

Different Effects of High Temperature Acclimation on Bleaching-Susceptible and Tolerant Corals

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Abstract

Although prior exposure to high light is reported to make the coral *Goniastrea aspera* more tolerant to bleaching, effects of prior exposure to high temperature have not been studied. We examined the influence of prior exposure to high temperature on the photoinhibition-sensitivity of photosynthetic activity in the zooxanthellae of two bleaching-susceptible corals, *Stylophora pistillata* and *Montipora digitata*, and two bleaching-tolerant corals, *Pachyseris rugosa* and *Pavona divaricata* from Okinawa, Japan. Coral fragments were acclimated either at 24 or 31°C for 5 days, and then exposed to high light (1,200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 2 h at 24 or 31°C. Bleaching-susceptible species acclimated at 31°C showed significant increase in photosynthetic activity (P_{max}) and total chlorophyll content, while bleaching-tolerant species showed the opposite pattern. The rate of dark respiration (R) was significantly higher in fragments acclimated at 31°C than in those acclimated at 24°C in all investigated coral species. F_v/F_m decreased to a similar extent after high light treatment at 31°C in corals acclimated at 24 and 31°C in both tolerant and susceptible species. P_{max} and chlorophyll content of bleaching-susceptible corals

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showed a more pronounced decline after high light exposure at 31°C than those of bleaching-tolerant species. The extent of reduction in P_{\max} and chlorophyll content was significantly higher in fragments of *S. pistillata* and *M. digitata* acclimated at 31°C than in those acclimated at 24°C. Significant decrease in R was evident only in susceptible species acclimated at 31°C and exposed to high light at 31°C. These results suggest that mechanisms underlying the thermal acclimation of photosynthesis vary with coral species. A high photosynthetic acclimation potential to temperature might be associated with a response of P_{\max} but not F_v/F_m . Susceptibility of *S. pistillata* and *M. digitata* to synergistic effects of high light and elevated temperature depends on a decreased capacity of their zooxanthellae to cope with photodamage, although the role of the host can not be excluded.

Keywords: Reef-building corals, bleaching, temperature acclimation, zooxanthellae, photosystem II, oxygen evolution, chlorophyll

1. Introduction

Reef-building corals consist of animal host and symbiotic dinoflagellates (zooxanthellae). Bleaching is a general stress response in corals exposed to a wide variety of stressors and refers to a change in coloration of coral tissue through the loss of zooxanthellae, or their photosynthetic pigments (Glynn, 1993). In most cases, wherever coral reef bleaching was reported, it occurred during the summer season when conditions favor localized heating in combination with high intensities of solar irradiance (Glynn, 1993; Hoegh-Guldberg, 1999; Wilkinson, 2000). Recent studies have shown that exposure to elevated temperatures acts to reduce the photosynthetic rate of dinoflagellate symbionts (Hoegh-Guldberg and Smith, 1989; Warner et al., 1996) and might predispose their photosynthetic apparatus to further damage by excess light thus creating a state photoinhibition (Fitt and Warner, 1995; Lesser, 1996; Jones et al., 2000). The thermal sensitivity of symbiotic algae of corals is believed to be the underlying cause of the phenomenon of coral bleaching (Iglesias-Prieto et al., 1992; Jones et al., 1998). However, the importance of host in the maintenance of the intact coral symbiosis under the stress conditions also has not been ignored (Jokiel and Coles, 1990; Porter et al., 1989; Brown et al., 2002). Coles and Jokiel (1977) reported that coral bleaching at abnormally high temperatures correlated with respiratory rates, and that increase in respiration of corals with temperature leads to a diminishing autotrophic capacity at the coral-algal association level.

