

## Photosynthesis and light utilization in the Caribbean coral *Montastraea faveolata* recovering from a bleaching event

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### Abstract

Coral bleaching—the loss of symbiotic dinoflagellates—is initiated when corals are exposed to sea surface temperatures above the regional summer average and has been responsible for massive coral mortality episodes. The ability of symbionts to recolonize the host after bleaching may be critical in determining if a colony will recover or experience mortality. Here, following the serendipitous bleaching of specimens of *Montastraea faveolata* of known photosynthetic, spectroscopic, and genetic characteristics, we describe changes in photosynthesis and light utilization during the recovery process. Fully recovered *M. faveolata* had minimum quantum requirements ( $1/\Phi_{\max}$ ) that are very close to the theoretical minimum, indicating that symbiotic corals are not only one of the most efficient light collectors in nature, but also use this energy with maximum efficiency. Analyses of the photosynthetic responses of *M. faveolata* throughout the recovery process indicate that during the early stages, the symbiont population exhibited characteristics consistent with acclimation to higher irradiance relative to fully recovered corals. The absorption spectra of bleached samples showed contributions of chlorophyll *b* (Chl *b*) associated with a biomass increase of the endolithic algae. The propagation of endolithic algae after bleaching may provide partial protection to the surviving symbionts from excessive radiation by reducing the reflectivity of the skeleton. Changes in the relative abundances of different symbiotic algae between recovered and unbleached colonies did not result in significant variations in photosynthetic and light utilization characteristics.

Symbioses between phototrophic dinoflagellates (zooxanthellae) and invertebrates play a very significant role in coastal tropical marine ecosystems. In coral reefs, symbiotic scleractinian corals are among the most important primary producers and are responsible for the formation and maintenance of the reef structure (Muscatine and Weis 1992). The success of scleractinians as reef-builders can be attributed to the energetic advantages of harboring photosynthetic dinoflagellates of the genus *Symbiodinium*. On one hand, the translocation of photosynthates and its utilization by the coral host provides, in some instances, more than 100% of the basal metabolic requirements of the intact association (Muscatine and Weis 1992). On the other

hand, there is considerable evidence linking the photosynthetic activity of the symbionts with the high calcification rates characteristic of reef-building corals (Goreau and Goreau 1959). In this context, the efficient harvesting and use of solar energy by the symbiotic dinoflagellates is essential not only for the survival of individual corals, but for the construction and maintenance of the reef itself. The importance of documenting the capacity of corals for efficient light utilization has long been recognized (Dubinsky et al. 1984). Unfortunately, because of the technical difficulties in assessing the spectroscopic properties of intact coral surfaces, most of the assessments of the maximum quantum yields of photosynthesis in corals are not consistent with the expected high efficiency in light utilization (Enriquez et al. 2005). This information is critical to understanding the abilities of different coral species to colonize the entire photic zone (Falkowski et al. 1990).

Chlorophyll *a* (Chl *a*) densities are highly variable among coral species and are dependent on the prevalent environmental conditions. For example, in the course of photoacclimation, pigment densities in some coral species may experience up to five-fold changes (Falkowski and Dubinsky 1981; Porter et al. 1984; Dubinsky et al. 1990). These changes result from variations in pigment content

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### Acknowledgments

We thank two anonymous reviewers for their helpful criticism and A. Banaszak for her editorial assistance.

This research was supported by a Dirección General de Asuntos del Personal Académico de la Universidad Nacional Autónoma de México (DGAPA-UNAM) grant (IN218599) to S.E. and a grant (32517-T) from the Consejo Nacional de Ciencia y Tecnología (CONACYT) to R.I.-P. A. R.-P. was supported by a pre-doctoral fellowship from CONACYT (95358).

per cell without major modifications in cell densities. In contrast, corals exposed to elevated nutrient concentrations experience similar increases in Chl *a* density, but mainly because of symbiont proliferation (Muscatine et al. 1989; Dubinsky et al. 1990). Recently, variations in Chl *a* density resulting from seasonal variations in symbiont densities have been documented (Fitt et al. 2000).

Probably the most dramatic decreases in photosynthetic pigment density experienced by corals results from the phenomenon known as coral bleaching. Coral bleaching is characterized by the loss of pigmentation by either the loss of symbionts and/or reductions in the cellular concentrations of photosynthetic pigments (Hoegh-Guldberg 1999). Although this phenomenon occurs when corals are exposed to several environmental extremes, massive bleaching episodes are positively correlated with elevated sea surface temperatures above the regional summer average (Hoegh-Guldberg 1999). Depending on the severity of the thermal stress, coral bleaching may be a reversible phenomenon or may result in massive coral mortality episodes with catastrophic ecological consequences (Hoegh-Guldberg 1999). Although there is controversy regarding the actual site of thermal damage (Warner et al. 1999; Tchernov et al. 2004; Smith et al. 2005), coral bleaching appears to be initiated by the temperature-dependent inactivation of the photosynthetic function of the symbionts (Iglesias-Prieto et al. 1992; Jones et al. 1998). Once photosynthesis is impaired, light plays a major role in modulating the disruption of the symbiosis (Hoegh-Guldberg 1999). Under thermal stress, the excitation energy collected by the light harvesting complexes cannot be stored photochemically and may increase the formation rate of free radicals, which further damage the photosynthetic apparatus of the symbionts and surrounding animal tissues (Lesser 1996).

The genus *Symbiodinium* is a diverse assemblage of several distantly related subgeneric clades, each consisting of an unknown number of types or species (Rowan 1991; Lajuenesse 2004). Some coral species form symbiosis with one particular alga, whereas others associate simultaneously with more than one algal type (Rowan 1991). *Montastraea faveolata*, one of the most abundant reef-building corals in the Caribbean, possesses polymorphic populations of *Symbiodinium* (Rowan and Knowlton 1995). Although changes in the relative abundances of each algal taxon in *M. faveolata* have been correlated with irradiance and stress gradients, the physiological traits of each different combination have not been directly characterized (Rowan and Knowlton 1995; Rowan et al. 1997; Toller et al. 2001). It has been postulated that coral bleaching can promote a rapid response to environmental change by enabling corals to modify their symbiont populations (Buddemeier and Fautin 1993). The abilities of different symbionts to recolonize the host after bleaching may be critical in determining if a particular colony will be able to recover or experience mortality. Despite its importance, the physiological process of recovery from coral bleaching has received very little attention (Jones et al. 2000; Nakamura et al. 2003).

