

## Response of *Millepora alcicornis* (Milleporina: Milleporidae) to two bleaching events at Puerto Morelos reef, Mexican Caribbean

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**Abstract:** Two naturally occurring colonies of *Millepora alcicornis* were monitored during 1997 and 1998, both years in which this species bleached in the Mexican Caribbean. One colony (HL) was naturally exposed to a high light environment and another nearby colony (LL) was exposed to 5.9 times lower light levels due to shadowing by a pier. For 10 days in August 1997, seawater temperatures in the surrounding reef lagoon rose up to 1.5°C above the 6-year August average. The HL colony bleached to white during this period, whereas, the LL colony remained dark-brown colored. The HL colony recovered its normal dark-brown coloration (reversible bleaching) within several weeks, during which time the seawater temperatures returned to average. The following year, for 10 days, seawater temperatures rose up to 3°C above the 7-year August average and both colonies bleached to white and neither colony recovered (irreversible bleaching). Both colonies were rapidly overgrown by algae and hydroids and, as of June 2003, no recovery has taken place. Prior to the 1997 bleaching, experiments using solar radiation showed that the quantum yield of photosystem II charge separation of branches from HL and LL colonies were affected for several hours by exposure to ultraviolet radiation (UVR, 280 to 400 nm), but recovered by the same evening, suggesting that UVR does not have long-term effects on photochemistry in *M. alcicornis*. *In situ* effective quantum yield of photosystem II charge separation ( $\Delta F/F_m'$ ) measurements before the 1998 bleaching event indicate that both colonies were healthy in terms of the physiological status of their endosymbionts. During and after the 1998 bleaching event, both colonies showed a reduction in  $\Delta F/F_m'$  and consequently an increase in excitation pressure on photosystem II. The data suggest that temperature is not the only factor that causes bleaching and that solar radiation may play an important role in coral bleaching.

**Key words:** chlorophyll fluorescence, thermal stress, light stress, coral bleaching, ultraviolet radiation, symbiotic dinoflagellates, *Millepora*.

*Millepora alcicornis* L., 1758, also known as fire coral, is a common reef inhabitant of the Mexican Caribbean, overgrowing many substrates including gorgonians and piers. In recent years, colonies of *M. alcicornis* have undergone bleaching during the warmer months in the Mexican Caribbean. Bleaching can result from (1) expulsion of symbiotic algae from the host, (2) loss of algal photosynthetic pigments, or (3) by direct damage to host tissue (Fig. 1) (Iglesias-Prieto *et al.* 1992,

Lesser 1996, Warner *et al.* 1996, 1999, Jones *et al.* 1998). In any case, normal pigmentation of coral tissue is generally lost and the white calcium carbonate skeleton of the coral is visible through the transparent host tissue. In some coral species, color is retained due to the presence of animal pigments (Iglesias-Prieto, Enríquez and Banaszak, pers. obs.). While the mechanisms causing bleaching have not been completely elucidated, there are a number of hypotheses as to how bleaching is initiated.

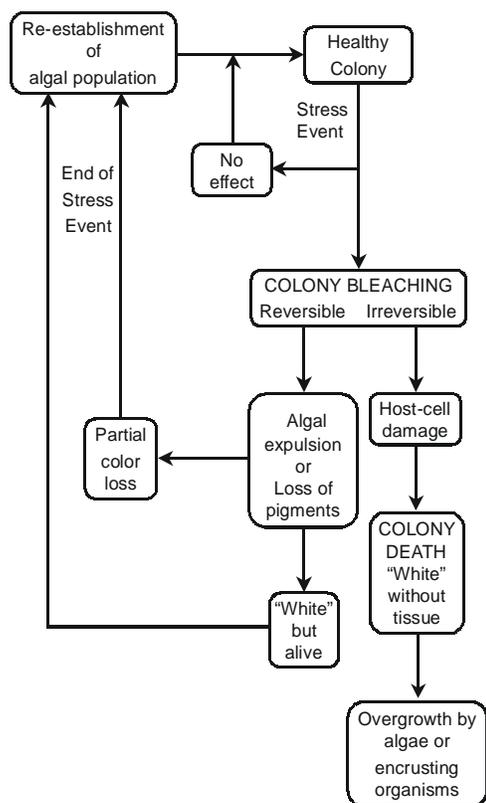


Fig. 1. Proposed scheme for the sequence of events leading to reversible and irreversible bleaching and the potential outcomes.