Different symbiotic associations might have different levels of tolerance to bleaching (Marshall and Baird, 2000; Loya et al., 2001). The differential bleaching responses have been attributed to either genetic differences in symbiotic algae (Rowan et al., 1997; LaJeunesse, 2001) or to the experience

(acclimation) of corals to stress conditions (Brown et al., 2000, 2002). It was shown that pre-exposure to high solar radiation makes the coral *Goniastrea aspera* more tolerant to bleaching (Brown et al., 2002). One possible explanation for differences in the susceptibility of hermatypic corals is that zooxanthellae within different hosts develop differential tolerance to high temperature through acclimation mechanisms. Clausen and Roth (1975) showed that responses of *Pocillopora damicornis* differed depending on the times of the year (and therefore with different thermal histories) when the thermal tolerance of the coral was studied. Al-Sofyani and Davies (1992) found that respiration rates of *Echinopora gemmacea* in the Red Sea did not change with a 6°C increase in seawater temperature, suggesting acclimation for this species, while respiration rates of *Stylophora pistillata* indicated no such acclimation. The authors also reported that *S. pistillata* had a higher photosynthetic rate and a higher metabolic expenditure under high light, high temperature (30°C) when compared with the low temperature conditions (24°C).

However, studies that have demonstrated how prior exposure to high temperature might influence bleaching response of corals are scarce. Therefore, the present study was undertaken to investigate the effect of prior exposure to high temperature on the photochemical efficiency of PSII (F_v/F_m), maximal photosynthetic activity (P_{max}) and chlorophyll content in algal symbionts, zooxanthellae density and respiration rates of two bleaching-susceptible corals, *Stylophora pistillata*, *Montipora digitata* (Loya et al., 2001), and two tolerant corals, *Pachyseris rugosa*, *Pavona divaricata* (Sakai, personal communications), corals. After prior exposure to high temperature, we compared photoinhibition sensitivity of photosynthesis and the respiration changes in these species during exposure to high light at normal and high temperatures. We hypothesize that response of photo-physiology of zooxanthellae symbionts to high temperature prior exposure are different in bleaching-susceptible and tolerant corals. Prior exposure to high temperature under weak light makes corals more susceptible to photoinhibition by affecting photosynthetic activity of their symbionts. Different acclimation responses to high temperature can stipulate the differences in photoinhibition-sensitivity of corals with different susceptibility to bleaching.

2. Material and Methods

Collection and maintenance of corals

Colonies of *Stylophora pistillata* (Esper 1797), *Montipora digitata* (Dana 1846), *Pachyseris rugosa* (Lamarck 1801) and *Pavona divaricata* (Lamarck 1816) were collected from a depth of 0.5–1 m at the reef of Aka Island, the

eastern reef of Sesoko Island and at Bise, Okinawa, Japan. Colonies were kept in an outdoor tank supplied with running seawater for two months until use to stabilize their physiological parameters and to avoid the possible effect of photoacclimation to low light on their response to the subsequent temperature acclimation. During this period, seawater temperature in the tank was 22–23°C and incident photosynthetically active radiation (PAR) did not exceed 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The tank was shaded by black plastic mesh to reduce the light intensity to 10% of incident PAR.

Experimental design

The experiments were conducted in April, 2003. Branches about 3 cm in length were taken from colonies of *Stylophora pistillata* and *Montipora digitata*, and fragments about 4 cm² of tissue surface area were removed from *Pachyseris rugosa* and *Pavona divaricata*. Four replicate colonies were used for each species. Coral fragments were prepared from each coral colony, and mounted on glass slides using rubber bands. Fragments from four replicate colonies were divided into 2 groups and placed into each of two aquaria containing aerated seawater. The seawater temperature in the aquarium for the first and second groups was maintained at 24 and 31°C, respectively. The temperature was regulated using automatic thermostats ($\pm 0.5^\circ\text{C}$, IC Thermostat, EX-003) and aquarium heaters. The coral fragments were illuminated at a light intensity of 140 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with four white fluorescent lamps (National, FL20SS-N/18) during day time (12 h). Half of the seawater in the aquaria was replaced every second day with fresh seawater adjusted to respective temperatures during the experimental period.

After a 5 d acclimation period, the initial measurements of chlorophyll a fluorescence (F_v/F_m), photosynthesis light response curve, the rate of dark respiration, chlorophyll content and zooxanthellae density were performed with four fragments each from different colonies for all species. Then remaining fragments were placed in transparent plastic chambers (30 cm \times 40 cm) containing aerated seawater and used as the experimental units. The chambers with experimental fragments were put into one of the water baths whose temperature was regulated at 24 and 31°C by temperature control units (EYELA, Thermister Tempet T-80). The experimental fragments were exposed to 1,200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of PAR for 2 h as shown in Fig. 1. PAR treatment was carried out using two 500-Watt incandescent lamps (NIKKO, Tokyo, Japan). Visible irradiance was measured using a light meter (LICOR, LI-250). F_v/F_m , maximal photosynthesis (P_{max}), the rate of dark respiration, chlorophyll content and zooxanthellae density were measured before and immediately after 2 h high light exposure.

