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Physiological Responses of *Acropora cervicornis* to Increased Solar Irradiance†

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ABSTRACT

The effects of increased UV radiation (UV-B [280–320 nm] + UV-A [320–400 nm]; hereafter UVR) on the growth, production of photosynthetic pigments and photoprotective mycosporine-like amino acids (MAAs) were studied in the threatened Caribbean coral *Acropora cervicornis* transplanted from 20 to 1 m depth in La Parguera, Puerto Rico. The UVR exposure by the transplanted colonies was significantly higher than that at 20 m, while photosynthetically active radiation (PAR) only increased by 9%. Photosynthetic pigments, quantified with HPLC, as well as linear extension rates and skeletal densities, were significantly reduced 1 month after transplantation to 1 m depth, while MAAs increased significantly despite immediate paling experienced by transplanted colonies. While these colonies showed a significant reduction in photosynthetic pigments, there were no significant reductions in zooxanthellae densities suggesting photoacclimation of the coral's symbionts to the new radiation conditions. The results suggest that while corals might be able to survive sudden increases in UVR and PAR, their skeletal structure can be greatly debilitated due to a reduction in the photosynthetic capacity of their symbionts and a possible relocation of resources.

INTRODUCTION

Clear water is one of the most important requirements for optimal coral reefs development. Nevertheless, clear oligotrophic waters, such as those found over many coral reef areas, are notably transparent to UV radiation (UVR) (1–6). Penetration of UVR, particularly UV-B depends strongly on the optical properties of the water itself, and the dissolved and suspended organic materials (7). In the tropics, penetration of UVR is also enhanced due to a low solar zenith angle and hence a shorter atmospheric path length, and to the thinness of the ozone layer (8–10).

Despite the high UVR levels reaching tropical reef waters, these shallow-water ecosystems have thrived at low latitudes for millions of years. The notable success of reef-building corals and other cnidarians containing symbiotic algae might be attributed to their ability to tolerate UVR. The plant and animal fractions may be unable to offset independently the high metabolic cost of UVR amelioration on shallow reefs, but can do so as a symbiotic unit (3). This suggests that corals and other shallow-water sessile zooxanthellate organisms have evolved mechanisms to protect their tissues from the damaging effects of UVR. Indeed, one such photoprotective mechanism exists in the form of mycosporine-like amino acid compounds (MAAs) (11). These photoprotective compounds are presumably formed within the zooxanthellae cells *via* a series of chemical reactions known as the shikimate pathway (12) and are later transferred to their animal host. The MAAs function as a broad-band filter believed to provide protection from the high energy wavelengths of the UV region of the spectrum (13). The photoprotective function of MAAs has been inferred from numerous experiments where their concentration was correlated with changes in UVR (13–16). Certain MAAs also serve as antioxidants for protection from photo-oxidative stress induced by free radicals and other active oxygen species (14). To date, approximately 20 MAAs are known from marine organisms (see 15–19 for reviews).

Regardless of the apparent protection from UVR afforded by MAAs, reef corals and other reef organisms are susceptible to physiological damages caused by these harmful wavelengths. The harmful effects of UVR in coral reef and other marine fauna and flora were reviewed by Shick *et al*. (20) and include damage to DNA and proteins (21) and photo-oxidation of chlorophyll (22). Specifically, middle UV radiation (UV-B) is known to disrupt many photosynthetic processes including the electron transport system, photosystem II reaction centers and pigment stability in marine algae (22,23). Both UV-A and UV-B can also reduce algal growth rates (23). Additionally, UVR may induce coral bleaching, inhibit skeletal growth, decrease carbon fixation and reduce photosynthetic pigment concentration, and affect coral survival (24–29). For example, early works by Coles and Jokiel (24) and Jokiel and York (25,26) showed the effects of UVR on several physiological processes in Pacific reef corals, such as *Montipora verrucosa* and *Pocillopora damicornis*, and microalgae, including growth rates and photosynthetic capacity. Gleason and Wellington (29) reported bleaching signs in the Caribbean reef-builder *Montastraea annularis* 7 days after transplantation from 24 to 12 m, presumably as the resulting increase in UVR.

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Similarly, a study on the Great Barrier Reef showed significant reductions in photosynthesis in colonies of *Acropora microphthalma* exposed to enhanced UVR after transplantation from 30 to 2 m depth (30).

The objective of this work was to explore the detrimental effects by increased UVR to some physiological functions of the threatened Caribbean shallow-water branching coral *Acropora cervicornis* Lamarck. Field manipulations entailing transplantation of colonies to deeper or shallower depths were implemented to assess the effects of changing irradiance regimes, and physiological responses including changes in skeletal linear extension rates, skeletal density, MAA accumulation and transient bleaching following contrasting field manipulations were quantified.

MATERIALS AND METHODS

Species studied. The range of *A. cervicornis* includes the tropical Atlantic regions of the Caribbean Sea, the Bahamas and the Gulf of Mexico. In Puerto Rico, this species is mainly found in shallow clear reef bottoms between 0 and 10 m, but it is not uncommon to find populations at depths of up to 25 m in clear shelf-edge waters. Extensive population decreases through its geographical region, caused by a variety of factors (31), have led to the listing of this species, along with its congener *Acropora palmata*, as a threatened species in the Endangered Species Act (70CFR24359).

Study sites. San Cristóbal Reef (17°56′41′N; 067°04′38′W) is located on La Parguera insular shelf platform, approximately 3 km from the Magueyes Island Field Station (University of Puerto Rico, Mayagüez Campus, Department of Marine Sciences) on the southwest coast of Puerto Rico. The Old Buoy site is located at La Parguera shelf edge (17°53′11′N; 066°59′51′W) approximately 10 km south of Magueyes Island. The site is characterized by a spur and groove topography and depth ranges from 18–25 m. Relatively calm and clear water is found year long at both study areas.

Experimental setup. To assess the effects of UVR on some physiological aspects of *A. cervicornis*, we conducted a series of experiments in which colonies of the species were exposed to either none or increased UVR levels.

UV-exclusion experiment (UV-E hereafter). The experiment was conducted during 6 April to 3 July 2001. Eighteen colonies of *A. cervicornis* were subjected to depleted UVR and reduced PAR levels received at actual colony living depths (1 m) at San Cristóbal Reef. Additionally, six colonies were placed in a 4 m^2 quadrat spaced 5 m away from the location of the treatments and used as controls. The controls were exposed to unfiltered ambient irradiance. All colonies were collected on-site at a 5 m distance to their nearest intraspecific neighbor to reduce pseudoreplication due to clone mates. All colonies (controls and treated) were tied to 1.25 cm diameter PVC tubes placed on cement platforms to avoid contact with the bottom sand. All other physical factors remained unaltered (salinity, water motion, temperature, *etc.*). To block UVR and some of the PAR received at the site, three hemispherical open-ended steel rod frames (1 m width × 1 m length × 1 m height) covered by a 4 mm thick Hyzod® SR-Polycarbonate Sheet (DSM Sheffield Plastics) were constructed. This material excludes 99% of wavelengths below 400 nm (UVR) and 12% of PAR (Fig. 1). All frames were located at the same depth as the control colonies (1 m). Six colonies were located under each covered frame. All frames were aligned to the mean sun azimuth angle calculated for the experimental period to ensure that direct solar radiation reached the colonies only after passing through the filter.

UV-supplementation experiment (UV-S hereafter). A UV-supplementation experiment was conducted during 20 March to July 31 2003 consisting of exposing colonies of *A. cervicornis* to an increase in UVR and PAR in the field by transplanting ten colonies from the La Parguera shelf edge (20 m depth) to the back-reef area of San Cristóbal Reef at 1 m depth. Ten colonies of *A. cervicornis* living at San Cristóbal Reef at 1 m were also transplanted to the shelf edge at 20 m. Additionally, ten colonies were used as controls at San Cristóbal (1 m depth) and ten colonies were used as controls at the shelf edge (20 m depth). All colonies studied (controls and transplants) were tied to plastic-coated cyclone fence wires to avoid contact with the bottom sand. The cyclone fence wires were held in place with cement blocks and cleaned on a weekly basis.

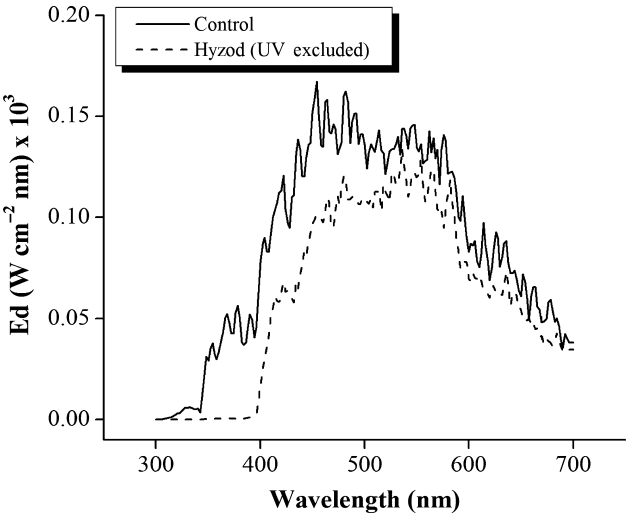

Figure 1. Unweighted UV irradiance measured in both treatments (control and UV-excluded) at 1 m depth at San Cristóbal Reef in La Parguera, Puerto Rico.

