Distribution of mycosporine-like amino acids in the tissues of Hawaiian Scleractinia: A depth profile

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ABSTRACT: The tissues of five species of Hawaiian hermatypic corals were found to contain concentrations of mycosporine-like amino acids (MAAs) inversely correlated to depth and dose of UV radiation. Four of the six depth profiles conducted resulted in highly significant (P < 0.01) linear relationships between MAA concentration and UV radiation level. Montipora verrucosa, Montipora patula, Porites compressa, Porites damicornis and Axinella qeniei were collected at a series of depths in and outside of Kaneohe Bay, Oahu, HI and analyzed using HPLC to identify and quantify the UV-absorbing compounds. Eight known and two unknown compounds with absorption maxima ranging from 313 to 360 nanometers were separated and quantified. Spectroradiometric measurements were made simultaneously during sampling to quantify and analyze the light regime at the collection sites.

INTRODUCTION
Hermatypic corals have evolved to flourish in environments characterized by clear, oligotrophic water. The transparency of this water results in an extremely low attenuation coefficient, and organisms living there are exposed to large fluxes of UV radiation (Jerlov, 1950). UV-A (wavelengths 320 - 400 nm) and UV-B (280 - 320 nm) radiation has been found to cause substantial physiological damage to organisms exposed at irradiance levels commonly emitted by the sun (Calkins, 1982), especially at low latitudes where the ozone layer is thinner allowing higher levels to reach the Earth's surface. Targets of adverse effects in biological systems include the information carrying nucleic acids and proteins. The recognition of UV radiation as a selective pressure within a coral reef community has been well documented (Jokiel, 1980). Behavioral adaptations such as avoidance, diurnal migration and cryptic lifestyles are not available to sessile organisms, specifically corals that need to be exposed to the sun for their photosynthetic zooxanthellae symbionts. This lifestyle presupposes the existence of a protective mechanism against the sun's damaging rays. First discovered in corals by Shibata (1969), a group of water soluble compounds (since termed Mycosporine-like Amino Acids or MAAs) absorbing in the 320 nm range have been found to perform this function. These compounds have been identified and quantified in many marine and terrestrial organisms (Chalker & Dunlap, 1990). Dunlap et al. (1986) conducted a study of the bathymetric distribution of MAAs in corals of Australia and found a considerable decline in the concentration of S-320 compounds in Acropora spp. with depth. This pattern was presumed to be an adaptation to the amount of UV radiation found at those depths. In this study, quantitative analysis of light regime has been correlated with concentration of seven described MAAs in five species of corals. Corals were collected along a depth gradient at two sites varying drastically in spectral quality, one inside of a barrier reef and one on an open-ocean island. A comprehensive baseline survey of the MAAs occurring in Hawaiian corals was also conducted in this study.

MATERIALS AND METHODS

Coral Collection
Coral reefs were collected using snorkeling and SCUBA techniques on the windward side of Oahu, Hawaii (21°N, 157°W) during the summer months of June and July. Corals were collected at two sites; inside Kaneohe Bay at a site called the Silver Reef, and outside of the bay in open ocean water at Moku Manu Island. The conditions inside the bay are characterized by a high attenuation of light due to a high level of particulate matter. The open ocean site at Moku Manu displays relatively transparent, oligotrophic water with a low level of attenuation of light through the water column. The spectral irradiance (300-700 nm) was quantitatively measured at both sites using a LiCor LI-1800/W scanning spectroradiometer (LiCor, Lincoln, Nebraska). The cosine-corrected collector and sensors were programmed to scan from 300-700 nm in 2 nm intervals. All measurements of ambient solar irradiance were made at approximately 12 pm. For each depth
three scans were taken and the mean reported in units of mW m⁻² nm⁻¹. The instrument was deployed and coral samples simultaneously collected using SCUBA. *Montipora verrucosa* was collected inside Kane’ohe Bay (Silver Reef) at 1.5 m, 3 m, 4.6 m, 6.1 m, 7.6 m and 9.1 m, and at Moku Manu at 9.1 m, 12.2 m, 15.2 m, 18.3 m, and 21.3 m. *Pocillopora meandrina* and *Montipora patula* were collected at 6.1 m, 9.1 m, 12.2 m, 15.2 m, 18.3 m, and 21.3 m at Moku Manu. *Porites compressa* and *Pocillopora damicornis* were collected at 1.5 m, 3 m, 4.6 m, 6.1 m, 7.6 m and 9.1 m at Silver Reef. Branches of each colony were harvested and placed in plastic bags for transport back to the lab where they were immediately frozen at -50°C until the time of extraction.

**Analysis of Mycosporine-like amino acids by HPLC**

Coral samples were thawed, cleaned of epiphytes, broken into small pieces, and placed in a known volume of 100% HPLC grade Methanol to extract overnight at -20°C. The extraction and analysis of MAAs were performed according to the procedures in Dunlap and Chalker (1986). Dunlap et al. (1986), and Shick et al. (1992). Samples were extracted in 5 cm³ HPLC grade 100% methanol. Individual MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column protected with an RP-8 guard, in an aqueous mobile phase including 0.1% acetic acid and 45% methanol. Detection of peaks was by UV absorbance at 313 and 340 nm. Identities of peaks were confirmed by co-chromatography with standards of Mycosporine-glycine, shinorine, porphyrin-334, palythine, asterina-330, palythinol, and palythene. Peaks were integrated and quantification of individual MAAs was accomplished using the quantitative standards listed and by on-line diode array spectroscopy. All MAAs were normalized to μmol MAA/mg protein using the soluble protein from an aliquot of the extracted sample. Protein was analyzed using the procedure described in Lowry et al. (1951).

**Statistical analysis**

Linear regression analysis was conducted to assess the possibility of an explanatory-response relationship between MAA concentration and UV radiation level. A linear relationship between the explanatory variable, UV radiation level, and the response variable, concentration of MAAs, was hypothesized. It was hypothesized to be linear because UV radiation (and light) has been shown to attenuate exponentially with depth, and the hypothesized notion that MAA concentration would also decrease exponentially with depth. Since these two variables are hypothesized to vary together in an exponential manner with depth, it follows that there would be a linear relationship between the two variables themselves.

**RESULTS**

The concentrations of MAAs in all five species of coral analyzed in this study clearly display an inverse relationship with depth. In all samples, there was a single MAA high in concentration relative to the others detected which showed this decreasing pattern most vividly. *Montipora verrucosa*, the species that was collected at both Kane’ohe Bay and Moku Manu, revealed the same pattern at both sites (Fig. 1). The MAA highest in concentration in this species was palythine, which showed a decrease of around two orders of magnitude from the shallowest to deepest collection. Concentrations of MAAs in *Pocillopora meandrina* (Fig. 2) and *Montipora patula* (Fig. 3) from Moku Manu were plotted both against depth (m) and UV dose (W m⁻²). Concentrations of MAAs decreased with UV dose as they did with depth. Mycosporine-glycine was found in high concentrations in *P. meandrina*, whereas in *M. patula*, palythine was the most abundant MAA. *Porites compressa* collected inside Kane’ohe Bay (Fig. 4) showed a two orders of magnitude decrease in asterina, but an unknown MAA showed an unusual pattern peaking in concentration at 5 m (approximately midway between surface and bottom).

The spectral data collected at the two sites (Figs. 5 & 6) show that radiation in the lower PAR wavelengths and the UV portion of the spectrum are attenuated at much shallower depths inside the bay than outside. The amount of integrated energy reaching the bottom of Kane’ohe Bay at 10 m was 0.59 W m⁻², and at Moku Manu the value at this depth was 15.8 W m⁻². At Moku Manu (21 m) there was 6.86 W m⁻² of total UV reaching the bottom on the day of collection. The attenuation of light through a depth of 21 m at Moku Manu seems to be similar to the attenuation of light in only 10 m of water column inside Kane’ohe Bay.

The results of the linear regression analysis of the relationship between MAA concentration and UV radiation level (Table I) are highly significant for four of the six depth profiles: *M. patula*
(p<.001), *P. meandrina* (p=.003), *M. verrucosa* at the Kane‘ohe Bay site (p=.005) and *P. compressa* (p=.005). R-squared values were also very high, signifying a strong causal relationship between the explanatory variable, UV dose, and the response variable, concentration of MAAs.

**Table I. Linear regression analysis.**

<table>
<thead>
<tr>
<th>Depth profile</th>
<th>Site</th>
<th>ANOVA F</th>
<th>P value</th>
<th>Rsq</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. patula</em></td>
<td>MM</td>
<td>457.9</td>
<td>.000</td>
<td>99.1</td>
</tr>
<tr>
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<td>MM</td>
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<tr>
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<tr>
<td><em>M. verrucosa</em></td>
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<td>.005</td>
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</tr>
<tr>
<td><em>P. damicornis</em></td>
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<td>.052</td>
<td>85.2</td>
</tr>
<tr>
<td><em>P. compressa</em></td>
<td>KB</td>
<td>32.14</td>
<td>.005</td>
<td>88.9</td>
</tr>
</tbody>
</table>

* = P value of less than .01 significance level

**DISCUSSION**

The concentration of MAAs in the tissues of four species of corals have been found to be inversely related to the amount of UV radiation reaching the coral colonies. *Montipora verrucosa* sampled over a 23 m depth gradient outside the bay showed a similar two-orders of magnitude decrease in concentration of palythina as the sample collected inside the bay where the water is only 10 m deep. The spectral irradiance data, showing a similar level of attenuation of UV radiation at both sites even though the water column was twice as deep inside the bay, supports the speculation that the corals are responding to some sort of cue associated with light regime, and not simply depth. This study supports the idea that MAAs are indeed acting as UV radiation blockers in coral tissues because corals of the same species were found to contain very different concentrations of MAAs at similar depths when sampled from areas differing drastically in light regime.

The results of the linear regression analysis between MAA concentration and UV light level are very supportive of the hypothesis that this is indeed an explanatory-response relationship. This study supports the hypothesis that the corals are responding to the level of UV radiation, resulting in the accumulation of higher concentrations of MAAs in high UV environments. There is need for caution, however, because many parameters of the marine environment decrease with depth, and pinpointing which one is explicitly responsible will be quite difficult. It has been suggested that increased water motion and its association with increased metabolism may act as a cue to increase MAA production (Jokiel & Lesser, 1994). However, it still remains to be confirmed that the coral is harvesting MAAs from the symbiotic algae, and not either obtaining them through diet or producing MAAs themselves. Presently the only known origin of MAAs is the Shikimate pathway, a complex metabolic tree that produces the aromatic amino acids, plastoquinones, vitamins E and K, and many more compounds in photosynthetic microorganisms and higher plants (Bentley, 1990). Considering the diversity of non-symbiotic organisms that contain MAAs, they must be obtaining these compounds through diet, just as most animals obtain the essential amino acids. A non-symbiotic coral, *Tubastra eococcinea*, does contain high levels of MAAs.
Figure 1. *Montipora verrucosa* MAA concentrations versus depth for Kane'ohe Bay (A) and Moku Manu (B).
Figure 2. _Pocillopora meandrina_ MAA concentrations from Moku Manu plotted both against depth (m) and UV dose (W m$^{-2}$).
Figure 3. Montipora patula MAA concentrations from Moku Manu were plotted both against depth (m) and UV dose (W m⁻²).
Figure 4. Portes compressa MAA concentrations versus depth in Kane`ohe Bay.