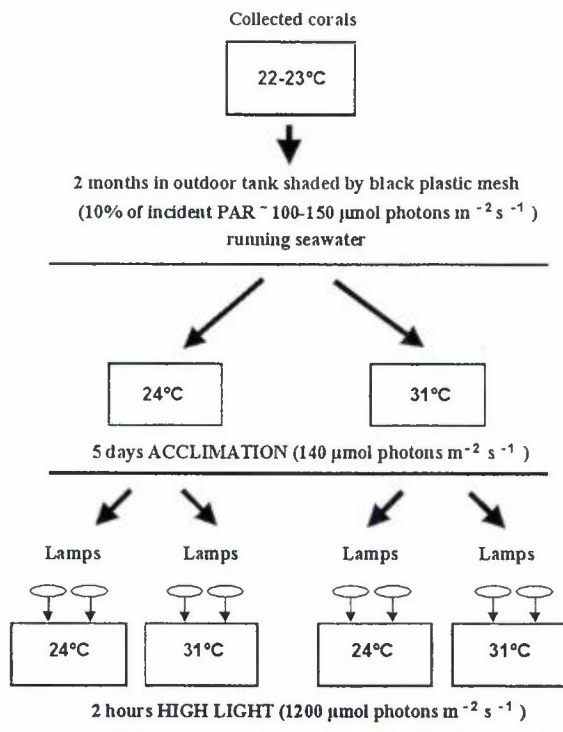


Figure 1. Schematic diagram of the experimental protocol. Coral fragments were acclimated at 24 or 31°C for 5 d. Then they were exposed to high light (1,200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 2 h. Chlorophyll fluorescence (F_v/F_m), maximal photosynthetic activity (P_{max}), the rate of dark respiration (R), zooxanthellae density and chlorophyll content were measured before and after high light treatment.

Chlorophyll a fluorescence measurements

Chlorophyll fluorescence was measured using a pulse-amplitude-modulation fluorometer (MINI-PAM, Waltz, Germany). Coral fragments were taken from the experimental chamber or aquarium, placed in a custom-made black box with filtered (0.45 μm) seawater and dark adapted for 20 min. The initial fluorescence (F_0) was measured by exposing the coral to weak red light (<1 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Maximum fluorescence (F_m) was determined by applying a 0.8 s saturation pulse of intense white light (8,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The ratio of variable (F_v) to maximum fluorescence (F_m), F_v/F_m , was used as an indicator of the maximum potential quantum yield. The dark adapted quantum yield (F_v/F_m , Schreiber et al., 1994), provides a good approximation of the maximum photochemical efficiency of photosystem II (PSII) (Oquist et al., 1992). The measurements of chlorophyll fluorescence were done at the respective treatment temperatures, 24 and 31°C.

Oxygen flux measurements

Coral fragments were placed into a transparent chamber (37.5 ml) fitted with a Clark-type electrode (Oxi 197S, Germany) and a magnetic stirrer. The chamber containing filtered (0.45 μm) seawater (FSW) was kept in the water baths whose temperature was regulated at corresponded temperatures of specific treatment, 24 or 31°C. A slide projector halogen lamp was used as the light source. Fragments were exposed for 30 min to an initial dark respiration period, and then photosynthesis light response curve (P versus I curve) was recorded by incubating coral fragments at different light intensities for 5-30 min each. Light intensity was varied between 30 to 1,300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ by using neutral density filters. Oxygen levels under experimental conditions were never below 80% of saturation.

This procedure was used for fragments before (initial controls) and after 2h high light treatment. The hyperbolic tangent function [$P_{\text{net}} = P_{\text{max}} \tanh(\pm\alpha I/P_{\text{max}})$] was used to analyze the P versus I curves (Jassby and Platt, 1976). Curve fitting provided estimates of the maximum photosynthetic activity, P_{max} , and the minimum irradiance required to saturate photosynthesis, I_k . Dark respiration was added to net photosynthesis to calculate gross primary production, which was then standardized to surface area of the samples. The surface area of coral fragments was determined by the paraffin-wax technique (Stimson and Kinzie, 1991).