UVR and PAR measurements. Irradiance measurements during the UV-E experiment at the control and UV-excluded treatment were obtained with an Optronic OL-754 spectroradiometer (Optronic Laboratories) attached to a submersible integrating sphere connected through a fiber optics cable. Underwater irradiance scans were obtained between 280–700 nm at 2 nm intervals at noon on randomly selected days during the course of the experiment. Surface irradiance measurements were obtained with a ground-based radiometer (Biospherical Instruments GUV-511) located at Magueyes Island. This radiometer measures UVR in four discrete bands of 305, 320, 340 and 380 nm, and has an additional channel that integrates downwelling irradiance in the visible region (400–700 nm: PAR). The spectral resolution for each band is 10 nm for 305 and 320 nm, 8 nm for 340 nm, and 9 nm for 380 nm. The radiometer averages and records irradiance measurements every 5 min. The penetration of UVR and PAR was measured at the Old Buoy site and San Cristóbal Reef during the UV-S experiment at the beginning, middle and end of the experiment. Total UVR levels at the sampling sites were obtained with a Solar Light radiometer connected to an underwater 2π cosine collector. This radiometer measures unweighted UVR in a single band (bandwidth: 280–400 nm). Measurements were recorded at 1 m intervals between surface and 10 m depth and every 2 m between 10–20 m depth at the Old Buoy site and at 0.5 m intervals at San Cristóbal Reef. Data from the GUV-511 were compared with the onsite data obtained just above the surface with the Solar Light radiometer using equations for integrating daily solar radiation in the UV-B and UV-A regions (32) and adding the results to obtain an estimate of total UVR. A *t*-test showed no significant differences between total UVR measured with the Solar Light and estimated total UVR from the GUV-511 ($P < 0.01$) for the sampling dates. PAR measurements were obtained at the same depth intervals with a LiCor 182 s cosine collector connected to a LiCor 1400 data logger. Additional 2π cosine collectors were located on the boat to correct for atmospheric changes during the sampling. Therefore, Ed underwater measurements were normalized by simultaneous above-water Ed measurements. Sampling was conducted at similar times at both sites during all sampling dates for consistency. Both measurements (UVR and PAR) were later used to obtain diffuse vertical attenuation coefficient (Kd) values for both sites. Kd for UV-A, UV-B and PAR was calculated using:

$$Kd = 1/(Z_2 - Z_1)[\ln(Ed_2/Ed_1)] \quad (1)$$

where Ed_1 and Ed_2 are the downwelling irradiances measured at depths Z_1 and Z_2, respectively (7).

Other physical parameters. A HOBO® Temperature Data Logger (Onset Computer Corporation) recorded water temperature data on an hourly basis at San Cristóbal Reef during both UV-E and UV-S experiments and at the shelf edge during the latter. The data were recovered at approximately monthly intervals. A one-way ANOVA was used to test for differences in temperature between sites.

Skeletal growth analysis. Two growth parameters were measured during both experiments: skeletal linear extension rates and skeletal density. A plastic tie was attached to one of the main branches of each colony during the transplanting date in both, colonies to be transplanted to a different depth and the controls at each depth (1 and 20 m) to measure the skeletal linear extension rates at the end of the experiment. The length from the plastic tie to the tip of the branch was measured on-site with a Vernier caliper to the nearest 0.1 mm at the beginning of the experiment and used as reference. Length was also measured at the end of the experiment and the difference was considered as the linear growth for the experimental period. Linear extension rates were then calculated by dividing the linear growth by the number of experimental days. Skeletal density was measured using a liquid displacement technique. The tissue-free piece of each colony used for the pigment analysis (see below) was left to dry for a week at room temperature and weighed to the nearest 0.0001 g in an analytical balance. Each coral piece was covered with a previously weighed aluminum foil piece to avoid penetration of water into the coral skeleton. Then, it was quickly submerged in a test tube with deionized water and the volume recorded to the nearest 0.1 mL. The skeletal density was obtained by dividing the weight of the piece by the volume of displaced water.

Pigment analysis—sampling and tissue extraction. One branch from each colony was removed during each of the three sampling dates (beginning, 1 month after and end of the experiment). All samples were transported in sterilized Whirl-Pak plastic bags in a dark container. Each branch was broken into three pieces. The proximal piece (closer to the base of the branch) was used for histological analysis of the gonads (J.L. Torres, unpublished data), the middle piece was used for zooxanthellae density and the distal piece was used for the pigment analysis. The white tip of each branch was discarded as it usually either lacks zooxanthellae or pigments (33). To avoid differences in the concentration of pigments with increasing distance from the branch tip (34), this procedure was kept constant for each branch. Individual samples were then placed into 40 mL glass vials. Samples were extracted following established procedures (35–39) using 20 mL of HPLC grade menthanol:tetrahydrofuran (80:20, vol/vol) solution at 4°C in the dark for 24 h followed by a second 20 min extraction to remove the remaining pigments. Photosynthetic pigments were separated from MAAs following the procedures of Corredor *et al*. (38) using Sep-Pak C_{18} 900 mg cartridges, and later removed from the cartridge by injecting 4 mL of the same extraction solvent. Individual pigments were quantified using reversed-phase HPLC. Both photosynthetic pigment and MAA extracts were kept in 4 mL HPLC vials and stored at −70°C until HPLC analysis was performed.

HPLC analysis—MAAs. Mycosporine-like amino acids were separated following published extraction protocols (37,38). Briefly, MAAs were separated, by injecting 30–80 μL into a guarded Phenosphere® 250 × 4.6 mm C_8 column connected to a Shimadzu LC-10AT liquid chromatograph coupled to a Shimadzu SPD-M10AV diode-array detector, using an isocratic solution consisting of 55% MeOH, 0.1% acetic acid and 44.9% DIW ran at 0.8 mL/min for 20 min. Due to high concentrations, especially by samples from San Cristóbal Reef, some samples were diluted 1:10 using the extracting solvent before injection. Representative samples were later sent to Dr. Daniel Gleason (Georgia Southern University) for individual MAAs identification. Samples were co-chromatographed with standards obtained from *Porites astreoides* from St. Croix, *Lissoclinum patella* and *Porphyra* sp. from Australia and *Acanthopleura elegantissima* from Pacific Grove, California. Following identification of individual MAAs, their concentrations were obtained using an extension of Beer's Law and published molar extinction coefficients: mycosporine-glycine, ϵ_{307} = 28 100 (40); palythine, ϵ_{320} = 36 200 (41); porphyra-334, ϵ_{334} = 43 200 (42); shinorine, ϵ_{334} = 44 668 (43); and palythene, ϵ_{360} = 50 000 (44). The molar extinction coefficients of asterina-330 and usijirene have not been reported yet. We used the molar extinction coefficients of palythinol (ϵ_{330} = 43 000) (44) for asterina-330 (45), and that of palythene for estimating the concentration of usujirene. Peaks were detected at 313, 334 and 360 nm depending on the pigment wavelength absorption maxima (λ_{max}). Peaks were baseline corrected and integrated before concentrations were calculated using the molar extinction coefficients. The concentrations were corrected using the calculated extraction efficiencies. Extraction efficiencies were determined for all MAAs following established procedures (36). The MAA concentrations were normalized to soluble protein and expressed as nmol mg prot^{-1} (38).

HPLC analysis—photosynthetic pigments. Photosynthetic pigments were separated using a modification of an earlier described procedure (46). The gradient system consisted of 80:20 methanol:ammonium acetate (pH 7.2, vol/vol), 90:10 acetonitrile:water and 100% ethyl acetate with a Symmetry® C_{18}, 25 cm × 3.9 mm-inner diameter, 5 μm particle size column at a constant flow rate of 1.0 mL min^{-1}. Eluting peaks were detected using the absorbance spectra at 436 nm for carotenoids and chlorophylls (47). Peaks were integrated, and quantification of individual pigments was accomplished using peak areas and calibration factors determined with authentic standards of chlorophyll *a* and lycopene (Sigma Co.). Individual pigments were identified using published spectra and their respective peak maxima (46,48). Photosynthetic pigments and zooxanthellae concentrations were normalized to coral tissue area determined by the aluminum foil technique (49) and their concentration was expressed as μg cm^{-2}. Photosynthetic pigments were also normalized to zooxanthellae densities and their concentration was expressed as picogram zooxanthellae per cell.

Zooxanthellae analysis. The tissue of the middle piece of the sampled branch was fixed using a 10% formalin in seawater solution for 24 h and rinsed in DIW for another 24 h. The fixation process started at similar hours during the sampling dates to avoid any influence from the diel patterns of cell division (50). Subsequently, the sample was decalcified using a 10%HCl 0.7%EDTA solution and the tissue cylinder was ground with a mortar and pestle and homogenized at 7000 rpm using a tissue homogenizer (Biospec Products, Inc.). The slurry was decanted into a 50 mL centrifuge tube with 5 mL of DIW, centrifuged at 5000 rpm for 15 min, and the supernatant discarded. The remaining pellet containing the zooxanthellae was resuspended in 2 mL of DIW until analysis. Counts were performed in triplicate in a Reichert haemocytometer and averaged. The percentage of dividing cells was calculated for an estimation of the Mitotic Index (MI) (51).

