

REVIEW

ULTRAVIOLET RADIATION-ABSORBING MYCOSPORINE-LIKE AMINO ACIDS IN CORAL REEF ORGANISMS: A BIOCHEMICAL AND ENVIRONMENTAL PERSPECTIVE¹

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Key index words and abbreviations: coral reefs; MAAs, mycosporine-like amino acids; PAR, photosynthetically active radiation (400–700 nm); PC, phosphatidylcholine; PC-OOH, phosphatidylcholine hydroperoxide; ROS, reactive oxygen species; UVA, ultraviolet-A radiation (320–400 nm); UVB, ultraviolet-B radiation (280–320 nm); UVR, environmental ultraviolet radiation (295–400 nm)

The clear waters surrounding tropical coral reefs typically are oligotrophic, yet these reefs are highly productive and support dense populations of marine organisms. This paradox is resolved in part because many coral reef invertebrates accommodate unicellular autotrophs (“zooxanthellae,” *Symbiodinium* spp.; Prochlorales, *Prochloron* sp.; cyanobacteria) within their tissues. These photoautotrophic symbioses entail an exchange of nutrients between the endosymbionts and the animal hosts. Organic compounds produced by the microalgal partners are released to the hosts for their nutrition while inorganic metabolic wastes are recycled to fertilize the algae (Muscatine 1990).

The requirement for photosynthetically active radiation (PAR, 400–700 nm) also exposes such algal-invertebrate symbioses and other primary producers to high levels of environmental ultraviolet radiation (UVR = 295–400 nm) in tropical waters (Smith and Baker 1979, Fleischmann 1989). These waters are often low in UV-absorbing particulate and dissolved organic matter, and sea level fluences of solar UV are high because of the short atmospheric path length and thinness of the stratospheric ozone layer above the tropics (Baker et al. 1980). Photoautotrophic symbiosis thus presents an evolutionary challenge by precluding the morphological development of an optically opaque barrier (such as hair, scales, and feathers in higher vertebrates) and potentially allows damaging UV radiation to reach vulnerable biomolecules in both partners.

The problem is exacerbated because the tissues are hyperoxic (>250% air saturation: Kühl et al.

1995), with most photoautotrophic corals, sea anemones, and tridacnid clams typically producing more O₂ than the symbiosis consumes in respiration (Fig. 1) (Mangum and Johansen 1982, Chalker et al. 1985, Shick 1990). These conditions are conducive to photooxidative stress in the symbiosis (D’Aoust et al. 1976) via the photodynamic production of cytotoxic reactive oxygen species (ROS), including singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), and oxygen-centered radicals (superoxide, O₂⁻, hydroperoxyl, HOO[•], and hydroxyl, HO[•]) (Dykens and Shick 1982, DiGiulio et al. 1989, Tyrrell 1991, Dykens et al. 1992, Shick 1993).

Biochemical defenses against direct photochemical damage caused by solar UVB radiation and effects of UVA (and UVB) mediated by reactive oxygen species include UV-absorbing compounds (sunscreens), water-soluble reductants (glutathione, ascorbate, urate) in the cytosol, lipid-soluble antioxidants (α -tocopherol, β -carotene) residing in membranes, and the antioxidant enzymes glutathione peroxidase, glutathione reductase, ascorbate peroxidase, catalase, and superoxide dismutase. These cellular defenses in corals and other tropical symbioses are often inducible under conditions of UV exposure (Jokiel and York 1982, Scelfo 1986, Lesser et al. 1990, Shick et al. 1991, Kinzie 1993, Banaszak and Trench 1995b, Lesser 1996) and have been implicated in the prevention of UV damage and oxidative stress in marine algal-invertebrate endosymbiosis (Matta and Trench 1991, Dykens and Shick 1982, Dykens et al. 1992, Shick et al. 1995). In contrast to antioxidant enzymes, little is known about the biochemistry of small-molecule antioxidants (reductants) for maintaining redox balance in photoautotrophic symbioses (e.g. Dunlap and Yamamoto 1995), and even less is known about the relative importance of mechanisms for repair of UV photodamage (Carlini and Regan 1995).

Effects of Environmental UV Radiation on Coral Symbiosis

Because sunlight is a major factor influencing the growth of coral reefs (Chalker et al. 1986, 1988a), considerable interest has developed in the physio-

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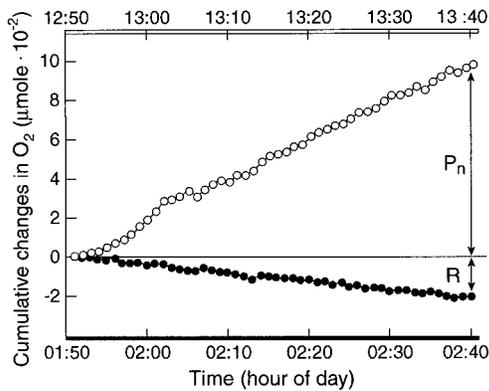


FIG. 1. Photosynthesis-respiration diagram: cumulative oxygen production (P_n) during peak daylight (\circ) and consumption during nocturnal respiration (\bullet) by the coral, *Acropora formosa*, having the endosymbiotic algae, *Symbiodinium* sp. P_n is the cumulative measurement (rate) of net oxygen production during peak daylight, P_g is gross photosynthesis, and R is the cumulative consumption of oxygen measured during dark respiration; $P_n = P_g + R$, where R is a negative value. Maximum net hourly rate of photosynthesis (P_n) was $1250 \text{ mmole O}_2 \cdot \text{h}^{-1}$, the dark respiration rate was $-243 \text{ mmole O}_2 \cdot \text{h}^{-1}$, and the maximum hourly P_g/R ratio was 6.14. Data from Chalker et al. 1985.

logical and ecological consequences of the exposure of reef organisms to solar UV. Jokiel (1980) was the first to demonstrate that cryptic reef epifauna were killed when acutely exposed to ambient levels of UV radiation in shallow water; he suggested that the community structure of coral reefs was affected by the relative UV tolerances of their constituent species. Jokiel and York (1982) were later to provide physiological evidence that UV exposure reduces skeletal growth in a reef-building coral, *Pocillopora damicornis*, and reduces the photosynthetic capacity of endosymbiotic zooxanthellae isolated from various invertebrate hosts. UV inhibition of endosymbiont photosynthesis from a variety of coral species has since been reported (Gleason 1993, Kinzie 1993, Masuda et al. 1993, Banaszak and Trench 1995a, Shick et al. 1995, Lesser 1996). Likewise, UV inhibition of photosynthesis by zooxanthellae *in hospite* (within the host) and *in vitro* for reef anthozoans other than corals has been measured (Lesser and Shick 1989, Shick et al. 1991). Gleason and Wellington (1995) have documented depth-related variations in the UVB sensitivity of planula larvae of the coral *Agaricia agaricoides*. Planulae from colonies growing at 3 m were found significantly more UV resistant than planulae from 24-m depth. This differential UV sensitivity was attributed to the effects of UVB rather than UVA or visible light (PAR), and has profound implications for coral reproductive success and recruitment.