Determination of chlorophyll content and zooxanthellae density

Coral fragments were rinsed gently with FSW and then coral tissue was removed from the coral skeleton by Water-Pick with FSW (Johannes and Wiebe, 1970). The coral blastate (70-120 ml) was then homogenized with a potter homogenizer and a 30 ml aliquot was filtered under vacuum through a Whatman GF/C glass fiber filter (47 mm in diameter). The glass fiber filters with adsorbed zooxanthellae were immersed in an aqueous solution of 90% acetone and placed in a refrigerator for two days. The solution with sample was shaken daily. The absorbance of acetone extracts was measured at 630, 663 and 750 nm using a Hitachi U-2001 spectrophotometer. The concentrations of chlorophyll a and c₂ were determined using the equations of Jeffrey and Humphrey (1975).

For measurements of zooxanthellae density, 30 ml of the homogenate was centrifuged at 3,000 g for 15 min. The pellet was re-suspended and the volume was adjusted to 1 ml. Counting was made on eight 1 mm² squares on a hemocytometer for each sample. Only healthy-looking zooxanthellae (Titlyanov et al., 1996) were counted. Microscopic observations were made under 400 \times magnification using a Nikon OPTIPHOT-2 microscope. Zooxanthellae

density was expressed as number of cells per unit surface area. The density and chlorophyll content of zooxanthellae were measured for four coral fragments from the four respective colonies in each treatment.

Statistical analyses

The absolute values and relative data of F_v/F_m , P_{max} , the rate of dark respiration (R) and total chlorophyll content were arcsine transformed prior to analysis of variances (ANOVA). The transformed absolute values of F_v/F_m , P_{max} , R and chlorophyll content were used for comparisons among initial and after stress data for each species. The transformed relative data of F_v/F_m , P_{max} , R and chlorophyll content were used to compare between species and among treatments. The Post-hoc Tukey Honest Significance Differences (HSD) test was employed for multiple comparisons of means at $P < 0.05$.

3. Results

Effects of acclimation temperatures on physiological parameters

Fragments of *Stylophora pistillata*, *Montipora digitata*, *Pachyseris rugosa* and *Pavona divaricata* acclimated at 24°C under low light for 5 d did not show any significant change in either chlorophyll content, zooxanthellae density, photochemical efficiency (F_v/F_m), maximal photosynthetic activity (P_{max}), maximum irradiance required to saturate photosynthesis (I_k), or dark respiration (R) compared with those acclimated in an outdoor tank for two months at 22–23°C. However, fragments acclimated at 31°C showed significant changes in some parameters compared with those acclimated at 24°C (Table 1).

Fragments of bleaching susceptible species *S. pistillata* and *M. digitata* acclimated at 31°C had significantly higher P_{max} and total chlorophyll content, and became irradiance saturated at light levels half of those acclimated at 24°C, while fragments of bleaching-tolerant species *P. rugosa* and *P. divaricata* acclimated at 31°C had slightly but significantly lower P_{max} , total chlorophyll content and the threshold for irradiance saturated photosynthesis than those acclimated at 24°C.

The rate of dark respiration (R) was affected by acclimation temperature in all investigated coral species and was significantly higher in fragments acclimated at 31°C than those acclimated at 24°C. The F_v/F_m and zooxanthellae density values did not differ in either corals between those acclimated at 24 and 31°C.

Table 1. Physiological parameters in four reef-building corals after 5 d acclimation at 24 and 31°C under 140 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Mean \pm SD (n=4). Asterisks represent values significantly different from 24°C-acclimated corals (ANOVA, *P<0.05, **P<0.01, ***P<0.001).

