Glutamine synthetase activity in the symbiotic dinoflagellate *Sympodium* microadestatum

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Abstract

Few studies on the nitrogen metabolism of coral-zooxanthellae symbioses have examined the zooxanthellae enzymes involved. The purpose of this study was to examine the activity of the asparagine assimilating enzyme glutamine synthetase in freshly isolated and cultured zooxanthellae. Glutamine synthetase activity was found in both freshly isolated *Pocillopora damicornis* zooxanthellae and cultured *Zanclus zoanthus* zooxanthellae. Attempts to determine the Km for ammonium for glutamine synthetase were unsuccessful. The addition of methionine sulfoximine, an irreversible inhibitor of glutamine synthetase, to chamber incubations of intact *P. damicornis* symbioses completely inhibited the uptake of ammonium by the symbioses and resulted in the excretion of ammonium into the medium by the coral. These results indicate the enzyme glutamine synthetase plays an important role in the nitrogen metabolism of coral-algal symbioses.

Introduction

Most studies on the nitrogen metabolism of coral-zooxanthellae symbioses focus on the uptake of exogenous inorganic nitrogen by the symbiotic association (Kawaguti, 1953; Tranekier, 1973; D’Elia and Webb, 1977; Muscatine and D’Elia, 1977; Watabe and Watabe, 1979; and Muscatine et al., 1979). D’Elia and co-workers (D’Elia and Watabe, 1977; D’Elia et al., 1983; and Sommier and D’Elia, in press) studied in detail the kinetics of nitrate and ammonium uptake from seawater by freshly isolated zooxanthellae, cultured zooxanthellae and intact coral-zooxanthellae symbioses. They showed that the nutrient uptake kinetics of freshly isolated and cultured zooxanthellae are similar to those of free-living marine phytoplankton, and follow Michaelis-Menten kinetics. Uptake by intact coral symbioses, however, is best described by a modified Michaelis-Menten expression with an additional term representing a diffusive component of the uptake process.

In addition to the uptake of nutrients from seawater, it has been proposed that nitrogenous waste from the coral host may provide a second route by which zooxanthellae obtain nitrogen. Yonge and Nicholls first suggested in 1931 that zooxanthellae take up ammonium waste products from the coral. Lewis and Smith (1977) proposed that the zooxanthellae convert this ammonium into amino acids which are then translocated to the coral host. 14C labelling experiments with isolated zooxanthellae and intact coral-zooxanthellae symbioses show alanine is the amino acid translocated to the coral host and preincubation of corals with S-14C ammonium increases the fraction of alanine translocated (Lewis and Smith, 1974).

Less attention has been given to the zooxanthellae enzymes involved in the assimilation of inorganic nitrogen. Crossland and Barnes (1977) reported high activities of nitrite reductase and glutamate dehydrogenase and low but consistent nitrate reductase activity in zooxanthellae isolated from two Australian reef corals. Using 14C-labelled ammonium chloride, sodium nitrate and urea, Summons and Osmond (1981) were able to demonstrate incorporation of 14C into the amino acid pool of isolated zooxanthellae. They also found that the pathway of this incorporation was via the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT).

Presented in this paper are the first measurements of the activity of glutamine synthetase in zooxanthellae freshly isolated from *Pocillopora damicornis* and in zooxanthellae cultured from the Caribbean zoanthid *Zoanthus sp.*, and the effect of an irreversible inhibitor of glutamine synthetase, methionine sulfoximine (Sontio et al., 1969; and Sontio and Meister, 1973), on ammonium uptake by intact coral heads are also reported.

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materials and methods

Culture of Zooxanthellae

Zooxanthellae isolated from the Caribbean zoanthid *Stephanozoa sp.* were maintained in 150 ml batch cultures bubbled with air. Air-bubbling prevented the zooxanthellae from growing in sheets adhering to the glass walls of the culture containers and results in thick homogenous cell suspensions. Cells were grown in 0.22 micron Millipore filtered 33% artificial seawater enriched with Provasoli’s FS-I media with sodium nitrate as the sole nitrogen source (Provasoli, 1968). Cultures were maintained in an Environator growth chamber at 27°C with 75 μE·m⁻²·s⁻¹ illumination provided by GE cool white fluorescent lights on a 12:12 h light:dark cycle. Light intensities were measured with a Biospherical Quantum Irradiance Meter (San Diego, Ca.).

Isolation of Zooxanthellae

Colonies of *Pocillopora amicrosia* were collected just prior to use from the south fringing reef of Coconut Island, Hawaii in 1-2 m of water. Coral heads were cleaned of coralline algae and gently rinsed with 0.45 micron Millipore filtered seawater (MSW). The coral tissue and zooxanthellae were excised from the corallus using the Water Pik method (Johannes and Wiebe, 1970) and filtered through 2 layers of surgical gauze and then through a layer of 20 micron Nitex screening. The resulting coral-zooxanthellae suspension was centrifugally washed (1000 rpm for 3 min in an IEC International Portable Refrigerated Centrifuge PR-2) 8-10 times with 0.45 micron MSW. Prior to assay for glutamine synthetase activity, freshly isolated zooxanthellae or cultured zooxanthellae were centrifugally washed (1000 rpm for 3 min) 3 times in a 0.3 M sucrose and 1 mM Hepes, pH 7.8, solution and resuspended in the same solution to a concentration of approximately 3 μg Chl a ml⁻¹.

Glutamine Synthetase Assay

Whole cell glutamine activity was determined using the biosynthetic radioactive assay modified from Proctor and Sailer (1979) which follows the conversion of 14C-glutamate plus ammonia to 14C-glutamine. Zooxanthellae and water controls were incubated with 50 μM glutamate; 100 mM magnesium chloride; 20 mM ATP; 1.0 mM NADH; and 0.18 μCi of 1-(14)C)glutamate (in Nabiavi, TCH Pharmaceuticals, Inc., Irvine, Ca. and at The Pennsylvania State University (PAS): New England Nuclear, Boston, MA.) for 10 min at 25° C. The reaction was stopped by the addition of 2.0 ml of cold water and the reaction mixtures were placed on Dowex 1 x 8 CL- 100 (Sigma Chemical Co., St. Louis, Mo.) ion exchange columns. The eluant was collected and after the addition of 10 ml scintillation cocktail, the activity of 14C-glutamine was counted in a Beckman LS-230 Liquid Scintillation Counter and at PAS on a Tri-Carb liquid scintillation system (Nuclear-Chicago Corp., Des Plaines, Ill.).