Here, following the serendipitous bleaching of several specimens of *M. faveolata* of known photosynthetic and

spectroscopic characteristics as well as symbiont identity, we describe in detail changes in photosynthesis, light absorption, and utilization during the process of recovery.

## Material and methods

*Coral collection and maintenance*—As part of an ongoing research project, fragments of three individual colonies of the Caribbean scleractinian *M. faveolata* were collected with a hammer and chisel from the back-reef in Puerto Morelos, Quintana Roo, Mexico (5.0-m depth). The samples were transported immediately to the laboratory, where each of the three individual samples were fragmented into eight nubbins of approximately 3-cm diameter and placed in polyvinyl chloride bases. The samples were placed in a 600-liter running seawater aquarium screened with a netting layer to remove 50% of the natural illumination and an ultraviolet (UV)-opaque acrylic sheet. Water motion was maintained in the aquarium with 21 water jets generated by a 370 watts (1/2 horsepower) pump. A constant flux of 13 liters of seawater per minute, equivalent to 31 aquarium volumes per day, was maintained throughout the experiment. The constant replacement of seawater resulted in synchronous temperature oscillations between the aquarium and the reef lagoon adjacent to the laboratory. Nubbins were maintained in the aquarium for 5 months as part of a different experiment. During this period, three replicates of each colony were used as experimental subjects, and their spectroscopic and photosynthetic traits were routinely assayed; another five fragments from each colony were maintained as backup material.

*Coral bleaching and recovery conditions*—On 12 September 2004, Hurricane *Ivan* threatened the coast of Quintana Roo. In preparation for the contingency, the netting and UV shield were removed, and the flow of seawater into the aquarium and the internal water circulation system were suspended. The netting and the UV shield were replaced on 16 September, and the laboratory seawater system was reestablished on 18 September. Immediately after the hurricane contingency, all of the nubbins presented obvious discoloration. Although the internal conditions in the aquarium during this period were not measured, corals in the aquarium experienced dramatic increases in photosynthetically active radiation (PAR), UV dose, and temperature, as well as stagnant water for 3 days. All of these factors individually and in synergy are known to produce stressful conditions in corals, leading to coral bleaching (Warner et al. 1999; Nakamura and Van Woesik 2001). Previous tests indicate that the suppression of water flow and the removal of the PAR and UV screens resulted in increases of up to 4°C in the average temperature of the tank relative to the adjacent lagoon water (Winters and Iglesias-Prieto, unpubl. data). On 22 September 2004, once the stressful conditions in the experimental tank were removed, the documentation of the recovery processes of the bleached *M. faveolata* samples was initiated.

*Reflectance spectra of corals and absorption estimates*—Reflectance spectra of each of the original nine experimen-

tal nubbins were determined once a week during recovery from bleaching. The spectra were obtained between 400 and 750 nm with 1-nm resolution using a 4800S Lifetime spectrofluorometer (SLM-Aminco) equipped with a red-sensitive photomultiplier tube (R955, Hamamatsu). Samples were submersed in seawater in a small glass container with a black bottom. Homogeneous illumination was provided by an incandescent light source placed  $\approx 25$  cm above the coral surface at a  $45^\circ$  angle. The reflected light was collected with a 2-mm-diameter waveguide attached to the spectrometer. The detector waveguide was placed underwater, 1 cm away from the sample at an angle of  $45^\circ$  to the coral surface. The field of view of the detector waveguide was  $\approx 0.4$  cm<sup>2</sup>. Reflectance was expressed as the ratio of the radiance measured from the coral surface relative to the radiance obtained from a reference white-diffusing surface. Coral absorbance was calculated from reflectance spectra, assuming that transmission through the thick skeleton of the specimens is negligible, using the following expression

$$D_e = \log(1/R) \quad (1)$$

where  $D_e$  represents the estimated absorbance and  $R$  the measured reflectance. This approximation is based on the high reflectivity of the aragonite coral skeletons. Clean coral skeletons have reflectance in the red and near infrared close to 96% of the incident light (Enríquez et al. 2005). Comparison of the absorbance estimated from the reflectance spectra and those measured directly in transmission mode are in close agreement, particularly in the red part of the spectrum (Enríquez et al. 2005). The estimated absorption spectra were corrected by subtracting the apparent  $D_e$  at 750 nm to minimize the effect of the small amount of radiation transmitted through the skeleton.

**Pigment analyses**—Considering the small number of samples available and to conserve the nine original nubbins throughout the entire recovery process, destructive procedures were practiced in only a limited number of samples. For pigment analyses, 5 backup nubbins with different colorations and a sample collected directly from the reef were selected. Symbiotic dinoflagellates were isolated from coral fragments using a recirculating WaterPik (Teledyne). Coral slurries were centrifuged at  $400 \times g$  for 3 minutes in a clinical centrifuge. Pellets containing the algae were resuspended in 15 mL of filtered seawater in a tissue homogenizer and concentrated by centrifugation. Photosynthetic pigments were extracted in acetone, and the concentration of Chl *a* was determined spectroscopically (Jeffrey and Humphrey 1975).

**Estimation of the Chl *a* specific absorption coefficient**—The in vivo Chl *a* specific absorption coefficient  $a_{chl\ a}^*$  was calculated by the relation  $a_{chl\ a}^* = 1n10De/\rho$ , where  $\rho$  is the pigment content per projected surface area (in mg m<sup>-2</sup>) (Enríquez et al. 2005), and  $D_e$  represents the estimated absorbance as calculated from the reflectance spectra using Eq. 1. The specific absorption coefficient was calculated

only for the red Chl *a* absorption band at 675 nm. At this wavelength the reflectance of the skeleton is close to one, without interference from the absorption of accessory algal and animal pigments.