Species/Parameter	24°C	31°C
<i>Stylophora pistillata</i>		
F _v /F _m	0.68 \pm 0.02	0.71 \pm 0.015
Gross P _{max} ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	1.26 \pm 0.06	2.19 \pm 0.30**
Dark respiration ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	-0.41 \pm 0.01	-1.05 \pm 0.23**
Chl (a+c2) (pg cell ⁻¹)	14.42 \pm 0.27	18.07 \pm 0.67**
Zooxanthellae density (10 ⁶ cm ⁻²)	0.83 \pm 0.17	0.94 \pm 0.10
I _k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	191 \pm 5	108 \pm 10***
<i>Montipora digitata</i>		
F _v /F _m	0.57 \pm 0.02	0.51 \pm 0.06
Gross P _{max} ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	1.04 \pm 0.02	1.57 \pm 0.06***
Dark respiration ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	-0.31 \pm 0.05	-0.59 \pm 0.04*
Chl (a+c2) (pg cell ⁻¹)	6.25 \pm 0.01	11.01 \pm 0.02**
Zooxanthellae density (10 ⁶ cm ⁻²)	1.035 \pm 0.095	0.836 \pm 0.105
I _k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	310 \pm 7	118 \pm 4***
<i>Pachyseris rugosa</i>		
F _v /F _m	0.53 \pm 0.05	0.51 \pm 0.05
Gross P _{max} ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	1.95 \pm 0.05	1.80 \pm 0.06*
Dark respiration ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	-0.81 \pm 0.05	-1.02 \pm 0.03*
Chl (a+c2) (pg cell ⁻¹)	6.68 \pm 1.52	3.99 \pm 0.69*
Zooxanthellae density (10 ⁶ cm ⁻²)	1.27 \pm 0.21	1.12 \pm 0.06
I _k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	330 \pm 8	390 \pm 5*
<i>Pavona divaricata</i>		
F _v /F _m	0.66 \pm 0.03	0.61 \pm 0.02
Gross P _{max} ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	1.76 \pm 0.07	1.61 \pm 0.02*
Dark respiration ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$)	-0.61 \pm 0.05	-0.71 \pm 0.03*
Chl (a+c2) (pg cell ⁻¹)	17.52 \pm 1.20	10.38 \pm 1.91**
Zooxanthellae density (10 ⁶ cm ⁻²)	1.82 \pm 0.06	1.77 \pm 0.08
I _k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	425 \pm 10	466 \pm 5*

Changes in dark-adapted F_v/F_m after high light exposure

Values of F_v/F_m, P_{max}, R, chlorophyll content and zooxanthellae density after high light exposure were compared with those of initial measurements before stress treatment.

The 2 h high light ($1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) exposure induced a significant reduction in photochemical efficiency, F_v/F_m , in all the investigated coral species. Temperature during high light exposure did not affect the extent of reduction in F_v/F_m in both bleaching-tolerant and susceptible coral species independently of their temperature acclimation conditions (Fig. 2).