Figure 5. Spectral data collected at the Silver Reef, Kane`ohe Bay.
supporting the idea that corals can obtain MAAs through diet. The mechanism that is responsible for storing appropriate concentrations of MAAs in response to light regime is completely unknown. If corals harvest MAAs from their zooxanthellae, it is possible that increased concentrations of MAAs are a result of an increased rate of photosynthesis due to higher PAR and higher water motion (lower diffusion factor), and it is simply coincidental that high levels of MAAs are found in the tissues of corals that are found closest to the surface in high water motion, high UV environments.

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LITERATURE CITED


UV-absorbing compounds in the coral *Pocillopora damicornis*: interaction effects of light, water flow and UV radiation.

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ABSTRACT: A direct relationship exists between dose of solar ultraviolet (UV) radiation and concentration of mycosporine-like amino acids (MAAs) in the Hawaiian reef coral *Pocillopora damicornis*. However, MAA concentration is also influenced by flow regime and flux of photosynthetically active radiation (PAR). High fluxes of UV radiation in reef environments are correlated with high PAR and high water velocity, because all three parameters diminish exponentially with increased depth. This correlation is further strengthened along horizontal gradients on reefs. The clearest water is typically found on outer reefs growing in oceanic waters. These ocean reefs typically experience high water velocities due to ocean swell when compared to the more turbid lagoon reefs exposed only to small wind-driven waves. Flow-modulated photosynthetic rate appears to be a major factor affecting the observed changes in MAA concentration when this coral is grown under different flow regimes and identical fluence rates of UV and PAR radiation. High PAR and/or high water velocity significantly enhance the effect of increased UV radiation on MAA concentration. Thus, observed differences and changes in MAAs under different environmental conditions might not directly reflect differences or changes in UV radiation flux unless all other parameters are equivalent.

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Response of a Pacific stony coral to short-term exposure of ultraviolet and visible light

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ABSTRACT: Colonies of the Pacific stony coral Montipora verrucosa were transplanted from 10m depth to an in-situ respirometer at 0.5 m depth. The corals were exposed for 1 to 2 days to full sun or 30% sun, without UV-A or UV-B, with UV-B but not UV-B, or with both UV-A and UV-B. Metabolic measurements were taken continuously for each coral and levels of chlorophyll and MAAs were determined at the culmination of the experiment. No significant interaction between ultraviolet (UV) radiation effects and visible irradiance (photosynthetically active radiation = PAR) effects was observed. Corals exposed to full sun showed significantly lower maximum net photosynthesis rates, respiration rates, and photosynthetic efficiency, and net PAR ratios, compensation point, and saturation point were unchanged. These results suggest that increased visible irradiance was detrimental to both the photosynthetic algae and to the coral tissue. Maximum net photosynthesis rates and chlorophyll a levels were lower in corals exposed to UV-B, but respiration rates remained the same. This may indicate that UV was damaging to the photosynthetic algae but not the coral tissue. There was no significant difference between effects of UV-A and effects of UV-A + UV-B for any response variable. These results are important because they indicate that corals respond dramatically even to very short-term exposure to both increased visible irradiance and to increased UV irradiance.

INTRODUCTION
Tropical coral reefs are regularly exposed to high levels of visible irradiance, or photosynthetically active radiation (PAR, 400 nm - 700 nm), and ultraviolet radiation (UV-A, 320 nm - 400 nm and UV-B, 280 nm - 320 nm) (Cullen & Neale, 1993; Gleason, 1993). While it has long been recognized that high visible irradiance reaches these reef organisms, historically it was believed that UV radiation was not a significant influence. Although high levels of UV reach low latitude ocean surfaces due to the thinness of the ozone layer and the low zenith angle of the sun (Baker et al., 1980), it was believed that these short wavelengths were attenuated rapidly and efficiently by the water and, therefore, did not reach reef organisms (Smith & Baker, 1979). However, it is now well known that UV radiation penetrates to considerable depth in tropical oceans (Janov, 1950; Jerlov, 1968; Smith & Baker, 1979; Fleschmann, 1989).

Concern is mounting over the potential increase in UV radiation reaching coral reefs as ozone depletion continues (Hader and Worrest, 1991). Reef organisms may not be able to adapt quickly enough to survive the changing conditions. On shorter time scales, episodic events such as unusually calm periods may result in dramatic water column clearing as witnessed at bleaching locations in the Caribbean in 1987 and 1990 (Goenaga et al., 1987; Gleason & Wellington, 1993). These water column clearing events can provide for greater exposure of reef organisms to both UV radiation and visible irradiance.

Reef organisms can employ three main defense mechanisms against UV radiation: avoidance, protection, and repair. The sessile nature of stony corals coupled with the dependence of the coral-zooxanthellae symbiosis on solar radiation necessitates that corals be exposed to UV radiation. Therefore, corals are left with two options: protect themselves, and be capable of repair should damage occur.

In shallow water marine environments, it is believed that many sessile invertebrates employ UV absorbing compounds to protect themselves from the damaging effects of UV radiation. These compounds, formerly known as "S-320" (Shibata, 1969), are collectively known as mycosporine-like amino acids (MAAs) with absorption maxima in the 310 - 360 nm range (Hirata et al., 1979; Tsujino et al., 1980; Karentz et al., 1991). It has been suggested that hermaphrodite corals synthesize or accumulate their own suites of MAAs as protection against this radiation (Duniap & Chalker, 1986; Duniap et al., 1986). Jokiel and York (1982) observed a decrease in these compounds when UV was blocked from Pocillopora damicornis, and Maragos (1972) observed decreased concentrations as depth increased.

UV radiation has been implicated in damaging organisms both in terrestrial and aquatic systems (Harm, 1980; Wood, 1987; Cullen & Neale, 1993). Worrest et al. (1981a; b) correlated altered species compositions and standing crops of algae with increased long-term UV dosage. Lasser & Shick (1989) reported 30% lower growth rates in zooxanthellae from Aiptasia pallida acclimated under high visible light conditions with UV radiation than those acclimated under high visible light conditions without UV or acclimated in low light conditions. Jokiel and York (1982; 1984) also found reduced growth rates in a number of algal species, including zooxanthellae, when exposed to visible light with UV-A + UV-B radiation. There is also evidence of UV induced
photosynthetic inhibition (Sisson, 1986; Lesser & Shick, 1989). Studies of this pho-toinhibition suggest UV damages or destroys chlorophyll and/or chloroplasts (Gessner & Diehl, 1951; Smith et al., 1960; Hader & Worrest, 1991). Lesser and Shick (1989) found reduced levels of chlorophyll in Aiptasia pallida in the presence of ultraviolet radiation. It has been suggested that increased UV radiation has been instrumental in causing widespread bleaching observed in tropical oceans (Fisk & Done, 1985; Harriott, 1985; Oliver, 1985; Goenaga et al., 1988). However, the evidence is strictly correlational and is confounded by increases in visible irradiance.

The shorter wavelength, higher energy UV-B radiation, is considered more biologically damaging than UV-A (Cullen & Need, 1993). Bothwell et al. (1994) discovered that UV-B disrupts many photosynthetic processes including pigment stability, electron transport system, and photosystem II reaction centers. Despite the belief that UV-B is more damaging than UV-A (Calkins & Thordardottir, 1980), numerous studies of UV effects have not investigated these components independently (but, see Jokiel & York, 1984). It is important to consider that while UV-B photons may be more damaging per photon than UV-A, there are much greater fluxes of UV-A in the ocean than UV-B. Bothwell et al. (1994) concluded that although UV-B is more disruptive, higher photon flux in UV-A usually produces the majority of inhibition of photosynthesis in algae.

There is some debate regarding the relative contribution of visible irradiance and UV radiation in damaging reef organisms. Brown et al. (1984) speculate that the bleaching patterns observed in corals in Thailand result from longer wavelength, photosynthetically active radiation (PAR) and that UV radiation played a minimal role, if any at all. They contend that there is increasing evidence that high levels of PAR negatively affect algal photosynthetic systems (review in Powles, 1984). Contrastingly, Jokiel and York (1984) consider that the role of PAR in pho-toinhibition has been overestimated and that long-term photoinhibition effects are primarily caused by UV radiation. They discovered that algae in their study could rapidly photosynthesize to increased PAR (92% surface irradiance), but the addition of UV resulted in growth photoinhibition. Their study agreed with previous studies (Steemann-Nielsen, 1982; Steemann-Nielsen et al., 1982; Prazell & Matick, 1983) that showed that some microalgae can rapidly physioadapt to high levels of visible light (<24 hrs). This debate can only be settled by further, non-correlational research on the relative impacts of these two light components. In addition to the impact of UV radiation or visible irradiance on reef organisms, there may be an interaction between these two effects. It is possible that the combination of these two factors produces greater detrimental effects than either of the two acting alone. It is equally possible that one factor may ameliorate the effects of the other.

The experiments in this study were designed to test for acute effects of short-term exposure of the Hawaiian stony coral, Montipora verrucosa to ecologically realistic levels of increased visible Irradiance, increased UV radiation (both UV-A and UV-B), and the interaction of the two. The specific questions addressed were: (1) Does the metabolic ability of the Hawaiian stony coral, Montipora verrucosa, change with increased visible irradiance and/or UV-A and/or UV-B radiation? (2) Does chlorophyll content change with increased visible irradiance and/or UV-A and/or UV-B radiation? (3) Do MAA levels change with increased irradiance and/or UV-A and/or UV-B radiation?

MATERIALS AND METHODS

Collection Site and Study Organism

This study was conducted at the Hawaii Institute of Marine Biology (HIMB), Coconut Island, Kaneohe, Oahu during the summer of 1994. All coral pieces were collected off the Coconut Island's Lighthouse Dock from large colonies of platy Montipora verrucosa at a depth of 10 m. A pair of coral pieces were taken from the same location on each colony; one piece of the pair was used in the experimental treatments while the other piece of the pair was used immediately for lab analysis to obtain initial estimates for chlorophyll a levels, and MAA levels. A total of 36 pieces from 18 colonies was used for this experiment.