Protein assay analysis. Protein analysis was performed using a Bio-Rad soluble protein determination kit. The procedure is similar to that described by Bradford (52). Protein standards were prepared with lyophilized bovine gamma globulin. The solid portion of each sample was transferred to 15 mL centrifuge tubes with 3 mL NaOH 1 *N* and heated for 30 min at 90°C to solubilize the proteins. Samples were allowed to cool to room temperature for approximately 1 h and then neutralized with 3 mL HCl 1 *N*. A hundred microliter aliquots of each solution was assayed with 500 μL of an alkaline copper tartrate solution (reagent A), and 4 mL of a dilute Folin reagent (reagent B) and shaken in a vortex mixer. After 15 min the absorption was recorded at 750 nm in a Shimadzu UV–visible UV-1601 spectrophotometer. Weight of soluble protein was obtained using a calibration curve prepared during the procedure.

Statistical analysis. For both experiments, data were tested for normality and equality of variances using a Bartlett's Test with Bonferroni 95% confidence intervals. Data were log-transformed whenever unequal variances were found to comply with the statistical testing assumptions. Based on the experimental design, a fully nested ANOVA was used to test for statistical differences in linear extension rates, skeletal density, and concentration of photosynthetic pigments and MAAs among and between replicates of the same treatment and among treatments. Where statistical differences were found, a Tukey test with pairwise comparisons was performed to distinguish where these differences were present (53). Statistical significance was set at ≤ 0.05.

RESULTS

UVR and PAR measurements

The experimental time period for both experiments coincided with the highest UVR reaching La Parguera reef platform surface waters as measured by the Magueyes Island UV monitoring station (Fig. 2). Data from September to December of 2001 were not available due to instrument problems. With respect to the UV-E experiment, the polycarbonate material absorbed 99% of the entire incident UVR at 1 m as well as 12% of the entire incident PAR (Fig. 1). Estimated daily UV radiation doses (kJ m^{-2} day^{-1}) are shown in Table 1. The downwelling irradiance for total UVR (Ed_{UVR}) at the shelf edge (Fig. 3) and at San Cristóbal Reef (Fig. 4) shows an exponential decay with depth similar to that of Ed_{PAR}, Ed_{UVR} and Ed_{PAR} measurements at both experimental depths (1 and 20 m) showed a 2.5-fold increase in UVR but only a 9% increase in PAR levels experienced by the colonies transplanted from 20 to 1 m depth.

The vertical attenuation coefficients measured at the beginning and end of the UV-S experiment for total UVR (Kd_{UVR}) were approximately two to three times higher than those of PAR (Kd_{PAR}) at the San Cristóbal back-reef area (Table 2). In contrast, both Kd_{UVR} and Kd_{PAR} values were lower at the shelf edge indicating a higher water transparency compared with the nearshore reef at least during sampling dates. Nevertheless, both Kd coefficients show some variations at different times of the year (Table 2). The results are

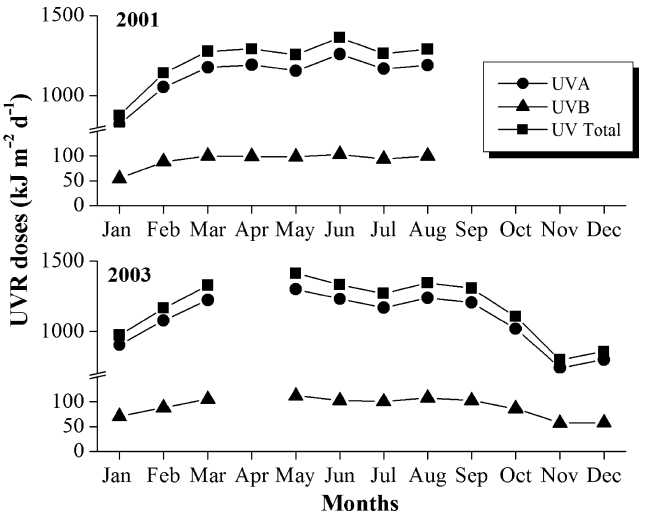

Figure 2. Average UVR doses received at the ocean surface calculated from the data collected by the GUV-511 radiometer at Magueyes Island, La Parguera, Puerto Rico: (a) 2001, (b) 2003. Data for April 2003 could not be obtained due to instrumental problems.

Table 1. Estimated daily UV radiation doses (kJ m^{-2} day^{-1}) received at sampling depth (control) and under the UV-depleted treatment during the UV-E experiment.

Treatment	UV-B (kJ m^{-2} day^{-1})	UV-A (kJ m^{-2} day^{-1})	UVT (kJ m^{-2} day^{-1})
Control	13.6	360.1	373.7
Hyzod (UV-depleted)	0.1	6.4	6.5

UVT = total UVR (UV-A + UV-B).

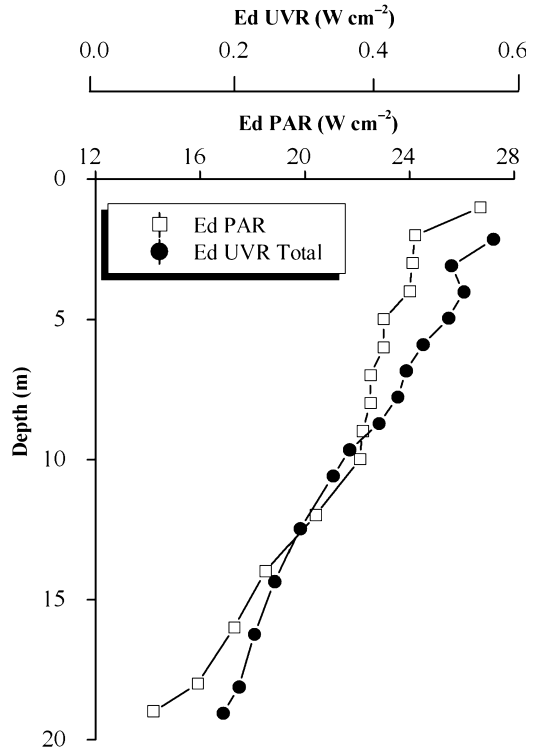

Figure 3. Average downwelling irradiance (Ed_{PAR} and Ed_{UVR}) measured at the shelf edge site in La Parguera, Puerto Rico.

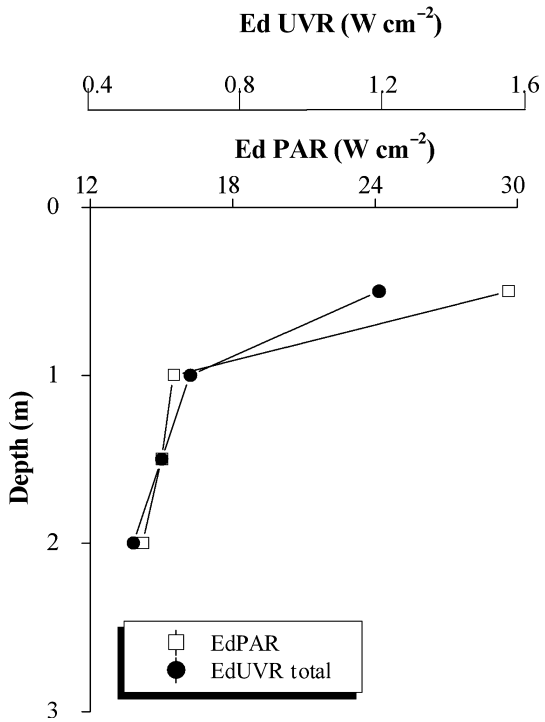

Figure 4. Average downwelling irradiance (Ed_{PAR} and Ed_{UVR}) measured at the back-reef lagoon of San Cristóbal Reef in La Parguera, Puerto Rico.

Table 2. Diffuse vertical attenuation coefficients (Kd) measured for total UVR and photosynthetically active radiation (PAR) at study sites at the beginning and end of the experimental period during the UV-S experiment.

	Kd (UVR) (m^{-1})		Kd (PAR) (m^{-1})	
Study site	20 March 2003	31 July 2003	20 March 2003	31 July 2003
San Cristóbal Reef (1 m)	0.27	0.19	0.09	0.09
Old Buoy (20 m)	0.13	0.16	0.06	0.08

Table 3. Temperature measurements during the UV-E (March–July 2001) and UV-S experiments (March–July 2003).

	Temperature (°C)		
Location	Minimum	Maximum	Average (±1 SD)
UV-E experiment			
Control	24.8	27.9	26.5 ± 0.6
Hyzod	24.4	27.5	26.1 ± 0.5
UV-S experiment			
San Cristóbal Reef (1 m)	24.9	28.3	26.1 ± 0.3
Old Buoy shelf edge site (20 m)	23.8	27.7	25.4 ± 0.2

Numbers for average temperature represent the average ±1 SE.

comparable to those of Dieppa-Ayala (54) and Detrés *et al.* (55) who found similar Kd values in oligotrophic waters off La Parguera between March and June and no significant variations in Kd_{PAR}. Continuing studies on the characterization of the underwater light spectrum at the present study site and other sites in La Parguera have found similar Kd_{UVR} and Kd_{PAR} values (56; Armstrong *et al.*, unpublished data).

Other physical parameters

Temperature was recorded at 1 h intervals during both experiments (Table 3). No significant differences were found among temperature means (ANOVA, $P = 0.621$), minima (ANOVA, $P = 0.485$), or maxima (ANOVA, $P = 0.693$) recorded in both the UV-E and UV-S experiments. At least for these experiments, temperature was discarded as a possible environmental factor influencing the response of the *A. cervicornis* colonies transplanted to either 1 or 20 m depth.