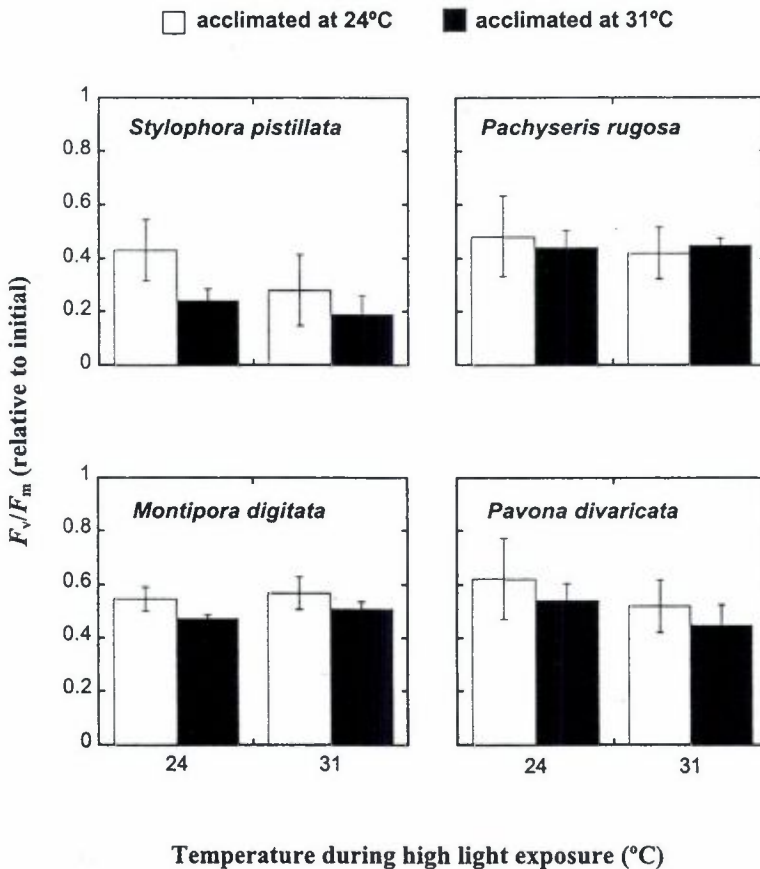


Figure 2. Effects of temperature during high light exposure ($1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, for 2 h) on the photochemical efficiency of PSII (F_v/F_m) in zooxanthellae of four scleractinian corals preacclimated at 24 and 31°C. The relative F_v/F_m , which was normalized to initial values measured with the same fragments before high light exposure, is shown. Means \pm SD ($n=4$).

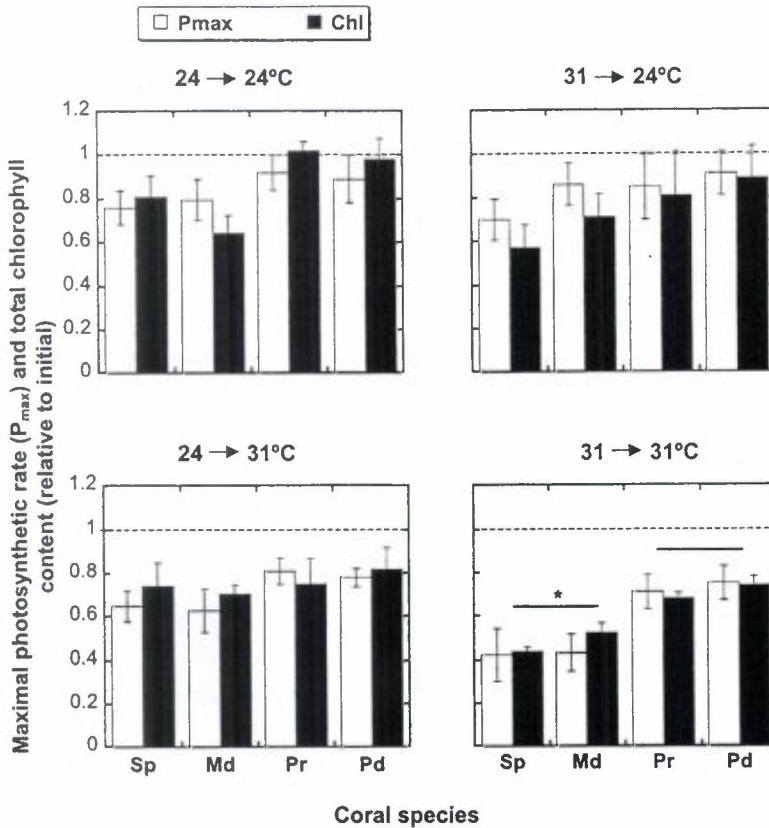


Figure 3. Effects of temperature during high light exposure ($1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, for 2 h) on the maximal photosynthetic rate (P_{max}) and total chlorophyll content in zooxanthellae of four scleractinian corals preacclimated at 24 and 31°C. The relative P_{max} which was normalized to initial values measured with the same fragments before high light exposure and relative chlorophyll content which was normalized to values of control fragments that experienced the same temperature treatment without high light exposure, are shown. Sp - *Stylophora pistillata*; Md - *Montipora digitata*; Pr - *Pachyseris rugosa*; Pd - *Pavona divaricata*. Means \pm SD ($n=4$). Asterisk represents significant difference between species (Tukey-Kramer HSD test, $p < 0.05$).