There has been growing concern since the early 1980s over the tropic-wide phenomenon of mass coral "bleaching", which in severe cases results in mortality (Oliver 1985, Drollet et al. 1995). Bleaching (via the photodestruction of algal pigments or

expulsion of endosymbionts from the host) is a response to various physical environmental stressors, especially elevated seawater temperature, and in some cases, solar UVR (reviewed in Glynn 1996, Shick et al. 1996, Brown 1997). While there have been many reports concerning the occurrence and ecological impacts of coral bleaching, the molecular and cellular processes leading to bleaching remain unknown, although oxidative stress has been implicated as a signal or proximal cause (Lesser et al. 1990, Lesser 1997, Nii and Muscatine 1997).

Mycosporine-like Amino Acids in Marine Organisms

Sessile symbioses, sedentary invertebrates, and benthic algae and cyanobacteria occupying the light-intense environment of coral reefs have evolved a physicochemical strategy to reduce UV damage. Shibata (1969) discovered the presence of UV-absorbing substances in corals, observing that aqueous extracts of five *Acropora* species, one *Pocillopora* species, and a cyanobacterium from the Great Barrier Reef contained large quantities of a water-soluble material ("S-320") having a broad absorbance maximum (λ_{max}) at approximately 320 nm. Sivalingam et al. (1974) reported similar absorption peaks in 70 species of marine algae representing four phyla. Maragos (1972) demonstrated that the absorbance by S-320 in colonies of *Porities lobata* is inversely proportional to depth, presumably in compensation to the ambient levels of solar UV radiation prevailing in their environment; the relationship appears to be a general one (Kuffner et al. 1995), and one such example of depth-dependent photoacclimatization in *Acropora formosa* (Dunlap et al. 1986) is provided in Figure 2. This correlation may explain why corals collected from depths of 1–2 m were more resistant to artificial near-UV (UVA and UVB) exposure than conspecifics growing at greater depths (Siebeck 1988), although other depth-dependent defenses such as antioxidant enzyme activities may also be involved (Shick et al. 1995).

S-320 has since been identified in corals to comprise a family of mycosporine-like amino acids (MAAs) (Dunlap and Chalker 1986), which were previously known metabolites found in a variety of marine organisms (Nakamura et al. 1982). MAAs are characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid and have UV-absorption maxima in the range 310–360 nm (Fig. 3), extending across much of the spectrum of potentially damaging UVA and UVB wavelengths. The parent class of mycosporines was originally discovered as fungal metabolites associated with light-induced sporulation (Favre-Bonvin et al. 1976). These metabolites were later postulated to provide UV protection to fungal spores while exposed to solar radiation during dispersal by atmospheric transport (Young and Patterson 1982).

MAAs have been identified in taxonomically di-

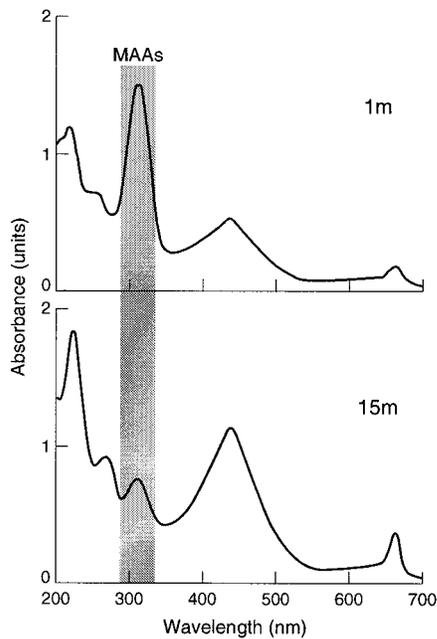


FIG. 2. UV-visible light absorption spectra of 20% tetrahydrofuran in methanol (v:v) extracts of 6-cm tips of *Acropora formosa* collected from depths of 1 and 15 m at Davies Reef, Great Barrier Reef, Australia. The shaded portion of the spectra corresponds to the absorption of UV light by endogenous MAAs in tissue extracts. Data from Dunlap et al. 1986.

verse organisms, including a marine heterotrophic bacterium (Arai et al. 1992), cyanobacteria (Garcia-Pichel and Castenholtz 1993, Garcia-Pichel et al. 1993, Karsten and Garcia-Pichel 1996), microalgae (Okaichi and Tokumura 1980, Carreto et al. 1990a, Vernet and Whitehead 1996, Shashar et al. 1997), and macroalgae (Takano et al. 1979, Tsujino et al. 1980, Sekikawa et al. 1986, Banaszak and Lesser 1995, Carroll and Shick 1996). Within nonsymbiotic marine invertebrates, MAAs have been identified in echinoderms (Nakamura et al. 1981, Chioccaro et al. 1986b, Chalker et al. 1988b, Shick et al. 1992, Adams and Shick 1996, Carroll and Shick 1996), a mussel (Chioccaro et al. 1986a), a sea hare (Carefoot et al. 1998), brine shrimp (Grant et al. 1985), and in an ascidian (Kobayashi et al. 1981). MAAs are also found in the eyes, skin, and reproductive tissues of tropical and temperate fishes (Chioccaro et al. 1980, Dunlap et al. 1989, Thorpe et al. 1993, Mason et al. 1998). MAAs are common in microalgal-invertebrate symbioses on coral reefs and elsewhere, including: a sponge (Bandaranayake et al. 1996), scleractinian corals (Dunlap and Chalker 1986, Dunlap et al. 1986, Gleason and Wellington 1993, 1995, Kuffner et al. 1995, Shick et al. 1995, Wu Won et al. 1995, 1997, Teai et al. 1997a, b) and their eggs (Krupp and Blanck 1995) and mucus (Drollet et al. 1997), sea anemones (Shick et al. 1991, Stochaj et al. 1994, Banaszak and Trench 1995b), octocorals (Aliño 1989, Shick et al. 1991), a zoanthid (Ito and Hirata 1977, Takano et al.