Studies of symbiotic dinoflagellates in culture (Iglesias-Prieto *et al.* 1992, Lesser 1996) and *in hospite* (Warner *et al.* 1996) have suggested that damage to photosystem II by elevated temperatures or high levels of solar radiation may lead to bleaching. Damage has been shown to specifically occur at the D1 protein of photosystem II (Warner *et al.* 1999) and to the primary carboxylating enzyme, RUBISCO (Jones *et al.* 1998). Measurements of quantum yield of charge separation at photosystem II ( $\Delta F/Fm'$ ), therefore, are a very useful parameter in detecting changes in physiological status of endosymbionts.

Evidence has also been gathered about environmental factors that induce bleaching events, which in some years have reached global proportions. The primary environmental factor presently correlated with massive

bleaching events is increased seawater surface temperature. Other factors such as exposure to photosynthetically active radiation (PAR, 400-700 nm) and ultraviolet radiation (UVR, 280-400 nm) (Glynn 1996) and a reduction in water motion (Enríquez and Iglesias-Prieto, unpubl. data) have been considered as secondary factors that act in synergy with increased temperature (Fig. 1). Our results support temperature as the primary factor related to bleaching in *M. alvicornis*, but that synergism with exposure to solar radiation plays a role in a reversible bleaching episode. However, this synergism is not significant during an irreversible bleaching event. We discuss a possible mechanism linking bleaching and mortality in *M. alvicornis*.

## MATERIALS AND METHODS

### Location of *Millepora alvicornis* colonies:

A colony of *Millepora alvicornis* exposed to full solar radiation throughout the day (Fig. 2A) was found in front of the pier belonging to the Universidad Nacional Autónoma de México marine station located in Puerto Morelos (20°52'N, 86°52'W). The colony, completely over-growing a wood piling measuring 0.3 m wide and 1.8 m high, the top of which was located at 0.1 m depth, was named high light (HL). Another *M. alvicornis* colony (LL) was found over-growing a PVC pipe measuring 0.2 m wide and 1.8 m high, with the top of the colony at 0.1 m depth, located within 5 m of the HL colony. The LL colony experienced 5.9 times lower total daily irradiance due to shadowing for most of the day (Fig. 2A) by the same pier. *In situ* PAR measurements were obtained using a LICOR data logger attached to a spherical  $4\pi$  quantum sensor (LI-193SA, USA) at 30 cm depth, oriented towards the north of each colony and fixed parallel to the colonies at 5 cm distance. Light data were sampled at an average of 40 measurements at 10 min intervals over a 24 hr cycle. Temperature data were collected daily in the reef lagoon within 25 m of the colonies (Fig. 2B) and average monthly temperatures were calculated from 1992 to 1997 for 1997 data and 1992 to 1998 for 1998 data.