Nutrient Uptake Experiment with an Inhibitor of Glutamine Synthetase, Methionine Sulfoximine

Coral heads for nutrient uptake experiments in the presence or absence of methionine sulfoximine (MSX), an inhibitor of glutamine synthetase, were carefully collected and cleaned, as above, just prior to use. The coral heads were then placed on a raised perforated platform inside a 7.0 liter capacity plexiglass chamber filled with 0.45 micron MSW. A magnetic stir bar under the platform provided vigorous mixing. The entire chamber was set in a cooled waterbath which maintained the temperature inside the chamber at 27 ± 0.5°C. Illumination of 80 μE·m⁻²·s⁻¹ was provided by a 150 W Flood light suspended directly above the chamber. The coral heads were allowed to acclimate in the chamber for 30 min prior to the start of the experiment. The seawater in the chamber was enriched with 5 μM nitrate and 5 μM ammonium at the start of the experimental period and duplicate seawater samples for analysis of nitrate and ammonium were taken at 30 min intervals through a small port one inch from the base of the chamber.

Nutrient Analyses of Seawater Samples

Seawater samples obtained as above were filtered through precultured (500 ° C for 4 hr) GFC glass fiber filters and stored and frozen immediately in linear
Chlorophyll a Determination

In Hawaii, chlorophyll a was measured by extracting samples in 100% acetone (−4°C) in the dark. Absorbances were measured on a Beckman DB-2 spectrophotometer and chlorophyll a concentrations calculated using the equation of Jeffrey and Humphrey (1975). Chlorophyll a determinations on Zoanthus zoanthellae were done at 65°C using 4:1 (v:v) methanol: tetrahydrofuran to extract rhodophytes were done at 65°C using 4:1 (v:v) methanol: tetrahydrofuran to extract the pigments. Absorbances were measured on a Packard DB-2a spectrophotometer using the following equation (R. F. Simmons, personal communication):

\[ \text{ug Chl a ml}^-1 = 11.6 A(653) - 0.6 A(630) \]

Results

Zoanthus zoanthellae freshly isolated from Pocillopora damicornis yielded consistent glutamine synthetase activity at one half the rate measured in cultured zoanthellae from Zoanthus spp. (see Table 1). Controls of animal tissue from the zooxanthellate coral Tubastrea triangulosa and the isolated animal fraction from P. damicornis showed no GS activity.

Table 1. Glutamine synthetase activity from various sources. NA = no activity detected.

<table>
<thead>
<tr>
<th>Source</th>
<th>GS Activity (μM Gln produced per g Chl a h^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoanthus sp. zoanthellae</td>
<td>0.47</td>
</tr>
<tr>
<td>Pocillopora damicornis zoanthellae</td>
<td>0.19</td>
</tr>
<tr>
<td>Pocillopora damicornis animal</td>
<td>NA</td>
</tr>
<tr>
<td>Tubastrea triangulosa animal</td>
<td>NA</td>
</tr>
</tbody>
</table>

During initial attempts to determine the Kₐ of glutamine synthetase in Zoanthus zoanthellae for ammonium it was found that the GS activity was relatively constant regardless of the concentration of ammonium supplied in the assay mixture. Even with no added ammonium GS activity was 60-85% of the maximum activity obtained with the addition of 10 mM ammonium. This problem of high GS activity with no ammonium present in the assay mixture was believed to be due to a high endogenous level of nitrogen in the zoanthellae. In an attempt to reduce the endogenous pool of nitrogen, the Zoanthus zoanthellae were transferred to nitrogen-free media and were assayed for GS activity with zero added ammonium and 10 mM added ammonium. It was found that the level of GS activity measured was 0-0.05 of the maximal activity obtained with 10 mM ammonium even after 24 h starvation in nitrogen-free media. The activity with 10 mM ammonium remained the same. It proved impossible to determine a Kₐ for ammonium for the enzyme in Zoanthus zoanthellae. Pocillopora damicornis zoanthellae appeared to also contain high levels of endogenous nitrogen and attempts to starve them were unsuccessful. GS activity declined rapidly when these P. damicornis zoanthellae were incubated in nitrogen-free seawater, indicating the freshly isolated zoanthellae did not remain healthy under these conditions.

Nutrient uptake experiments on whole P. damicornis coral heads in the presence of 0.5 mM methionine sulfoximine showed that ammonium uptake is rapidly and completely inhibited by the glutamine synthetase inhibitor (results in Fig. 1). Ammonium uptake in completely stopped within 60 min of the addition of MSX and ammonium excretion occurs after that time. Control corals with no added methionine sulfoximine continued to take up ammonium and nitrate at an almost linear
rate throughout the entire incubation period. Methionine sulfoximine had no effect on the uptake of nitrate.

Fig. 1. Nitrate and ammonium concentrations in the medium during incubations of Pocillopora damicornis. Methionine sulfoximine, an irreversible inhibitor of glutamine synthetase, added at t = 60 min.

Discussion

Cultured Zoanthas zooxanthellae and zooxanthellae freshly isolated from Pocillopora damicornis appear to have high endogenous level of nitrogen which interferes with the determination of a $K_m$ for ammonium of glutamine synthetase. It is possible to reduce the endogenous level of nitrogen in cultured cells by transferring them to nitrogen-free seawater and starving them for at least 24 h but residual GS activity with no added ammonium remains. Zooxanthellae freshly isolated from P. damicornis and transferred to nitrogen-free seawater did not remain healthy and it was not possible to starve them for nitrogen to determine a $K_m$ for ammonium. Wilkerson and Muscatine (in press) also reported negative cooperativity of Alastasia pulchella zooxanthellae GS for ammonium, even after dialyzing the enzyme extract against RPSN. They also attribute the residual GS activity with no added ammonium to high endogenous levels of ammonium in the enzyme preparation. Recent work indicates that a second enzyme activity may be interfering with the GS assay making it impossible to determine a $K_m$ for ammonium. However, further experimentation is needed to confirm this possibility.

That no GS activity was detected in the animal fraction from P. damicornis or the aposymbiotic coral Turbinaria cocinea may be a function of the assay system used. The biosynthetic radioactive assay used here, while one of the most sen-
active assays for GS activity, has specific requirements for pK and metal ion cofactors. The assay conditions used here were optimized for zooxanthellae, and it is possible that the actual enzyme may have different pK optima and metal ion requirements. It would be premature to rule out the presence of glutamine synthetase in the tissues of corals based on the data presented in this paper.

Preliminary experiments show that ammonium uptake by the intact *P. damicornis* symbiont is readily inhibited by the glutamine synthetase inhibitor methionine sulfoximine, but the inhibitor had no effect on the uptake of nitrate. That ammonium uptake can be inhibited by methionine sulfoximine indicates glutamine synthetase plays an important role in the nutrition of symbiotic corals. Methionine sulfoximine should prove to be a useful tool in further studies of coral nutrition.