1978a, b, Hirata et al. 1979, Uemura et al. 1980), a scyphozoan jellyfish (Banaszak and Trench 1995b), a tridacnid clam (Ishikura et al. 1997), and an ascidian (Lesser and Stochaj 1990, Dionisio-Sese et al. 1997).

MAAs are one of nature's sunscreens, with 19 structurally distinct MAAs presently identified in marine organisms (Fig. 3), together with several biochemically related gadusols (Grant and Plack 1980, Plack et al. 1981, Grant et al. 1985). MAAs occur in organisms from tropical and subtropical coral reefs to the Antarctic Ocean. MAAs are found over a broad taxonomic distribution of Antarctic marine organisms (Karentz et al. 1991), where they may protect benthic species (Karentz et al. 1997) and the planktonic food web of the southern oceans from extreme fluctuations in UV radiation under the seasonal "ozone hole" (Dunlap et al. 1995, Helbling et al. 1996, Riegger and Robinson 1998).

The MAA content of 51 species of macrophytes (19 Rhodophyta, 11 Phaeophyta, 8 Chlorophyta, and 3 Cyanophyta) was surveyed from Kaneohe Bay, Hawaii (Banaszak and Lesser 1995). MAAs were detected in 46 (90%) of the species examined. Mycosporine-glycine and shinorine were the most frequently encountered MAAs, both being found in 28 of the species studied. Porphyra-334 was found in 16 species, palythine occurred in 10 species, asterina-330 was found in 8 species, and palythinol was the MAA least often detected (3 species). Similarly, two of these MAAs, porphyra-334 and shinorine, were the most abundant among eight species of Antarctic algae (five Rhodophyta, one Phaeophyta, one Chlorophyta, and one Bacillariophyta) (Karentz et al. 1991).

Rhodophyte and phaeophyte species were predominant on patch reefs in Kaneohe Bay, and chlorophytes when found were present in low biomass. The observation that predominant red and brown algae on these exposed reefs have more diverse and higher concentrations of these UV-absorbing MAAs than the other algae is consistent with their greater exposure and resistance to UVR. In this study, the authors noted the presence of two unknown compounds that consistently occurred across algae in all divisions, although they did not give the UV-absorption spectra and λ_{\max} for these unknown metabolites.

UV Photoacclimation and UV Protection

Jokiel and York (1982) demonstrated that long-term exclusion of solar UV from the coral *Pocillopora damicornis* caused a decrease in the tissue concentrations of S-320, which suggests that S-320 compounds are synthesized in response to UV light and not some other bathymetrically distributed environmental factor. Conversely, Scelfo (1986) found that both UV and longer wavelengths induced increases in S-320 in corals translocated from depths of 3 and 10 m to aquaria at sea level. Although there are no