**Photosynthesis versus irradiance measurements**—The photosynthetic versus irradiance curves (P–E) of the nine experimental nubbins were determined weekly with a laboratory-made respirometer. The instrument consists of three independent water-jacketed chambers equipped with Clark-type O<sub>2</sub> electrodes (Hansatech). Three individually controlled magnetic stirrers produced agitation in each chamber. Temperature within the chambers was maintained at 27°C with an external recirculating water bath (Fisher Scientific). This temperature was chosen to compare changes in the photosynthetic performance during recovery from bleaching with data collected previously on the same samples. Light was provided by halogen lamps placed 15 cm above the chambers. The maximum output of the lamps was attenuated by combinations of different neutral density filters made of glass covered with black plastic mesh. Light intensities in the interior of the chambers were determined with the light sensor of a Diving PAM (Waltz) previously calibrated against a cosine-corrected sensor (Li-Cor). Corals were exposed sequentially to 0, 6, 20, 40, 51, 96, 604, and 1,392  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ . Dark respiration was obtained by averaging the values obtained at the beginning and at the end of the incubation. The electrodes were calibrated with air- and N<sub>2</sub>-saturated filtered seawater. Oxygen tension within the incubation chambers was maintained between 20% and 60% saturation by bubbling with N<sub>2</sub> gas. Data were captured with a computer equipped with an analog/digital converter. The P–E curve parameters were obtained by a nonlinear fitting to a hyperbolic tangent function. Quantum yields for oxygen evolution were calculated from the initial slope of the P–E curve and the absorptance (fraction of incident light captured by the intact coral) of each individual nubbin. Absorptance was estimated as  $1-R$ , considering the high reflectivity of the skeleton and assuming that transmittance through the thick skeleton was negligible.

**Genetic analyses**—To document possible changes in the composition of the symbiont populations during the recovery process, the identity of the symbionts was determined at various times during recovery using the nuclear small subunit ribosomal RNA (ssrDNA) genes as markers. Algal cells were isolated from coral tissue by a flow of pressurized filtered seawater with 5 mmol L<sup>-1</sup> ethylene diamine triacetic acid. Algal cells were ground in liquid nitrogen and resuspended in 1 mL of DNA extraction buffer to isolate DNA (Rowan 1991). Nuclear ssrDNA genes were amplified using zooxanthellae specific primers, and their restriction fragment length polymorphism (RFLP) patterns after *Taq* I digestion were compared with standards obtained from cultured algae of known clade type (Lajeunesse and Trench 2000).

Table 1. *M. faveolata* range of variation of Chl *a* density, estimated absorption at the Chl *a* absorption peak in the red ( $D_e$ ), and Chl *a* specific absorption coefficients of the coral nubbins used for spectroscopic calibration ( $a_{chl\ a}^*$ ). Samples 1–3 were collected during the first week of October 2004, whereas samples 4 and 5 were collected in early November 2004. Sample 6 is a coral collected from the reef at 5.0 m in December 2004.

Sample	Chl <i>a</i> density (mg m <sup>-2</sup> )	$D_e$ log (1/ $R_{675}$ )	$a_{chl\ a}^*$ (m <sup>2</sup> mg <sup>-1</sup> Chl <i>a</i> )
1	5.4	0.23	0.099
2	9.5	0.35	0.086
3	16.7	0.58	0.081
4	20.5	0.84	0.096
5	32.4	1.22	0.088
6	77.3	0.99	0.030

## Results

**Spectroscopic properties**—The combined use of spectroscopic, physiological, and genetic tools enabled the estimation of changes in the patterns of light utilization and photosynthesis of *M. faveolata* throughout the process of recovery from bleaching. We used reflectance spectra to reconstruct the absorption properties of intact corals. The six samples used to validate this approach presented a 14-fold variation in Chl *a* density ranging from 5.4 mg Chl *a* m<sup>-2</sup> to 77.3 mg Chl *a* m<sup>-2</sup> (Table 1). Chl *a* density presented a linear correlation with the estimated absorption ( $r^2 = 0.987$ ,  $p < 0.01$ ) in the Chl *a* density range displayed by the nubbins in the experimental tank (5.4–32.5 mg Chl *a* m<sup>-2</sup>). The  $a_{chl\ a}^*$  of the symbionts from *M. faveolata* at different recovery stages showed very little variation, with values close to 0.09 m<sup>2</sup> mg<sup>-1</sup> Chl *a*, the only exception being the most pigmented coral collected from the reef with  $a_{chl\ a}^*$  values of 0.03 m<sup>2</sup> mg<sup>-1</sup> Chl *a* (Table 1).

The reflectance spectra of corals taken at different times during the recovery process show dramatic increases in pigmentation. The average reflectance in the PAR region (400–700 nm) of bleached corals was  $0.47 \pm 0.07$ , whereas the reflectance of recovered specimens acclimatized to the light conditions prevalent in the aquarium was  $0.23 \pm 0.06$  (Fig. 1A). Reconstruction of the absorption spectra from the reflectance spectra showed consistent results. Bleached *M. faveolata* nubbins have absorption at the Chl *a* maximum in the red part of the spectrum at 675 nm of  $0.36 \pm 0.05$ , whereas recovered specimens presented values of  $0.76 \pm 0.02$  ( $n = 9$ ), insignificantly different from those obtained from the same nubbins before bleaching ( $0.74 \pm 0.02$ ) (Figs. 1B, 2A). The reconstructed absorption spectra of the coral surfaces show some spectral differences during the recovery process. Analyses of the absorption in the red part of the spectrum indicate a clear contribution from chlorophyll *b* (Chl *b*), resulting from the reduction in the population density of symbiotic algae in bleached specimen (Fig. 1B,C). As expected, the absorption properties of coral surfaces during recovery are progressively dominated by dinoflagellates. Derivative analysis of the absorption spectra indicates the presence, only in bleached specimens, of an absorption band consistent with Chl *b*, with

a maximum around 650 nm (Fig. 1C). Difference spectra of bleached and recovered samples indicate the presence of a Chl *a* long-wavelength absorption band characteristic of the antenna of the endolithic algae *Ostreobium* sp. (data not shown) (Koehne et al. 1999). The propagation of endolithic algae in bleached specimens resulted in dramatic changes in the spectroscopic properties of the skeleton (Fig. 1D). Comparison of the reflectance spectra of skeletons cleaned with pressurized water indicate that unbleached corals have a reflectance of approximately 0.95 at 675 nm, whereas a coral recovering from bleaching and possessing a large population of endolithic algae has a reflectance at the same wavelength of 0.26 (Fig. 1D). The recovery of coral pigmentation showed a lag phase during the initial 3 weeks in which we did not detect any significant increments in the light absorption capacity of the colonies. The absorption in the Chl *a* maximum at 675 nm recovered its original values after 35 days (Fig. 3A).