Changes in P_{max} , chlorophyll content and zooxanthellae density after high light exposure

The P_{max} and total chlorophyll content decreased significantly after 2 h high light exposure in all species except *Pachyseris rugosa* and *Pavona divaricata* exposed to high light at 24°C independently of their temperature pre-exposure regimes (Fig. 3). There were no significant differences in the

reduction of P_{\max} and chlorophyll content between investigated species acclimated at different temperatures and exposed to high light at 24°C. The extent of reduction in these parameters differed significantly (ANOVA, $p < 0.05$) only between coral species acclimated at 31°C and exposed to high light at 31°C. The decline in P_{\max} and chlorophyll content was significantly higher in *Stylophora pistillata* and *Montipora digitata* than in *P. rugosa* and *P. divaricata*. They did not differ significantly (ANOVA, $p < 0.05$) between these species acclimated at 24°C (Fig. 3). Exposure to high light at 31°C revealed significant (ANOVA, $p < 0.05$) differences in the reduction of P_{\max} and chlorophyll content between fragments of bleaching-susceptible coral species acclimated at 24 and 31°C (Table 2). Fragments of *S. pistillata* and *M. digitata* acclimated at 31°C showed a more pronounced decline in these parameters when compared to those acclimated at 24°C (Fig. 3). The extent of reduction in P_{\max} and chlorophyll content did not differ significantly (ANOVA, $p < 0.05$) between bleaching-tolerant coral species acclimated at 24 and 31°C (Table 2).

There were no significant (t-test, $p < 0.05$) differences in zooxanthellae density before and after high light exposure in all coral species investigated (data not shown).

Changes in dark respiration(R) after high light exposure

A 2 h exposure to high light at 24°C showed no significant effect (t-test, $p < 0.05$) on the R rate of investigated corals independently of their acclimation temperature regimes (Fig. 4). Exposure to high light at 31°C revealed significant (ANOVA, $p < 0.05$) differences in the level of R between coral species. After 2 h light exposure, fragments of *Stylophora pistillata* and *Montipora digitata* acclimated at 24 and 31°C showed a significant (t-test, $p < 0.05$) increase and decrease in the R rate, respectively, when compared to the initial values. In contrast, *Pachyseris rugosa* and *Pavona divaricata* exposed to high light at 31°C did not show any significant (t-test, $p < 0.05$) deviations in this parameter before and after stress independently of acclimation temperature.

4. Discussion

The results of our experiments clearly demonstrated differences in photo-physiological response to pre-exposure to high temperature between bleaching-susceptible corals, *Stylophora pistillata* and *Montipora digitata*, and tolerant corals, *Pachyseris rugosa* and *Pavona divaricata*. *S. pistillata* and *M. digitata* showed a significant increase in the maximal photosynthetic activity (P_{\max}) in response to high temperature acclimation. These changes are similar to those

Table 2. Summary of statistical results of comparisons of the reduction in maximal photosynthetic activity (P_{max}) and total chlorophyll content after 2 h high light exposure at different temperatures among coral fragments acclimated at 24 and 31°C (Tukey HSD test). ** $P < 0.01$, *** $P < 0.001$, NS - no significant difference.

	24->24°C		31->24°C		24->31°C	
	P_{max}	Chl	P_{max}	Chl	P_{max}	Chl
<i>Stylophora pistillata</i>						
31->24°C	NS	NS				
24->31°C	NS	NS	NS	NS		
31->31°C	***	**	***	NS	***	**
<i>Montipora digitata</i>						
31->24°C	NS	NS				
24->31°C	NS	NS	NS	NS		
31->31°C	***	***	***	**	***	**
<i>Pachyseris rugosa</i>						
31->24°C	NS	NS				
24->31°C	NS	**	NS	NS		
31->31°C	**	**	NS	NS	NS	NS
<i>Pavona divaricata</i>						
31->24°C	NS	NS				
24->31°C	NS	NS	NS	NS		
31->31°C	**	***	NS	NS	NS	NS

Note. 24->24°C fragments acclimated at 24°C and exposed to high light at 24°C; 31->24°C fragments acclimated at 31°C and exposed to high light at 24°C; 24->31°C fragments acclimated at 24°C and exposed to high light at 31°C; 31->31°C fragments acclimated at 31°C and exposed to high light at 31°C.

found in a number of symbiotic cnidarians (Coles and Jokiel, 1977; Jacques et al., 1983; Hoegh-Guldberg and Smith, 1989) as well as in phytoplankton (Li and Morris, 1982; Schofield et al., 1998) and macroalgal species (Davison, 1990) after acclimation to high temperature. The increased P_{max} might be related to the temperature-dependent nature of carbon metabolism and explained by either increased activity of enzymes of the reductive pentose cycle (Li et al., 1984) or increased chlorophyll content (Table 1), an indication of the increase in absorption cross section areas of photosynthetic reaction centers (Weykam and Wiencke, 1996), or both.