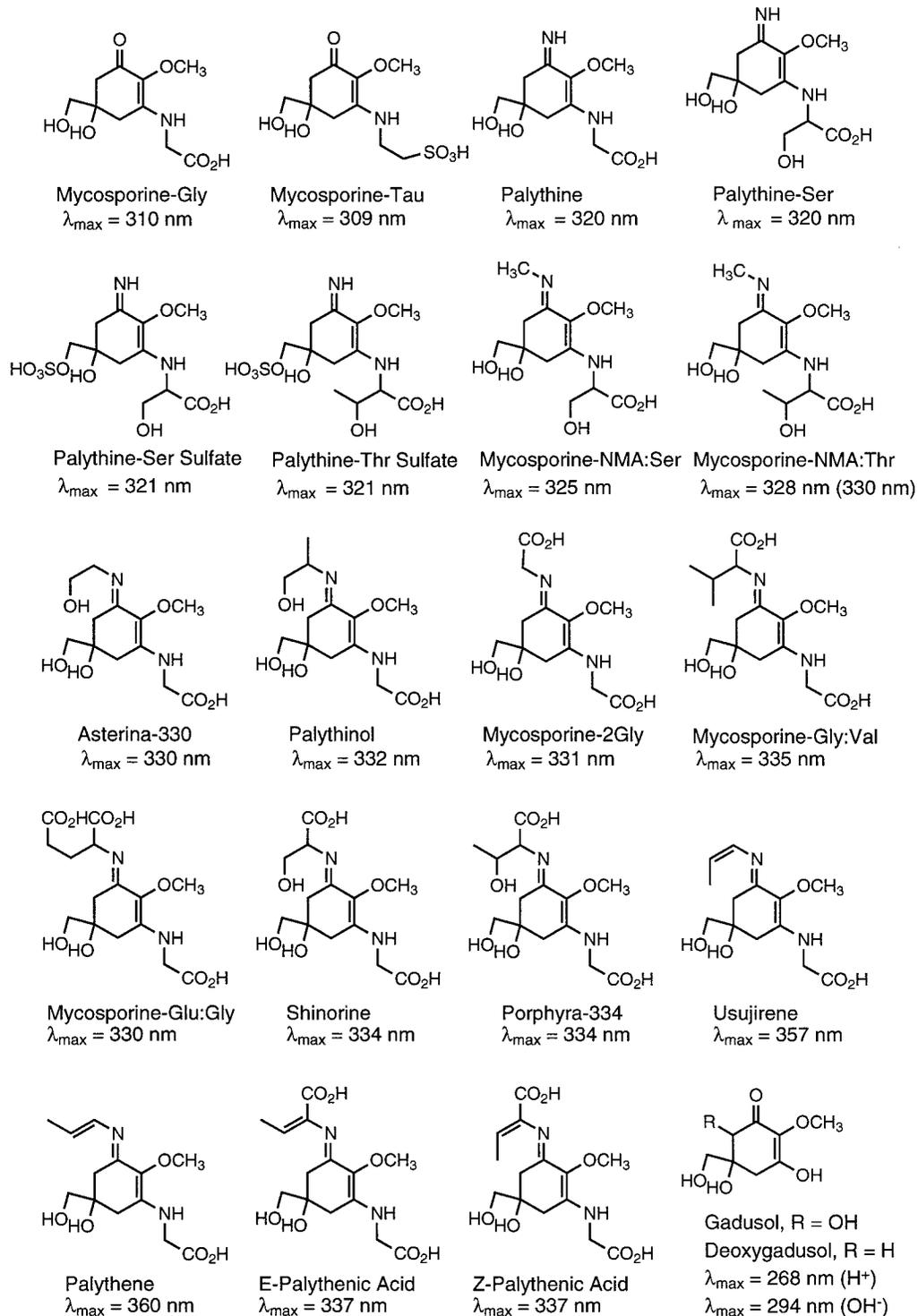


FIG. 3. Molecular structures and wavelengths of maximum absorption (λ_{\max}) of mycoporinelike amino acids, and biosynthetically related gadusols, identified in marine organisms.

studies of the responsiveness of MAA levels in symbiotic corals to precisely controlled spectral irradiance, research on the free-living dinoflagellate *Alexandrium excavatum* is relevant. Carreto et al. (1990b) showed that sunlight is more effective than tungsten illumination in causing MAA synthesis in

this dinoflagellate, that red and green wavelengths are ineffective, that blue light induces MAA synthesis (but less so than full-spectrum sunlight at the same total irradiance), and that UVA radiation is highly effective. Assuming that MAAs in corals are produced by their symbiotic dinoflagellates (see be-

low), these results, together with the greater attenuation of UV than of blue wavelengths with depth in seawater (see, e.g. figures in Lesser 1995), suggest that UV irradiance is the principal determinant of the depth-dependent relationship of MAA concentration in corals. Other depth-related factors, including PAR and water motion, can also influence MAA synthesis via effects on coral metabolism (Jokiel et al. 1997).

The UV-photoprotective function of MAAs is inferred from their efficient UVA/B-absorbing properties (molar extinction coefficients, $\epsilon \approx 28000$ – 50000), together with the often observed, depth-dependent relationship of MAA concentrations in coral tissues whereby shallow-water corals contain higher concentrations of MAAs or S-320 than do conspecifics living at greater depth (Fig. 2) or in shaded habitats (Maragos 1972, Dunlap et al. 1986, Scelfo 1986, Gleason and Wellington 1993, Kinzie 1993, Shick et al. 1995). Similar photoacclimatization has been noted in other coral reef anthozoans and algae (Olson 1986, Wood 1989). A notable exception is that no significant differences in MAA concentrations were found over a depth gradient of 4 to 17 m in the octocoral *Clavularia* sp. (Shick et al. 1991).

Jokiel and York (1982) demonstrated that long-term exclusion of solar UV from the coral *Pocillopora damicornis* caused a decrease in MAA tissue concentrations, and transplantation of *Montipora verrucosa* to shallower depth increased the tissue concentrations of these compounds in specimens acclimatized to full solar UV after only 13 days (Scelfo 1986). However, not all anthozoans respond in this manner. After an initial increase, attributed to the physical disturbance of transplantation, Scelfo (1985) found no significant increase in the levels of MAAs in the Hawaiian zoanthid *Zoanthus pacificus* maintained at high UV levels for 78 days, although concentrations did decline in individuals shielded from UV. Similarly, the reef anemone *Phyllodiscus semoni* showed a significant loss in mycosporine-glycine after prolonged shielding from UV light (Shick et al. 1991), although in another study, zooxanthellate and naturally apozooxanthellate (functionally lacking zooxanthellae) specimens of the temperate sea anemone *Anthopleura elegantissima* acclimated with and without simulated solar UV for 28 days did not show any regulation of MAA concentrations in response to UVR (Stochaj et al. 1994). Likewise, no significant increase in S-320 concentration was observed in the reef-building coral *Stylophora pistillata* 4 weeks after transplantation from 30 to 5 m (Gattuso 1987). A lesser ability for some corals to change MAA concentrations with increasing levels of UV exposure may account for their mortality when transplanted from greater to lesser depths without UV shielding (Scelfo 1986, Vareschi and Fricke 1986).

Unlike *Phyllodiscus semonii* (Shick et al. 1991), MAA or S-320 concentrations in *Palythoa caribaeorum* (Lesser et al. 1990), *Anthopleura elegantissima* (Sto-

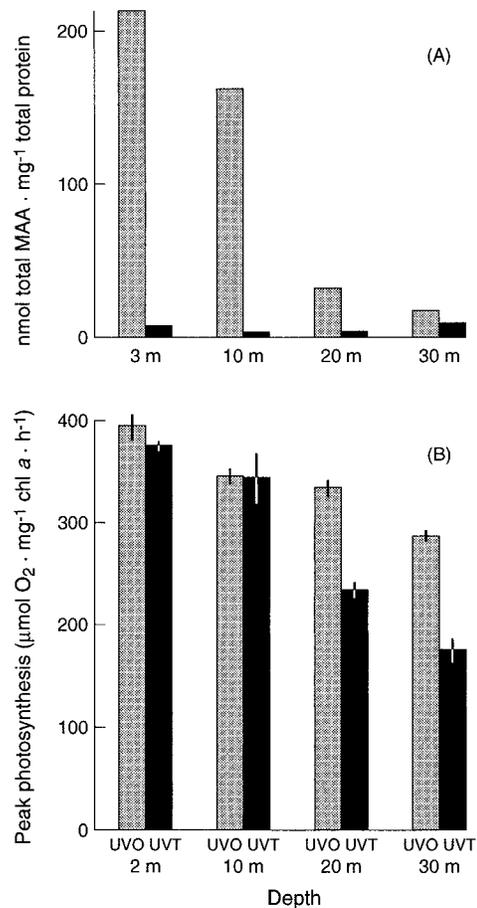


FIG. 4. (A) Total concentrations of mycosporine-like amino acids (nmol MAAs · mg⁻¹ protein) in the host coral tissues (gray bars) and in isolated zooxanthellae (black bars) from *Acropora microphthalma* colonies collected at 3-, 10-, 20-, and 30-m depths. (B) Peak rates of photosynthesis (μmol O₂ · mg⁻¹ chl a · h⁻¹) in paired branches of corresponding colonies in (A) moved to 1-m depth for acute measurements of photosynthesis in UV-opaque (UVO) and UV-transparent (UVT) chambers. Data from Shick et al. (1995).