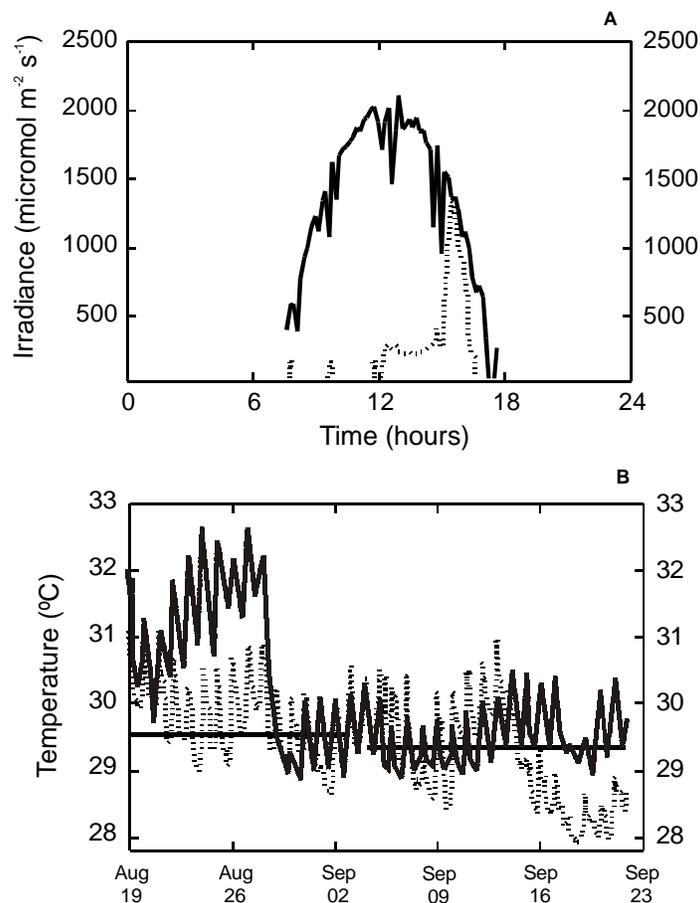


Fig. 2. (A) Irradiance profiles experienced by the high light (black line) and the low light colony (dashed line) colonies, (B) temperature profiles taken in Puerto Morelos reef lagoon in August and September 1997 (dashed line) and August and September 1998 (black line). Horizontal bars represent average August and September temperatures.

**Effect of ultraviolet radiation (UVR) on photochemical efficiency:** To determine the effect of UVR on photochemical efficiency in *M. alcicornis*, nine branches (5 cm long) of the HL colony and six branches of the LL colony were collected from the same depth (0.2 m) by snorkeling during mid-morning on March 11 1997 and protected by black plastic bags containing seawater. All branches were transferred to an aquarium with flowing seawater and covered with neutral density screening to approximate *in situ* PAR conditions at the HL site. On the same evening, six branches each of HL and LL colonies were anchored individually into Plasticine on acrylic sheets and

fixed with plastic pegs to ceramic tiles, which facilitated removal of branches for fluorescence measurements without undue stress. The branches were put into two 20 l acrylic tanks, which were placed on an outdoor seawater table equipped with flowing seawater, directly to the acrylic tanks and the seawater table, to maintain the tanks at ambient seawater temperature. Both tanks were covered with neutral density screening to approximate *in situ* PAR conditions at the HL site using solar radiation with an unobstructed, 360° view of the sun. The tanks were fitted with lids; one transmitted UVR and PAR (6 mm thick Plexiglas G UVT Acrylic, 50% half width maximum at

282 nm) and the other transmitted only PAR (6 mm thick Plexiglas G UF-3 Acrylic, 50% half width maximum at 380 nm).

Algae were freshly isolated (FIA) from the three remaining HL colony branches using a Water Pik and 0.45  $\mu\text{m}$  Millipore filtered seawater, centrifuged at 1000 g for 2 min and re-suspended in seawater three times to remove host tissue, with the final resuspension in 1 liter seawater. FIA suspensions were placed in bags and floated in the acrylic tank fitted with the lid that transmitted only PAR. Fluorescence measurements were made of HL branches ( $n=3$ ), LL branches ( $n=3$ ) and FIA ( $n=3$ ) with a high temporal resolution fluorometer (Plant Efficiency Analyzer, PEA, Hansatech, UK) on the same evening. Measurements were continued the next day from morning to night at approximately 90 min intervals. Rain suspended the experiments on the morning of March 13 so final measurements were taken. These fluorescence measurements also served as an indicator of the physiological status of the endosymbionts prior to the 1997 August bleaching event.

**In situ fluorescence measurements:** *In situ* chlorophyll fluorescence measurements were recorded each hour for 24 hours using a Diving-PAM (WALZ, Germany) for HL and LL colonies on October 9 to 10 1997, June 22 to 23 1998 and August 10 to 11 1998. Diurnal cycle measurements were begun before sunrise and continued for 24 hr.

Measurements were also made at mid-afternoon on August 19, 22 and 28 1998 and September 1, 4 and 7 1998. Diurnal cycle and mid-afternoon measurements were taken in exactly the same position by placing the PAM measuring probe over 1  $\text{cm}^2$  acrylic tags fixed to each colony at a depth of 0.2 m. Light measurements were taken at the same time as PAM measurements using a cosine-corrected PAR sensor aligned normal to the surface of the colonies at each measuring point. Measurements of  $F$ ,  $F_m'$  and  $\Delta F/F_m'$  are automatically calculated by the Diving PAM. Excitation pressure was calculated using the method of Maxwell *et al.* (1995).