Experimental Design

The collected pieces of M. verrucosa were transported approximately 300 m in shaded fresh seawater to the site of the in-situ respirometer in the evening before each experimental run. The
The respirometer was located on a suspended platform at a constant depth of 0.5 m. Six pieces of *M. verrucosa* were collected at a time. One piece from each pair was randomly placed in each of the three chambers of the respirometer, while the other pair member was taken to the lab for immediate processing.

The treatments were established in a 2(PAR) x 3(UV) x 2(Days) incomplete factorial design. The three UV treatments were established by placing filters over each respirometer chamber. One filter only allowed PAR to pass, one filter allowed PAR + UV-A to pass, and the third filter allowed PAR + UV-A + UV-B to pass. Two visible irradiance treatments were crossed with this set of three UV treatments. Neutral density screening was used to create two PAR levels: full light intensity and simulated 10 m light intensity (30% surface intensity at the coral collection site). The factorial design is incomplete in that a second day of treatment was applied only to the corals exposed to the full light PAR treatment. All combinations of treatments were replicated 3 times.

**Metabolic Measurements**

Photosynthetic measurements were made using the suspended respirometer. The experimental corals were placed inside sealed 2.3 liter plexiglass chambers for the duration of the experiment. The chamber lids were quartz and therefore transparent to UV-A and UV-B radiation. Each chamber was connected to a submerged impeller pump which fully exchanged all the water in the chambers every hour. Temperature readings taken periodically inside the chambers showed that this flushing rate prevented significant heating inside the chambers (< 0.5 °C higher than surrounding water). Uniformity of oxygen levels throughout the chamber was achieved by rotating stir bars below a perforated pedestal that held the coral. Oxygen production (photosynthesis) and consumption (respiration) were measured by YSI oxygen probes and recorded every 4 minutes by an Omnidata datalogger. Light was measured by a LICOR light meter and 4pi steradian spherical sensor attached to the respirometer and recorded every 4 minutes.

All the oxygen data was downloaded from the datalogger to a computer immediately after each run. The recorded voltage readings were converted into oxygen (ppm) and light (uE m² s⁻¹) units. Rates of oxygen consumption and production were calculated and plotted against the irradiance values to develop light saturation curves. The curves were fit to the data using the following model:

\[ P = R + (P_{\text{max}} - R)(1 - e^{-\alpha l}) \]

This model yielded the following response variables: \( P_{\text{max}} \), \( R \), \( \alpha \), \( l_c \), and \( l_s \). \( P_{\text{max}} \) is the maximum net photosynthesis rate achieved by the coral. It is measured as the horizontal asymptote of the light saturation curve. \( R \) is the nighttime respiration rate for the coral. The initial slope (\( \alpha \)) at the compensation irradiance (\( l_c \)) of the light saturation curve is termed the photosynthetic efficiency. It is the irradiance level at which the coral produces enough oxygen to compensate for its respiration and there is a net production of oxygen. \( l_s \) is the saturation irradiance. It is the irradiance at which the coral reaches its maximum net photosynthesis (\( P_{\text{max}} \)).

Three metabolic response variables (\( l_c \), \( l_s \), net P:R) were independent of normalizations. Three metabolic response variables (\( R \), net \( P_{\text{max}} \), alpha) were normalized in three ways: per cm² surface area, per gram wet weight, and per microgram chlorophyll a.

**Surface Area Determination**

After removal from the chambers, each coral was taken to the lab in seawater and surface area was determined. Each coral was video taped and projected surface areas were then calculated. This value was then multiplied by 2 to determine the surface area of the coral involved in photosynthesis and respiration because *M. verrucosa* has tissue on both the top and underside of the plate.

**Photosynthetic Pigment Determination**

One pair member was analyzed for chlorophyll immediately after collection from the field. As this piece was taken from an area of the colony immediately adjacent to the experimental coral piece, its chlorophyll level served as an estimate of the pre-treatment chlorophyll level of the pair member.

Chlorophyll levels were determined photometrically. A small plug was taken from the middle of each piece using a 1 cm diameter cork borer and then ground in 90% acetone. The ground
coral and solvent were placed in a dark refrigerator to extract overnight. The tubes were then 
spun in a refrigerated centrifuge at 3500 x g and the absorbance of the supernatant was then 
measured on a scanning spectrophotometer at 750 nm, 663 nm, and 630 nm. Chlorophyll a 
values were determined using the equations of Jeffrey and Humphrey (1975).

**MAA Assays**

MAA levels were determined using HPLC. A small plug was taken from the center of each 
coral with a 1 cm diameter cork borer and then placed in a -50°C freezer. When the experiment 
was completed, all frozen samples were sent to Dr. Michael Lesser (University of New Hampshire) 
where the samples were then extracted using methanol. The extract was separated using an 
HPLC and the peaks were quantified and identified using standards. All the MAAs were 
normalized by protein. Protein values were determined using the Lowery method (Lowery, 1951).

**Methods of Statistical Analysis**

The response of an individual coral piece to a treatment effect may be influenced by 
pretreatment factors. Hence, a covariate analysis with these factors (ANCOVA) might provide a 
more powerful test than a simple non-covariate parametric test (ANOVA). Covariates were only 
used when the covariate model was significantly different than the reduced, non-covariate model. 
The choice between different significant covariate models follows the method outlined by C. L. 
Mallows (1973) (also see the description under PROC REG in SAS/STAT User's Guide, Volume 
2, 1990). Independence of the covariates was tested by a linear correlation procedure (Diloro, 

Comparisons of treatment means were tested using t-tests, but only if the treatment effect 
first tested significant under an F-test. The significance level for all tests was 5%. All statistical 
analyses were carried out using a PC SAS package.

**RESULTS**

**Covariate Analysis**

The set of potential covariates were: MAA, chlorophyll, respiration normalized by surface 
area, respiration normalized by chlorophyll, and respiration normalized by wet weight. Each of 
these covariates represents a "before treatment" factor. The respiration rates of each 
experimental coral were measured during the night prior to exposure to the UV and PAR 
treatments. The chlorophyll and MAA estimates for each experimental coral were obtained from 
the bioassay of the corresponding pair member.

Chlorophyll and MAA were evaluated as covariates for all metabolic response variables. The 
respiration rates were only used for the metabolic response variables with the same normalization. 
Table I shows the correlation analysis for the covariates. The only covariates that showed a 
significant correlation were R by wet weight and R by surface area (correlation coefficient = 0.73, 
p=0.001). Since these two covariates were always used separately in any analysis, all covariate 
models tested used a set of independent covariates. Tables II, III, and V show the covariates used 
in the analyses. Of all the covariates tested, only two were used: Chi-before and MAA-before. An 
ANOVA procedure was used to analyze the following response variables: R normalized by Chi 
using Chi-before as covariate, net P_max normalized by Chi using MAA-before as covariate, and net 
P_max normalized by surface area using MAA-before and Chi-before as covariates. All other 
response variables were analyzed using an ANOVA procedure.

**Assay Analysis - Chi and MAA**

Within 10 m light corals - UV effects:

Corals under shaded conditions were run for 1 day. For the MAA analysis, the 
before treatment chlorophyll level was a significant covariate, but there was no significant 
UV effect (p=0.58). For the chlorophyll a analysis, no covariate tested significant, and 
there was no significant UV effect (p=0.61).
Within full light corals - UV effects:

Corals exposed to full visible light were run for 2 days. No covariates tested significant in either the MAA analysis or the chlorophyll a analysis. There was no significant UV effect for total MAA levels (p=0.16) or chlorophyll a level (p=0.08).

The covariates used and the significance levels of the treatment effects are summarized in Table VII, and the means and standard errors are provided in Table VIII.

Metabolic Analysis Within Day 1 - UV and PAR Effects

There was no observed interaction between the UV treatment effects and the PAR treatment effects for any of the metabolic response variables. Significance levels for both unnormalized and normalized response variables are provided in Tables II and III, respectively. The means and standard errors are provided in Table IV.

UV Effects:

Compensation irradiance ($I_c$), saturation irradiance ($I_s$), and net P:R ratio did not show a significant UV effect (p=0.59, 0.39, and 0.52, respectively). There was no significant differences between the means for each of these response variables among the different UV treatments (Table IV).

Of the metabolic response variables normalized by surface area, wet weight, and chlorophyll, the only significant UV effect occurred with net $P_{max}$ normalized to surface area (p=0.02, Table III). The corals that were shielded from UV had a higher net $P_{max}$ than those exposed to UV-A (Table IV, Figure 1). However, this design could not detect a significant difference between those exposed to UV-A + UV-B from those shielded from UV or those receiving UV-A-only (means and t-groupings - Table IV).

The light saturation curves shown in Figure 2 provide an overall view of the UV treatment effects on the metabolism of M. verrucosa. The higher net $P_{max}$ for corals receiving only visible light is evident.

PAR Effects:

There was no significant PAR effect on $I_c$, $I_s$, and P:R (p=0.57, 0.12, and 0.46, respectively, Table II, means - Table IV). There was a highly significant PAR effect for each of the metabolic response variables for each of the three normalizations. The 10m light corals showed significantly higher net $P_{max}$, R and alpha values (all p < 0.05 - Table III, means and t-groupings - Table IV). Figure 1 shows mean values and 95% confidence intervals of net $P_{max}$ normalized by surface area. The same trend was evident for the other two metabolic response variables normalized by surface area, as well as for all metabolic response variables normalized by wet weight and chlorophyll.

Figure 3 shows the light saturation curves for the 2 PAR treatments, irrespective of UV treatment. They clearly indicate the effects of increased visible irradiance on the photosynthetic ability of M. verrucosa.

Metabolic Analysis Within Full Light Treatment - UV and Day Effects

There was no interaction observed between the UV treatments and the day of exposure for any of the metabolic response variables (Table V).
UV Effects:

When full light corals from the three UV treatments were compared for the first and second day of exposure, saturation irradiance (Iₘ) and net P:R ratio were not different among the UV treatments (p=0.72, and 0.33, respectively, Table V). However, there was a significant UV effect for compensation irradiance (p=0.01, Table V). Corals shielded from UV had significantly lower compensation points than those exposed to UV-A (means and t-groupings, Table VI). However, it was not possible to distinguish the compensation point of corals exposed to UV-A + UV-B from that of corals exposed to UV-A or shielded from UV (Table VI). Figure 4 shows the lower compensation irradiance for corals shielded from UV. Maximum net photosynthesis, respiration, and photosynthetic efficiency normalized to surface area, wet weight, and chlorophyll did not show significant UV effects (p values - Table V, means and standard errors - Table VI). Figure 5 shows the light saturation curves for the full light corals for both days of exposure to the different UV treatments. Although corals shielded from UV appear to have a higher photosynthesis, this was not significant.

