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

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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, UVA and UVB screened 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 zooxanthella 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 zooxanthella 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; Szmant & Gassman, 1990). The gravity of this phenomenon has lead 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., 1993; 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.* (1993), 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 coral 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 plating morphology of *Montipora verrucosa*, at a depth of 10 m at the LP site. A smaller subfragment with dimensions approximating 100 cm^2 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 transplantion 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)[1].

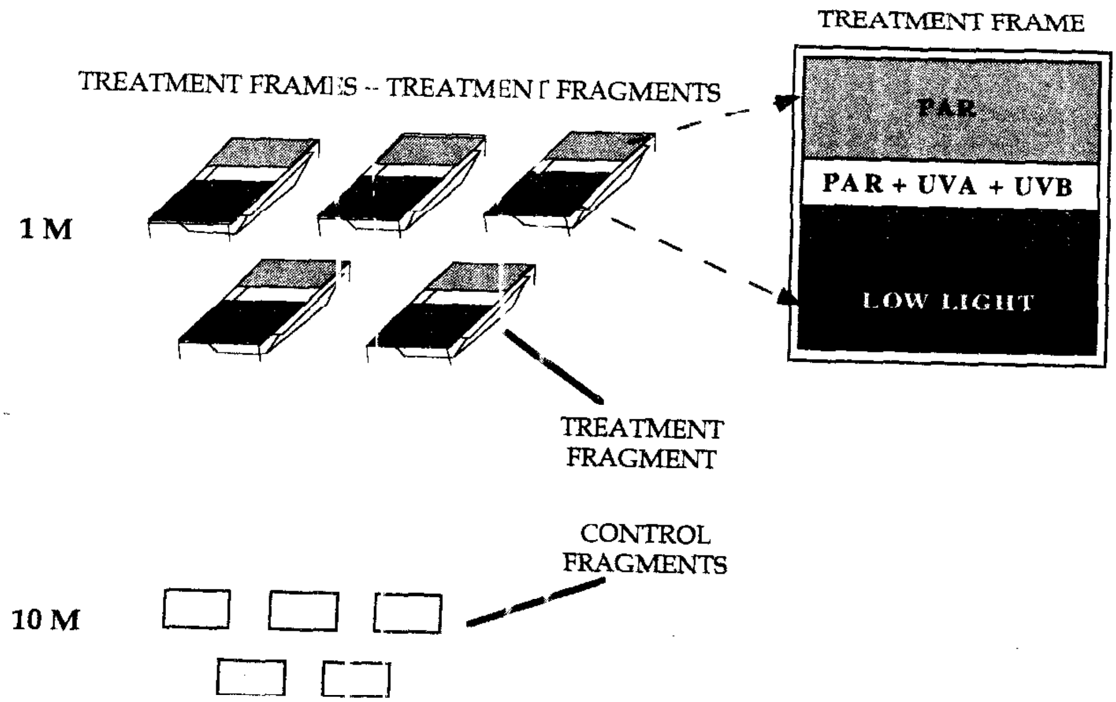

Figure 1: Summary of methodology. Uneven bleaching was induced in Hawaiian *Montipora verrucosa* coral fragments by transplanting them from 10 m (low light) to 1 m (high-light) and centrally placing them under a treatment frame for 9 days. Each frame consisted of a PAR, PAR + UV-A + UV-B, PAR + UV-A transparent strip as well as a low-light control which reduced total irradiance (PAR + UV-A + UV-B) by 70% 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.

[1] See Gulko *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 nm 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 66% greater at 1 m than at 10 m respectively. Treatment fragments were transplanted to the BTN 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 BTN and at the 10 m LP site using two $HOBO_{TM}$ 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 BTN average temp. = 26.95 ± 1.01°C)(Jokiel & 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 cm^2 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 ug/cm^2.

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).

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 mm^3 hemocytometer and reported as the average number of zooxanthellae per cm^2. The amount of chlorophyll *a* per zooxanthellae was determined by dividing the amount of chlorophyll *a* per cm^2 by the total number of zooxanthellae per cm^2 and was reported in ng of chlorophyll *a* per zooxanthellae.

The mean lipid, chlorophyll *a*, zooxanthellae or chlorophyll *a* per zooxanthella 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 zooxanthella 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).

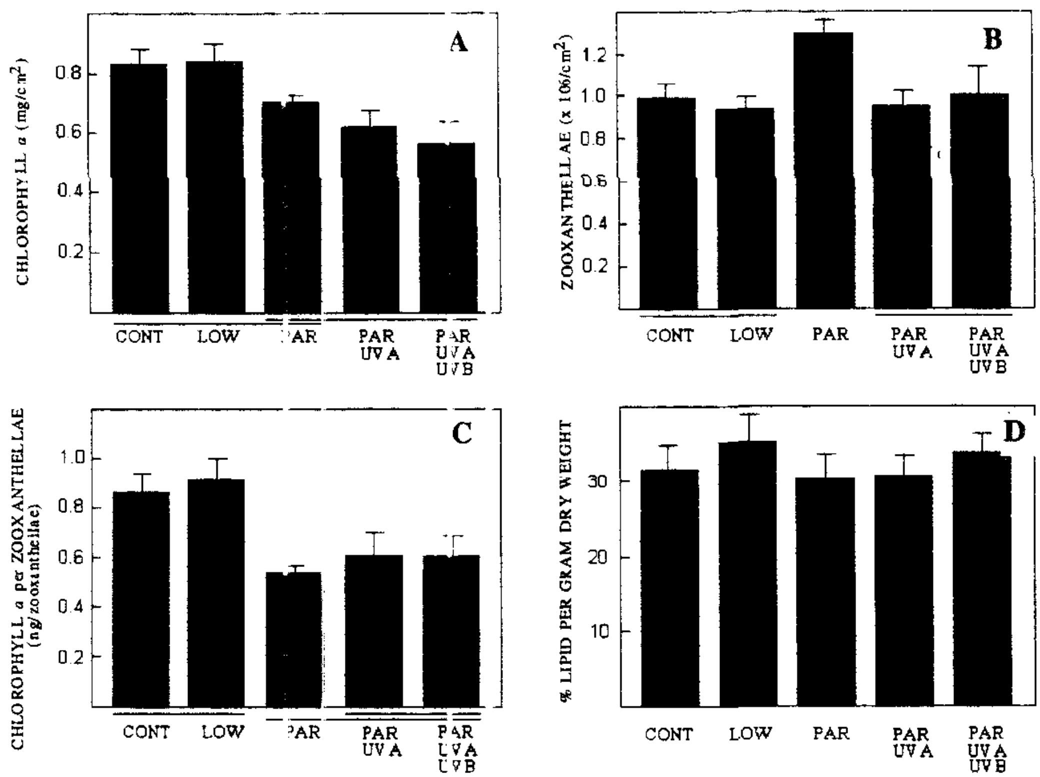

Figure 2: Mean (±SE) A. chlorophyll *a* (mg/cm^2), B. zooxanthellae (x10^6 /cm^2), C. chlorophyll *a* per zooxanthella (ng/zooxanthellae) and D. percent lipid per gram dry weight levels in each of the four treatment regions and control fragments of the Hawaiian coral, *Montipora verrucosa*, after 9 days. Treatments underlined 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 + UVA 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 primarily 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, gametes 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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