**Cell counts and chlorophyll determination:** Each date that *in situ* measurements were

made, fragments were removed from each colony using a hammer and a fine-pointed chisel and transported in plastic bags to the laboratory for algal cell count and chlorophyll analysis. The samples were isolated by Water Pik, as described above, and an aliquot was used to make replicate cell counts in a hemocytometer. Mitotic indices were calculated using the ratio of dividing cells to total cell count. Surface areas of fragments used for cell count determination were estimated using the aluminum foil technique. Chlorophyll concentration was determined after resuspension of the algal pellet in acetone followed by dimethylsulfoxide (DMSO). The supernatants were combined, centrifuged and the absorbance read on a spectrophotometer (Aminco, USA). Chlorophyll *a* content was calculated using the equation of Jeffrey and Humphrey (1975).

## RESULTS

Two naturally occurring colonies of the branching fire-coral *Millepora alcicornis*, one growing in a high light (HL) environment and the other in a low light (LL) environment, were monitored during 1997 and 1998. During August 1997, seawater temperatures rose up to 1.5°C above the monthly average of 29.5°C (calculated for 1992-1997) for about 10 days (Fig. 2B) and the HL colony bleached to white but showed evidence of healthy polyp extension. The LL colony consistently had a dark-brown color and no signs of bleaching were evident. During this period, colonies of *M. alcicornis* bleached throughout the Puerto Morelos reef lagoon (Rodríguez-Martínez and Iglesias-Prieto, pers. obs.). On return to average seawater temperatures, HL regained its normal dark-brown coloration, so we characterize this event as reversible bleaching (Fig. 1). The pattern of recovery in *M. alcicornis* was evident lagoon-wide (Rodríguez-Martínez and Iglesias-Prieto, pers. obs.).

During August 1998, seawater temperatures rose up to 3°C above the monthly average (calculated for 1992-1998) for about 10 days (Fig. 2B) and both colonies bleached to white and showed no signs of polyp extension. The HL colony lost color approximately one week