Day Effects:

There was no significant difference for saturation irradiance or net P:R ratio between the first day and second day of exposure for the full light corals (p=0.14, and 0.70, respectively, Table V). However, the compensation irradiance was significantly lower during the first day of exposure and increased during the second day of exposure (p=0.002, Table V, means and t-groupings - Table VI, Figure 4).

Of the three variables normalized to surface area, wet weight, and chlorophyll, only respiration normalized to surface area showed a significant day effect (p=0.02, Table V, means and t-groupings, Table VI). Figure 6 shows that respiration rates were significantly higher on the second day of exposure.

DISCUSSION

Assay Analysis - Chl and MAA:

UV Effects:

The lack of a UV effect for chlorophyll or total MAA levels for full light or 10 m light corals should be considered in the context that the exposure time was only two days and one day, respectively. Further studies using larger sample sizes may determine whether or not chlorophyll levels and total MAA levels change during short-term exposures to increased UV irradiance.

Previous studies have found that corals shielded from UV for an extended time tend to lose their MAAs, while corals exposed to higher levels of UV for an extended time tend to increase their MAAs (Jocht & York, 1982; Scalfio, 1985). Kinzie (1993) found that M. verrucosa acclimated in PAR + UV had higher levels of these compounds than those acclimated in PAR only. Although these changes occurred after multiple weeks of exposure, it is not yet known how quickly corals of this species will change MAA levels. This experiment did not uncover any changes in MAA levels in 2 days.

Metabolic Analysis: Within Day 1 - UV and PAR Effects:

The observation that no interaction occurred between UV radiation and visible irradiance after one day of exposure suggests that the detrimental effects of either treatment were not exacerbated or ameliorated by the other treatment. A previous study with freshly isolated zooxanthellae from the zoanthid, Palythoa caribaegorum have indicated that there can be a synergistic effect between these two factors (Lasser et al., 1990).

UV Effects:

In this experiment, only one of the metabolic response variables, the maximum net photosynthesis rate, showed a significant UV effect after one day of exposure. The observation that net Pₑ was highest in those corals shielded from UV suggests that UV radiation may be damaging the photosynthetic components of zooxanthellae. These results are consistent with previous studies. For example, Kinzie (1993) found enhanced
photosynthetic ability in full sun by *Montipora verrucosa* acclimated to PAR + UV compared to corals acclimated to PAR only. Lesser and Shick (1989) found UV exposure decreased net $P_{\text{net}}$ in freshly isolated zooxanthellae but not cultured zooxanthellae from *Aiptasia pallida*.

The inability to detect a difference in net $P_{\text{net}}$ between corals receiving only visible light from those exposed to UV-A + UV-B allows 2 interpretations. First, increased levels of UV-B may ameliorate the effects of increased UV-A. Second, the experimental design was not sufficient to detect the difference. The first interpretation seems unlikely, and perhaps a follow-up study with an increased sample size would be able to make a distinction.

The lack of a UV treatment effect on the respiration rates indicates that UV is not affecting the coral tissue and is consistent with results obtained by Kinzie (1993). One day of exposure to increased UV did not significantly change the irradiance necessary for the corals to reach compensation ($I_c$) or to achieve saturation ($I_s$). It is important to consider that the UV effects observed occurred after very short-term exposures to naturally occurring levels of UV radiation.

**PAR Effects:**

Powles (1984) provides a review of evidence that high levels of PAR affect algal photosynthetic systems, causing photoinhibition and subsequently photodestruction at elevated doses over prolonged time. In this experiment, similar detrimental effects of increased PAR were observed after only 1-2 days of exposure. Net $P_{\text{net}}$, respiration rates and photosynthetic efficiency were all significantly lower in corals exposed to full visible irradiance. These results suggest that significant increases in PAR (perhaps due to water column clearing events) may interrupt the proper functioning of both the host coral and the zooxanthellae.

These results contrast with previous work by Jokiel and York (1984), who found remarkably high tolerances to PAR in the dinoflagellate, *Symbiodinium microadriaticum* (a symbiotic coral zooxanthellae). This alga demonstrated growth photoinhibition to increased levels of UV but, even at full surface intensity, visible irradiance produced no inhibitory effects.

**Metabolic Analysis Within Full Light Treatment - UV and Day Effects:**

The corals exposed to full visible irradiance were run for a second day to allow comparison of changes from the first day of exposure to the second day of exposure for the different UV treatments.

**UV Effects:**

Since corals exposed to UV-A had higher compensation irradiance than those shielded from UV, it suggests that UV-A is stressful to corals. However, the low sample size of the experiment did not allow any distinction to be determined between UV-A effects and UV-B effects or between PAR only and UV-A + UV-B.

**Day Effects:**

I speculate that the higher compensation irradiance and higher respiration rates observed during the second day of exposure are due to cumulative stress from the high levels of visible irradiance.

**CONCLUSION**

Exposing colonies of *Montipora verrucosa* that were photoadapted to light levels at a 10 m depth, to dramatically increased visible irradiance, appeared to detrimentally impact both the photosynthetic zooxanthellae as well as the coral tissue. These colonies exhibited decreased maximum net photosynthesis rates, respiration rates, and photosynthetic efficiency. Colonies exposed to dramatically increased UV irradiance did show a metabolic response, but did not respond to the same degree as to the increased visible irradiance. The response to the increased UV appeared to be limited to the symbiotic algae. Corals shielded from UV had higher maximum net photosynthesis rates but no other differences in metabolic response variables were
observed. Significantly, the treatment effects observed in this experiment occurred following exposure to natural levels for only 1 to 2 days.

Table I. Pearson correlation coefficients for analysis for covariates (* indicates significantly correlated, p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>MAA- before</th>
<th>Chl- before</th>
<th>R by SA</th>
<th>R by Wgt</th>
<th>R by Chl</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA- before</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl- before</td>
<td>0.23</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R by SA</td>
<td>0.08</td>
<td>-0.23</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R by Wgt</td>
<td>-0.23</td>
<td>-0.27</td>
<td>0.73 (p=0.001 *)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>R by Chl</td>
<td>-0.007</td>
<td>-0.003</td>
<td>0.26</td>
<td>0.14</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table II. Metabolic data - comparison of first day of exposure for all treatments. Significance levels from ANOVAs for UV and visible light treatment effects for normalization independent variables (* indicates p < 0.05).

<table>
<thead>
<tr>
<th>Response Var.</th>
<th>Covariates</th>
<th>UV Effect</th>
<th>PAR Effect</th>
<th>UVxPAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>none</td>
<td>0.59</td>
<td>0.57</td>
<td>0.06</td>
</tr>
<tr>
<td>L</td>
<td>none</td>
<td>0.39</td>
<td>0.12</td>
<td>0.99</td>
</tr>
<tr>
<td>P:R</td>
<td>none</td>
<td>0.52</td>
<td>0.46</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table III. Metabolic data - comparison of first day of exposure for all treatments. Significance levels from ANOVAs or ANCOVAs for UV and visible light treatment effects for variables normalized to surface area (SA), wet weight (Wgt), and chlorophyll (Chl) (* indicates p < 0.05).

<table>
<thead>
<tr>
<th>Response Var.</th>
<th>Covariates</th>
<th>UV Effect</th>
<th>PAR Effect</th>
<th>UVxPAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>R by SA</td>
<td>none</td>
<td>0.88</td>
<td>0.0004 *</td>
<td>0.34</td>
</tr>
<tr>
<td>R by Wgt</td>
<td>none</td>
<td>0.91</td>
<td>0.003 *</td>
<td>0.62</td>
</tr>
<tr>
<td>R by Chl</td>
<td>Chl</td>
<td>0.29</td>
<td>0.0002 *</td>
<td>0.73</td>
</tr>
<tr>
<td>P by SA</td>
<td>MAA+Chl</td>
<td>0.02 *</td>
<td>0.0021 *</td>
<td>0.84</td>
</tr>
<tr>
<td>P by Wgt</td>
<td>none</td>
<td>0.71</td>
<td>0.0002 *</td>
<td>0.96</td>
</tr>
<tr>
<td>P by Chl</td>
<td>MAA</td>
<td>0.23</td>
<td>0.0001 *</td>
<td>0.13</td>
</tr>
<tr>
<td>Alpha by SA</td>
<td>none</td>
<td>0.86</td>
<td>0.001 *</td>
<td>0.74</td>
</tr>
<tr>
<td>Alpha by Wgt</td>
<td>none</td>
<td>0.86</td>
<td>0.0004 *</td>
<td>0.91</td>
</tr>
<tr>
<td>Alpha by Chl</td>
<td>none</td>
<td>0.70</td>
<td>0.01 *</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Table IV. Metabolic data - comparison of first day of exposure for all treatments. Mean values for metabolic response variables, sample sizes, and standard errors (letters represent t-test groupings. Values in different groups are significantly different at p < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variable: ( I_n )</th>
<th>Variable: ( I_n )</th>
<th>Variable: ( P:R )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  SE</td>
<td>N  Mean  SE</td>
<td>N  Mean  SE</td>
</tr>
<tr>
<td>PAR-only</td>
<td>6  66.08  4.56</td>
<td>6  451.13  65.08</td>
<td>6  4.17  0.88</td>
</tr>
<tr>
<td>PAR+UV-A</td>
<td>6  72.02  6.16</td>
<td>6  361.13  32.55</td>
<td>6  3.62  0.67</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6  66.40  6.04</td>
<td>6  361.00  37.00</td>
<td>6  4.42  1.10</td>
</tr>
<tr>
<td>10m Light</td>
<td>9  56.19  3.42</td>
<td>9  413.33  18.63</td>
<td>9  4.99  1.00</td>
</tr>
<tr>
<td>Full Light</td>
<td>9  59.48  5.44</td>
<td>9  344.78  36.98</td>
<td>9  4.82  0.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>normalized by ( SA )</th>
<th>normalized by ( Wgt )</th>
<th>normalized by ( Chi )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  SE</td>
<td>Mean  SE</td>
<td>Mean  SE</td>
</tr>
<tr>
<td>PAR-only</td>
<td>6  -4.79  0.77</td>
<td>-10.20  1.58</td>
<td>-0.367  0.06</td>
</tr>
<tr>
<td>PAR+UV-A</td>
<td>6  -4.78  0.40</td>
<td>-10.99  1.54</td>
<td>-0.529  0.06</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6  -4.52  0.75</td>
<td>-10.50  1.90</td>
<td>-0.563  0.06</td>
</tr>
<tr>
<td>10m Light</td>
<td>9  -5.86 a  0.36</td>
<td>-13.25 a  1.13</td>
<td>-0.628 a  0.05</td>
</tr>
<tr>
<td>Full Light</td>
<td>9  -3.53 b  0.30</td>
<td>-7.88 b  0.69</td>
<td>-0.358 b  0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>normalized by ( SA )</th>
<th>normalized by ( Wgt )</th>
<th>normalized by ( Chi )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  SE</td>
<td>Mean  SE</td>
<td>Mean  SE</td>
</tr>
<tr>
<td>PAR-only</td>
<td>6  24.7 x  2.15</td>
<td>50.76  8.69</td>
<td>2.2  0.24</td>
</tr>
<tr>
<td>PAR+UV-A</td>
<td>6  16.4 y  2.11</td>
<td>41.12  11.43</td>
<td>1.7  0.24</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6  19.1 xy  2.06</td>
<td>45.13  12.95</td>
<td>2.3  0.24</td>
</tr>
<tr>
<td>10m Light</td>
<td>9  27.4 a  1.73</td>
<td>64.55 a  7.13</td>
<td>2.9 a  0.20</td>
</tr>
<tr>
<td>Full Light</td>
<td>9  12.7 b  1.73</td>
<td>26.79 b  4.43</td>
<td>1.2 b  0.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>normalized by ( SA )</th>
<th>normalized by ( Wgt )</th>
<th>normalized by ( Chi )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  SE</td>
<td>Mean  SE</td>
<td>Mean  SE</td>
</tr>
<tr>
<td>PAR-only</td>
<td>6  0.85  0.007</td>
<td>0.140  0.018</td>
<td>0.006  0.0004</td>
</tr>
<tr>
<td>PAR+UV-A</td>
<td>6  0.061  0.010</td>
<td>0.141  0.029</td>
<td>0.006  0.0012</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6  0.061  0.012</td>
<td>0.144  0.035</td>
<td>0.007  0.0016</td>
</tr>
<tr>
<td>10m Light</td>
<td>9  0.081 a  0.005</td>
<td>0.157 a  0.022</td>
<td>0.008 a  0.0010</td>
</tr>
<tr>
<td>Full Light</td>
<td>9  0.044 b  0.003</td>
<td>0.097 b  0.057</td>
<td>0.004 b  0.0007</td>
</tr>
</tbody>
</table>
Table V. Metabolic data - comparison of first day versus second day of UV exposure by full light corals. Significance levels from ANOVAs for metabolic response variables for UV treatment effect and day effect (* indicates p < 0.05).