Skeletal growth analysis

The linear extension rates (mm d^{-1}) of *A. cervicornis* were significantly higher in colonies growing under UV-depleted conditions compared with those of colonies growing at ambient levels of UVR (control colonies) (0.46 ± 0.02, 0.39 ± 0.02, respectively; fully nested ANOVA, $P \ll 0.001$; Fig. 5a) during the UV-E experiment. This represents an increase in skeletal extension of up to 22% for this species under no UVR conditions. No differences were found in the linear extension rates of *A. cervicornis* between colonies within replicates of the same treatment and between replicates within treatments (fully nested ANOVA, $P = 0.489$).

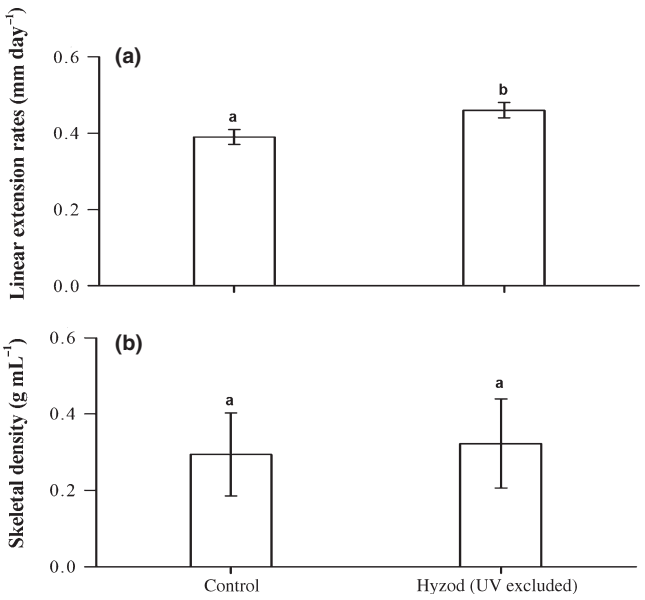

Figure 5. (a) Average linear extension rates of *Acropora cervicornis* measured after 88 days of exposure during the UV-E experiment. (b) Change in skeletal density of *A. cervicornis* measured after 88 days of exposure during the UV-E experiment. Similar superscripts indicate no significant differences among treatment means at $P < 0.05$ (Tukey test with pairwise comparisons).

There were no differences in the skeletal density of *A. cervicornis* colonies exposed to normal *vs* 99% depleted UVR conditions (fully nested ANOVA, $P = 0.732$; Fig. 5b) during the UV-E experiment. No differences were found in the skeletal density of *A. cervicornis* between colonies within replicates of the same treatment and between replicates within treatments (fully nested ANOVA, $P = 0.641$).

In contrast to the results of the UV-E experiment, significant differences were found during the UV-S experiment in the linear extension rates of those colonies transplanted to different depths *vs* the controls at those depths (Fig. 6a; fully nested ANOVA, $P < 0.0001$). The colonies of *A. cervicornis* grew significantly less when transplanted from 20 to 1 m depth compared with the control colonies at 20 m (0.30 ± 0.004 *vs* 0.83 ± 0.0004, respectively; Tukey test, $P < 0.0001$). No significant differences were found among individual colonies of the same treatment (fully nested ANOVA, $P = 1.000$).

Colonies transplanted from 20 to 1 m also developed a significantly less dense skeleton compared to the control colonies at 1 m (Fig. 6b; Tukey test, $P < 0.001$). No significant differences were found in the skeletal density among colonies left on-site as controls at San Cristóbal and the Old Buoy shelf edge site (fully nested ANOVA, $P = 0.990$).

Pigment analysis—MAAs

A significant reduction was found in the total concentration of MAAs in colonies exposed to excluded UVR (Fig. 7; fully nested ANOVA, $P \ll 0.0001$) during the UV-E experiment. No differences in total MAAs were found between replicates among treatments (fully nested ANOVA, $P = 0.938$). Total MAAs were reduced to 16% of that of the control colonies of

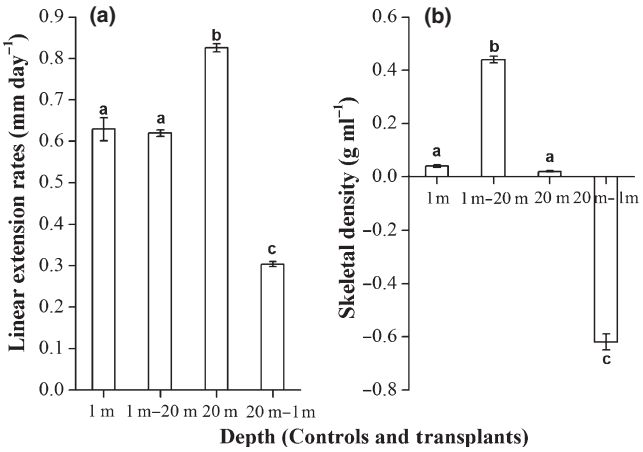

Figure 6. (a) Average linear extension rates of *Acropora cervicornis* 115 days after transplanting the colonies to the different sites during the UV-S experiment. (b) Change in skeletal density of *A. cervicornis* 115 days after transplanting the colonies to the different sites. Vertical lines in both graphs represent ±1 SE ($\alpha = 0.05$). Similar superscripts indicate no significant differences among treatment means at $P < 0.05$ (Tukey test with pairwise comparisons).

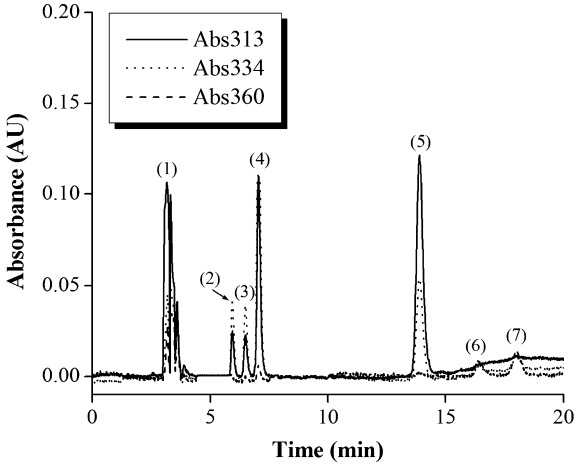

Figure 8. Representative chromatogram showing the individual mycosporine-like amino acids found in *Acropora cervicornis*. (1) mycosporine-glycine (λ_{max} = 310 nm); (2) porphyra-334 (λ_{max} = 334 nm); (3) shinorine (λ_{max} = 333 nm); (4) asterina-330 (λ_{max} = 330 nm); (5) palythine (λ_{max} = 320 nm); (6) usujirene (λ_{max} = 357 nm); and (7) palythene (λ_{max} = 360 nm).

A. cervicornis in those colonies in the excluded UVR environment (64.53 ± 6.6 nmol mg protein^{-1}, compared to 400.92 ± 35.7 nmol mg protein^{-1} in the control colonies).

The MAA composition was consistent during the UV-E and UV-S experiments. A representative chromatogram showing the different MAAs found in *A. cervicornis* is shown in Fig. 8. In order of dominance, the following MAAs were identified in *A. cervicornis*: palythine (λ_{max} = 320 nm), asterina-330 (λ_{max} = 330 nm), mycosporine-glycine (λ_{max} = 310 nm), palythene (λ_{max} = 360 nm), shinorine (λ_{max} = 333 nm), usujirene (λ_{max} = 357 nm) and porphyra-334 (λ_{max} = 334 nm). All the MAAs showed a systematic decrease through the UV-E experiment in colonies exposed to reduced levels of UVR. By the end of the experiment, only one colony in the UVR excluded treatment contained a small amount of shinorine, whereas none of the other MAAs was detected. In contrast, the control colonies showed an increase in all the MAA concentrations, associated with an increase in both solar day and UVR doses as the experiment approached summertime (Fig. 9).

During the UV-S experiment, colonies of *A. cervicornis* normally living at 1 m depth at San Cristóbal Reef contained 40–88 times higher total MAAs than those living at 20 m at the shelf edge with a steady increase as summertime approached. There was a significant difference among transplants (fully nested ANOVA, $P \ll 0.0001$). In contrast, those colonies transplanted to deeper reef zones reduced drastically their respective total (Fig. 10) and individual (Fig. 11) MAA concentrations. Colonies transplanted from 1 to 20 m showed no significant difference in total MAAs compared to the controls at 20 m (fully nested ANOVA, $P = 0.994$). The colonies transplanted from 20 to 1 m, despite the almost immediate paling experienced (see below), showed an approximately 40-times increase by the first month of exposure and 88-times higher total MAAs by the end of the experiment compared to control colonies at 20 m. Total MAAs in these colonies also showed a significant increase compared to the controls at 1 m (fully nested ANOVA, $P < 0.001$). There were no significant differences in total MAAs between colonies among replicates of the same treatment or between replicates of the same treatment (fully nested ANOVA, $P = 0.992$).

While the colonies transplanted to deeper areas lost their longer wavelength UV-absorbent MAAs (*i.e.* palythene and usujirene), the colonies transplanted to 1 m showed a higher increase in all MAAs especially those absorbing near the UV-B region (see Fig. 11). The extraction efficiencies for all MAAs ranged between 0.95 and 0.98 during both experiments.