**Photosynthetic responses**—Bleached *M. faveolata* nubbins presented a 4.6-fold reduction in the initial slope of the P–E curve ( $\alpha$ ) relative to values observed in the same samples before exposure to the stressful conditions ( $0.0123 \pm 0.002$  and  $0.056 \pm 0.002$  mol O<sub>2</sub> mol quanta<sup>-1</sup>, respectively). The kinetics of the recovery showed a significant increase after only 7 days, although full recovery required 5 weeks. A completely different pattern was observed for the recovery of the maximum photosynthetic capacity ( $P_{max}$ ). As a result of coral bleaching,  $P_{max}$  in *M. faveolata* nubbins experienced a 4.8-fold reduction relative to the original values ( $2.50 \pm 0.50$  and  $12.16 \pm 1.05$   $\mu$ mol O<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, respectively). This parameter showed values statistically indistinguishable from those obtained before bleaching after recovering for 21 days, remaining at a relatively constant level for the rest of the experiment (Fig. 3C). The minimum quantum requirements ( $1/\Phi_{max}$ ) for O<sub>2</sub> evolution of the symbiotic dinoflagellates inhabiting *M. faveolata* show a dramatic increase as a result of bleaching. Samples analyzed shortly after bleaching showed  $1/\Phi_{max}$  of  $38.1 \pm 7.3$  quanta absorbed per O<sub>2</sub> evolved, whereas samples before the stress exhibited values of  $15.4 \pm 2.3$  quanta absorbed per O<sub>2</sub> evolved. Recovery of  $1/\Phi_{max}$  occurred without any lag phase, reaching values indistinguishable from the originals after 5 weeks (Fig. 3D).

**Symbiont identification**—Analyses of the RFLP patterns of the amplified nuclear *ssrDNA* genes indicate that although the *Symbiodinium* populations inhabiting the three original colonies of *M. faveolata* were polymorphic with algae in clades A and C, clade C symbionts appear to be dominant (Fig. 3). After bleaching, the symbiont populations in one colony were dominated by algae in clade D, whereas the algal populations of the two other colonies appear to have higher relative abundances of clade A *Symbiodinium*.

## Discussion

**Spectroscopic properties**—The results presented in this study illustrate the potential of using reflectance spectra to

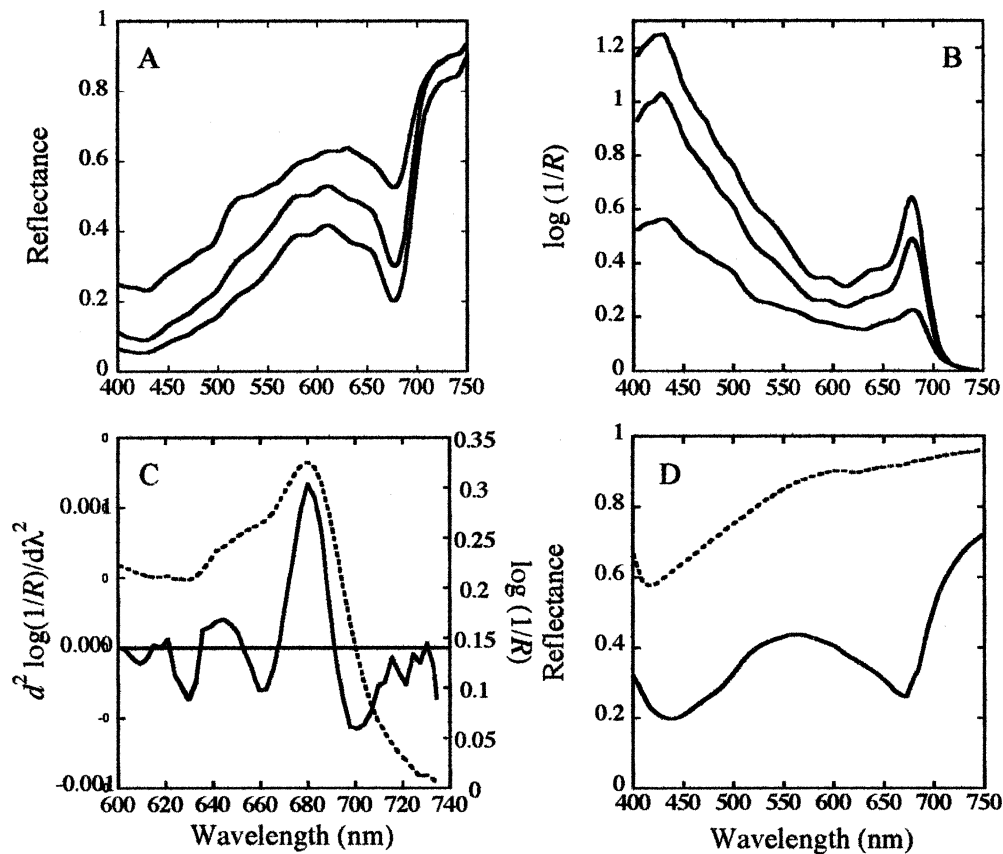


Fig. 1. Changes in the spectroscopic characteristics of *M. faveolata* during the process of recovery. (A) Reflectance spectra of three of the experimental nubbins at different stages of recovery. The spectrum with the highest reflectance was captured during the first week of recovery, the intermediate during week three, and one with the lowest reflectance on week six. (B) Estimated absorption spectra of the same samples shown in panel A. The spectra were calculated from the information presented in panel A. (C) Second-derivative analysis of the absorption spectrum of a bleached coral during the early stages of recovery (solid line). The absorption spectrum is shown for comparison (broken line). (D) Comparison of the reflectance spectra of an unbleached coral skeleton (broken line) and a coral after recovery from bleaching. Animal tissue was removed from the samples with a flow of pressurized water.