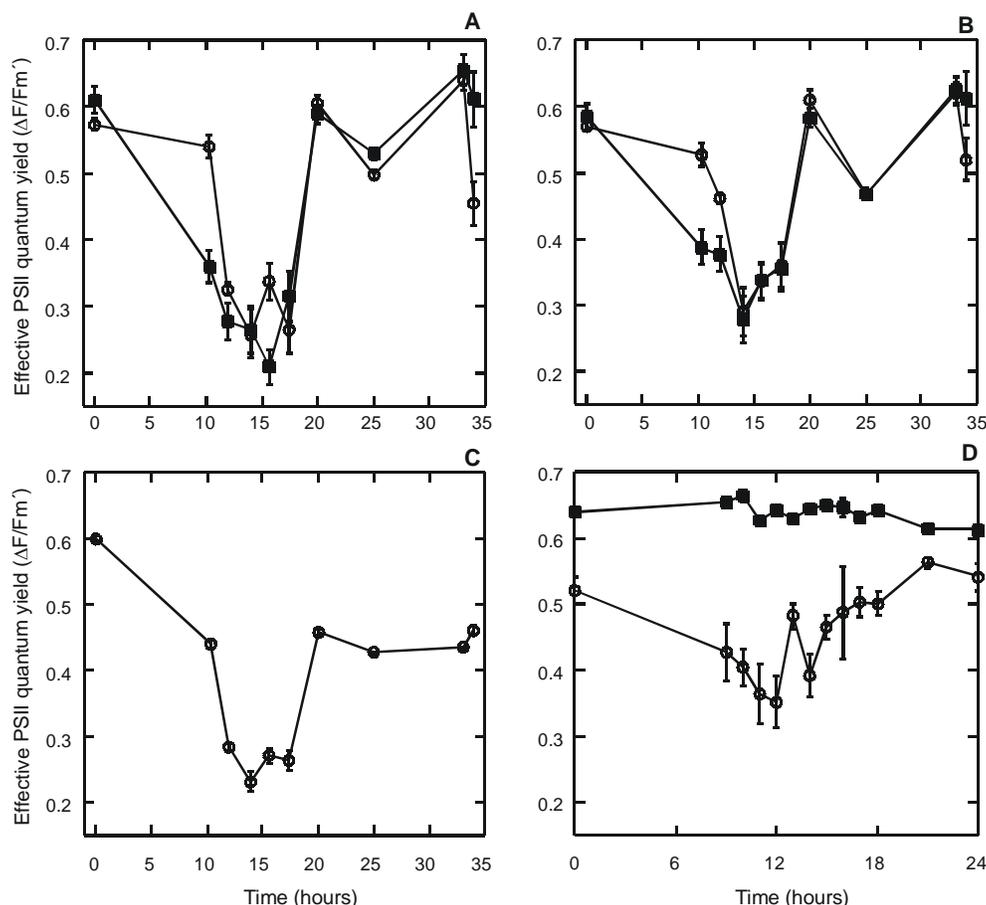


Fig. 3. Diurnal cycles of quantum yields of photosystem II charge separation obtained during 1997. Open circles represent data for colony branches exposed only to photosynthetically active radiation (PAR, 400 to 700 nm). Closed circles represent data for branches exposed to PAR and ultraviolet radiation (UVR, 290 to 400 nm). (A) High light colony in March, (B) Low light colony in March, (C) Freshly isolated symbionts from the high light colony exposed to only PAR in March (open circles), (D) High light (open circles) and low light (closed squares) colonies in October. Vertical bars indicate standard errors of the mean.

earlier than the LL colony. Neither colony recovered and both sites were rapidly overgrown by algae and hydroids and remain overgrown as of June 2003. We characterize this event as irreversible bleaching (Fig. 1). During this period, massive *M. alcicornis* mortality was observed throughout the Puerto Morelos reef lagoon (Rodríguez-Martínez, pers. obs.).

Prior to the 1997 reversible bleaching event, in March, we determined that although UVR has short-term effects (hours) on the efficiency of photochemistry, as evidenced by a greater

reduction of  $\Delta F/F_m'$  for HL and LL branches exposed to UVR, compared with those exposed to only PAR, there do not appear to be any long-term effects of UVR damage. Both colonies recovered on the same evening from any effects of UVR (Fig. 3A, B). FIA also exhibited rapid recovery after exposure to only PAR (Fig. 3C). These data show that both colonies were healthy, in terms of the physiological status of their endosymbionts, prior to the August 1997 bleaching event. In October 1997, the HL colony exhibited similar  $\Delta F/F_m'$  values to those obtained in March 1997 (Fig. 3).