**Literature Cited**


Effect of varying solar radiation intensities and ultraviolet radiation on growth rates of *Symbiodinium microadriaticum* isolated from different hosts

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Abstract

Growth rates of zooxanthellae isolated from five different hosts were determined at five levels of incoming solar radiation. The various strains differed significantly in their growth rates, as well as in their abilities to adapt to low and high irradiance. However, recent evidence including isoenzyme patterns (Swanberg and Trench 1980 a, c), sterol composition (Wothers et al. 1982), and effect on growth rates of experimentally infected hosts (Kashie and Chee, 1979) indicates considerable genetic diversity among *S. microadriaticum*. Since zooxanthellae are associated with their hosts for a large part of the life cycle in some species and the entire life cycle in others, the genome of the symbiont contributes significantly to the genetic potential of the consortium (Harrison and Siegel, 1985; Marulis, 1976). In effect, the establishment of a symbiosis may be viewed as a parasexual event involving the recombination of genes, thus allowing one or both of the partners to exploit previously unavailable habitats (Marulis, 1980). Conversely, the metabolic needs of a symbiont may render some previous host environments unsuitable. Therefore genetic diversity in zooxanthellae may influence under what conditions their hosts can live.

The ability of different strains of *S. microadriaticum* to adapt to varying light regimes may be a trait which has such an ecological effect. One possibility is that animal species which live only in high light environments are restricted due to the inability of their zooxanthellae to adapt to low irradiances found in shaded areas of deep water. Since it has been demonstrated that zooxanthellae contribute significantly to the nutrition of their hosts via translocation of photosynthate (Muscatine and Porter, 1977; Muscatine et al., 1981), animals which inhabit low light environments could suffer from a lack of reduced carbon if zooxanthellar photosynthesis decreases with decreasing irradiance. However, several marine phytoplankton have been shown to adapt to low irradiance by varying degrees by increasing the concentration of photosynthetic pigments, allowing maximum gross photosynthesis and growth rate to remain constant in the face of decreasing irradiances (Chan, 1978; Prezelin and Sweeney, 1978; Falkowski, 1980). Studies on low light adaptation in *S. microadriaticum* have been done primarily on intact coral symbioses, and have yielded conflicting results. Prezelin and Porter (1976 a, b) demonstrated that maximum gross photosynthesis remains almost constant in the coral *Porites* *nana* at low irradiances (10 and 25 µmol quanta m⁻² s⁻¹), while Muscatine and Porter (1977) found a very large decrease in algal production between colonies of *Stylophora pistillata* exposed to 5.9 and 5.5 µmol quanta m⁻² s⁻¹. Other workers have documented increases in the amount of photosynthetic pigments in corals kept at low light in the laboratory (Salañez, 1976), collected from shaded environments (Falkowski and Prezelin, 1978; Titlyanov et al., 1980), and collected from deep water (Buskey, 1979; Titlyanov et al., 1980) relative to their high light and shallow water counterparts.
However, work by Titlyanov et al. (1980) and unpublished observations of R. Garnock, J. F. Pitt and J. F. Barter (in Trench, ms.) revealed constant or decreasing pigment concentrations with depth in other species. In the only study to compare species with different depth ranges, Pedalius (1976), using a series of decreasing irradiances, showed that zooxanthellae from *Leptogorgia incrustans*, a deep water species, could increase their chlorophyll content under low irradiances at which the shallow species, *Cyclogorgia perpallida*, could not longer adapt. This supports the hypothesis that different strains of *S. microadriatica* have different capacities for photoadaptation, thereby setting limits on host bathymetric range.

A second possibility is that animals living in low light environments may have to tolerate lowered photosynthetic rates due to an inability to withstand high irradiances, especially in the ultraviolet region of the spectrum (280-400 nm). Ultraviolet radiation (UV) which penetrates clear tropical ocean water (Jerlov, 1950) is harmful to many organisms (Orbach, 1969), although some appear more resistant than others (Jokiel and York, 1982). Jokiel and York (1982) have shown that zooxanthellae isolated from the "sunloving" species *Cassiopea sp.* exhibit a faster growth rate under high UV than do those from *Aiptasia sp.* a species which favors low light environments, while without UV their growth rates were almost identical. Again, response of zooxanthellae to irradiance appears to affect preferred host environments.

In this study, the photoadaptive abilities of *S. microadriatica* isolated from several hosts with different preferences for high and low light environments were assessed, and attempts made to correlate this ability with host habitat. Growth rates (%) were used as a measure of productivity. Growth rates of five strains of *S. microadriatica* in culture were compared at varying solar radiation intensities. Four of the five strains were examined for growth rates with and without UV.

Materials and Methods

Cultures of *S. microadriatica* were generously provided by Richard York of the Hawaii Institute of Marine Biology. All strains had been in culture for at least 2 years. Host species, location of collection, algal clone number, and irradiance preference of the hosts are listed in Table 1.

Table 1. Strains of *S. microadriatica* used.

<table>
<thead>
<tr>
<th>Host Species</th>
<th>Location of collection</th>
<th>Clone #</th>
<th>Irradiance preference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aiptasia pulchella</em> (anemone)</td>
<td>Kaneohe Bay, Oahu, HI</td>
<td>KBE</td>
<td>low</td>
</tr>
<tr>
<td><em>Cassiopea enigmatic</em> (scyphozoan)</td>
<td>Kaneohe Bay, Oahu, HI</td>
<td>KBE</td>
<td>high</td>
</tr>
<tr>
<td><em>Melitae filosa</em> (nudibranch)</td>
<td>Kaneohe Bay, Oahu, HI</td>
<td>not a clone</td>
<td>low</td>
</tr>
<tr>
<td><em>Montipora verrucosa</em> (scleractinian coral)</td>
<td>Kaneohe Bay, Oahu, HI</td>
<td>FB12</td>
<td>wide range</td>
</tr>
<tr>
<td><em>Tridaca marina</em></td>
<td>Kaneohe Bay, Oahu, HI</td>
<td>Fos channel</td>
<td>high</td>
</tr>
</tbody>
</table>

Determined from site of host collection and field observations.

Experiments were conducted in a continuous flow water bath maintained at 24.5±1°C under full natural solar irradiance. The water bath was subdivided into 12 compartments which were covered with various materials in order to produce the desired light regimes. Throughout each experiment, incoming solar radiation was continuously measured near the test site using an Epply pyranometer.
For the first experiment, which tested growth at various intensities of visible light only, compartments were covered with UV-stabilized polycarbonate (Soba and Hess Tufnol) sheets, which had been darkened with varying amounts of black spray paint. Polycarbonate blocks almost all UV but is highly transparent to visible light. Panels with transmittances of 6.6, 14.5, 20.6, 29.1, 43.5, and 88.5 percent were prepared in duplicate. Percent transmittance as measured with a Lambda Instruments 1-165 quartz meter varied as an average of 1.93% (range ± 0.3 to 0.6%) within a panel and ±0.75% (range ± 0.1 to 1.3%) between duplicates. Positions of the panels on the water bath were randomly assigned. Algae were grown in half strength "f" medium (Guillard and Ryther, 1962). Counts of each culture were made and densities adjusted to approximately 10^4 cells ml^-1. Thirty replicate counts were made to determine starting densities. Aliquots were added to sterile acid-washed screwcap test tubes, and 6 ml aliquots dispensed into sterile acid-washed screwcap test tubes, and 6 ml aliquots dispensed into sterile acid-washed screwcap test tubes, and 6 ml aliquots dispensed into sterile acid-washed screwcap test tubes, and 6 ml aliquots dispensed into sterile acid-washed screwcap test tubes, and 6 ml aliquots dispensed into sterile acid-washed screwcap test tubes. The caps were screwed on as tightly as possible and the tubes placed horizontally in submerged racks in order to prevent shading which may have occurred had they been placed upright in test tube racks. Three replicates of each of the five zoanthellae cultures were placed in each of the 12 compartments, giving a total of six replicates of each combination of algae and light. At the end of 12 days, the tubes were collected and cell densities determined using a Spier-Levy eosinophil counter. Doublings day^-1 were calculated according to the equation:

\[
doublings \, day^{-1} = \frac{\ln n^2 - \ln n}{\text{no. days}} \\ln 2
\]

Eight replicate counts of each tube were made.