reconstruct the absorption properties of intact coral surfaces. The description of the light harvesting capacity of the symbionts in intact corals has been difficult because of the complex nature of coral surfaces (Falkowski et al. 1990). Most of the descriptions of the absorption properties of corals have been made using freshly isolated symbionts (Dubinsky et al. 1984; Wyman et al. 1987; Lesser et al. 2000), thereby ignoring the effect of the skeleton. Recently, two different approaches to overcome this limitation have been reported (Enríquez et al. 2005; Stambler and Dubinsky 2005). Although the results and conclusions of the two studies differ, the calculated light collection efficiencies for the symbiont pigments are significantly higher than those obtained using freshly isolated dinoflagellates. Stambler and Dubinsky (2005) used an integrating sphere to determine the fraction of the incident radiation captured by the symbiotic dinoflagellates of several corals species, whereas Enríquez and coworkers (2005) used thin laminae of *Porites branteri* and the opal

glass technique (Shibata 1959) to determine, in transmission mode, the absorption properties of the symbionts. Unfortunately, the preparation of thin lamina can be used in only a limited set of coral species. In contrast, good quality reflectance spectra are relatively easy to acquire (Hochberg and Atkinson 2000) from corals with diverse morphologies. Considering the high reflectivity of the skeleton in the red and the near infrared part of the spectrum (Fig. 1D), the reflectance spectra in this region are dominated by the absorption of the symbiotic dinoflagellates. The approach used here may introduce a small error by overestimating absorption, because we did not fully account for a small percentage of the incident radiation that penetrates the skeleton. It is important to note that as the reflectance of the skeleton decreases in the blue part of the spectrum, the quality of the calculated absorption is progressively degraded.

Coral pigmentation is primarily caused by the presence of symbiotic dinoflagellates and different combinations of

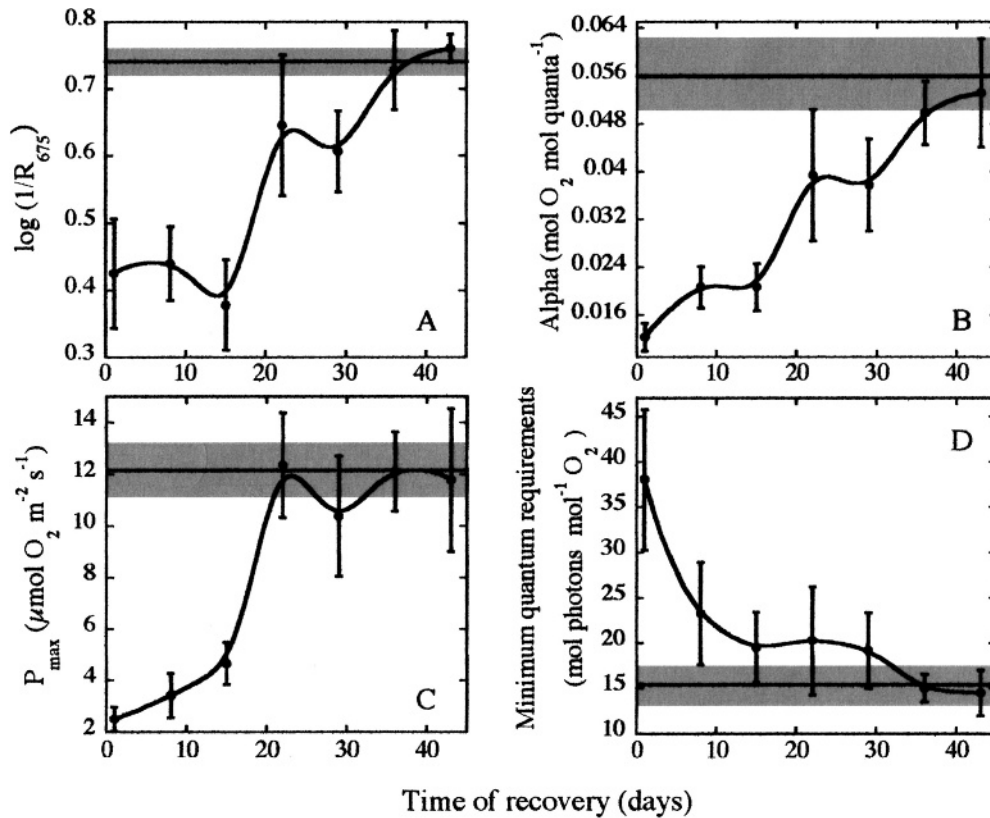


Fig. 2. *M. faveolata*. Analyses of the changes in photosynthesis and light utilization efficiencies during recovery from bleaching. Symbols represent averages of nine replicates and error bars are  $\pm 1$  SEM. The solid horizontal lines represent the averages for the nine replicates before the onset of bleaching, whereas the shaded areas represent  $\pm 1$  SEM. (A) Changes in the estimated absorption at 675 nm. (B) Changes in the initial slope of the P-E curve ( $\alpha$ ). Note that the numbers of photons represent incident and not absorbed radiations. (C) Changes in photosynthetic capacity ( $P_{\max}$ ). (D) Variations throughout the recovery process of the minimum quantum requirements  $1/\Phi_{\max}$ . In this case the reference to photons implies absorbed radiation.

animal pigments (Dubinsky et al. 1984; Mazel et al. 2003). Multiple scattering of the highly reflective aragonite skeleton further modifies the optical properties of corals (Schlichter et al. 1988; Enríquez et al. 2005; Köhl et al. 1995). Multiple scattering by coral skeletons produces a local irradiance enhancement that increases the probability of absorption by photosynthetic pigments. Schlichter and coworkers (1988) proposed, based on ultrastructural analyses of the deep-water scleractinian *Leptoseris fragilis*, that pigment granules located near the symbionts may serve as reflectors, increasing the probability of absorbing solar radiation, although no experimental evidence supporting this role was provided. Direct estimates using irradiance microprobes indicate light enhancement factors close to two for normally pigmented corals (Köhl et al. 1995), and by a factor of five in bleached corals (Köhl, pers. comm.).

A theoretical analysis of this phenomenon indicates that for a flat reflective surface the local irradiance enhancement can be equivalent to three times the external light field (Enríquez et al. 2005). The enhancement may be larger if we consider a more complex surface, although the problem

cannot be addressed analytically. In this context, as a result of coral bleaching, multiple scattering by the skeleton produces internal light fields that are potentially extremely high. How do symbiotic algal populations recover after thermal stress under such extreme light environments?