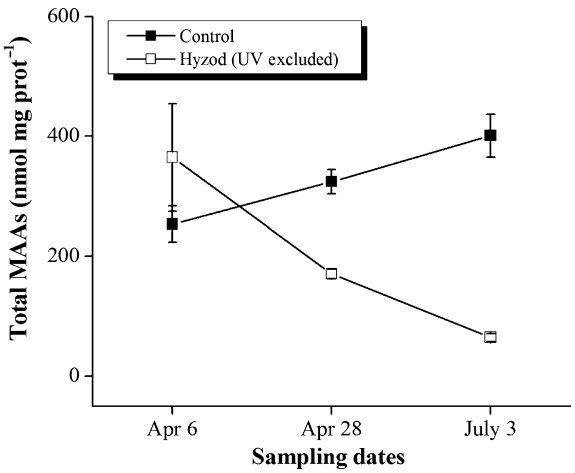

Figure 7. Total mycosporine-like amino acids concentration in *Acropora cervicornis* (nmol mg protein^{-1}) measured at the beginning, 1 month later, and at the end of the experimental period during the UV-E experiment. Vertical lines represent ±1 SE ($\alpha = 0.05$).

Pigment analysis—photosynthetic pigments

During the UV-E experiment, there were no significant differences in total photosynthetic pigment concentration

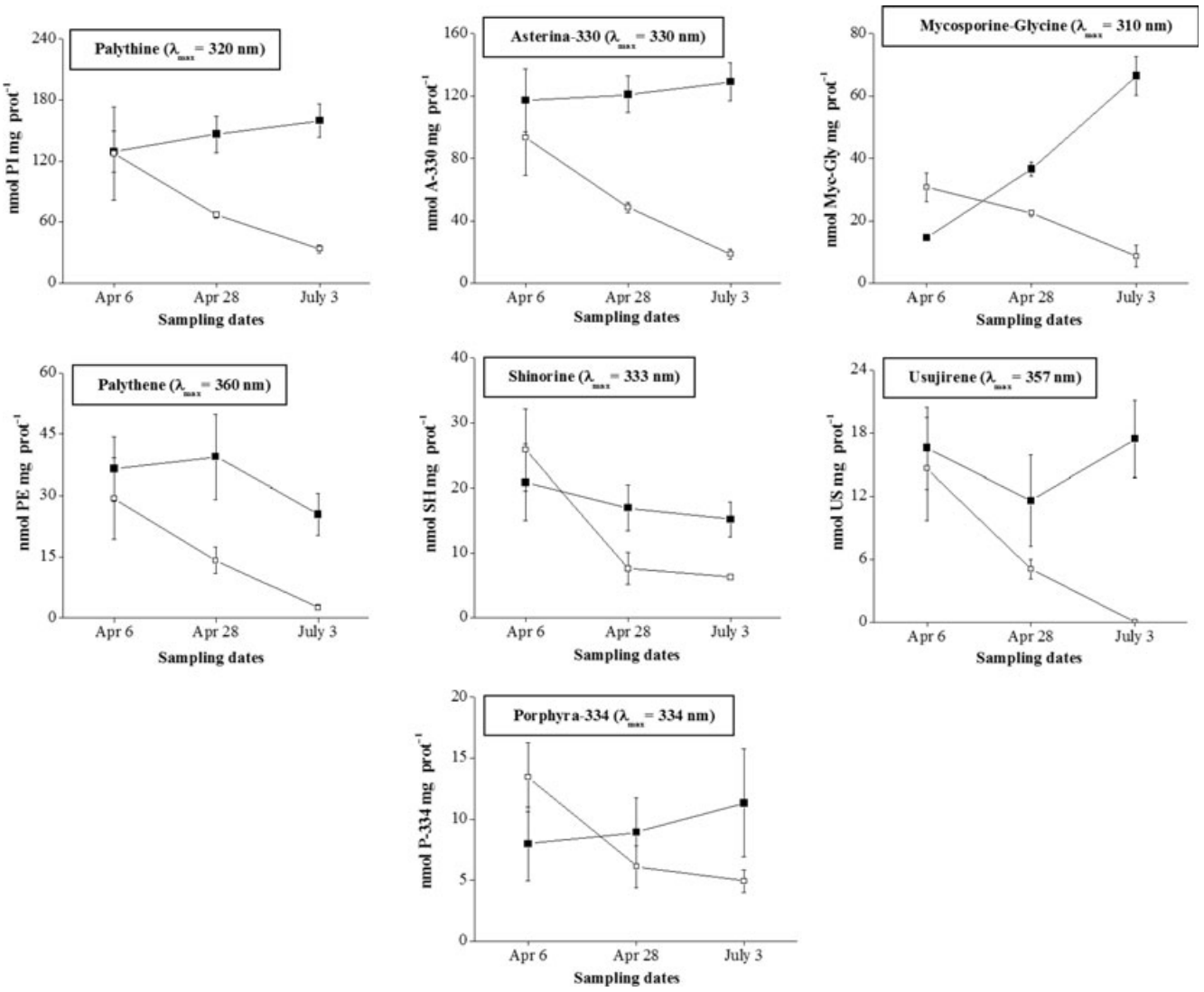

Figure 9. Individual concentration of the seven mycosporine-like amino acids (MAAs) identified in *Acropora cervicornis* as measured at the beginning, 1 month later, and at the end of the experimental period during the UV-E experiment. Black squares indicate average concentration of any given MAA in the control treatment and open squares indicate average concentration of any given MAA in the UV-excluded treatment. Vertical lines represent ± 1 SE ($\alpha = 0.05$).

among treatments normalized either by host tissue area or by zooxanthellae cell between replicates (fully nested ANOVA, $P = 0.634$; fully nested ANOVA, $P = 0.966$, respectively) nor among treatments (Fig. 12a; fully nested ANOVA, $P = 0.805$; Fig. 12b; fully nested ANOVA, $P = 0.711$, respectively). Both the control and the UV-depleted colonies showed a slight decrease after 1 month of exposure; yet, by the end of the experiment all the colonies had experienced increases in their corresponding pigment concentrations.

During the UV-S experiment, colonies of *A. cervicornis* transplanted from 20 to 1 m depth showed immediate "bleaching" signs (*i.e.* paling) just 1 day after the transplant. This was reflected in a significant decrease in total photosynthetic pigments per coral tissue area (Fig. 13a; fully nested ANOVA, $P < 0.001$) and per zooxanthellae cells (Fig. 13b; fully nested ANOVA, $P < 0.0001$). Individually, colonies transplanted to 1 m showed decreased concentrations of major photosynthetic pigments (*i.e.* chlorophyll *a*, chlorophyll c_2, peridinin, diadinoxanthin and β,β-carotene) both normalized to coral tissue area and zooxanthellae cell (data not shown). Other pigments found in lesser concentrations were diatoxanthin, P-457, diadinochromes I and II, zeaxanthin, chlorophyll *a* allomer and chlorophyll *a* epimer. In contrast, those colonies transplanted from shallow to deeper areas showed an increase in total photosynthetic pigment concentration (see Fig. 13a,b) as well as in the major photosynthetic pigment concentrations (data not shown).

Zooxanthellae analysis

Zooxanthellae densities (normalized to host tissue area) were not significantly different between replicates (fully nested ANOVA, $P = 0.741$) nor among treatments (fully nested ANOVA, $P = 0.169$) during the UV-E experiment. No

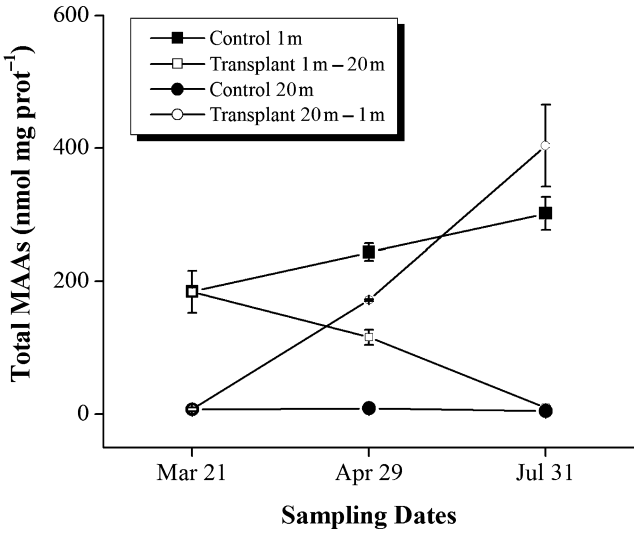

Figure 10. Total mycosporine-like amino acids concentration in *Acropora cervicornis* (nmol mg protein^{-1}) measured at the beginning, 1 month later, and at the end of the experimental period during the UV-S experiment. Vertical lines represent ± 1 SE ($\alpha = 0.05$).

differences were found also in the zooxanthellae MI between replicates (fully nested ANOVA, $P = 0.539$) nor among treatments (fully nested ANOVA, $P = 0.057$).

Similarly, during the UV-S experiment, no significant differences were found in zooxanthellae densities between replicates (fully nested ANOVA, $P = 0.981$) nor among treatments (fully nested ANOVA, $P = 0.168$). No significant differences were also found in the zooxanthellae MI between replicates (fully nested ANOVA, $P = 0.194$) nor among treatments (fully nested ANOVA, $P = 0.173$). Control colonies at deeper areas varied their zooxanthellae concentrations during the experiment, while those colonies at shallower areas showed a constant decline in zooxanthellae densities as summer approached.