![Absorption characteristics of filters used in the experiment. Dashed line represents relative distribution of solar radiation at the surface of the earth.](image-url)
The second experiment was designed to verify patterns of low light adaptation found in Experiment 1. One "sun-loving" strain (C. medusa), one "shade-loving" strain (A. pulchella), and the strain with a wide irradiance tolerance (G. verrucosa) were used. Four tubes of each strain were grown at 5.6 and 43.5% incident irradiance. Since in the first experiment growth rates and light saturation in all strains between 20.6 and 43.5% was chosen in order to produce a maximum growth rate against which to scale growth inhibition at low irradiance. Cultures were prepared as in the first experiment, except that starting densities were approximately 10^9 cells ml^-1. Also, due to diatom contamination in 5% of the tubes in the first experiment, which was thought to have been introduced throughout the water bath, tubes were incubated in an upright position with their caps out of the water. Since there were fewer tubes in this experiment and they were widely spaced within each compartment, shading was minimized. Again, tubes were collected and cells counted after 12 days.

Variations in UV tolerance between the strains of G. microsclerotypa were also tested. For this experiment, one portion of each of the water bath was covered with clear polycarbonate to block out the majority of UV radiation, and the other part covered with UV transparent polycarbonate (Kodak Chemical Corp.) which is highly transparent to all UV and visible wavelengths. Spectral transmission curves for both materials are measured in a Beckman DB-3 spectrophotometer as shown in Fig. 1. The level of photosynthetically available irradiance was the same in both treatments (Jodie and York, 1984 in press). After adjusting culture densities to approximately 10^8 cells ml^-1, 10 ml aliquots of each culture were dispensed into sterile UV transparent quartz tubes and the tubes stoppered with cotton and capped with parafilm. Since a lack of the expensive quartz tubes prevented testing all five strains, G. verrucosa was omitted. After inoculation, the tubes were placed in their respective treatment chambers in racks slanted at approximately a 45° angle. Tubes were collected and cells counted after 15 days. Data were analyzed using a standard analysis of variance followed by either Scheffe's multiple-comparison procedure or Duncan's multiple range test.

Results

Different strains of zoanthellae exhibited significantly different responses to the range of solar radiation intensities to which they were exposed, despite a high degree of variability (Fig. 2). During the first experiment, average daily incoming solar irradiation was 471.56 cal cm^-2 day^-1. Growth rates were essentially constant at all light levels between 10.5 and 43.5%, except for zoanthellae from P. pilosa which showed a significant (p < 0.05) decrease at 43.5%, and those from G. verrucosa whose growth rate decreased at 20.6% compared to 43.35%. The latter decrease may be an aberrant point since it seems unlikely that there would be a decrease at an intermediate light level. Algae from A. pulchella, C. medusa, and G. pilosa displayed lower growth rates (p < 0.05) at the highest light level tested, while those from P. verrucosa and T. sarsii did not appear to be perturbed by 20.6% incident irradiance. All strains except G. verrucosa grew at significantly (p < 0.05) lower rates under 6.5% incident light, however the data for C. medusa must be just within the limits of significance, as the percent decrease at low light for this strain is very similar to that of G. verrucosa algae (Table 2). Table 2 presents the rate of doubling day^-1 for each strain of G. microsclerotypa at 6.6 and 80.5% relative to that at 43.35%, which was arbitrarily chosen to represent maximum growth rate.

All maximum growth rates proved to be significantly different from one another (p < 0.05) except in the comparison of zoanthellae from T. sarsii and C. medusa. The latter two strains had the fastest maximum growth rates, followed by P. pilosa, A. pulchella, and G. verrucosa algae.

In the second experiment, involving only zoanthellae from A. pulchella, C. medusa, and G. verrucosa and light levels of 6.6 and 43.5% incident irradiance, doubling day^-1 were much lower in general than those in the first experiment due to the high starting densities of the cultures. Average daily irradiance for this period was only slightly lower than during the first experiment (391.21 cal cm^-2 day^-1). Nevertheless, similar results were obtained for the A. pulchella and C. medusa strains. Again, growth of A. pulchella algae was greatly inhibited by low light, while that of the C. medusa algae was not (Tables 2 and 7). In contrast, G. verrucosa showed more low light-dependent inhibition in this experiment than in the previous one. This may have been due to a reduction in the actual irradiance "seen" by the algae, which would have been lowered compared to the first experiment by self-shading. These algae may show a steep slope in the growth rate vs. irradiance curve when irradiances lower than those
Fig. 2. Comparison of growth rates of zooxanthellae isolated from various hosts under different intensities of solar irradiance.
used in these experiments are included, causing a small decrease in light to produce a sharp drop in growth rate. Another discrepancy between the second and first experiments is the relative rate of growth of *A. pulchella* and *C. medusa* algae at 43.5% incident irradiance, whereas in the first experiment *A. pulchella* grew more slowly than *C. medusa*. In the second the growth rate of *C. medusa* was slightly lower than *A. pulchella*. This may be easily explained by the portion of the growth curve of the algae over which these experiments were performed. That is, since the second experiment began at densities much closer to the maximum density, the results imply that *A. pulchella* zoochlorellae have a slower growth rate than those from *C. medusa*, but reach a similar or slightly higher maximum density.

UV radiation had a profound effect on the growth of all strains of *S. microcalicium*. While all strains grew at very similar rates when not exposed to UV, only algae from *C. medusa* grew at all when full UV was present (Table 9). The growth rate of these algae under UV was 22.6% of that without UV exposure. In addition, the cells of all strains were much larger in the culture grown with UV than in those grown with visible light only.

Table 2. Growth of *S. microcalicium* strains under high and low irradiance.

<table>
<thead>
<tr>
<th>Host</th>
<th>Low at 9.6% incident irradiance</th>
<th>High at 43.5% incident irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td>First exp.</td>
<td>Second exp.</td>
<td></td>
</tr>
<tr>
<td><em>A. pulchella</em></td>
<td>52.0%</td>
<td>62.1%</td>
</tr>
<tr>
<td><em>C. medusa</em></td>
<td>92.0%</td>
<td>81.1%</td>
</tr>
<tr>
<td><em>P. dilgosa</em></td>
<td>73.6%</td>
<td>88.6%</td>
</tr>
<tr>
<td><em>F. virgincosa</em></td>
<td>93.3%</td>
<td>92.4%</td>
</tr>
<tr>
<td><em>T. maxima</em></td>
<td>85.5%</td>
<td>97.6%</td>
</tr>
</tbody>
</table>

Table 3. Experiment 2: Growth rates (doublings/day).