The  $a_{chl a}^*$  estimated for *M. faveolata* samples with different Chl *a* densities are consistent with those obtained for *P. branneri* in a previous study (Enríquez et al. 2005) and significantly larger than those reported for freshly isolated symbionts from different corals including *M. faveolata* (Wyman et al. 1987; Dubinsky et al. 1990; Lesser et al. 2000). The data presented here confirm, on the one hand, that it is feasible to estimate algal absorption solely from reflectance measurements of the intact corals, and on the other hand, that symbionts inside corals are capable of absorbing light more efficiently than symbionts in isolation (Enríquez et al. 2005). The two samples with the lowest Chl *a* densities exhibited  $a_{chl a}^*$  that are approximately half those reported for *P. branneri* with similar densities. The absorption spectra of *M. faveolata* samples in the early stages of recovery showed contributions of Chl *b* consistent with a biomass increase of the endolithic algae (Fig. 1B,C).

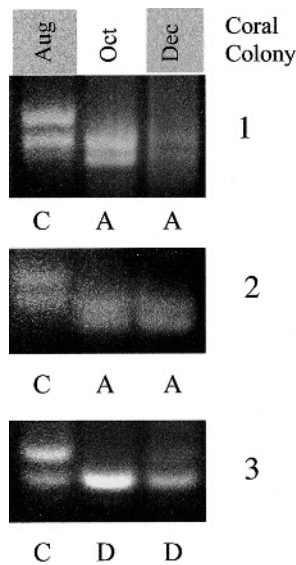


Fig. 3. RFLP patterns of 18S ssrDNA before and after bleaching. Polymerase chain reaction–amplified DNA was extracted from dinoflagellates freshly isolated from three colonies (1, 2, 3), digested to completion with *Taq* I endonuclease, and the products separated in a 1.5% agarose gel. The first lane (August) corresponds to DNA extracted from corals before the bleaching event, the second lane corresponds to DNA extracted from corals immediately after the bleaching event (October), and the third lane corresponds to DNA extracted once recovery was complete (December). The letters underneath each lane denote the predominant clade (*sensu* Rowan 1991).

Enríquez et al. (2005) show that the total flux absorbed by a particle above a flat scattering surface ( $\Phi_{abs}$ ) can be described as  $\Phi_{abs} = (1 + 2R)\Phi_{abs}^{(i)}$ , where  $\Phi_{abs}^{(i)}$  represents the incident flux, and  $R$  is the reflectance by the surface. It has been observed that after bleaching the abundance of endolithic algae rapidly increases, responding to increases in the availability of solar radiation (Fine and Loya 2002; Fine et al. 2005). The propagation of endolithic algae after bleaching may provide partial protection from excessive radiation to the small surviving symbiont population by reducing the reflectivity of the skeleton. Endolithic algae may play a very important role during the recovery from bleaching, not only as a source of photoassimilates (Fine and Loya 2002) but also by reducing the light stress on the recovering symbiont population. Full understanding of the role played by these cryptic organisms requires further investigation.

**Photosynthetic responses**—One of the first signs of thermal stress in symbiotic dinoflagellates is a reduction in their photosynthetic function (Iglesias-Prieto et al. 1992). Recovery from bleaching requires, therefore, the reestablishment of the photosynthetic function of the symbiotic dinoflagellates. Consistent with analyses of the recovery of the quantum yield of charge separation (*Fv/Fm*) of coral symbionts after exposures to elevated temperatures (Jones et al. 2000), our data indicate that the maximum quantum yields for oxygen evolution quickly recover from  $0.026 \pm$

$0.006$  to  $0.043 \pm 0.010$  mol O<sub>2</sub> mol photon<sup>-1</sup> after one week. These data correspond to  $1/\Phi_{max}$  values that indicate higher light utilization efficiencies than those reported for healthy *M. faveolata* individuals from the Dry Tortugas (Lesser et al. 2000) and *M. annularis* from Jamaica (Wyman et al. 1987). Considering that *Fv/Fm* in dinoflagellates growing under nitrogen sufficiency is around 0.650 (Rodríguez-Román and Iglesias-Prieto 2005) and a theoretical  $1/\Phi_{max}$  value for linear electron transport of 8, the maximum operative  $1/\Phi_{max}$  for these organisms should be close to 12.5 photons absorbed per O<sub>2</sub> evolved. In contrast with most of the previously reported  $1/\Phi_{max}$  for corals (Wyman et al. 1987; Dubinsky et al. 1990; Lesser et al. 2000), the data presented here indicate high light-utilization efficiencies. Fully recovered *M. faveolata* presented  $1/\Phi_{max}$  that are very close to the theoretical minimum ( $14.5 \pm 2.4$ ,  $n = 9$ ) (Fig. 2D), indicating that symbiotic corals are not only one of the most efficient light collectors in nature (Enríquez et al. 2005), but that they also use this energy with maximum efficiency.

Considering that the internal light fields of a coral colony change dramatically depending on the Chl *a* density (Enríquez et al. 2005), symbionts will be exposed to different light environments during the recovery process. Acclimatization to different light environments has been documented in corals (Falkowski and Dubinsky 1981; Porter et al. 1984) and cultured symbiotic dinoflagellates (Iglesias-Prieto and Trench 1994). This process involving the rearrangement of the photosynthetic apparatus results in characteristic modifications of the P–E curve. Analyses of the photosynthetic responses of *M. faveolata* throughout the recovery process indicate that during the early stages, the symbiont population exhibited characteristics consistent with acclimation to higher irradiance relative to fully recovered corals. In contrast to the rapid initial recovery of  $1/\Phi_{max}$ , increases in coral pigmentation were not detected during the first three weeks of this study (Fig. 2A,D). Similar results were reported for bleached *Stylophora pistillata*, where increases in cell Chl *a* densities occurred after a three-week period of stasis (Nakamura et al. 2003). During this period, the photosynthetic capacity per unit area ( $P_{max}$ ) showed small increases (Fig. 2C). Coincident with the first detectable increases in coral pigmentation,  $P_{max}$  returned to values indistinguishable from the originals, whereas the values for  $\alpha$  and the absorption at 675 nm remained relatively low (Fig. 2). These characteristics, in conjunction with higher  $1/\Phi_{max}$  relative to fully recovered corals, suggest that symbionts during the first five weeks of the recovery process are acclimated to higher internal light fields than symbionts in fully recovered corals.

