymes (Fig. 13). Such defenses in the zooxanthellae of *Phyllodiscus semoni* appear very robust compared with those in some other cnidarians (see Lesser and Shick, 1989a,b; Lesser et al., 1990).

Several conclusions emerge from the foregoing discussion. First, the rapid and pronounced changes in the concentrations of S-320 compounds seen in some hermatypic corals are not universal among cnidarians. The smaller response of individual MAAs in *Clavularia* sp. during acclimatization and lack of difference over a depth gradient, together with the relatively high concentrations of MAA present, suggest that these compounds are constitutive in this symbiosis.

The stoloniferan *Clavularia* sp., an opportunistic, "weedy" species, occurs within a relatively narrow bathymetric range (B.E. Chalker, personal communication, and our own observations), and may monopolize much of the available space on hard substrates in this range, which includes most of the depths at which UV radiation is expected to have its biological effects (e.g., Jerlov, 1950). The success of *Clavularia* sp. within this range may be due to its capacity for rapid vegetative proliferation, its constitutively high levels of defense against UV radiation, and the capacity of its zooxanthellae for depth-dependent photoadaptation. Long-term acclimatization of colonies of the species to visible and ultraviolet irradiances prevailing at its minimum depth of occurrence results in a pronounced decrease in photosynthesis by its zooxanthellae, which also have comparatively low levels of defenses against forms of active oxygen. This suggests that the restriction of *Clavularia* sp. to depths greater than about 3–4 m is related in part to the relative intolerance of its zooxanthellae to UV exposure.

Conversely, the actiniarian *Phyllodiscus semoni* typically occupies shallow reef-flat habits on the southern Great Barrier Reef (I.D. Lawn, Heron Island Research Station, personal communication), and occurs down to at least 5 m on offshore reefs in the Coral Sea (Shick, personal observation). Acclimatization of the anemone to visible and UV fluxes typical of its shallow water habitat results in significant increases in the levels of UV-absorbing MAAs, particularly in its zooxanthellae. Acclimatization to solar UV enables its zooxanthellae to maintain high rates of photosynthesis even in the presence of UV fluxes that inhibit those from unacclimatized anemones. Zooxanthellae from *Phyllodiscus semoni* do not show photoinhibition at irradiances of PAR (1,000 μmol photons $m^{-2}s^{-1}$) that inhibit the relatively "shade-loving" zooxanthellae in *Aiptasia* spp. (see Jokiel and York, 1982; Muller-Parker, 1984; Lesser and Shick, 1989a). Moreover, both the animal and especially the zooxanthellae in *P. semoni* exhibit particularly high activities of antioxidant enzymes, which increase during acclimatization to UV exposure. This suite of characters probably contributes to the success of the symbiosis in shallow water.

This study has demonstrated that acclimatization to solar UV may elicit very different responses in various zooxanthellae anthozoans. It reports the first controlled experiments on the modulation of identified mycosporine-like amino acids by exposure to solar UV, and provides the first evidence that MAA concentrations in zooxanthellae are associated with differential sensitivity of their photosynthesis to UV. The results provide clues to the sites of UV effects on photosynthesis, which may vary in zooxanthellae isolated from the different hosts.

Acknowledgements

long-term acclimatizations would have ended prematurely and disastrously. D.G. Fautin aided in the identification of *Phyllodiscus semoni*. The masters and crew of the *R.V. Lady Basten* provided their characteristically excellent logistical support and hospitality, and the AIMS workshop personnel (particularly G. McNaughton and J.-C. Collingwood) demonstrated again why their artisanship and cooperation are world-famous. B.E. Chalker and an anonymous reviewer provided thoughtful comments on the manuscript.

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