<table>
<thead>
<tr>
<th>Host</th>
<th>6.6% incident irr.</th>
<th>43.5% incident irr.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. pulchella</em></td>
<td>0.062±0.018</td>
<td>0.132±0.009</td>
</tr>
<tr>
<td><em>C. medusa</em></td>
<td>0.113±0.018</td>
<td>0.124±0.023</td>
</tr>
<tr>
<td><em>P. dilgosa</em></td>
<td>0.087±0.002</td>
<td>0.114±0.015</td>
</tr>
</tbody>
</table>

Table 4. Growth with and without UV radiation (doublings/day).

<table>
<thead>
<tr>
<th>Host</th>
<th>Visible only</th>
<th>Visible + UV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. pulchella</em></td>
<td>0.121±0.017</td>
<td>0.00</td>
</tr>
<tr>
<td><em>C. medusa</em></td>
<td>0.121±0.010</td>
<td>0.00</td>
</tr>
<tr>
<td><em>P. dilgosa</em></td>
<td>0.120±0.009</td>
<td>0.027±0.013</td>
</tr>
<tr>
<td><em>T. maxima</em></td>
<td>0.129±0.010</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Discussion

Photoadaptive capacities of zooxanthellae were not shown to be correlated with host habitat. In general, algae from "shade-loving" animals had a higher percent decrease in growth rate at low irradiance levels than those from "sun-loving" animals. Since in intact symbioses, algal growth rate is rigidly controlled (Trench, 1979), changes in growth rates of algae in culture are likely to appear as variations in amount of organic material translocated to the host and/or stored in the algae. Indeed, it has been shown (McCloskey and Muscatine, ms.) that the percentage of fixed carbon translocated decreases from 39% to 11% in 2. pinctadae at 5 and 35 m, respectively, while maximum gross photosynthesis also decreased. These results suggest that animals which live at low irradiances, in the evolution of their symbioses, not selectively chosen algae which are better adapted to low light levels. Thus, these associations which are often found at low irradiances may possess other mechanisms for low light adaptation besides those of the zooxanthellae for keeping photosynthetic rate constant.

Adaptations to decreasing irradiances, and thus most likely decreasing translocation, may include a drop in algal and/or animal respiration rate and increased heterotrophic feeding. That respiration rates of corals decline with depth has been well documented (Zwolska, et al., 1980; Spencer-Bowers, 1980; McCloskey and Muscatine, ms.). This may be explained by the observations of Fitt, et al. (in press) and Svoboda and Ormond (1980) who have shown that respiration rate in sponges is correlated with the amount of xenogenous nutrients. Both McCloskey and Muscatine (ms.) and Szewalski, et al. (1980) however, found that the decline in coral respiration was not enough, to compensate for the lowered photosynthetic rate. This implies, then, that heterotrophy must be increasing with depth in these animals. It has been established that only these invertebrates can feed heterotrophically on zooxanthellae (Porter, 1978; Jouanne, et al., 1978) and bacteria (Bisello, 1971; Sorokin, 1978) and assimilate dissolved organic carbon (Murdoch and Lenhoff, 1980; Trench, 1974; Sorokin, 1973). Therefore, a shift in the autotrophy:heterotrophy ratio may be accomplished with such ease that animals have not had to distinguish between zooxanthellae which perform differently at low irradiances. Changes in the algal density per animal tissue may also occur at low irradiances, although a clear trend in this area has not been established. Several workers have found no differences in algal numbers with irradiance (Peady, 1976; Svoboda and Ormond, 1980; Flinker and Dufilloy, 1981), others have found increased numbers (Lutzonov et al., 1981; Rouch, 1976; Bisello, this volume), while still others have shown a decrease (Dusan, 1979; McCloskey and Muscatine, ms.). A decrease in algal density at low light levels could help to keep photosynthetic rate constant by reducing self-shading.

There also appeared to be no correlation between habitat preference of hosts and growth inhibition of zooxanthellae by high irradiance levels, as it. piliusa also responded similarly to the two "sun-loving" strains. The photoinhibition which did occur, except for M. modusa algae, less than growth inhibition by low light, and so can probably be explained by the same adaptations on the part of the host.

Tolerance of UV radiation by the different strains of zooxanthellae also did not appear in this study to be related to host habitat, since the only strain able to grow in the presence of UV was the "shade-loving" M. piliusa zooxanthellae. Preceding cell densities been lower, a lower resolution of UV tolerance among the strains might have been perceived. Ability to withstand high levels of UV has been linked to the pigment S-320, so called because of its absorption peak at 320 nm (Shibata, 1969; Jokiel and Yorke, 1982). S-320 has been detected in the corals Pocillopora sp. and five species of Acropora (Shibata, 1969), P. dianorina (Jokiel and Yorke, 1982), and M. verrucosa (Scolto, this volume), and in a cyanobacteria (Shibata, 1969). In addition, increasing S-320 concentration in shallow water has been reported for P. dianorina (Jokiel and Yorke, 1982) and M. verrucosa (Scolto, this volume), and so is thought to function as a UV shield. Whether it is produced by the animal or algal partner of the corals has not been ascertained. If it is produced by the animal, and so protects both partners, this may explain why zooxanthellae show such severe UV inhibition in culture (Jokiel and Yorke, in press). Furthermore, differential production of S-320 by different invertebrates could account for some of the differences in UV tolerance between strains of S. microadriatica. In other words, although M. piliusa generally inhabits low
light environments, if the animal produces no UV protectant, then the algae may require more UV resistance than algae in a "sun-loving" animal which does produce a UV screen. The observation that cell size is greatly increased in UV exposed cells suggests that not only photosynthesis but cell division may be inhibited.

To thoroughly assess the effect of low irradiances on marine invertebrate-alga associations, changes in productivity, translocation, respiration of algae and animal, number of algae per host tissue area, and heterotrophic feeding must be examined. The results of this study imply that although zooxanthellae productivity is kept constant over a wide range of irradiances, animals which are found in shaded or deep water environments may be exercising other photoadaptive mechanisms as well. Resistance of zooxanthellae to UV is low, and may have evolved in response to the amount of UV protection afforded by the host. This problem requires further examination. In summary, although strains of S. microadriatica isolated from different hosts do exhibit different responses to varying solar radiation intensities and UV radiation, these differences may not be of critical importance to the host in symbiont selection.

Literature Cited


Symbiodinium microadriaticum Tredenthal, a revised taxonomic description, ultrastructure. J. Phycol. 5:341-350.