**Symbiont identification**—The genus *Symbiodinium* is a diverse and divergent group composed of several subgeneric clades containing an undetermined number of species (Rowan 1991; Baker 2004; Lajuenesse 2004). In addition to the genetic diversity of the group, distinct types of cultured *Symbiodinium* present significant physiological and biochemical traits, including photosynthetic responses (Iglesias-Prieto and Trench 1994) and sensibility to thermal stress (Tchernov et al. 2004), although most of these traits

cannot be directly correlated to any particular clade designation. Recently it has been demonstrated that the differential photoacclimatory abilities of different *Symbiodinium* types explain the vertical distributions of their respective hosts (Iglesias-Prieto et al. 2004). In this context, the abilities of different symbionts to propagate in the extreme light environment of a bleached coral may be dependent on their photoacclimatory potential and not necessarily on their thermal tolerance.

*M. faveolata* possesses polymorphic populations of *Symbiodinium* (Rowan and Knowlton 1995; Rowan et al. 1997; Toller et al. 2001) and changes in the relative abundance of each algal type have been correlated with light intensity gradients. *Symbiodinium* clade A inhabiting *M. faveolata* has been associated with high irradiance environments and is considered a stress tolerant generalist. The changes in the relative abundances of the different algal types as indicated by the variations in the intensities of their respective RFLP bands (Fig. 3) exhibited by the symbionts of two of the three colonies analyzed are consistent with the interpretation that the particular *Symbiodinium* clade A present in the tissues of *M. faveolata* appears to be adapted to high irradiances and stress. Similarly, one of the three *M. faveolata* colonies analyzed here was dominated by clade D *Symbiodinium* during the recovery phase. *Symbiodinium* in this clade has been identified also as stress tolerant (Baker 2004; Rowan 2004). However, it should be noted that the lack of taxonomic resolution of the genetic marker employed here prevents any generalizations regarding the physiological characteristics of all clade A and D *Symbiodinium* genotypes (Lajuenesse 2004; Tchernov et al. 2004). Recently, variations in the physiological traits of the holosymbiont associated with the presence of different algal types have been reported (Rowan 2004; Goulet et al. 2005). In contrast, the data presented here indicate that despite the changes in the relative abundances of their symbionts, recovered *M. faveolata* colonies exhibited indistinguishable photosynthetic light absorption and utilization traits relative to those observed before bleaching.

Recovery from coral bleaching may be the result of two nonexclusive processes: the recolonization of the original population of symbionts and/or the acquisition and subsequent propagation of symbionts from the external environment (Baker 2004). Changes in the relative abundances of different algal types after coral bleaching have been interpreted as possible mechanisms to select for thermally tolerant genotypes (Buddemeier and Fautin 1993; Baker 2004). Considering that recovery from bleaching occurs once the source of stress has been removed, the results presented here indicate that changes in the relative abundances of symbionts after a coral bleaching event may be the result of a transient selection process for symbionts capable of surviving and propagating under the extreme photic environment of a nonpigmented coral. Our results stress the importance of the recovery process for understanding coral bleaching, because the capacity of the symbionts to recolonize their hosts after bleaching may be critical in determining the abilities of the host to survive a coral bleaching event.

## References

- BAKER, A. C. 2004. Flexibility and specificity in coral-alga symbiosis: Diversity, ecology and biogeography of *Symbiodinium*. *Annu. Rev. Ecol. Evol. Systemat.* **34**: 661–689.
- BUDDEMEIER, R. W., AND D. G. FAUTIN. 1993. Coral bleaching as an adaptive mechanism. *BioScience* **43**: 320–326.
- DUBINSKY, Z., P. G. FALKOWSKI, J. W. PORTER, AND L. MUSCATINE. 1984. Absorption and utilization of radiant energy by light- and shade-adapted colonies of the hermatypic coral *Stylophora pistillata*. *Proc. Roy. Soc. Lond. B.* **222**: 203–214.
- , N. STAMBLER, M. BEN-ZION, L. R. MCCLOSKEY, L. MUSCATINE, AND P. G. FALKOWSKI. 1990. The effect of external nutrient resources on the optical properties and photosynthetic efficiency of *Stylophora pistillata*. *Proc. Roy. Soc. Lond. B* **239**: 231–246.
- ENRÍQUEZ, S., E. R. MÉNDEZ, AND R. IGLESIAS-PRIETO. 2005. Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnol. Oceanogr.* **50**: 1025–1032.
- FALKOWSKI, P. G., AND Z. DUBINSKY. 1981. Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. *Nature* **289**: 172–174.
- , P. L. JOKIEL, AND R. A. KINZIE. 1990. Irradiance and corals, p. 109–131. *In* Z. Dubinsky [ed.], *Coral reefs. Ecosystems of the world*. Elsevier.
- FINE, M., AND Y. LOYA. 2002. Endolithic algae: An alternative source of photoassimilates during coral bleaching. *Proc. Roy. Soc. Lond. B* **269**: 1205–1210.
- , E. MEROZ-FINE, AND O. HOEGH-GULDBERG. 2005. Tolerance of endolithic algae to elevated temperature and light in the coral *Montipora monasteriata* from the southern Great Barrier Reef. *J. Exp. Biol.* **208**: 75–81.
- FITT, W. K., M. E. 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**: 677–685.
- GOREAU, T. F., AND N. I. GOREAU. 1959. The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. *Biol. Bull.* **117**: 239–250.
- GOULET, T. L., C. B. COOK, AND D. GOULET. 2005. Effect of short-term exposure to elevated temperatures and light levels on photosynthesis of different host-symbiont combinations in the *Aiptasia pallida* *Symbiodinium* symbiosis. *Limnol. Oceanogr.* **50**: 1490–1498.
- HOCHBERG, E. J., AND M. J. ATKINSON. 2000. Spectral discrimination of coral reef benthic communities. *Coral Reefs* **19**: 164–171.
- HOEGH-GULDBERG, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshwat. Res.* **50**: 839–866.
- IGLESIAS-PRIETO, R., V. H. BELTRÁN, T. C. LAJUENESSE, H. REYES-BONILLA, AND P. E. THOMÉ. 2004. The presence of different algal symbionts explains the vertical distribution patterns of two dominant hermatypic corals. *Proc. Roy. Soc. Lond. B.* **271**: 1757–1763.
- , J. L. MATTA, W. A. ROBINS, AND R. K. TRENCH. 1992. Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. *Proc. Natl. Acad. Sci. USA* **89**: 10302–10305.
- , AND R. K. TRENCH. 1994. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Mar. Ecol. Progr.* **113**: 163–175.