chaj et al. 1994, Banaszak and Trench 1995b), *Acropora microphthalma* (Shick et al. 1995), *Lissoclinum patella* (Lesser and Stochaj 1990, Dionisio-Sese et al. 1997), and tridacnid clams (Ishikura et al. 1997) occur predominately or entirely in the animal tissue rather than in their algal symbionts; details regarding *A. microphthalma* are presented in Figure 4A. A common feature of these studies was that photosynthesis in symbiont cells freshly isolated from their invertebrate host was strongly inhibited by UV radiation, whereas photosynthesis by symbionts *in hospite* from shallow-water colonies was largely unaffected by UV exposure. In the case of *A. microphthalma*, peak rates of photosynthesis in colonies from 2 and 10 m transferred to 1 m were unaffected by the higher levels of ambient UV irradiance at 1 m, whereas photosynthesis in colonies transferred from 20 and 30 m having significantly lower concentrations of MAAs in host tissues, showed 30 and 38%

inhibition of peak photosynthetic rates, respectively (Fig. 4B). Results from these studies suggest that MAAs in host tissues (and external mucus: Drollet et al. 1997) are the first line of defense against solar UV damage providing protection to their endosymbiotic algae. This barrier also extends to providing UV photoprotection to zooxanthellae lacking MAAs in the siphonal mantle of *Tridacna crocea* (Ishikura et al. 1997), to MAA-deficient *Prochloron* sp. living beneath the tunic of *L. patella* (Dionisio-Sese et al. 1997), and to endolithic chlorophytes (*Ostreobium* sp.) residing beneath the coral tissue within the calcified skeleton (Shashar et al. 1997). Optical considerations may provide a mechanistic explanation for the predominant occurrence of the sunscreen within the hosts' cells rather than in the smaller endosymbionts: lower, less osmotically disruptive concentrations of MAAs in the former would nevertheless afford greater protection because UV would be attenuated over a longer optical pathlength (see Garcia-Pichel 1994).

Sources of MAAs in Coral Reef Organisms

An autotrophic advantage of MAA biosynthesis in symbioses is yet to be fully established. Heterotrophy is important in many corals (Sebens et al. 1996), but the principal source of carbon for most zooxanthellate species is generally autotrophic (Muscatine 1990). Since MAA biosynthesis (in fungi) involves the shikimic acid pathway (Favre-Bonvin et al. 1987), a biochemical route not present in animal tissues (Bently 1990), the origin of MAAs in microalgal-invertebrate symbiosis is assumed to be the algal partner. This is surely the case in the tropical scyphozoan *Cassiopeia xamachana*, where the polyp stage, when rendered apozooxanthellate in culture, does not produce MAAs whether or not exposed to UV, whereas polyps and free-living medusae hosting endosymbionts do contain MAAs (Banaszak and Trench 1995b). Moreover, zooxanthellae (*Symbiodinium microadriaticum*) isolated and cultured from this scyphozoan produce MAAs, particularly in response to UV.

Conversely, the occurrence of the same complement and concentrations of MAAs in naturally apozooxanthellate specimens of *Anthopleura elegantissima* as in symbiotic individuals suggests that zooxanthellae are not the source of MAAs in this species (Stochaj et al. 1994). This is shown conclusively by the observation that the zooxanthellae (*Symbiodinium californium*) associated with *A. elegantissima* do not synthesize MAAs in culture or *in hospite* (Banaszak and Trench 1995b). Similarly, chemical analysis of the siphonal mantle of *Tridacna crocea* showed significant amounts of MAAs, whereas none were detected in zooxanthellae freshly isolated from this tissue or in zooxanthellae cultured in the presence of UVA (Ishikura et al. 1997). However, in most studies of photoautotrophic symbiosis, the same suite of MAAs is usually found in the zooxanthellae as in the

animal tissue, indicating an exchange of these compounds between host and symbiont (when present), although the direction of such transfer has never been determined. Furthermore, an accounting of all MAAs (endosymbiotic, dietary, or a contribution of both) has not been fully established for any one example of a marine algal-invertebrate symbiosis.

There is little doubt that symbiotic zooxanthellae inhabiting different coral species may themselves be taxonomically distinct (Blank and Trench 1985; reviewed by Rowan, this issue) and that genotypic differences among zooxanthellae can even occur across a single coral colony (Rowan and Knowlton 1995). The aforementioned biochemical diversity among *Symbiodinium* species in their ability to synthesize MAAs may reflect this genotypic diversity, although there has been no systematic study of this and no direct link has been established between genetic differences in interspecific colony associations and their MAA concentrations. Such differentiation, however, might have profound effects on the biochemical response to environmental stress, including inter- and intraspecific differences in the sensitivity of coral colonies to bleaching (Brown 1997, Rowan et al. 1997). For example, small genetic differences in symbiont associations may affect the biochemical (MAA) response to the synergy of temperature and photooxidative stress in coral symbioses (Lesser 1996).