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Relationship between solar radiation and pigmentation of the coral *Montipora verrucosa* and its zooxanthellae

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Abstract

Colonies of the reef coral *Montipora verrucosa* were transplanted from shallow (10 m) and deep (3 m) depths to a shallow aquarium (20 cm). Corals were exposed to solar radiation with and without ultraviolet (UV) under full intensity and shaded conditions. *M. verrucosa* collected from 3 m had a much higher concentration of a UV absorbing pigment (S320) than the colony taken from 10 m. The 10 m colony did not survive at shallow depth in full sunlight, whereas *M. verrucosa* transplanted from moderate depth was capable of tolerating the increased intensity in light intensity and UV radiation. A significant increase in the concentration of UV absorbing pigment was evident for all surviving corals and was greater for corals in full sunlight than for those in the shade. Corals exposed to ultraviolet radiation had at least double the amount of S320 pigment as corals receiving the same intensity of solar radiation but without UV.

Introduction

Hermatypic corals most inhabit shallow water in order to receive adequate sunlight for their endosymbiotic zooxanthellae (Symbiodinium sp.). In shallow water, the corals often are exposed to substantial amounts of solar ultraviolet radiation because the clear tropical oceanic waters covering the reefs are highly transparent to short wavelengths. In some cases, 80% of the surface UV irradiance penetrates to a depth of one m. Decreasing to 11% at 10 m (Jorlov, 1950, 1968). Ultraviolet radiation is detrimental to many organisms, as these wavelengths damage DNA and chloroplasts and denature proteins (Giese, 1964; Steenman Nielsen, 1964; Haldal et al., 1972). The ability of hermatypic corals to tolerate solar UV radiation suggests that these organisms have evolved a protective mechanism (Jorlov, 1960).

Pigments which reflect, fluoresce or absorb ultraviolet radiation may effectively protect the coral and its endosymbionts. Shibata (1969) extracted from several species of corals, a water soluble pigment (S320) having an absorbance maximum at 320 nm. Jorlov and York (1982) demonstrated that the production of this pigment in direct response to UV and may serve a protective function. They observed an increase in growth rate and a decrease in S320 concentration for *Pocillopora damicornis* when solar UV was blocked. Siebeck (1981) found that the UV tolerance of several scleractinian corals varied between and within species, in relation to the location of the individuals' habitat. His study showed that UV tolerance decreases as the depth of habitat increases. Other researchers have found that the concentration of S320 decreases with depth (Maragos, 1972; Chalker, personal communication).

The purpose of the present study was to investigate the photodynamic capabilities of the coral *Montipora verrucosa* collected from different depths and to determine the effects of solar UV radiation upon the production of the UV absorbing pigment (S320). The effect of solar UV radiation upon the endosymbiotic zooxanthellae was also assessed.

Materials and Methods

This study was conducted on Coconut Island in Kaneohe Bay, Oahu, Hawaii (21° 26' N, 157° 48' W) during July and August of 1983. The effect of ultraviolet radiation upon *Montipora verrucosa* colonies taken from different depths was determined by measuring changes in pigment concentration (S320 and chlorophyll a). Two large colonies were collected from moderate (3 m) and deep (10 m) depths and transplanted to a shallow aquarium (20 cm). Each colony was broken into smaller colonies, which were then randomly divided and placed into different light...
treatments. Corals were exposed to solar radiation with and without ultraviolet under full intensity and shaded conditions.

The experiment was conducted in continuous flow aquaria located in full sunlight. One aquarium was covered with UV-stabilized polycarbonate filter (Kohm and Hass, Truñaak brand). This material blocked UV radiation (400 nm) but transmitted approximately 90% of visible light energy. The other aquarium was covered with Allied Chemical Corp. alizarin brand fluoroalkylcarbon film which transmitted both UV and visible wavelengths at about 80%. The spectral curves for these filters were measured with a Beckman DB-G grating spectrophotometer and are illustrated in Fig. 1 (Johel, 1982). Neutral density screen was used to shade half of each aquarium, reducing the light intensity to approximately 40%.

Photosynthetically active radiation (PAR) was measured with a Li-Cor Li 180 B integrating quantum meter. Simultaneous light measurements were taken within each treatment and above the aquaria. The present surface irradiance of PAR for the shaded treatment receiving UV (2=39%, SD=3%) was comparable to the treatment lacking UV (2=39%, SD=3%). A two sample t-test (Zar, 1976) indicated that the sample means for the two treatments were not significantly different (0.29>p>0.44). The percent surface irradiance of PAR for the full intensity sunlight treatment receiving UV (2=65%, SD=17%) was not significantly different from the full sun treatment without UV (2=65%, SD=16%) (Z=3.85 , p<0.001).

The percent surface irradiance was also determined in the field at several depths along the reef slope near the collection sites. Measurements were taken along a vertical depth transect at 0.25 m, 0.55 m, 3.0 m, 4.5 m and 7.5 m. A linear regression analysis using arcsine transformed data indicated that the slope was not equal to zero (p<0.0001). The percent surface irradiance for the 10 m site was inverse of predicted, however, the extrapolation of points is considered invalid. The percent surface irradiance of PAR for the 3 m and 10 m sites were determined to be 44% and 11% respectively. The linear regression curve for the 1 irradiance along the coral reef slope is illustrated in Fig. 2.

Samples were taken on five occasions during a 36 day period, and were analyzed immediately. In preparation for analysis, the coral was first rinsed thoroughly with filtered seawater. A 1 cm diameter core borer was used to obtain the sample plugs, which were then trimmed and placed in a test tube with the appropriate extraction solvent.

To extract the S320 pigment, the coral plugs were placed in 10 ml of deionized water (Shibata, 1969). The pigment extractions were conducted in dim light at 25°C and allowed to extract for 5 hours. Samples were centrifuged at 1500 RPM at 4°C. The supernatant was analyzed in a Beckman DB-G grating spectrophotometer using a 1 cm quartz cuvette.

Samples analyzed for chlorophyll a content were prepared in the same manner as described above, except that they were placed in 5 ml of 100% acetone and crushed in a glass tissue grinder to improve extraction (Paragas, 1972). These data were then converted to pigment concentrations using the equations of Jeffrey and Humphrey (1975).

Results

UV Absorbing Pigment

Montipora verrucosa collected from moderate depth (3 m) had an initial S320 concentration (2=0.047 absorbance units cm-1, SD=0.02) 3 times greater than the deep colony (3 m) (2=0.005 absorbance units cm-1, SD=0.001). A Student's t-test indicated that the difference between sample means was very significant (0.001<p<0.05).

There was 100% mortality for M. verrucosa transplanted from 10 m and placed in full intensity sunlight. The corals exposed to the full solar spectrum (with UV) bleached and died within two days, whereas the corals receiving the same intensity PAR, but without UV, survived for three weeks. Corals in the shaded treatments showed a very significant increase in S320 concentration (p<0.001); corals receiving UV were significantly higher (p<0.001) than those not receiving UV. Fig. 3 shows the change in S320 concentration for the 10 cm colony in the different light treatments. The absorbance values are given in Table 1.
Fig. 1. Percent spectral transmission for the fluorohalocarbon and polycarbonate filters. Also shown is a spectral irradiance curve for incident light at the earth's surface.
Fig. 2. Depth profile for light measurements at field collection site.
Fig. 3. *P. verrucosa* transplanted from deep depth (10 m) to shallow aquaria. Change in S320 concentration under different light treatments.