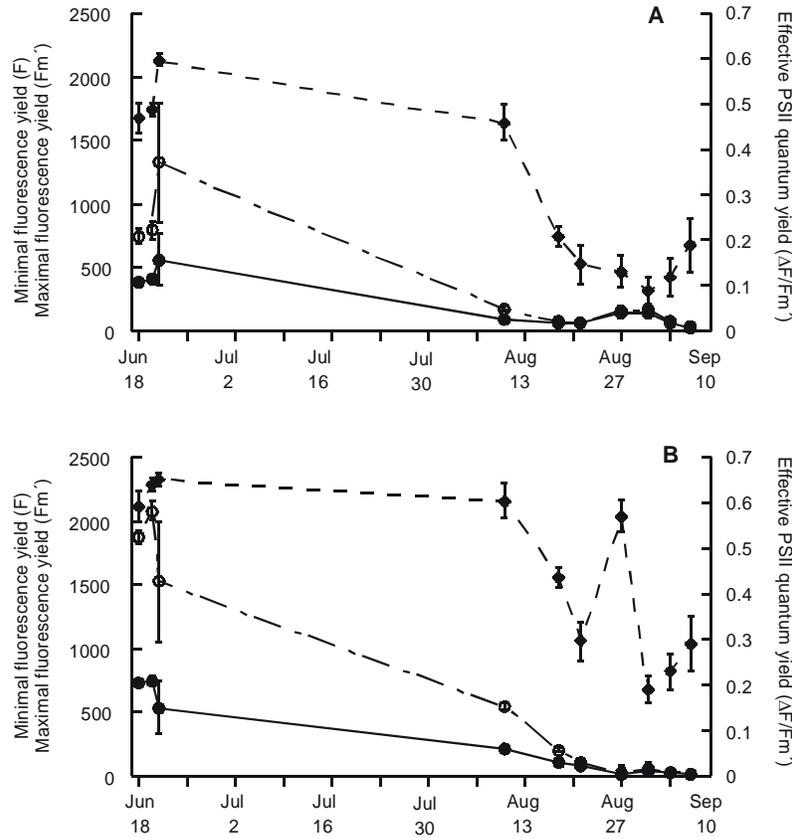


Fig. 4. Changes in chlorophyll fluorescence parameters over the period of the 1998 bleaching event for the high light colony (A) and the low light colony (B). Plotted are minimal fluorescence yield (F, closed circles), maximal fluorescence yield (Fm', open circles) and effective quantum yield of photosystem II charge separation ( $\Delta F/F_m'$ , closed diamonds). Vertical bars indicate standard errors of the mean.

Prior to the August 1998 high-temperature episode, in June (Fig. 4A, B), the HL and LL colonies exhibited similar patterns of  $\Delta F/F_m'$  as in 1997 (Fig. 3D). During and after the high-temperature episode (August and September data, respectively), the minimal and maximal fluorescence values as well as  $\Delta F/F_m'$  dropped dramatically (Fig. 4A, B) in both colonies and excitation pressures increased significantly (Fig. 5A, B).

Mitotic indices measured on six dates from June 22 to August 6, prior to the bleaching event, averaged  $26.24\% \pm 12.43$  for the HL colony and  $26.68\% \pm 15.16$  for the LL colony. Algal cell counts, mitotic indices and chlorophyll concentrations all reduced during the bleaching event to undetectable values.

## DISCUSSION

Monitoring of two colonies of the branching fire-coral *Millepora alcicornis* during two consecutive bleaching events, in 1997 and 1998, allowed us to describe the effects of thermal and light stress on the physiological status of their endosymbionts. In 1997, the HL colony bleached white, which was associated with a  $1.5^\circ\text{C}$  increase in seawater temperature above the monthly average. This HL colony experienced reversible bleaching as it completely recovered within several weeks, a pattern observed in *M. alcicornis* throughout the Puerto Morelos reef lagoon after seawater temperatures returned to average in late August.  $\Delta F/F_m'$  values taken two months after