Assuming that invertebrates are unable to synthesize MAAs (an assumption not yet tested experimentally), and although a bacterial source cannot be discounted (Arai et al. 1992), their trophic accumulation by nonsymbiotic reef consumers such as holothuroid echinoderms (sea cucumbers) may be the major pathway of UV acclimatization (Shick et al. 1992). The MAAs in holothuroids occur predominantly in their epidermal tissues and gonads, suggesting a photoprotective function against topical UV exposure and during reproduction. MAAs are also consistently present in gut tissues, implicating a dietary source. However, the MAAs in coral reef epiflora (benthic microalgae and filamentous cyanobacteria) consumed by holothuroids differ markedly from the complement of MAAs stored in the consumer's tissues; the principal MAA component in holothurian tissues is asterina-330, which is absent from dietary epiflora (Dunlap and Shick, unpubl.). It was discovered in studying the metabolic processes of MAA bioaccumulation in the holothuroid *Thelenota ananas* that the ubiquitous marine bacterium *Vibrio harveyi* (in this case isolated from the gut fluid of *T. ananas*) selectively hydrolyzes the hydroxyamino acid substituents of the algal MAAs, shinorine, and porphyra-334 to yield mycosporine-glycine, which was found in increasing quantities along the lumen of the digestive tract until its ultimate translocation to the body tissues (Dunlap and Shick, unpubl.). From these observations, we postulate that the conversion to asterina-330 is completed within

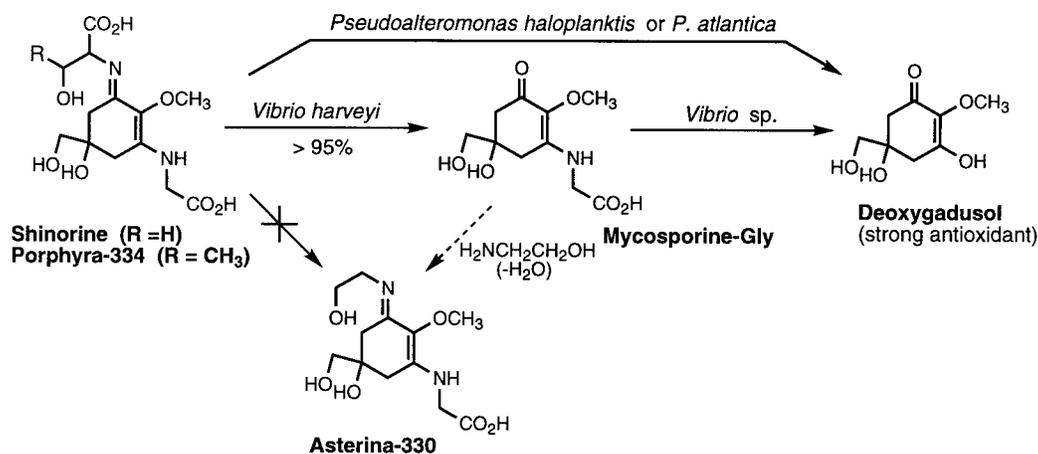


FIG. 5. Proposed scheme for the bioconversion of algal MAAs (shinorine and porphyra-334) to asterina-330 in tropical holothuroids, and conversion to the strong antioxidant, 4-deoxygadusol. Scheme in part from Dunlap et al. (in press) and Dunlap et al. (unpubl.).

the holothuroid's tissues via reamination of mycosporine-glycine by metabolic pools of ethanolamine (Fig. 5). The kinetic data for the conversion of shinorine to mycosporine-glycine in artificial seawater using a cultured strain of *V. harveyi* are presented in Figure 6 (conversion yield = 97.2%).

Direct measurements of MAA accumulation (and clearance) in nonsymbiotic, coral reef invertebrates via selective feeding experiments have not been attempted. However, experiments on the boreal sea urchin *Strongylocentrotus droebachiensis* feeding on a controlled diet of the rhodophyte *Mastocarpus stellatus* have clearly established the dietary origin of MAAs (principally shinorine) with little secondary modification in its ovaries and eggs (Carroll and Shick 1996), and a similar algal dietary origin has been demonstrated in the eggs of the sea hare *Aplysia dactylomela* (Carefoot et al. 1998). This may also be the case in coral reef asteroids (Chalker et al.

1988b) and echinoids (Adams and Carroll, unpubl.), the ovaries, eggs (but not sperm), and larvae of which contain high concentrations of MAAs.

Medaka fish (*Oryzias latipes*) accumulate asterina-330 and palythine (both present in an artificial diet containing *M. stellatus*) in their eyes, although they do not accumulate shinorine, the principal MAA in this diet (Mason et al. 1998). The experimental results on accumulation of these particular MAAs by medaka are consistent with the data on the MAA composition in the ocular lenses of wild-caught coral reef fishes (Dunlap et al. 1989), again suggesting a trophic passage of MAAs in coral reef communities, as well as a specificity in such bioaccumulation.

The functional role of MAA photoprotection in eggs of *S. droebachiensis* was confirmed by the inverse relationship between MAA concentration and UV-induced inhibition of the first cell division of embryos developing from dietarily manipulated eggs having high and low concentrations of MAAs (Adams and Shick 1996). These data fit the biooptical model of Garcia-Pichel (1994) for predicting the efficiency of UV-absorbing, intracellular sunscreens in unicells, which model may also explain why MAAs are not accumulated to the extent they are in eggs by picoplankton ($\leq 1\text{-}\mu\text{m}$ radius) or by invertebrate spermatozoa of similar size: the very high concentration of MAAs required to provide effective UV protection in cells less than a few micrometers in diameter would be osmotically infeasible in metabolically active cells. Thus, the vulnerability of unprotected sperm to UVR may help explain the prevalence of nocturnal spawning in corals and other reef animals (Gulko 1995).

Furthermore, the concentration of shinorine (the principal MAA in the eggs) did not appreciably change during either short-term UV exposure of sea urchin eggs *in vivo*, or long-term exposure of shinorine in aqueous solution (Adams and Shick 1996), indicating the functional photostability of

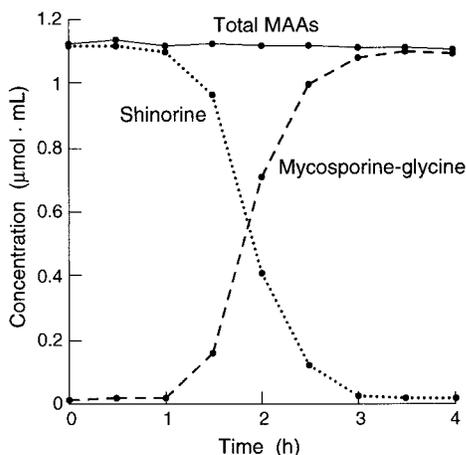


FIG. 6. Bioconversion of shinorine, prepared from *Mastocarpus stellatus* extract, in filter-sterilized ($0.22\text{-}\mu\text{m}$ pore size) seawater inoculated with cultured *Vibrio harveyi* (strain RML20) isolated from the enteric fluid of *Theleota ananas*. Data from Dunlap et al. (unpubl.).