Fig. 4. *P. verrucosa* transplanted from moderate depth (3 m) to shallow aquaria. Change in S320 concentration under different light treatments.
### Table 1. S320 pigment (absorbance units/cm²) for the corals transplanted from moderate (3 m) and deep (10 m) into the shallow aquarium (mean ± SD) (n=4).

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Full Sun With UV</th>
<th>50% Sun With UV</th>
<th>Full Sun Without UV</th>
<th>50% Sun Without UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.003 ± 0.02</td>
<td>0.0005 ± 0.001</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0</td>
<td>±0.017 ± 0.005</td>
<td>±0.036 ± 0.045</td>
<td>0.001 ± 0.019 ± 0.015</td>
<td>0.004 ± 0.006</td>
</tr>
<tr>
<td>11</td>
<td>0.012 ± 0.016</td>
<td>±0.014 ± 0.057</td>
<td>±0.001 ± 0.007 ± 0.017</td>
<td>±0.006 ± 0.008</td>
</tr>
<tr>
<td>18</td>
<td>0.010 ± 0.015</td>
<td>±0.034 ± 0.034</td>
<td>±0.001 ± 0.007 ± 0.017</td>
<td>±0.018 ± 0.018</td>
</tr>
<tr>
<td>36</td>
<td>±0.023 ± 0.055</td>
<td>±0.050 ± 0.022</td>
<td>±0.016 ± 0.073</td>
<td>±0.004 ± 0.004</td>
</tr>
</tbody>
</table>

### Table 2. Results of two level ANOVA applied to change in S320 pigment concentration for moderate depth colony relative to light intensity and UV.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Intensity</td>
<td>0.066</td>
<td>1</td>
<td>0.182</td>
<td>40.30</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>UV treatment</td>
<td>0.458</td>
<td>1</td>
<td>0.003</td>
<td>279.97</td>
<td>0.0001</td>
</tr>
<tr>
<td>Intensity*UV treatment</td>
<td>0.023</td>
<td>1</td>
<td>0.002</td>
<td>16.26</td>
<td>0.0026</td>
</tr>
<tr>
<td>Error</td>
<td>0.020</td>
<td>12</td>
<td>0.002</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0.567</td>
<td>15</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

### Table 3. Results of two level ANOVA applied to change in S320 pigment concentration in *P. verrucosa* colonies in shaded treatments relative to original habitat depth and UV.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original Depth of Coral</td>
<td>0.120</td>
<td>1</td>
<td>0.120</td>
<td>152.91</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>UV</td>
<td>0.235</td>
<td>1</td>
<td>0.235</td>
<td>284.23</td>
<td>0.0001</td>
</tr>
<tr>
<td>Depth*UV</td>
<td>0.003</td>
<td>1</td>
<td>0.033</td>
<td>0.33</td>
<td>0.5710</td>
</tr>
<tr>
<td>Error</td>
<td>0.010</td>
<td>12</td>
<td>0.000</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0.567</td>
<td>15</td>
<td>--</td>
<td>--</td>
<td>0.5901</td>
</tr>
</tbody>
</table>
**A. verrucosa** transplanted from moderate depth showed a highly significant increase in S320 concentration for each treatment (p<0.001). Light intensity and UV have a significant effect as indicated by a two level ANOVA (Table 2). There is evidence of an intensity x UV interaction. Corals in full sunlight attained higher concentrations of S320 than those in shaded treatments and were higher for colonies exposed to UV than for those receiving PAR only. The change in pigment concentration during the experimental period is shown in Fig. 4 and the absorbance values are given in Table 1.

A comparison of the corals maintained in reduced sunlight showed that the colonies from moderate and deep depths respond differently and that the response also varies with respect to the presence or absence of ultraviolet radiation. UV appears to affect the two colonies equally, for there is insufficient evidence of a colony depth x UV interaction. These effects are indicated by a two-level ANOVA (Table 3).

The relative increase of pigment concentration for both colonies in each treatment was obtained from the ratio of final S320 concentrations to initial concentration. The greater increase in pigment concentration was observed for the colony transplanted from 10 m to the reduced sunlight treatment with UV (63 fold increase). The 3 m colony showed the least amount of increase in both treatments lacking UV radiation. A six fold and seven fold increase was measured for the shaded and full sunlight conditions, respectively. These results are summarized in Table 4.

**Photosynthetic pigment**

Montipora verrucosa collected from 10 m had a higher chlorophyll a (chl a) content (F=1.8 pg cm^-2, SD=0.022) than the 3 m colony (F=1.44 pg cm^-2, SD=0.032). A student's t-test indicated that this difference was highly significant (F=0.002<p<0.001).

**Montipora verrucosa** colonies transplanted from 10 m to full intensity sunlight bleached and died. The bleached color indicated chloroplast damage and is a sign of zooxanthellae death. Corals in the UV treatment lost their algal symbionts immediately while the corals not exposed to UV took approximately 10 days for loss to occur. Having lost their endosymbionts, coral death quickly ensued. 10 m corals placed in the shaded treatments showed an initial decline in chl a content, but increased again after two weeks. By the end of the experiment, no net change in chl a concentration was evident for the corals in the no-UV treatment (F=0.1<p<0.1), while the corals exposed to UV decreased significantly (F=0.001).

In full intensity sunlight, the 3 m colony showed no significant change in chl a concentration when in the treatment receiving UV (F=0.1<p<0.5), but decreased slightly when UV was blocked (0.05<p<0.02). Under shaded conditions, chl a content increased for both the with- and without-UV treatments (0.01<p<0.001 and 0.02<p<0.01, respectively). A two level ANOVA indicates that light intensity and UV effect the chl a concentration of the moderate depth and colour (Table 5). There is not an intensity UV interaction - and consequently no synergistic effects of intensity and UV. Fig. 5 illustrates the change in chl a concentration for both coral colonies in the various treatments. The concentration values are given in Table 6.

A two level ANOVA applied to the change in chl a concentration in **Montipora verrucosa** colonies in shaded treatments indicates that there is a significant difference in response relative to colony type (moderate vs deep habitat depth) and UV. There is evidence of an interaction between habitat depth of coral x UV suggesting that UV effects chl a production of the colonies differently. These results are given in Table 7.