<table>
<thead>
<tr>
<th>Response Var.</th>
<th>Covariates</th>
<th>UV Effect</th>
<th>Day Effect</th>
<th>UVxDay Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L_1 )</td>
<td>none</td>
<td>0.013 *</td>
<td>0.002 *</td>
<td>0.781</td>
</tr>
<tr>
<td>( L_2 )</td>
<td>none</td>
<td>0.723</td>
<td>0.137</td>
<td>0.991</td>
</tr>
<tr>
<td>( P:R )</td>
<td>none</td>
<td>0.332</td>
<td>0.703</td>
<td>0.921</td>
</tr>
<tr>
<td>R by SA</td>
<td>none</td>
<td>0.111</td>
<td>0.022 *</td>
<td>0.891</td>
</tr>
<tr>
<td>R by Wgt</td>
<td>none</td>
<td>0.263</td>
<td>0.087</td>
<td>0.991</td>
</tr>
<tr>
<td>R by Chl</td>
<td>none</td>
<td>0.721</td>
<td>0.515</td>
<td>0.959</td>
</tr>
<tr>
<td>( P_{max} ) by SA</td>
<td>none</td>
<td>0.314</td>
<td>0.377</td>
<td>0.988</td>
</tr>
<tr>
<td>( P_{max} ) by Wgt</td>
<td>none</td>
<td>0.346</td>
<td>0.385</td>
<td>0.901</td>
</tr>
<tr>
<td>( P_{max} ) by Chl</td>
<td>none</td>
<td>0.333</td>
<td>0.39</td>
<td>0.907</td>
</tr>
<tr>
<td>Alpha by SA</td>
<td>none</td>
<td>0.074</td>
<td>0.302</td>
<td>0.948</td>
</tr>
<tr>
<td>Alpha by Wgt</td>
<td>none</td>
<td>0.203</td>
<td>0.372</td>
<td>0.494</td>
</tr>
<tr>
<td>Alpha by Chl</td>
<td>none</td>
<td>0.418</td>
<td>0.585</td>
<td>0.999</td>
</tr>
</tbody>
</table>
Table VI. Metabolic data - comparison of first day of exposure and second day of exposure by shaded corals. Mean values, standard errors, and standard deviations for metabolic response variables (letters represent least square groupings. Values in different groups are significantly different at p < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Variable: ( I_1 )</th>
<th>N</th>
<th>Variable: ( I_2 )</th>
<th>N</th>
<th>Variable: P:R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{Mean} )</td>
<td>SE</td>
<td>( \text{Mean} )</td>
<td>SE</td>
<td>( \text{Mean} )</td>
<td>SE</td>
</tr>
<tr>
<td>PAR-only</td>
<td>6</td>
<td>69.32 x</td>
<td>5.25</td>
<td>442.00</td>
<td>66.54</td>
<td>6.47 0.98</td>
</tr>
<tr>
<td>PAR+UV-A</td>
<td>6</td>
<td>97.98 y</td>
<td>8.14</td>
<td>390.33</td>
<td>53.07</td>
<td>2.57 0.55</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6</td>
<td>81.15 xy</td>
<td>9.89</td>
<td>373.17</td>
<td>52.80</td>
<td>3.68 1.30</td>
</tr>
<tr>
<td>10m Light</td>
<td>9</td>
<td>59.48 a</td>
<td>5.44</td>
<td>344.78</td>
<td>38.98</td>
<td>3.99 1.00</td>
</tr>
<tr>
<td>Full Light</td>
<td>9</td>
<td>96.56 b</td>
<td>6.24</td>
<td>458.89</td>
<td>50.42</td>
<td>3.49 0.87</td>
</tr>
</tbody>
</table>

Table VII. Respiration - comparison of first day of exposure and second day of exposure by shaded corals. Mean values, standard errors, and standard deviations for metabolic response variables (letters represent least square groupings. Values in different groups are significantly different at p < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>normalized by ( \text{SA} )</th>
<th>normalized by ( \text{Wgt} )</th>
<th>normalized by ( \text{Chl} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{Mean} )</td>
<td>SE</td>
<td>( \text{Mean} )</td>
<td>SE</td>
</tr>
<tr>
<td>PAR-only</td>
<td>6</td>
<td>-3.76 0.33</td>
<td>-8.23 0.93</td>
<td>-0.409 0.056</td>
</tr>
<tr>
<td>PAR+UV-A</td>
<td>6</td>
<td>-4.45 0.24</td>
<td>-10.21 1.20</td>
<td>-0.475 0.072</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6</td>
<td>-3.62 0.35</td>
<td>-8.22 0.69</td>
<td>-0.390 0.080</td>
</tr>
<tr>
<td>10m Light</td>
<td>9</td>
<td>-3.53 a 0.30</td>
<td>-7.88 0.69</td>
<td>-0.395 0.071</td>
</tr>
<tr>
<td>Full Light</td>
<td>9</td>
<td>-4.36 b 0.14</td>
<td>-9.89 0.80</td>
<td>-0.454 0.065</td>
</tr>
</tbody>
</table>

Table VII. Pmax - comparison of first day of exposure and second day of exposure by shaded corals. Mean values, standard errors, and standard deviations for metabolic response variables (letters represent least square groupings. Values in different groups are significantly different at p < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>normalized by ( \text{SA} )</th>
<th>normalized by ( \text{Wgt} )</th>
<th>normalized by ( \text{Chl} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{Mean} )</td>
<td>SE</td>
<td>( \text{Mean} )</td>
<td>SE</td>
</tr>
<tr>
<td>PAR-only</td>
<td>6</td>
<td>17.42 2.33</td>
<td>38.92 6.43</td>
<td>1.93 0.30</td>
</tr>
<tr>
<td>PAR+UV-A</td>
<td>6</td>
<td>11.29 2.40</td>
<td>25.16 5.17</td>
<td>1.14 0.23</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6</td>
<td>11.85 3.46</td>
<td>27.10 7.51</td>
<td>1.26 0.50</td>
</tr>
<tr>
<td>10m Light</td>
<td>9</td>
<td>11.93 1.74</td>
<td>26.79 4.43</td>
<td>1.24 0.21</td>
</tr>
<tr>
<td>Full Light</td>
<td>9</td>
<td>15.11 2.81</td>
<td>33.99 6.19</td>
<td>1.64 0.37</td>
</tr>
</tbody>
</table>

Table VII. Alpha - comparison of first day of exposure and second day of exposure by shaded corals. Mean values, standard errors, and standard deviations for metabolic response variables (letters represent least square groupings. Values in different groups are significantly different at p < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>normalized by ( \text{SA} )</th>
<th>normalized by ( \text{Wgt} )</th>
<th>normalized by ( \text{Chl} )</th>
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<td>( \text{Mean} )</td>
<td>SE</td>
<td>( \text{Mean} )</td>
<td>SE</td>
</tr>
<tr>
<td>PAR-only</td>
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<td>0.048 0.0024</td>
<td>0.105 0.0053</td>
<td>0.005 0.0004</td>
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<tr>
<td>PAR+UV-A</td>
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<td>0.086 0.0086</td>
<td>0.004 0.0004</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>6</td>
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<td>0.086 0.0086</td>
<td>0.004 0.0004</td>
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<tr>
<td>10m Light</td>
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<td>0.097 0.0053</td>
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<tr>
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<td>9</td>
<td>0.039 0.003</td>
<td>0.088 0.0080</td>
<td>0.004 0.0008</td>
</tr>
</tbody>
</table>

Table VII. Significance levels from ANOVAs and ANCOVAs for UV treatment effects for chlorophyll a (\( \text{ug cm}^{-2} \)) and total MAA (\( \text{nmol mg}^{-1} \cdot \text{protein} \)) for 10 m light corals after 1 day exposure and for full light corals after 2 days exposure.

<table>
<thead>
<tr>
<th>Response Var.</th>
<th>10m Light Corals - 1 day exposure</th>
<th>Full Light corals - 2 day exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Covariates</td>
<td>UV Effect</td>
</tr>
<tr>
<td>Chl a</td>
<td>none</td>
<td>0.61</td>
</tr>
<tr>
<td>Total MAA</td>
<td>Chl-before</td>
<td>0.58</td>
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</table>
Table VIII. Mean and standard errors for chlorophyll a (ug cm^-2) content and total mycosporine-like amino acids (nmol/mg protein) for 10m light corals and full light corals.