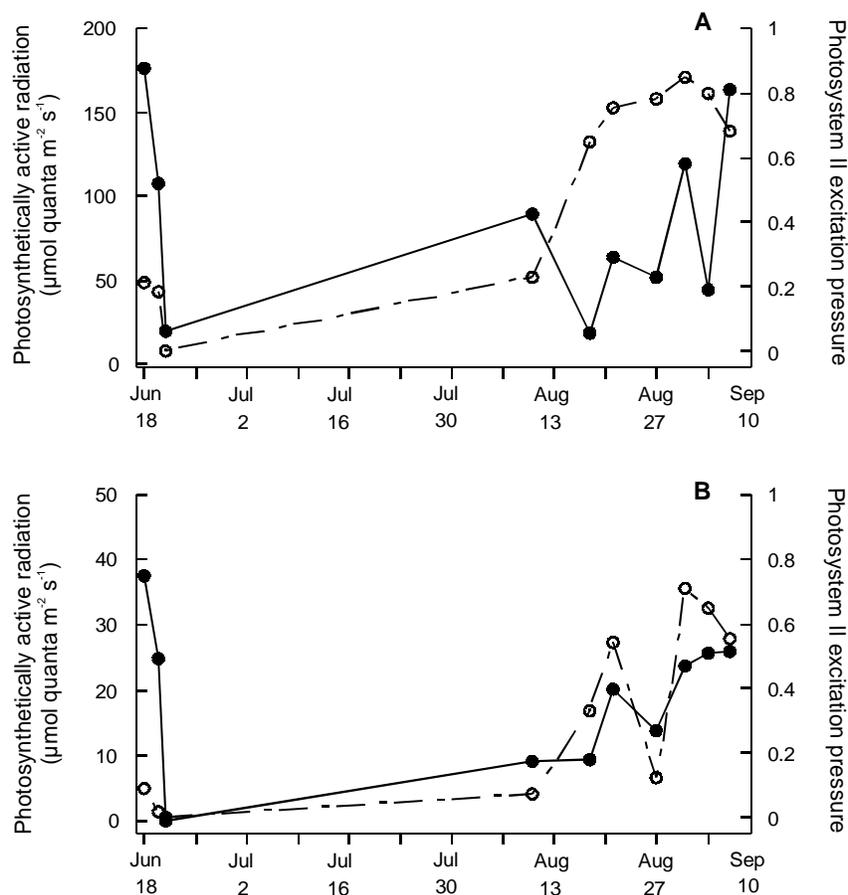


Fig. 5. Changes in the excitation pressure on photosystem II (open circles) during the 1998 bleaching event for the high light (A) and the low light colonies (B). Photosynthetically active radiation ( $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) measured at noon (dark circles) for the time period is also shown.

the 1997 bleaching event were similar to those obtained five months prior to the event and indicate that there was no lasting physiological effect, at least on endosymbiont photosynthesis. Our data also indicate that UVR does not have long-term damaging effects on *M. alci-cornis* colonies, as both HL and LL colonies recovered within hours of exposure to UVR.

The following year, both colonies bleached, again in August, associated with seawater temperatures up to  $3^{\circ}\text{C}$  above the monthly average. Prior to this bleaching episode, maximal  $\Delta F/F_m'$  values of each colony obtained during the early morning and late evening (Fig. 3D) differed significantly, by 15%, being  $0.56 \pm 0.008$  for the HL colony and  $0.66 \pm 0.009$  for

the LL colony. Low  $\Delta F/F_m'$  have been suggested to indicate chronic photo-inhibition due to nitrogen limitation (Kolber *et al.* 1988, Gorbunov *et al.* 2001), however, the exceptionally high mitotic index of the algal population in the HL colony at this time could not be maintained for such a long period under conditions of nitrogen starvation. In addition, the similarity of the mitotic indices for both colonies, suggests that the reduction in maximal  $\Delta F/F_m'$  in the HL colony could be an indication of photo-acclimation rather than photo-inhibition. The 37% diurnal reduction in effective photochemical efficiency, the so-called dynamic photo-inhibition, for the HL colony (Fig. 3D) would be increased to a hy-

pothetical 47% if the HL colony recovered nightly to the same maximal values as the LL colony. Maximizing photochemical efficiency would imply an additional cost of recovery, which is unnecessary under high light exposure and indicates that the colony has photo-acclimated to exposure to high light.

The deleterious effects of the 1998 bleaching event were irreversible for both colonies studied and coincided with massive mortality of *M. alcicornis* in the reef lagoon at Puerto Morelos. Neither colony studied has shown evidence of polyp extension nor recovery of its symbiont population as of June 2003 and both have been overgrown by filamentous and fleshy algae and hydroids. Decreases in effective  $\Delta F/F_m'$  and increases in excitation pressures mirrored the loss of chlorophyll and of symbiotic algae.

Together, these data indicate that thermally-induced inactivation of photosystem II plays an important role but is not the only factor inducing bleaching in *M. alcicornis*. The synergistic effect of irradiance was evidenced by the difference in responses found between the HL and LL colonies. The effect of irradiance needs to be considered in explaining the natural variability of responses to bleaching often found among colonies, even of the same species, particularly in reversible bleaching events. Excluding genetic variability, other environmental factors also need to be taken into account to explain the natural repertoire of coral responses to bleaching. The conditions that favor the appearance of bleaching events are characterized by high atmospheric stability, which is also associated with (1) higher light penetration into the water column (higher UV and PAR doses), and (2) a significant reduction in water motion that results in increased light penetration into the water column and has severe consequences for exchange rates of gases and solutes. Our study site is characterized by late-summer doldrums conditions that peak in August and result in lower light-attenuation coefficients (more light penetrating) at the same time as seawater temperatures peak. By September, the conditions become more wind-influenced and seawater temperatures diminish. However, in years when the temperatures are well above average, the