**Discussion**

*Montipora verrucosa* is most abundant between 2 and 10 m. Light intensity and UV penetration decrease with depth, thus colonies at different depths are exposed to very different light conditions. The responses of *M. verrucosa* and its zooxanthellae to transplantation suggests that colonies have certain photoadaptive capabilities. Zooxanthellae can photosynthesize over a wide range of light intensities and spectral qualities, thus extending the depth range of the coral
Fig. 5 a-d. *Montipora verrucosa* transplanted from moderate (3 m) and deep (10 m) depth to shallow aquaria. Change in chlorophyll a concentration under different light treatments.
Table 4. Relative increase in S320 concentration for Montipora verrucosa
\[
\frac{\langle S320\rangle_{\text{final}}}{\langle S320\rangle_{\text{initial}}}(\text{and } \% \text{ mortality})
\]

<table>
<thead>
<tr>
<th></th>
<th>Colonies from 10 m with UV</th>
<th>Colonies from 3 m without UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>without UV</td>
<td>with UV</td>
</tr>
<tr>
<td>Full sunlight</td>
<td>100% mortality</td>
<td>100% mortality</td>
</tr>
<tr>
<td>Reduced sunlight</td>
<td>60x</td>
<td>5x</td>
</tr>
</tbody>
</table>

*100% mortality occurred before 2nd sampling.
**This value was determined via the initial and 16th day absorbance values.

Table 5. Result of two level ANOVA applied to change in chlorophyll a concentration for moderate depth colony relative to light intensity and UV.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Intensity</td>
<td>1.34440</td>
<td>1</td>
<td>0.5171</td>
<td>32.73</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>UV treatment</td>
<td>0.19536</td>
<td>1</td>
<td>0.476</td>
<td>4.76</td>
<td>0.0498</td>
</tr>
<tr>
<td>Intensity x UV</td>
<td>0.01145</td>
<td>1</td>
<td>0.28</td>
<td>0.6072</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>0.46299</td>
<td>12</td>
<td>0.0383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>2.04225</td>
<td>15</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Chlorophyll a (µg/cm²) for the corals transplanted from moderate (3 m) and deep (10 m) into the shallow aquaria (Mean ±SD) (n=4).

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Full Sun With UV</th>
<th>Full Sun Without UV</th>
<th>50% Sun With UV</th>
<th>50% Sun Without UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.422±0.241</td>
<td>2.293±0.293</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.264±0.137</td>
<td>1.865±0.190</td>
<td>0.799±0.088</td>
<td>1.687±0.313</td>
</tr>
<tr>
<td></td>
<td>±0.275</td>
<td>±0.465</td>
<td>±0.726</td>
<td>±0.088</td>
</tr>
<tr>
<td>11</td>
<td>0.903±0.054</td>
<td>1.489±0.173</td>
<td>0.038±0.085</td>
<td>0.596±0.101</td>
</tr>
<tr>
<td></td>
<td>±0.197</td>
<td>±0.283</td>
<td>±0.039</td>
<td>±0.085</td>
</tr>
<tr>
<td>18</td>
<td>1.071±0.152</td>
<td>1.605±0.121</td>
<td>--</td>
<td>0.894±0.340</td>
</tr>
<tr>
<td></td>
<td>±0.136</td>
<td>±0.283</td>
<td></td>
<td>±0.273</td>
</tr>
<tr>
<td>36</td>
<td>1.368±0.143</td>
<td>2.150±0.172</td>
<td>--</td>
<td>1.029±0.243</td>
</tr>
<tr>
<td></td>
<td>±0.273</td>
<td>±0.332</td>
<td></td>
<td>±0.301</td>
</tr>
</tbody>
</table>

Table 7. Results of two level ANOVA applied to change in chlorophyll a concentration in P. verrucosa colonies in shaded treatments relative to colony type (from moderate vs. deep habitat depth) and UV.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original Depth of Coral</td>
<td>6.11326</td>
<td>1</td>
<td>111.86</td>
<td>p&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>UV</td>
<td>0.43957</td>
<td>1</td>
<td>2.6729</td>
<td>0.04</td>
<td>0.015</td>
</tr>
<tr>
<td>Depth*UV</td>
<td>1.46894</td>
<td>1</td>
<td>26.88</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>0.55583</td>
<td>12</td>
<td>0.0587</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8.67760</td>
<td>15</td>
<td>--</td>
<td>--</td>
<td>-1 6760</td>
</tr>
</tbody>
</table>

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Chong et al. (1983) report that the strain of S. *microadriatica* from S. *verrucosa* may photoadapt by changes in activities of CO₂-fixing enzymes or electron transport systems. They also observed light-induced changes in pigmentation. Algal photoadaptation was evident in the present study and was assessed by changes in chlorophyll a concentration. Colonies collected from an environment receiving ~1.3% surface PAR (10 m) had approximately double the chlorophyll a concentration per unit area as another colony receiving ~4% (3 m). When transplanted into an experimental treatment (~38% PAR), a change in pigment concentration was observed for both colonies. The colony from 3 m showed a net increase in chlorophyll a, having a relative increase of 97% PAR. The highest concentration of chlorophyll a at lower light levels most likely increases the efficiency of light capture.

Solar ultraviolet radiation is an important physical factor affecting shallow coral reef inhabitants. These wavelengths damage chloroplasts and chlorophyll, resulting in zooanthellae mortality and, if severe enough, will lead to coral death. This study demonstrates that S. *verrucosa* colonies adapt to UV by producing S320, a UV-absorbing pigment and suggests that this pigment may be essential to survival in a shallow reef environment.

The concentration of S320 is significantly higher in colonies from 3 m than the concentration observed at 10 m, having a ratio of 9:1. Assuming that UV is required to stimulate pigment production, the negative correlation of S320 with depth indicates that the corals are responding to attenuated levels of UV. Dunlap and Chalker (1986) reports similar findings for *Acropora* on the Great Barrier Reef.

The moderate depth colony was relatively well adapted to a high light intensity-high UV environment, whereas the deep colony was shade adapted with low UV exposure. The shade adapted colony had virtually no protective screening pigment. Consequently, a sudden increase in light intensity and UV resulted in coral death. However, the same colony, in the treatment not receiving UV also suffered 100% mortality, suggesting that strong intensity visible light (PAR) may be a factor. Yet, UV may still be a relevant factor because the cutoff point for the polycarbonate filter does not occur at exactly 400 nm. Radiation in the 380-400 nm range is transmitted and may be at a sufficient level to damage an unprotected coral. This may also explain the increase in S320 observed for all of the UV-blocked treatments. Alternately, S320 production may be stress-related. The results show a trend of higher pigment concentration with increased UV intensity; corals in the full intensity sunlight with a UV-transparent filter had the highest S320 concentration, while the shaded treatment with the UV-blocking filter had the lowest.

The decrease in chlorophyll a content following transplantation indicates UV incurred damage. After approximately 10 days, the chlorophyll a concentration increased and seemed to equilibrate. S320 increased immediately, providing a UV screen. The UV absorbancy pigment reached maximum level and equilibrated by the 10th day. Except for the extreme case (the deep colony transplanted to full sun), S. *verrucosa* was able to photoadapt to high light intensity and increased UV radiation within 2-3 weeks.

**Literature Cited**