<table>
<thead>
<tr>
<th>UV Treatment</th>
<th>10m LIGHT CORALS</th>
<th>FULL LIGHT CORALS</th>
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<tr>
<td>PAR-only</td>
<td>3</td>
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</tr>
<tr>
<td>PAR+UV-A</td>
<td>3</td>
<td>7.53</td>
</tr>
<tr>
<td>PAR+UV-A+UV-B</td>
<td>3</td>
<td>7.91</td>
</tr>
</tbody>
</table>

Figure 1. Significant UV and PAR treatment effects after one day of exposure for maximum net photosynthesis normalized by surface area. Means and 95% confidence levels.
Figure 2. Light saturation curves for all corals for first day of exposure to the three UV treatments.

Figure 3. Light saturation curves for first day of exposure to the two different PAR treatments.


Bleaching and lipids in the Pacific coral *Montipora verrucosa*

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Department of Biology, University of Houston, Houston, TX. 77204-5513

**ABSTRACT.** Endosymbiotically-derived fixed carbon is drastically reduced in bleached corals as a result of decreased chlorophyll *a* per *zooxanthellae* or the number of *zooxanthellae* per cm² of coral tissue. Under such conditions, corals may have to rely on other sources of energy including stored lipids. In this study, I investigated the relationship between coral bleaching and lipid concentrations when bleaching was induced by increased total solar irradiance. I hypothesized that bleached corals would have a lower lipid level than non-bleached corals and that progressively longer bleaching periods would result in successively lower lipid concentrations. Bleaching was induced via increased solar irradiance by transplanting fragments of *Montipora verrucosa* from 10 m to 1 m depth for 4, 8 or 14 days. Corals appeared paler in color after 3 days of exposure and chlorophyll *a* concentrations were significantly lower in all bleached corals after 4, 8 and 14 days of exposure. Chlorophyll *a* levels in bleached corals did not recover to normal levels after 10 or 6 days following exposure. Lipid levels in bleached corals fragments did not differ significantly from control fragments at any time during the experiment. These data indicate that *M. verrucosa* does not depend on lipid energy reserves during short bleaching periods. Decreased metabolism, increased heterotrophy, gamete reabsorption or some combination of these factors during the early stages of bleaching may compensate for the immediate decrease in photosynthetically-derived fixed carbon in this species. Future studies on the changes in lipids over a longer period of time is needed in order to fully assess the importance of lipid reserves in bleached corals.

**INTRODUCTION.** Over the past 15 years the incidence of widespread bleaching events has increased on coral reefs throughout the world. Coral bleaching is characterized by the loss of photosynthetic pigments and has been correlated to factors such as elevated seawater temperatures, increased ultraviolet radiation or combinations of the two (Coles & Jokiel, 1976; Jokiel, 1980; Hoegh-Gulberg & Smith, 1989; Cook et al., 1990; Glynn, 1993; Gleason & Wellington, 1993). Bleaching can result from interruption of coral growth, reduction of reproductive output and eventually death (Jokiel & Coles, 1977; Glynn & D'Croz, 1990; Szramt & Gassman, 1990). The magnitude of bleaching events and the rate of coral recovery varies within and between both sites and species (Brown & Suhrarscone, 1990; Fitt et al., 1993). The reasons for these variations in bleaching responses however are poorly understood. One physiological component that may account for inter- and intra-specific differences in bleaching susceptibility is lipid concentrations. Under standard physiological conditions, fatty acids and glycerol are synthesized by *zooxanthellae* from photosynthetically-fixed carbon and are translocated to the host where they are either metabolized or transformed and stored primarily in the form of wax esters and triglycerides (Batey & Patton, 1984). Lipids in corals are stored predominantly in the animal host with concentrations ranging from 29% of dry biomass in *Montastrea annularis,* to 30–40% of dry biomass in various Hawaiian species (Harland et al., 1992; Stimson, 1987). However, under bleached conditions corals may rely heavily on lipid stores due to decreases in the number of *zooxanthellae* and/or chlorophyll *a* per *zooxanthella* which lead to lower photosynthetic rates, and thus reduced quantities of translocated lipid. In the Caribbean, temperature-stressed corals showed 39–73% lower lipid concentrations than non-bleached conspecifics 6 months after the onset of the event (Porter et al., 1989). This lower lipid level was presumed to be the result of an estimated 50% decrease in translocated carbon. However, it is not known whether or not lipid levels decrease in corals bleached by increased solar irradiance nor how rapidly lipid levels change in response to bleaching. In this study I examined the initial effects bleaching by solar irradiation had on the total lipid content of the Pacific coral, *Montipora verrucosa.* I hypothesized that bleached corals would have a lower lipid level than non-bleached corals, and that progressively longer bleaching periods would result in successively lower lipid concentrations. Also, given that increased solar irradiance causes chlorophyll *a* levels to decrease, I expected lipid and chlorophyll *a* levels in corals to be correlated (Hoegh-Gulberg & Smith, 1989; Gleason & Wellington, 1993). Finally, I briefly monitored lipid and chlorophyll *a* levels in the corals following exposure to increased irradiance, since little is known about coral recovery following short bleaching periods.
MATERIALS AND METHODS

The experiment was conducted between July 12 and July 26, 1994, at the Lighthouse Point (LP) and Bridge to Nowhere (BTN) sites on Coconut Island, Hawaii. Fifteen coral colonies of the platelet morph of Montporella verrucosa were tagged at a depth of 10 m at the LP site. Two fragments with minimum dimensions of 15 cm² were broken off from each of these parent colonies. One fragment was transplanted to a depth of 1 m at the BTN site (treatment fragment) and the other fragment remained directly beside the parent colony as a control for transplantation (control fragment)(Fig. 1). Transplanted and control fragments were affixed to a uniform substrate plastic mesh platform. Treatment fragments were induced to bleach by exposing them to increased solar irradiance via transplantation from a low light (LP site at 10 m) to a high light site (BTN at 1 m) for 4, 8 or 14 days. Transplanted coral fragments were exposed to a 70% increase in average total irradiance over that observed at 10 m (based on integrated light measurements made every 2mm using a LiCor Li-1800UV underwater spectroradiometer between 300 - 700 nm). Integrated UVB (300 - 320 nm), UVA (320 - 400 nm) and photosynthetically active radiation (PAR 400 - 700 nm) levels were approximately 99%, 93% and 66% greater at 1 m than at 10 m respectively. Treatment fragments were transplanted to the BTN site to better replicate the low wave action conditions present at 10 m at the LP site (LP site is beside a dock with high boat traffic). These sites are separated by approximately 100 m, occur in the same small lagoon and have similar sedimentation and temperature regimes. Hourly temperature values were recorded both at the 1 m BTN and at the 10 m LP site using two HOBO brand miniature data loggers. The two sites differed by less than 1.0°C on average and neither temperature regime was high enough to induce bleaching (LP average temp. = 26.5 ± 2.0 °C and BTN average temp. = 27.0 ± 1.0°C)(Jokiel & Coles, 1977).

SAMPLING METHOD

Figure 1. Sampling schedule. Initially (day 0), two fragments each were broken off of each of 15 parent colonies at 10 m (squares = parent colonies). One fragment was transplanted to 1 m (circle = treatment fragment) and the other fragment was placed directly beside the parent colony as a control for transplantation (triangles = control fragments). After 4 days, 5 randomly chosen treatment fragments were returned to 10 m. The same process was repeated on day 8 and day 14. At each time interval, both fragments and the parent colonies were sampled and chlorophyll a and total lipid levels were determined.

Two subsamples from each fragment and parent colony were taken by drilling with a 1.25 cm cork borer through the coral plate. One subsample was analyzed for chlorophyll a concentrations and the other for total lipid levels in order to determine initial levels of both of these parameters. Since lipid and chlorophyll a levels did not vary significantly within a coral plate (determined prior to conducting the experiment), a single sample from each fragment and parent colony adequately
represented the concentrations of these parameters (F = 2.167, n = 6, p < 0.08; F = 1.596, n = 6, p < 0.194 respectively).

After four days (July 16), all of the treatment fragments, control fragments and the parent colonies were again sampled and analyzed for total lipid and chlorophyll a levels. Five of the fifteen treatment fragments were randomly selected, returned to their original sites at 10 m and subsequently monitored for signs of recovery. This procedure was repeated again on days 8 and 14 (July 20 and July 26) of the experiment thus returning all treatment fragments to their original depth (Fig. 1).

Chlorophyll a and lipid analyses were performed as follows: Chlorophyll a was extracted from fresh, finely-ground samples according to the method described by Jeffrey and Humphrey (1975) and reported in µg/cm². Lipids were extracted from finely-ground samples (samples had been frozen at -50°C for 1-2 weeks prior to extraction) in a chloroform-methanol (2:1,v:v) solution. Extracts were then washed once with 0.88% potassium chloride solution, three times with a methanol:water solution (1:1,v:v) and dried at 50°C for 24 hours before weighing. Animal tissue biomass was determined following lipid extraction by burning the skeleton and remaining tissue residue in a muffle furnace at 450°C for 6 hours. Lipid content in corals was reported as % lipid per gram dry tissue weight. This method differs slightly from Harland et al. (1992) where samples were decalcified prior to lipid extraction which can result in lipid loss during the decalcification process (triglycerides can hydrolyze in acid solutions and the glycerol component of the molecule is then soluble).

The data were analyzed by pairwise comparisons between parent colonies and control fragments as well as between control fragments and treatment fragments to determine if lipid and/or chlorophyll a levels had changed in treated fragments. The null hypothesis was that the difference between the above mentioned pairs was less than or equal to zero and was rejected at an alpha level of 0.05 by means of a paired ANOVA on each sampled date. Comparisons of the lipid and chlorophyll a levels in the treatment fragments that had recovered for 10, 6 and 0 days were examined by means of an ANOVA. The relationship between lipid and chlorophyll a levels was assessed by means of a correlation analysis.

RESULTS

Both the parent colonies and the control fragments were observed to have normal coloration throughout the experiment. Their chlorophyll a levels were not significantly different from each other at any time (Table 1)(Fig. 2). Treatment fragments were initially observed to have similar pigmentation as the controls but began to pale on the third day of exposure to high light (Table 1). They became progressively paler with increasing exposure time. Chlorophyll a levels were significantly lower in treatment fragments than in control fragments on days 4, 8 and 14 (Table 1)(Fig. 2).

During this interval, lipid levels in the parent colony, control fragment and treatment fragment samples did not differ (Table 1)(Fig. 2). Further indication that bleaching, as indicated by decreased chlorophyll a, was not accompanied by a decrease in lipid levels was revealed by a lack of a significant correlation between chlorophyll a and the percent lipid per gram dry weight (F=1.734, n=171, p<0.190) (Fig. 3).

Recovery in bleached fragments was assessed by directly comparing lipid and chlorophyll a levels in fragments that had bleached and recovered for 4 and 10 days, 8 and 6 days and 14 and 0 days, respectively. Lipid levels and chlorophyll a levels were not significantly different between treatment fragments that had recovered for 10, 6 and 0 days (F=0.124, p<0.884, n=15; F=0.243, p<0.788, n=15 respectively).
Table I: Results of pairwise comparisons. CHL a = chlorophyll a, P-CT = pairwise comparison between parent colonies and control fragments, CT-T = pairwise comparison between control fragments and treatment fragments. n = number of samples.