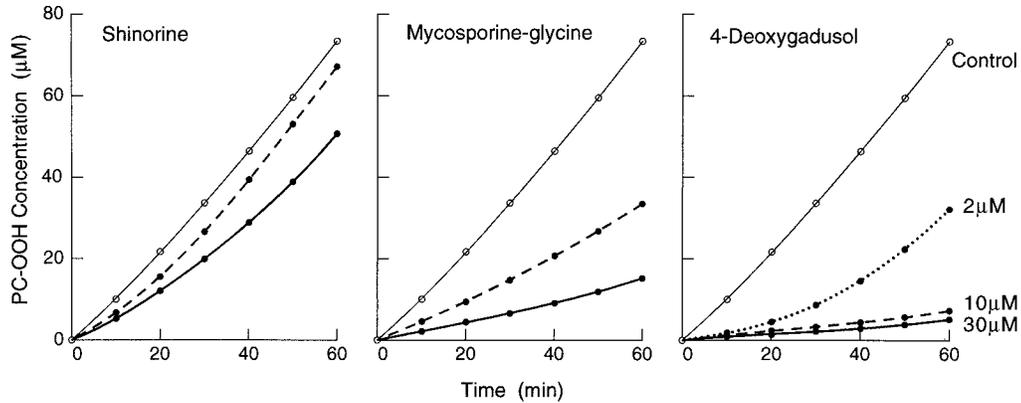


FIG. 7. Phosphatidylcholine (PC) peroxidation inhibition assay for purified shinorine, mycosporine-glycine, and 4-deoxygadusol. PC-OOH is the phosphatidylcholine hydroperoxide product of PC oxidation. Antioxidant activity is judged by the substrate-added reduction in the control rate of PC oxidation. Data from Dunlap and Yamamoto (1995) and Dunlap et al. (in press).

this MAA as a natural sunscreen. Such photooxidative robustness of MAAs released into seawater by phytoplankton may contribute not only to their persistence and attenuation of UV in the water column (Vernet and Whitehead 1996), but dissolved MAAs may also be available for uptake via integumentary transport in soft-bodied invertebrates, including larvae (reviewed by Wright and Ahearn 1997).

Antioxidant Function of Mycosporine-Glycine and Related Gadusols

Mycosporine-glycine is a predominant MAA in coral symbiosis on the Great Barrier Reef (Dunlap and Chalker 1986, Dunlap et al. 1986, Shick et al. 1991, 1995, Dunlap and Yamamoto 1995), Hawaii (Shashar et al. 1997, Jokiel et al. 1997), Tahiti (Teai et al. 1997a, b), Palau (Dionisio-Sese et al. 1997), and on Caribbean reefs (Gleason and Wellington 1995). As well as functioning as a natural sunscreen ($\lambda_{\max} = 310$ nm), this MAA also has moderate antioxidant activity (Dunlap and Yamamoto 1995) and may provide some protection against photooxidative stress in the hyperoxic tissues of algal-invertebrate symbioses. The presumed biochemical precursor of MAAs, 4-deoxygadusol (Fig. 3), is often observed in autotrophs and autotrophic symbioses (Dunlap, pers. observ.); it has strong antioxidant activity (Dunlap et al. 1998) and has been prepared via a "retrobiosynthetic" bacterial conversion of algal MAAs (Fig. 5) for commercial purposes (Masaki et al. 1996). The antioxidant capacities of mycosporine-glycine and 4-deoxygadusol, and the lack thereof in shinorine (showing a slight oxidative impurity), are demonstrated using the phosphatidylcholine (PC) peroxidation inhibition assay (Niki 1990) by which antioxidant activity is evaluated by the substrate-added reduction in the control rate of chemically initiated, free-radical hydroperoxidation of PC *in vitro* (Fig. 7). While the functional abilities, metabolic fate, and possible cycling of these antioxidants are largely unexplored in algal-invertebrate

symbioses, the oxidative robustness of imino-mycosporines is in keeping with their primary role as a stable biological sunscreen in coral reef organisms (Dunlap and Yamamoto 1995).

Conclusion and Prospects

The importance of MAAs as natural UV protectants in coral reef organisms will continue to attract scientific study of their biochemical responsiveness to environmental change. Future studies will define the importance of MAAs in the physiology, reproductive success, and community structure of tropical coral reef organisms. Many dogmas remain untested experimentally: is it true that invertebrates cannot synthesize MAAs?; are dietarily derived MAAs metabolically altered once within the cells of consumers?; do genotypic differences among zooxanthellae in their ability to synthesize MAAs affect the UV resistance and bathymetric distribution of their hosts?; do prevalence and biosynthesis of certain MAAs having different UV absorption maxima (and antioxidant activities) consistently correlate with the spectral irradiance, oxygen microenvironment, and thermal regime of the organisms containing them?; do the UV-absorbing and antioxidant properties of MAAs provide protection against coral bleaching? The evolutionary significance of MAA biosynthesis in autotrophs living on the early earth before the development of a protective ozone layer has not been considered. Comparison of extant photosynthetic bacteria, cyanobacteria, and microalgae having representatives in the early fossil record is one approach to this evaluation.

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Question (Adey): See preface before Adey's question to Rowan (1998): As stated in your introduction, it is widely accepted that the abundant symbiosis in reef environments is driven (for the algal partner) by nutrient stress. Considering the preface to this question, this seems unlikely. In the light of your review, could it be that animal (the host's) tissues provide some protection from UV? Could this also explain the apparent depth cline of *Symbiodinium* species in the same host? Is there any information on UV gradients in stony coral tissues?