DISCUSSION

Linear extension rates measured for *A. cervicornis* during the UV-E experiment increased significantly under a UV-depleted environment. Nonetheless, the results under both natural irradiance and UV-depleted treatments are similar to the earlier measurements of linear extension rates obtained for this species in the U.S. Virgin Islands along its depth range (57) and the Florida reef tract (58). In contrast, growth rates (*e.g.* linear extension rates and skeletal density) of *A. cervicornis* were negatively correlated with enhanced UVR daily doses in the present study. The *A. cervicornis* colonies transplanted from 20 to 1 m suffered a reduction in their skeletal linear extension rates by 66% during the experimental period compared to the control colonies at 20 m. This reduction was most likely mediated through a decrease in the possible photosynthates of the zooxanthellae as they suffered an almost immediate reduction in photosynthetic pigment content evidenced by the visually evident paling of these colonies and confirmed by HPLC analysis of their extracts. The results compare to those of Lesser (13) who found that UVR and high PAR irradiances promoted a reduction in the photosynthetic capacity of the symbiotic dinoflagellates of the Caribbean coral *Montastraea faveolata*. Furthermore, histological evidence suggests that these colonies may have survived by, among other ways, reabsorbing their reproductive structures as these were no longer seen following the transplantation and "bleaching" of the colonies (see 59; J.L. Torres, unpublished data).

Coral skeleton accretion can stop during a bleaching event (60). Results from *M. annularis* suggest that calcification, more than photosynthesis, is inhibited during bleaching events (61). A 63% reduction in mean annual growth in *M. annularis* after the mass bleaching event of 1987 in Florida was reported by Leder *et al.* (60). The significant reduction in linear extension rates and the increased fragility of the skeletons evidenced by the reduction in skeletal density after transplantation during the UV-S experiment to shallower waters are evidence of the detrimental effects that increased levels of UVR can cause to shallow-water scleractinian corals. The *A. cervicornis* colonies transplanted from 20 to 1 m underwent a significant reduction in the photosynthetic pigments of the symbionts resulting in a visible transient "bleaching" after just 1 day following the transplants. Hence, the changes in photosynthetic pigment concentrations are more likely due to a photoacclimation response by the corals caused by the increase in UVR and probably oxidative stress. This is further supported by the observation that only the sun-exposed sides of the colonies were pale while the bottom and shaded sides retained their coloration. Even severely bleached corals can retain at least 10% of their symbionts (62) and these can repopulate other areas within the colony later during recuperation. While there is a possibility of a recolonization of the pale branches by new zooxanthellae from the field, the fact that the lower sides of the branches did not bleach during the whole experiment is indicative of interpolyp translocation of zooxanthellae cells to repopulate the pale areas with new healthy cells.

The evidence here indicates that photoacclimatization (caused by enhanced UVR levels, and to a lesser degree by enhanced PAR), at least in the studied species, occurred mainly as a result of a decrease in the *Symbiodinium* photosynthetic pigments and not from an expulsion of the zooxanthellae cells. The reduction in photosynthetic pigment concentration indicates a possible defensive mechanism itself to reduce the chance of photodamage to the light-harvesting systems, especially PSII (see 63, 64), in the zooxanthellae cells.

Neither temperature nor PAR levels appear to be responsible for the responses attributed to increases in UVR. Temperatures were not significantly different between sites and PAR levels were only 9% higher at 1 m than those at 20 m. Whether small increases in PAR may influence the effects caused by two- to three-fold increases in UVR has been a matter of debate in the past (29). Gleason and Wellington (65) noted that the transplanted colonies, in their case of *Montastraea annularis*, were not living at the boundary of their visible light tolerance and that their studied species tolerates changes in PAR much more extreme than those introduced by their manipulations. Therefore, it seems highly improbable that such a small difference in PAR, compared to the greatly enhanced UVR, would have caused the results seen in their experiment. However, as no attempts were made to maintain PAR at pretransplant levels during the UV-S experiment, we

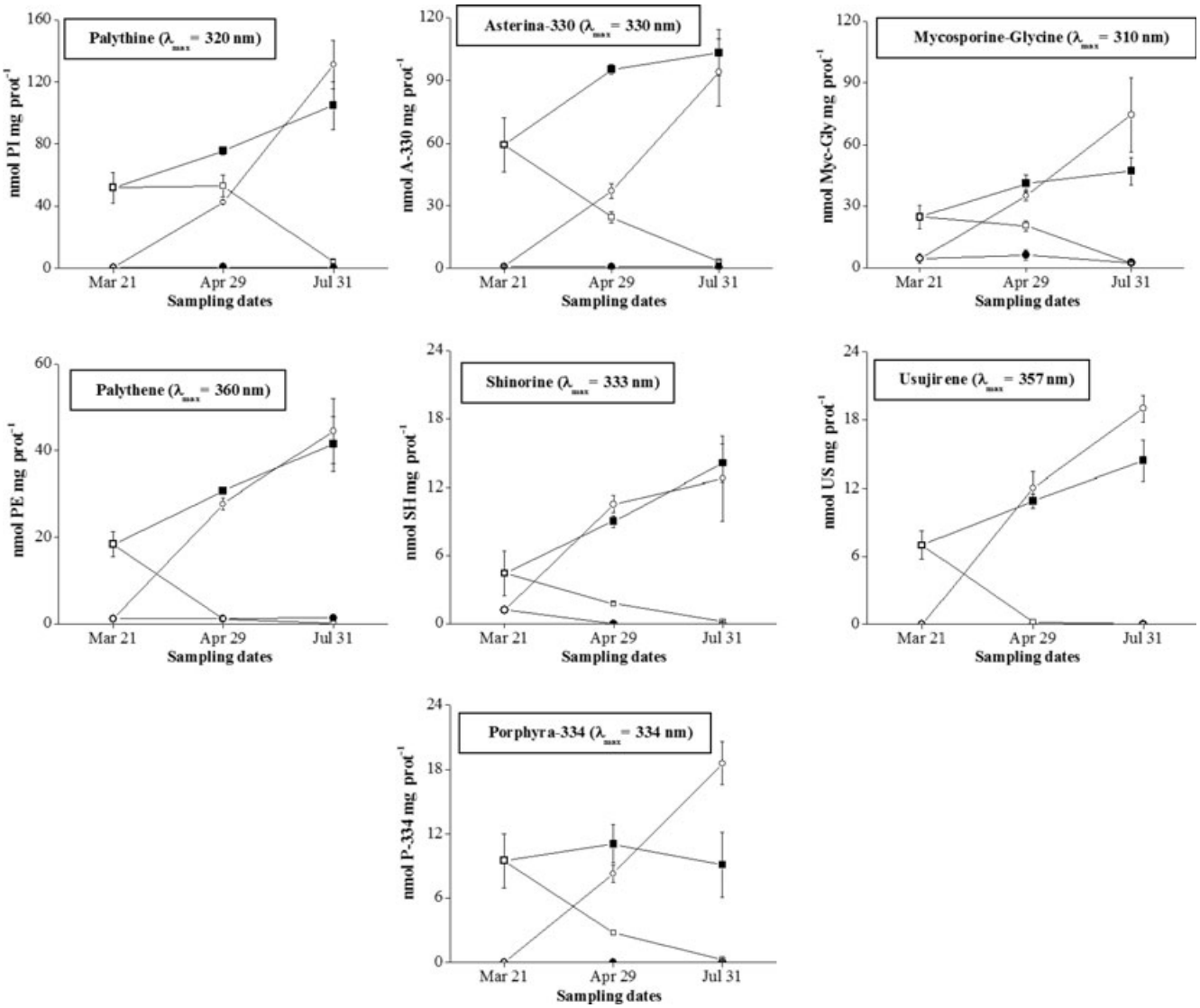

Figure 11. Individual concentration of the seven mycosporine-like amino acids (MAAs) identified in *Acropora cervicornis* as measured at the beginning, 1 month later, and at the end of the experimental period during the UV-S experiment. Black squares indicate average concentration of any given MAA in the control colonies at 1 m; open squares indicate average concentration of any given MAA in colonies transplanted from 1 to 20 m; black circles indicate average concentration of any given MAA in the control colonies at 20 m; open circles indicate average concentration of any given MAA in colonies transplanted from 20 to 1 m. Vertical lines represent ± 1 SE ($\alpha = 0.05$).

cannot completely eliminate any possible effects of the small increase in PAR on the coral responses.

UV-absorbing compounds (MAAs)

The significant 40-fold increase in total MAA concentration in *A. cervicornis* after 1 month of exposure to enhanced UVR levels and 88-fold increase by the end of the experimental period during the UV-S experiment is the largest increase in MAA concentrations documented in reef corals by increases in UVR levels either in the field or laboratory (but see 66). The significant increase in total MAAs in colonies transplanted from 20 to 1 m depth represents an immediate response to higher UVR levels for host and symbionts protection contrary to earlier results found in transplanted colonies of *M. annularis* in the Bahamas (29). Our findings are indicative that, at least in this species, the increase in MAAs is related to UVR protection. Similar increases have only been reported for the red alga *Chondrus crispus* (22). Our results support earlier findings of an inverse relationship between MAA concentration in four Hawaiian scleractinian corals (*M. verrucosa, M. patula, Pocillopora meandrina* and *Porites compressa*) with depth of occurrence and level of UVR (50).