<table>
<thead>
<tr>
<th></th>
<th>DAY 0</th>
<th></th>
<th>DAY 4</th>
<th></th>
<th>DAY 8</th>
<th></th>
<th>DAY 14</th>
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<tr>
<td></td>
<td>P&lt;</td>
<td>n</td>
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<td>n</td>
<td>P&lt;</td>
<td>n</td>
<td>P&lt;</td>
</tr>
<tr>
<td>CHL a</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>15</td>
<td>0.91</td>
<td>10</td>
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</tr>
<tr>
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<td>14</td>
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<td>15</td>
<td>0.00</td>
<td>10</td>
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</tr>
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<td></td>
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<td>0.33</td>
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<td>10</td>
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</tbody>
</table>

DISCUSSION

The upper surface of all treatment fragments appeared paler in color after 3 days of bleaching and remained pale throughout the experiment. This overall paler appearance was reflected in lower chlorophyll a levels. The decrease in pigment levels did not recover to pre-bleach levels during the course of the experiment irrespective of the length of exposure to high light. Lipids did not decrease in bleached fragments of *M. verrucosa* over the course of two weeks. Rather, a general trend towards increased lipid levels in the parent colony, control fragment and the treatment fragment was observed. *M. verrucosa* has a natural lunar cycle to its lipid levels that corresponds to spawning (Stimson, 1987). Since the experiment was initiated immediately following the July spawning, the observed trend in increased lipids seems to be a reflection of this natural cycle of reproduction. The lack of a significant correlation between chlorophyll a and the percent lipid per gram dry weight is consistent with the observation that decreases in chlorophyll a were not accompanied by decreases in lipid levels (Fig. 3). Studies by Fitt et al. (1993) showed that lipids in 3 bleached *Montastraea annularis* colonies were lower than in 3 unbleached colonies 6 months following bleaching. There are several possible reasons for the discrepancy between this study and that of Fitt et al. (1993). Lipids may be metabolized very slowly in bleached corals making decreases in lipid levels apparent only long after initial bleaching. However, shading experiments by Harriott (1993) indicated that lipid levels in the Hawaiian coral *Pocillopora damicornis* decreased in just one week. When corals were shaded, zooxanthellae were initially unable to maintain photosynthesis at the same level as when in full sunlight. Under these conditions, lipid stores were metabolized in order to fulfill the corals' daily energetic demands. This situation is analogous to bleaching in that bleached corals suffer from decreased photosynthetically-derived carbon as well. Based on this evidence, one might expect to observe a change in lipid levels in the first week of bleaching. But *M. verrucosa* is a much slower growing species than *P. damicornis* and predictions of a rapid response in *Montipora* based on the latter coral may be unrealistic. *Montipora verrucosa* in Hawai‘i lives in a more extreme habitat than Caribbean corals. Therefore it may be naturally more capable of coping with stresses such as bleaching making it difficult to detect any lipid responses after a short bleaching period. *M. verrucosa* spawns every month during the summer. Eggs released during spawning can be up to 70% lipid by dry weight (Arai et al., 1993). If stressed corals can resorb lipids from unreleased eggs, or can delay ripening eggs for spawning, then decreases in total lipid levels could possibly only be detectable over a longer period of time.

Corals may increase heterotrophy in order to supplement their nutritional demands. However, it is suspected that heterotrophy may only account for 10% of the corals diet in some species (Wellington, 1982). Lipid levels simply do not change when corals are bleached. In this study treated fragments were compared with genetically identical control fragments in a pairwise fashion. This is a very robust experimental method because it controls for genetic variation between corals. Paired comparisons such as these have not been used in any previous experiments which examined lipids in corals. I believe that my results are convincing evidence that lipids do not change within the first two weeks of bleaching. The hypothesis that lipid levels in
Figure 2. Average %lipid per gram dry weight (+ one standard error) and average chlorophyll a (ug/cm²) (+ one standard error) on day 0, 4, 8 and 14. Open squares, open diamonds and solid circles represent parent colonies, control fragments and treatment fragments, respectively, and are offset slightly from one another so that error bars do not overlap. The number of parent colonies, control fragments and treatment fragments are indicated in parentheses.
recently bleached corals would be lower, and that lipid content in corals would decrease as the length of the bleaching period increased, was rejected. No lipid response was detectable in bleached fragments over the course of the initial 2 weeks. Running the experiment for 1, 2 and 3 months would determine whether or not lipid levels change in bleached corals over a longer period of time. As well, monitoring gamete production and release over the same period in bleached and unbleached corals would reveal whether or not gametes are being resorbed or being prevented from developing during bleaching.

After natural bleaching events such as those observed in the Caribbean in 1983 and 1987, some coral species recovered more rapidly and more frequently than others. The ability to withstand and recover from prolonged bleaching events (i.e., several months) may yet be related to the amount of lipid stores, and may yield some insight into why some coral species recover more quickly from bleaching events than others. However, lipids do not appear to play a role in short-term coral bleaching and recovery.

ACKNOWLEDGMENTS: I would like to thank Dr. G. M. Wellington, Dr. D. Knupp, Dr. P. L. Jokiel, Dr. D. Gleason, Tom Wilcox and all the participants of the HMB summer program for their constructive criticism and support and Dr. M. Lesser for the use of his temperature data loggers and LICor 1800 spectroradiometer. This research was conducted as part of the 1994 Edwin W. Pauley Summer Program in Marine Biology: UV Radiation on Coral Reefs at the Hawaii Institute of Marine Biology and was supported by the Edwin W. Pauley Foundation and the University of Houston LGIA grant.

LITERATURE CITED:


Uneven bleaching within colonies of the Hawaiian coral *Montipora verrucosa*

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2. Hawai‘i Institute of Marine Biology, P.O. Box 1546, Kane‘ohe, HI, 96744

ABSTRACT: When a coral colony undergoes bleaching, the response may not be uniform across the entire colony. In this study, the bleaching response of *Montipora verrucosa* colonies varied significantly depending on the spectral quantity and quality of solar irradiance that each area of the colony was exposed to. In order to induce bleaching, five *M. verrucosa* colonies were transplanted from a low-light environment at 10 m to a high-light environment at 1 m. Four different light treatments were concurrently imposed on four distinct regions of each coral fragment. The four treatments included 1) elevated levels of PAR (photosynthetically active radiation: 400 - 700 nm), 2) elevated levels of PAR and UV-A (320 - 400 nm), 3) elevated levels of PAR, UV-A and UV-B (300 - 320 nm), and 4) a control treatment with PAR, UV-A and UV-B reduced to levels that mimicked radiation levels found at 10 m. At the completion of the nine-day treatment period, bleaching intensity was quantified by measuring the concentration of chlorophyll a, zooxanthellae density, and percent lipid per gram dry weight in samples from each treatment region of each coral fragment. Chlorophyll a per zooxanthellae was also calculated. Chlorophyll a concentrations decreased significantly in the two treatments that included elevated levels of PAR and UV (p < 0.013). Neither zooxanthellae densities nor lipid levels decreased significantly in any of the treatments. These results support the hypothesis that corals do not bleach uniformly when treated with different levels of solar irradiance. It also appears that *M. verrucosa*, when induced to bleach in this manner, responds by decreasing chlorophyll a concentrations, and not by expelling zooxanthellae.

INTRODUCTION

Pigment loss in scleractinian corals due to reduction in zooxanthellae density and/or the loss of photosynthetic pigment per zooxanthellae cell is a phenomenon known as coral bleaching. Over the past 15 years, the incidence of widespread bleaching events on coral reefs has increased throughout the world. Elevated temperature, ultraviolet radiation, total solar irradiance and sedimentation are among the environmental factors which have been found to cause bleaching in corals (Gleason & Wellington, 1993; Jokiel & Coles, 1990; Hoegh-Guldberg & Smith, 1989). Bleaching can result in the interruption of coral growth, reduction in reproductive output and, eventually, death (Jokiel & Coles, 1977; Glynn & D’Croz, 1990; Szram & Gassman, 1990). The gravity of this phenomenon has led to increased research examining the effects of bleaching on coral biology and ecology.

Uneven bleaching within a coral colony has been observed by several researchers (Fitt et al., 1990; Jokiel & Coles, 1990). Jokiel and Coles (1990) stated that "portions of coral colonies receiving the highest incident radiation bleach more readily than portions that are shaded." In a paper by Fitt et al. (1990), a large color photograph of a bleached caribbean coral, Montastrea annularis, clearly illustrates a mottled bleaching pattern. However, these studies did not quantitatively address the issue. We also observed uneven bleaching in experimentally manipulated *Montipora verrucosa* coral fragments. Each fragment had a wire identification tag wrapped around it and was induced to bleach via transplantation from a low-light site at 10 m to the high-light site at 1 m for one week. Initially, the coral fragments appeared to have bleached uniformly. Closer examination revealed that the area shaded by the wire tag was much darker than the adjacent, unshaded area.

Following this observation, we designed a study to empirically measure differential bleaching response within a colony by simultaneously exposing different areas of the plating coral, *M. verrucosa*, to varying levels of solar irradiance. We tested the hypothesis that uneven bleaching within a coral colony occurs as a result of different levels of incident solar radiation. Manipulations were also performed in order to determine which portion of the irradiance spectrum was inducing the response: PAR, UV-A, UV-B, or some combination of the three.

MATERIALS AND METHODS

The experiment was conducted between July 23 and August 1, 1994, at the Lighthouse Point (LP) and the Bridge to Nowhere (BTN) sites on Coconut Island, Hawai‘i. A coral
fragment with minimum dimensions of 30 cm x 10 cm was broken off from each of five separate colonies of the platting morphology of *Montipora verrucosa*, at a depth of 10 m at the LP site. A smaller subfragment with dimensions approximating 100 cm² was then broken off each larger fragment, tagged and placed back at the site of origin as a control for transplantation. The five larger fragments were exposed to increased light levels via transplantation to a depth of 1 m at the BTN site, and each centrally placed under a separate treatment frame for nine days (Fig. 1). Each frame was 50 cm x 50 cm and consisted of four adjacent treatment bands (Fig. 1):

1) A control treatment with integrated irradiance levels between 300 and 700 nm, similar to those found at 10 m (50 cm x 20 cm band of ultraviolet radiation-transparent (UVT) Plexiglas overlain with two layers of neutral density filter).
2) A high PAR + UV-A + UV-B treatment (300 - 700 nm) (a 50 cm x 5 cm band of UVT Plexiglas).
3) A high PAR + UV-A treatment (320 - 700 nm) (a 50 cm x 5 cm band of UVT Plexiglas overlain with UV-B opaque mylar).
4) A high PAR treatment (400 - 700 nm) (50 cm x 20 cm band of UV-opaque Plexiglas).  