Answer: In response to your preface, we suggest a different interpretation of our statement on nutrient exchange within symbiosis. We do not offer the opinion that coral symbiosis is driven to accommodate the algal partner under nutrient stress. Here, we refer to the accepted body of evidence that coral symbiosis involves a *mutualistic* exchange between the partners in which nutrients are largely conserved (Muscatine and Porter 1977), although external supplementation of inorganic nutrients is likely (Dubinsky and Jokiel 1994). This leads to the concept of an evolutionary cost to symbiosis, that is, the requirement for photosynthetically active radiation to reach algal cells within host tissues without damage to either partner by the UV component of solar radiation. Moreover, it should be recognized in your argument that algal endosymbionts residing within invertebrate tissues are not directly exposed to the "infinite availability" of ambient nutrients enjoyed by turf algal producers. In this sense, it is arguable that symbiosis is cultivated by the animal partner as a means to acquire autotrophic nutrition while utilizing the proton-pumping function of photosynthesis to regulate cellular pH and CO²/carbonate in favor of calcification.

Do coral tissues provide UV protection for the benefit of their algal symbionts? It is evident from tissue analyses in the coral *Acropora microphthalmia* that protein-specific MAA concentrations are predominantly associated with the animal host (Fig. 4A, our review). The same is true for algal symbiosis in *Tridacna crocea* (Ishikura et al. 1997). Presumably this "sunscreening" function is utilized by the animal host for its own protection while providing an inherent defence for their endosymbionts. The question is particularly relevant if algal symbionts need UV protection. This is likely given that it has been observed in several examples that photosynthesis by symbionts freshly isolated from shallow-water hosts is strongly inhibited at ambient levels of UV exposure, whereas photosynthesis by symbionts under equivalent conditions *in hospite* is largely unaffected (Masuda et al. 1993, Shick et al. 1995). Further investigation is warranted, as there is scant knowledge regarding the physiological ecology of "unprotected" free-living, prospective symbionts (necessary for infection of naturally apozooxanthellate embryos) exposed to environmental UV. A related question is whether free-living zooxanthellae

produce and retain MAAs for their own protection, and if so, whether a signal from the host subsequently causes them to release virtually all of their MAA production to the host's tissues once established in symbiosis.

We have asked the reverse of this question in the *Conclusion and Prospects* section of our review: "do genotypic differences among zooxanthellae in their ability to synthesize MAAs affect the UV resistance and bathymetric distribution of their hosts?" Iglesias-Prieto and Trench (1997) demonstrate a genotypic relationship with photosynthetic acclimation in symbiotic dinoflagellates of the genus *Symbiodinium*, but their study did not include examination of UV acclimation or adaptation. The need to link genetic diversity with biochemical and physiological functions in algal-invertebrate symbioses is strongly identified; we encourage further research in this pursuit.

Relevant data for transmission of visible wavelengths through endozoic algal layers in the brain coral *Favia* sp. are provided by Shibata and Haxo (1969). More recently, Kühl et al. (1995) have measured visible light fields in *Favia* sp. using a fiberoptic scalar irradiance microprobe connected to a custom-built, scanning light meter. Presumably, UV transmission in coral tissues could be measured with a similar device constructed with quartz optics. Recently, Shashar et al. (1997) have measured UV (300–400 nm) penetration through the tissues and skeleton of *Porites* sp. to the level of endolithic algae (94.0%–99.5% attenuation of ambient UV radiation), but gradients within tissues were not measured.

Question (Rowan): I consistently see that bleached corals (and other animals, such as giant clams) lose not only the pigments of their zooxanthellae, but also a variety of other pigments (colors) that are presumably in the animal tissues, and that presumably serve some function under "normal" conditions. What about MAAs in bleached symbioses? And, do you have any insights as to why the "visible" pigments disappear when zooxanthellae leave? Many invertebrates that lack zooxanthellae are colorful.

Answer: Coral bleaching occurs by the loss of zooxanthellae or their pigments from corals, either by degradation *in hospite* or expulsion of the algae, which may also involve loss of endodermal cells from the host coral (Gates et al. 1992, Brown 1997). Apparently, there have been no studies to compare MAA concentrations in bleached and unbleached conspecifics that may occur simultaneously at the same site. To our knowledge, the only published data on MAA concentrations in bleached corals are those in Drollet et al. (1997), where MAA concentrations in the mucus from partially bleached *Fungia repanda* did not change "noticeably" and where there was no significant change in MAA composition of the mucus compared with that from un-

bleached corals measured before and after the bleaching events. MAA concentrations were positively correlated with solar UV radiation at the site, with a lag time of 1 week, but concentrations were not correlated with water temperature.

Conversely, *Symbiodinium bermudense* cultured at high temperatures has lower concentrations of MAAs than cultures grown at lower, unstressful temperatures (Lesser 1996). Whether thermally bleached corals would have lower concentrations than unbleached colonies in the field should depend, *inter alia*, on the rates of MAA synthesis by zooxanthellae in colonies before they bleached, and on the persistence of preexisting MAAs (located largely in the host tissues [Shick et al. 1995]) after bleaching. Residence times of MAAs in several animal tissues following removal of dietary sources of MAAs are on the order of weeks to months (Adams and Shick 1996; Carroll et al. 1996; Mason et al. 1998), so that one might not expect large differences between bleached and unbleached colonies at a site unless MAA synthesis were depressed for a long period prior to bleaching. This could also be the case if mycosporine-glycine or the presumed MAA precursor 4-deoxygadusol were oxidatively vulnerable (Fig. 7, our review) to metabolic processes occurring at elevated temperatures prior to bleaching. To the extent that coral bleaching involves a loss of animal and not just algal biomass, total MAA content per colony would decline, but MAA per unit protein might not; there is some experimental evidence for this (Shick and Romaine, unpubl.).

Because animal tissues are lost during some forms of bleaching in corals, this would cause loss of the pigments they contain. This could include carotenoids (necessarily obtained from zooxanthellae or other nutritional sources) and carotenoproteins (Cheesman et al. 1967, Goodwin 1984), although presumably these would be those located specifically in detached endodermal cells. Other host-associated pigments such as the green fluorescent pigment localized in the coral ectoderm (Kawaguti 1944, 1973) might not be lost. The pink pigment pocilloporin, which is light-inducible (Takabayashi and Hoegh-Guldberg 1995), is also restricted to and apparently produced in the host tissue (Dove et al. 1995; Takabayashi and Hoegh-Guldberg 1995); however, it is not specified which cell layer contains pocilloporin, so it might or might not be lost when endodermal cells detach. After a hiatus of half a century, the functions of nonphotosynthetic pigments in corals are being reinvestigated experimentally.

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