- JEFFREY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*<sub>1</sub>, *c*<sub>2</sub> in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen* **167**: 191–194.
- JONES, R. J., O. HOEGH-GULDBERG, A. W. D. LARKUM, AND U. SCHREIBER. 1998. Temperature-induced bleaching of corals begins with impairment of the CO<sub>2</sub> fixation mechanism in zooxanthellae. *Plant Cell Environ.* **21**: 1219–1230.
- , S. WARD, A. Y. AMRI, AND O. HOEGH-GULDBERG. 2000. Changes in quantum efficiencies of Photosystem II of symbiotic dinoflagellates of corals after heat stress, and of bleached corals sampled after the 1998 Great Barrier Reef mass bleaching event. *Mar. Freshwat. Res.* **51**: 63–71.
- KOEHN, B., G. ELLI, R. C. JENNINGS, C. WILHELM, AND H.-W. TRISSL. 1999. Spectroscopic and molecular characterization of a long wavelength absorbing antenna of *Ostreobium* sp. *Biochim. Biophys. Acta* **1412**: 94–107.
- KÜHL, M., Y. COHEN, T. DALSGAARD, B. B. JØRGENSEN, AND N. P. REVSBECH. 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O<sub>2</sub>, pH and light. *Mar. Ecol. Progr.* **117**: 159–172.
- LAJUENESSE, T. C. 2004. “Species” radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Mol. Biol. Evol.* **22**: 570–581.
- , AND R. K. TRENCH. 2000. Biogeography of two species of Symbiodinium (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biol. Bull.* **199**: 126–134.
- LESSER, M. P. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnol. Oceanogr.* **41**: 271–283.
- , C. MAZER, D. PHINNEY, AND C. S. YENTSCH. 2000. Light absorption and utilization by colonies of the congeneric hermatypic corals *Montastraea faveolata* and *Montastraea cavernosa*. *Limnol. Oceanogr.* **45**: 76–86.
- MAZEL, C. H., M. P. LESSER, M. Y. GORBUNOV, T. M. BARRY, J. H. FARRELL, K. D. WYMAN, AND P. G. FALKOWSKI. 2003. Green-fluorescent proteins in Caribbean corals. *Limnol. Oceanogr.* **48**: 402–411.
- MUSCATINE, L., P. G. FALKOWSKI, Z. DUBINSKY, P. A. COOK, AND L. R. MCCLOSKEY. 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. *Proc. Roy. Soc. Lond. B* **236**: 311–324.
- , AND V. WEIS. 1992. Productivity of zooxanthellae and biogeochemical cycles, p. 257–271. *In* P. G. Falkowski and A. D. Woodhead [eds.], *Primary productivity and biogeochemical cycles in the sea*. Plenum Press.
- NAKAMURA, T., AND R. VAN WOESIK. 2001. Water-flow rates and passive diffusion partially explain differential survival of corals during 1998 bleaching event. *Mar. Ecol. Progr.* **212**: 301–304.
- , H. YAMASAKI, AND R. VAN WOESIK. 2003. Water flow facilitates recovery from bleaching in the coral *Stylophora pistillata*. *Mar. Ecol. Progr.* **256**: 287–291.
- PORTER, J. W., L. MUSCATINE, Z. DUBINSKY, AND P. G. FALKOWSKI. 1984. Primary production and photoadaptation in light-shade-adapted colonies of the symbiotic coral, *Stylophora pistillata*. *Proc. Roy. Soc. Lond. B* **222**: 161–180.
- RODRÍGUEZ-ROMÁN, A., AND R. IGLESIAS-PRieto. 2005. Regulation of photochemical activity in cultured symbiotic dinoflagellates under nitrate limitation and deprivation. *Mar. Biol.* **146**: 1063–1073.
- ROWAN, R. 1991. Molecular systematics of symbiotic algae. *J. Phycol.* **27**: 661–666.
- . 2004. Thermal adaptation in reef coral symbionts. *Nature* **430**: 742.
- , AND N. KNOWLTON. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc. Natl. Acad. Sci. USA* **92**: 2850–2853.
- , ———, A. BAKER, AND J. JARA. 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* **388**: 265–269.
- SCHLICHTER, D., H. W. FRICKE, AND W. WEBER. 1988. Evidence for PAR-enhancement by reflection, scattering and fluorescence in the symbiotic deep water coral *Leptoseris fragilis*. *Endocytobiosis and Cell Research* **5**: 83–94.
- SHIBATA, K. 1959. Spectrophotometry of translucent biological material: The opal glass transmission method. *Methods in Bio. Anal.* **7**: 77–109.
- SMITH, D. J., D. J. SUGGETT, AND N. R. BAKER. 2005. Is photo-inhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? *Global Change Biol.* **11**: 1–11.
- STAMBLER, N., AND Z. DUBINSKY. 2005. Corals as light collectors: An integrating sphere approach. *Coral Reefs* **24**: 1–9.
- TCHERNOV, D., M. Y. GORBUNOV, C. DE VARGAS, S. N. YADAV, A. J. MILLIGAN, M. HAGGBLOM, AND P. G. FALKOWSKI. 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc. Natl. Acad. Sci. USA* **101**: 13531–13535.
- TOLLER, W. W., R. ROWAN, AND N. KNOWLTON. 2001. Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. *Biol. Bull.* **2001**: 360–373.
- 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.
- WYMAN, K. D., Z. DUBINSKY, J. W. PORTER, AND P. G. FALKOWSKI. 1987. Light absorption and utilization among corals: A study in Jamaica, West Indies. *Mar. Biol.* **96**: 283–292.

Received: 8 August 2005

Accepted: 29 June 2006

Amended: 10 July 2006