Figure 1: Summary of methodology. Uneven bleaching was induced in Hawaiian *Montipora verrucosa* coral fragments by transplanting them from 10 m (low-light) to 1 m (high-light) and centrally placing them under a treatment frame for 9 days. Each frame consisted of a PAR, PAR + UV-A + UV-B, PAR + UV-A transparent strip as well as a low-light control which reduced total irradiance (PAR + UV-A + UV-B) by 75% in order to simulate the lower light levels at 10 m.

By placing each coral fragment under a separate treatment frame, different areas of each coral's surface were simultaneously exposed to the four different treatments. Transplanted fragments and treatment frames were affixed to a uniform substrate plastic mesh platform.

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1 See Guiko *et al.* (this volume) for scans and more information on the use of these filters.
Average total irradiance at the high-light site (BTM at 1 m) was 70% greater than that observed at the 10 m LP site (based on integrated light measurements made every 2 cm using a Li-Cor Li-1800UW underwater spectroradiometer between 300-700 nm). Integrated UV-B (300-320 nm), UV-A (320-400 nm) and photosynthetically active radiation (PAR 400-700 nm) levels were approximately 99%, 93% and 68% greater at 1 m than at 10 m respectively. Treatment fragments were transplanted to the BTM site to replicate the low wave action conditions present at 10 m at the LP site (LP site is near a dock with boat traffic). These sites are separated by approximately 100 m, occur in the same small lagoon, and have similar sedimentation and temperature regimes. Sediment was brushed off of the treatment frames daily for the duration of the experiment. Hourly temperature values were recorded both at the 1 m BTM and at the 10 m LP site using two HOBOH24T miniature data loggers. The two sites differed by less than 1°C, on average, and neither temperature regime was high enough to induce bleaching (LP average temp. = 26.47 ± 1.98°C and BTM average temp. = 26.95 ± 1.01°C) (Jackie & Coles, 1977).

After nine days, eight samples were taken from each of the four treatment bands within each coral fragment (for a total of 32 subsamples per fragment) and from the control fragment by drilling with a 1.25 cm² cork borer through the coral plate. Three samples were analyzed for chlorophyll a concentrations, three for total lipid levels, and two for zooxanthellae concentrations per treatment per coral.

Chlorophyll a was extracted twice from fresh, finely ground samples in 10 ml of 100% acetone at 4°C for 24 hours. Samples were then centrifuged for 10 minutes. The absorbance of the supernatant was measured using a spectroradiometer and the chlorophyll a concentrations were calculated according to the method described by Jeffrey and Humphrey (1975) and reported in µg/cm².

Lipids were extracted from finely ground samples (samples had been frozen at -50°C for 1-2 weeks prior to extraction) in a chloroform:methanol (2:1, v/v) solution. Extracts were then washed once with 0.88% potassium chloride solution, three times with a methanol:water solution (1:1, v/v) and dried at 50°C for 24 hours before weighing. Animal tissue biomass was determined following lipid extraction by burning the skeleton and remaining tissue residue in a muffle furnace at 450°C for 8 hours. Lipid content in corals was reported as % lipid per gram dry tissue weight. This method differs slightly from Harland et al. (1992) where samples were decalcified prior to lipid extraction which can result in lipid loss during the decalcification process (triglycerides can hydrolyze in acid solutions and the glyceral component of the molecule is then soluble).

In order to determine zooxanthellae concentrations, fresh samples were simultaneously decalcified in 10 ml of 10% acetic acid, preserved with a few drops of 4% formalin and stained with a few drops of Lugol's solution. Once decalcification was complete, samples were centrifuged on "high" setting for 10 minutes, the excess liquid was decanted off and the remainder was homogenized for 30 seconds before being resuspended into 10 ml of 4% formalin for long-term preservation. Four subsamples from each sample were counted using a 0.1 mm³ hemacytometer and reported as the average number of zooxanthellae per cm². The amount of chlorophyll a per zooxanthellae was determined by dividing the amount of chlorophyll a per cm² by the total number of zooxanthellae per cm² and was reported in ng of chlorophyll a per zooxanthellae.

The mean lipid, chlorophyll a, zooxanthellae or chlorophyll a per zooxanthellae levels for the transplanted control fragment and the low light control fragment were analyzed using a student's t-test. All of the data were then analyzed by pairwise model I ANOVA's between the control and the four treatments to determine if either lipid, chlorophyll a, zooxanthellae or chlorophyll a per zooxanthellae levels had changed in any of the treatments. In all cases the null hypothesis was rejected at an alpha level of 0.05.
RESULTS

On the first day of the experiment, all of the coral treatment and control fragments were uniformly dark brown in color. The portion of the coral treatment fragments positioned under the PAR + UV-A + UV-B, PAR + UV-A and PAR treatment bands began to visibly bleach after the fourth, fifth and seventh day respectively. At the end of the nine day experiment, the coral area under the low-light, PAR + UV-A + UV-B, PAR + UV-A and PAR treatment bands, were dark brown (like the control fragment), almost white, extremely light brown and medium brown in color respectively.

The control and low-light treatment were not significantly different from each other with respect to any of the variables measured. This indicated that breaking off a coral fragment had no significant effect and that changes in the proportionate amounts of PAR, UVA and UVB due to transplantation were negligible. Only changes in spectral quantity and quality had an effect on the manipulated coral fragments.

The degree of bleaching was determined by measuring the chlorophyll a and zooxanthellae concentrations. Relative to the control and the low-light treatment, a significant decrease in chlorophyll a was observed in the PAR + UVA and PAR + UVA + UVB treatments ($F = 4.894$, $p < 0.013$)(Fig. 2a).

Figure 2: Mean (±SE) A. chlorophyll a (mg/cm$^2$), B. zooxanthellae ($\times 10^6$ /cm$^2$), C. chlorophyll a per zooxanthellae (mg/zooxanthellae) and D. percent lipid per gram dry weight levels in each of the four treatment regions and control fragments of the Hawaiian coral, Montipora verrucosa, after 9 days. Treatments undefined with lines at the same level were not significantly different (alpha = 0.05). CONT = control fragment, LOW=low light treatment, PAR Treatment = PAR (400 - 700 nm), PAR + UV-A Treatment = PAR + UV-A (320 - 700 nm), PAR + UV-A + UV-B Treatment = PAR + UV-A + UV-B (300-700 nm).
These decreases in pigment levels in the PAR + UV-A and PAR + UV-A + UV-B treatments were not accompanied by any changes in zooxanthellae concentrations. Zooxanthellae levels were not significantly different in the PAR + UV-A and PAR + UVA + UVB treatments relative to the control and low-light treatments. However, zooxanthellae concentrations were significantly higher in the PAR treatment (F = 3.324, p < 0.047) (Fig. 2b). Consequently, the chlorophyll a per zooxanthella levels were significantly lower in the PAR treatment (F = 4.088, p < 0.025) (Fig. 2c).

Energy reserve levels were determined by measuring lipid levels. Lipid levels did not differ significantly between any of the treatments or control (F = 0.351, p < 0.789) (Fig. 2d).

**DISCUSSION**

Chlorophyll a, zooxanthellae and chlorophyll a per zooxanthella levels varied significantly in response to various light conditions within fragments of the coral, *Montipora verrucosa*. The portion of the coral fragments exposed to elevated levels of PAR + UVA + UVB, PAR + UVA, and PAR exhibited high- to low-levels of bleaching respectively. There was no bleaching in either the low-light control or the transplantation control.

The portions of the solar spectrum which induced the bleaching response were elucidated. While pigments levels did decrease in all elevated irradiance treatments (PAR, PAR + UVA, PAR + UVA + UVB), significant decreases were only detected in the two treatments that included ultraviolet radiation (Fig. 2a). Under elevated PAR conditions (UV excluded), *M. verrucosa* did not significantly lose chlorophyll a relative to controls, but the density of zooxanthellae increased, resulting in an overall decrease in the calculated value of chlorophyll a per zooxanthella (Fig. 2a, b, c). This evidence indicates that in Hawaiian *M. verrucosa*, PAR, as well as UV, causes chlorophyll a to decrease but that under elevated PAR conditions alone, the coral may be able to compensate for this by increasing the number of zooxanthellae. Perhaps the increase in zooxanthellae density in the absence of UV is a response to an increase in potential light harvest without an increase in the biologically damaging ultraviolet radiation.

Energy reserve levels were determined by measuring lipid levels. The percent lipid content per gram dry weight did not significantly differ between any of the treatments or control conditions. Under standard physiological conditions, fatty acids and glycerol are synthesized by zooxanthellae from photosynthetically-fixed carbon and translocated to the host where they are either metabolized or transformed and stored mainly in the form of wax esters and triglycerides (Batey & Patton, 1984). When corals bleach, chlorophyll a levels decrease; hence the amount of carbon fixed also potentially decreases and other sources of carbon have to be relied upon. Despite a dramatic decrease in photosynthetic pigment (chlorophyll a) in the bleached portions of the coral colonies, lipid reserves did not decrease in *M. verrucosa*. Other work by Grottoli-Everett (1995) shows that lipid levels in *M. verrucosa* do not change even after two weeks of bleaching. Perhaps the zooxanthellae are able to maintain a high level of fixed carbon production because at high-light levels, the chlorophyll a pigments are being fully saturated. Alternatively, decreased metabolism, increased heterotrophy, gametogenesis resorption or some combination of these factors during the early stages of bleaching are mechanisms by which bleached corals may be compensating for decreased photosynthetically-derived, fixed carbon.

Given that varying degrees of bleaching occurred in the three elevated irradiance treatments, and not in the low-light control treatment within fragments of *M. verrucosa*, we accept our hypothesis that uneven bleaching within a coral colony occurs as a result of different levels of solar irradiance. The results of this experiment suggest that PAR, UV-A and UV-B have a synergistic effect on bleaching in *M. verrucosa*, as the decrease in chlorophyll a concentration was greatest when all three sections of the spectrum were allowed through the filters, and respectively less in the treatments where UVB was screened out and the treatments where no UV was allowed through the filters. Furthermore, this study indicates that bleaching due to increased solar irradiance in the Hawaiian coral, *M. verrucosa*, results from a decrease in chlorophyll a per zooxanthella and not from a decrease in the number of zooxanthellae.

Differential bleaching responses within a coral colony are quantifiable. While uneven bleaching has been mentioned previously in the literature, this study is the first documented empirical evidence of this observation. When conducting experiments on bleached coral, researchers must be careful to take into account the heterogeneity involved in bleaching in order to avoid biased sampling.
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LITERATURE CITED