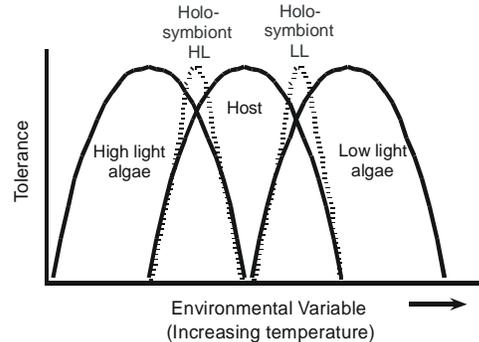


Fig. 6. Proposed model to explain the patterns of bleaching as a result of the different thermal tolerance limits of the holo-symbiont, the host and the high light (HL) and low light (LL) algal populations.

direct effect of thermal stress on host tissue overshadows other factors known to modulate bleaching potentially leading to coral mortality (irreversible bleaching).

Not all corals are equally sensitive to environmental pressures. Tolerance limits differ among species and also differ within a holo-symbiosis, i.e., between the algal and animal components, as shown in Fig. 6. The holo-symbiont occupies a limited range as defined by the overlapping tolerance of the coral and algal components, but will lie outside of the optimal tolerances of algal and host components (Fig. 6). The different photo-acclimatory states of HL and LL algal populations, as evidenced by *in situ*  $\Delta F/F_m'$  (e.g., Fig. 2D), suggest that the tolerance ranges of HL and LL algae are distinct (Fig. 6). As the environmental variable (e.g., temperature) increases in intensity, the thermal tolerance limit of HL algae would be exceeded prior to that of LL algae and would explain the different responses found between the *Millepora* colonies during the 1997 bleaching event. The combination of environmental parameters exceeded the thermal tolerance limit of the HL algal population resulting in bleaching of the colony. This bleaching event was reversible; the host survived because its thermal tolerance limit was not exceeded as indicated by healthy polyp extension and the host was able to recover its algal population within weeks. In 1998, the bleaching event resulted in mortality

of both colonies as the thermal tolerance limit of the host was exceeded as suggested by the loss of polyp extension in both colonies. We have no evidence as to whether the thermal tolerance limit of the LL algal population was also exceeded because even if host cells are damaged, the algae can survive independently in the water column.

More species of corals, particularly scleractinians, need to be studied to determine differences in susceptibility to bleaching under controlled conditions and to determine if factors other than temperature also contribute to modulate bleaching, such as, an increment in the intensity of PAR and UVR as well as a reduction in water motion.

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#### RESUMEN

Durante 1997 y 1998 dos colonias, HL (alta luz) y LL (baja luz) de *Millepora alcicornis* experimentaron blanqueamiento. Durante 10 días en 1997, la temperatura del agua sobrepasó 1.5°C el promedio de 1992-1997. Se observó que HL sufrió blanqueamiento, mientras que LL mantuvo su color. Al cabo de varias semanas, la colonia HL recuperó completamente su pigmentación natural (blanqueamiento reversible). En 1998 la temperatura sobrepasó 3°C el valor promedio durante 10 días. HL y LL su-

frieron blanqueamiento y ninguna logró recuperarse (blanqueamiento irreversible) y los dos fueron recolonizadas rápidamente por algas e hidroides y todavía (junio 2003) no hay señales de recuperación. La eficiencia fotoquímica ( $\Delta F/F_m'$ ) de ambas colonias sufrieron caídas significativas al exponerlas a radiación ultravioleta pero se recuperaron completamente sugiriendo que la luz ultravioleta no provocó daño permanente. Medidas *in situ* antes del evento de blanqueamiento del 1998, indicaron que ambas colonias estaban en óptimas condiciones con respecto a la fisiología. Durante y después del evento de blanqueamiento, ambas colonias mostraron una reducción en la eficiencia fotoquímica. Estos resultados indican que la temperatura no es el único factor que causa blanqueamiento y que la radiación solar debe también jugar un papel importante en el blanqueamiento de coral.

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