In addition to their function as UV-protection, MAAs act as antioxidants (14) against reactive oxygen species (ROS), which formation is typically mediated by UV-related oxidative stress in the hyperoxic media of coral tissues (30,67). The extremely rapid and continuous increase in MAAs in those colonies transplanted from 20 to 1 m depth combined with the reduction in photosynthetic pigments, and possibly photosynthetic capacity of the zooxanthellae, suggests that the formation of these compounds can be attributable to both factors

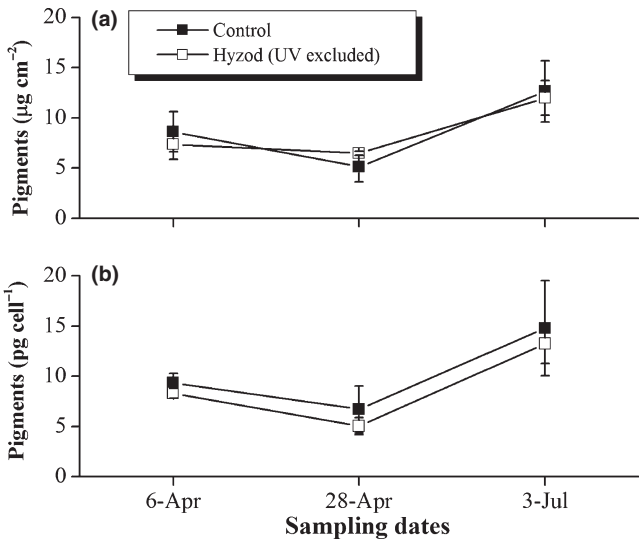

Figure 12. Total concentration of photosynthetic pigments (chlorophylls, carotenoids, and xanthophylls) normalized to (a) coral tissue area and (b) zooxanthellae density in *Acropora cervicornis* during the UV-E experiment. Vertical lines in both graphs represent ±1 SE ($\alpha = 0.05$).

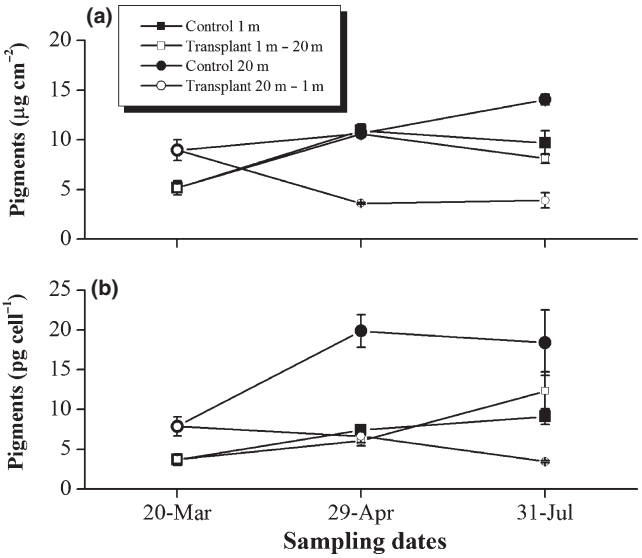

Figure 13. Total concentration of photosynthetic pigments (chlorophylls, carotenoids, and xanthophylls) normalized to (a) coral tissue area and (b) zooxanthellae density in *Acropora cervicornis* during the UV-S experiment. Vertical lines in both graphs represent ±1 SE ($\alpha = 0.05$).

(sudden increase in UVR and formation of ROS within the coral tissues).

It has been reported that high PAR levels can also induce the production of MAAs in corals (33) and marine diatoms (68). Colonies of *Montipora verrucosa* acclimated to ambient PAR and UVR showed higher levels of MAAs and lower zooxanthellae densities with no differences in chlorophyll *a* per host or symbiont cells, and Kinzie (69) suggested that the fact that these corals respond to increases in UVR levels by increasing their MAA concentrations indicates the active metabolism of these compounds whereas this expenditure of energy and resources was not incurred in low-UVR conditions. The results of the present study indicate that although UVR levels reaching deeper colonies (at 20 m) are minimal, the coral symbionts still produce some of these compounds, especially those that absorb in the UVB region (*i.e.* mycosporine-glycine, $\lambda_{max} = 310$ nm). Damaging UVR penetrates to at least 20 m depth in the clear insular shelf edge waters of Puerto Rico. PAR levels measured at those depths were slightly lower than those at 1 m. Hence, it is not surprising that colonies normally living at those depths, where small amounts of UVR and relatively high amounts of PAR still penetrate, may show at least a minimal level of UV-absorbing compounds.

The results obtained in the UV-E experiment present clear evidence that skeletal growth rates of the coral are significantly enhanced under an UVR-depleted environment despite the small decrease in PAR. Furthermore, no significant changes were seen in the concentration of photosynthetic pigments and zooxanthellae densities between UVR-shielded and unshielded colonies in the UV-E experiment. This result is comparable to that of Gleason and Wellington (29). The fact that MAA concentrations were significantly reduced in the UVR-depleted treatment probably contributed to the enhanced skeletal growth rates of the coral as there was less energy and resources spent in the protection of the host and symbionts.

Considering that coral reef calcification is predicted to decrease 20–60% by 2100, relative to preindustrial levels (70–75), and that increased UVR may induce the formation of less dense skeletons with reduced linear extension rates mediated through a reduction in the photosynthetic capacity of the host symbionts, the scenario for reef corals, especially for this threatened species, may be uncertain. This, added to the current status (threatened) of Caribbean acroporids (31; 70CFR24359), presents an alarming scenario for the future.

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REFERENCES

1. Jerlov, N. G. (1950) Ultraviolet radiation in the sea. *Nature* **166**, 111.
2. Smith, R. C. and K. S. Baker (1979) Penetration of UV-B and biologically effective dose-rates in natural waters. *Photochem. Photobiol.* **29**, 311–323.
3. Jokiel, P. L. (1980) Solar ultraviolet radiation and coral reef epifauna. *Science* **207**, 1069–1071.
4. Fleischmann, E. M. (1989) The measurement and penetration of ultraviolet radiation into tropical marine water. *Limnol. Oceanogr.* **34**(8), 1623–1629.
5. Madronich, S., R. L. McKenzie, L. O. Björn and M. M. Caldwell (1995) Changes in ultraviolet radiation reaching the Earth's surface. *Ambio* **24**, 143–152.

6. Booth, C. R. and J. H. Morrow (1997) The penetration of UV into natural waters. *Photochem. Photobiol.* **65**, 254–275.
7. Kirk, J. T. O. (1994) *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge University Press, New York.
8. Baker, K., R. C. Smith and A. F. S. Green (1980) Middle ultraviolet radiation reaching the ocean surface. *Photochem. Photobiol.* **32**, 367–374.
9. Cutchis, P. (1982) A formula for comparing annual damaging ultraviolet (DUV) radiation doses at tropical and mid-latitude sites. In *The Role of Solar Ultraviolet Radiation in Marine Ecosystems* (Edited by J. Calkins), pp. 213–228. Plenum Press, New York.
10. Frederick, J. E., H. E. Snell and E. K. Haywood (1989) Solar ultraviolet radiation at the Earth's surface. *Photochem. Photobiol.* **50**, 443–450.
11. Shibata, K. (1969) Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant Cell Physiol.* **10**, 325–335.
12. Shick, J. M., S. Romaine-Lioud, C. Ferrier-Pagès and J.-P. Gattuso (1999) Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. *Limnol. Oceanogr.* **44**(7), 1667–1682.
13. Lesser, M. P. (2000) Depth-dependent photoacclimatization to solar ultraviolet radiation in the Caribbean coral *Montastraea faveolata*. *Mar. Ecol. Prog. Ser.* **192**, 137–151.
14. Dunlap, W. C. and Y. Yamamoto (1995) Small-molecule antioxidants in marine organisms: Antioxidant activity of mycosporine-glycine. *Comp. Biochem. Physiol.* **112B**, 105–114.
15. Banaszak, A. T., M. P. Lesser, I. B. Kuffner and M. Ondrusek (1998) Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAS) in marine organisms. *Bull. Mar. Sci.* **63**(3), 617–628.
16. Dunlap, W. C. and J. M. Shick (1998) Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: A biochemical and environmental perspective. *J. Phycol.* **34**, 418–430.
17. Cockell, C. S. and J. Knowland (1999) Ultraviolet radiation screening compounds. *Biol. Rev.* **74**, 311–345.
18. Shick, J. M. and W. C. Dunlap (2002) Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.* **64**, 223–262.
19. Carreto, J. I., M. O. Carignan and N. G. Montoya (2005) A high-resolution reverse-phase liquid chromatography method for the analysis of mycosporine-like amino acids (MAAs) in marine organisms. *Mar. Biol.* **146**, 237–252.
20. Shick, J. M., M. P. Lesser and P. L. Jokiel (1996) Effects of ultraviolet radiation on corals and other coral reef organisms. *Global Change Biol.* **2**, 527–545.
21. Peak, M. J. and J. G. Peak (1990) Hydroxyl radical quenching agents protect against DNA breakage caused by both 365-nm and gamma radiation. *Photochem. Photobiol.* **51**, 649–652.
22. Karsten, U., L. A. Franklin, K. Lüning and C. Wiencke (1998) Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga *Chondrus crispus* (Rhodophyta). *Planta* **205**, 257–262.
23. Franklin, L. A., I. Yakovleva, U. Karsten and K. Lüning (1999) Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. *J. Phycol.* **35**, 682–693.
24. Coles, S. L. and P. L. Jokiel (1978) Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa* (Lamarck). *Mar. Biol.* **49**, 187–195.
25. Jokiel, P. L. and R. H. York (1982) Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. *Bull. Mar. Sci.* **32**(1), 301–315.
26. Jokiel, P. L. and R. H. York (1984) Importance of ultraviolet radiation in photoinhibition of microalgal growth. *Limnol. Oceanogr.* **29**, 192–199.
27. Siebeck, O. (1988) Experimental investigation of UV tolerance in hermatypic corals (Scleractinia). *Mar. Ecol. Prog. Ser.* **43**, 95–103.
28. Lesser, M. P. and J. M. Shick (1989) Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: Primary production, photoinhibition, and enzymatic defenses against oxygen toxicity. *Mar. Biol.* **102**, 243–255.
29. Gleason, D. F. and G. M. Wellington (1993) Ultraviolet radiation and coral bleaching. *Nature* **365**, 836–838.
30. Shick, J. M., M. P. Lesser, W. C. Dunlap, W. R. Stochaj, B. E. Chalker and J. Wu Won (1995) Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. *Mar. Biol.* **122**, 41–51.
31. Acropora Review Team (2005) Atlantic *Acropora* status review document. Report to National Marine Fisheries Service (NOAA). Southeast Regional Office. St. Petersburg, FL. 152pp.
32. Orce, V. L. and E. W. Helbling (1997) Latitudinal UVR-PAR measurements in Argentina: Extent of the ozone hole. *Global Planet Change* **15**, 113–121.
33. Oliver, J. K. (1984) Intra-colony variation in the growth of *Acropora formosa*: Extension rates and skeletal structure of white (zooxanthellae-free) and brown-tipped branches. *Coral Reefs* **3**, 139–147.
34. Jokiel, P. L., M. P. Leser and M. E. Ondrusek (1997) UV-absorbing compounds in the coral *Pocillopora damicornis*: Interactive effects of UV radiation, photosynthetically active radiation, and water flow. *Limnol. Oceanogr.* **42**(6), 1468–1473.
35. Chalker, B. E. and W. C. Dunlap (1981) Extraction and quantitation of endosymbiotic algal pigments from reef-building corals. *Proc. 4th Int. Coral Reef Symp.*, Manila **2**, 45–50.
36. Dunlap, W. C. and B. E. Chalker (1986) Identification and quantification of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. *Coral Reefs* **5**, 155–159.
37. Muszynski, F. Z., A. Bruckner, R. A. Armstrong, J. M. Morell and J. E. Corredor (1998) Within-colony variations of UV absorption in a reef building coral. *Bull. Mar. Sci.* **63**(3), 589–594.
38. Corredor, J. E., A. W. Bruckner, F. Z. Muszynski, R. A. Armstrong, R. García and J. M. Morell (2000) UV-absorbing compounds in three species of Caribbean zooxanthellate corals: Depth distribution and spectral response. *Bull. Mar. Sci.* **67**(2), 821–830.
39. Gleason, D. F. (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreoides*. *Limnol. Oceanogr.* **38**(7), 1452–1463.
40. Ito, S. and Y. Hirata (1977) Isolation and structure of a mycosporine from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* **28**, 2429–2430.
41. Takano, S., D. Uemura and Y. Hirata (1978a) Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* **26**, 2299–2300.
42. Takano, S., A. Nakanishi, D. Uemura and Y. Hirata (1979) Isolation and structure of a 334 nm UV-absorbing substance, porphyra-334 from the red alga *Porphyra tenera* Kjellman. *Chem. Lett.* **7**, 419–420.
43. Tsujino, I., K. Yabe and I. Sekikawa (1980) Isolation and structure of a new amino acid, shinorine from the red alga, *Chondrus yendoi* Yamada et Mikami. *Bot. Mar.* **23**, 65–68.
44. Takano, S., D. Uemura and Y. Hirata (1978b) Isolation and structure of two new amino acids, palythinol and palythene, from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* **49**, 4909–4912.
45. Banaszak, A. T. and R. K. Trench (1995) Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response to exposure to UV in Anthopleura elegantissima and Cassiopeia xamachana. *J. Exp. Mar. Biol. Ecol.* **194**, 233–250.
46. Wright, S. W., S. W. Jeffrey, R. F. C. Mantoura, C. A. Llewellyn, T. Bjørnland, D. Repeta and N. Welschmeyer (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.* **77**, 183–196.
47. Bidigare, R. R. (1991) Analysis of algal chlorophylls and carotenoids. In *Marine Particles: Analysis and Characterization* (Edited by D. C. Hurd and D. W. Spencer), pp. 119–123. Geophysical Monograph 63. American Geophysical Union, Washington, DC.
48. Jeffrey, S. W., R. F. C. Mantoura and S. W. Wright (1997) *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*. UNESCO Publishing, Paris.

49. Marsh, J. A. (1970) Primary productivity of reef building calcareous red algae. *Ecology* **51**, 255–263.
50. Jones, R. J. (1997) Zooxanthellae loss as a bioassay for assessing stress in corals. *Mar. Ecol. Prog. Ser.* **149**, 163–171.
51. Wilkerson, F. P., D. Kobayashi and L. Muscatine (1988) Mitotic index and size of symbiotic algae in Caribbean reef corals. *Coral Reefs* **7**, 29–36.
52. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.
53. Ott, R. L. and M. Longnecker (2001) *An Introduction to Statistical Methods and Data Analysis*. Duxbury, California.
54. Dieppa-Ayala, A. (1996) *Bio-optical Variability in Tropical Oceanic Waters*. MS thesis, University of Puerto Rico. 90pp.
55. Detrés, Y., R. A. Armstrong and X. M. Connelly (2001) Ultraviolet-induced responses in two species of climax tropical marine macrophytes. *J. Photochem. Photobiol. B, Biol.* **62**, 55–66.
56. García-Urueña, R. (2004) *Dinámica de Crecimiento de tres especies de coral en relación a las propiedades ópticas del agua*. Ph.D. thesis, University of Puerto Rico. 151pp.
57. Gladfelter, E. H., R. K. Monahan and W. B. Gladfelter (1978) Growth rates of five reef-building corals in the northeastern Caribbean. *Bull. Mar. Sci.* **28**(4), 728–734.
58. Shinn, E. A. (1966) Coral growth rate, an environmental indicator. *J. Paleontol.* **40**, 233–240.
59. Szmant, A. M. and N. J. Gassman (1990) The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastraea annularis*. *Coral Reefs*. **8**, 217–224.
60. Leder, J. J., A. M. Szmant and P. K. Swart (1991) The effect of prolonged "bleaching" on skeletal banding and stable isotopic composition in *Montastraea annularis*. *Coral Reefs* **10**, 19–27.
61. Goreau, T. J. and A. H. Macfarlane (1990) Reduced growth rate of *Montastraea annularis* following the 1987–1988 coral-bleaching event. *Coral Reefs* **8**, 211–215.
62. Fitt, W. K., F. K. McFarland, M. E. Warner and G. C. Chilcoat (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol. Oceanogr.* **45**(3), 677–685.
63. Warner, M. E., W. K. Fitt and G. W. Schmidt (1999) Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *Proc. Natl Acad. Sci. USA* **96**, 8007–8012.
64. Warner, M. E., G. C. Chilcoat, F. K. McFarland and W. K. Fitt (2002) Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Montastraea*. *Mar. Biol.* **141**, 31–38.
65. Gleason, D. F. and G. M. Wellington (1994) Ultraviolet radiation and coral bleaching: Reply to Dunne. *Nature* **368**, 697.
66. Yakovleva, I. and M. Hidaka (2004) Diel fluctuations of mycosporine-like amino acids in shallow-water scleractinian corals. *Mar. Biol.* **145**, 863–873.
67. Lesser, M. P. (1996) Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnol. Oceanogr.* **41**(2), 271–283.
68. Helbling, E. W., B. E. Chalker, W. C. Dunlap, O. Holm-Hansen and V. E. Villafañe (1996) Photoacclimation of Antarctic marine diatoms to solar ultraviolet radiation. *J. Exp. Mar. Biol. Ecol.* **204**, 85–101.
69. Kinzie, R. A. III (1993) Effects of ambient levels of solar ultraviolet radiation on zooxanthellae and photosynthesis of the reef coral *Montipora verrucosa*. *Mar. Biol.* **116**, 319–327.
70. Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R. M. Key, K. Lindsay, E. Maier-Reimer, R. Matear, P. Monfray, A. Mouchet, R. G. Najjar, G. K. Plattner, K. B. Rodgers, C. L. Sabine, J. L. Sarmiento, R. Schlitzer, R. D. Slater, I. J. Totterdell, M. F. Weirig, Y. Yamanaka and A. Yool (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681–686.
71. Pelejero, C., E. Calvo, M. T. McCulloch, J. F. Marshall, M. K. Gagan, J. M. Lough and B. N. Opdyke (2005) Preindustrial to modern interdecadal variability in coral reef pH. *Science* **309**, 2204–2207.
72. Kleypas, J. A., R. W. Buddemeier, D. Archer, J-P. Gattuso, C. Langdon and B. N. Opdyke (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **284**, 118–120.
73. Marubini, F., C. Ferrier-Pages and J. P. Cuif (2003) Suppression of skeletal growth in scleractinian corals by decreasing ambient carbonate-ion concentration: A cross-family comparison. *Proc. R. Soc. Lond., B, Biol. Sci.* **270**, 179–184.
74. Reynaud, S., N. Leclercq, S. Romaine-Lioud, C. Ferrier-Pages, J. Jaubert and J-P. Gattuso (2003) Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biol.* **9**, 1660–1668.
75. Müller, A., M. K. Gagan and J. M. Lough (2004) Effect of early marine diagenesis on coral reconstructions of surface-ocean $^{13}C/^{12}C$ and carbonate saturation state. *Global Biogeochem. Cycles* **18**, GB1033. DOI:10.1029/2003GB002112.