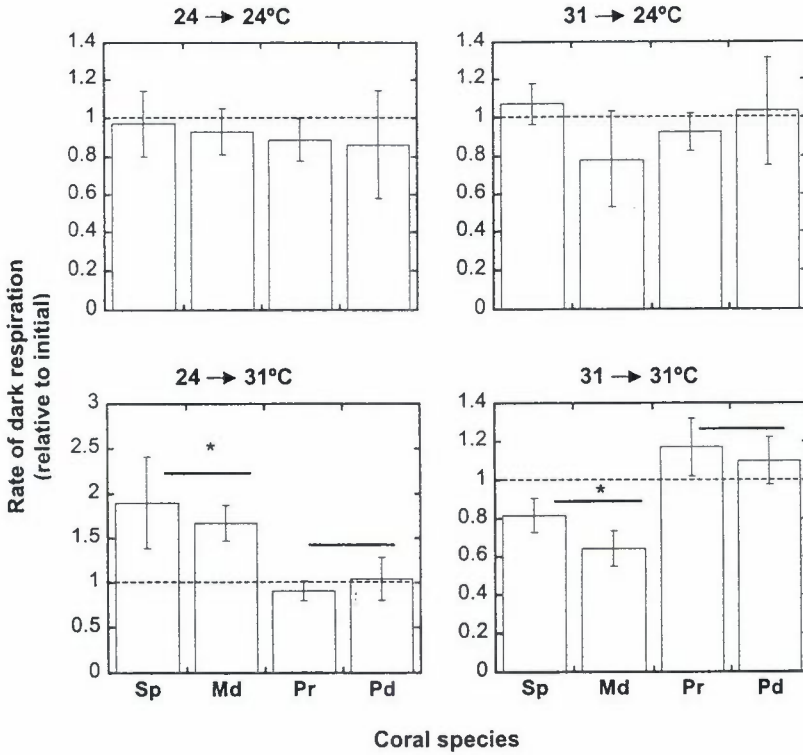


Figure 4. Effects of temperature during high light exposure ($1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, for 2 h) on the rate of dark respiration (R) in coral-algal association of four scleractinian corals preacclimated at 24 and 31°C. The relative R, which was normalized to initial values measured in the same fragments before high light exposure, is shown. Means \pm SD (n=4). Asterisks represent significant difference between species response (Tukey-Kramer HSD test, $p < 0.05$).

In contrast, *P. rugosa* and *P. divaricata* showed the opposite trend, acclimation at high temperature caused slight but significant decrease in P_{max} . Reduced photosynthetic performance in these coral species cannot be ascribed to irreversible heat inactivation of the photosynthetic apparatus of their endosymbionts because zooxanthellae exhibited high photosynthetic activity when returned to 24°C (data not shown) and the zooxanthellae density did not decrease in both species after 5 d acclimation at 31°C (Table 1). Similar decrease in the photosynthetic performance was observed in the experiments on terrestrial plants during different thermal preacclimation (Yamasaki et al., 2002) and explained by simultaneous increase in the rate of dark respiration

under the high temperature conditions. The results presented here also show that increase in the respiration rate occurs in both *P. divaricata* and *P. rugosa* acclimated at 31°C (Table 1).

In contrast, there were no significant differences in the photochemical efficiency of PSII, F_v/F_m , between fragments acclimated at 24 and 31°C for the none of the investigated coral species. Furthermore, the decrease in F_v/F_m under high light exposure was similar for corals pre-exposed at 31 and 24°C, with no significant differences in the high light reduction for all the investigated species. Down regulation of F_v/F_m is related to the redox state of the plastoquinone pool (Krause and Weis, 1991), which is, in turn, a function of coordinated activity between PSI and PSII (Allen et al., 1981). Thus, coral responses induced to minimize damage of PSII under high light were not sensitive to temperature.

Our results are consistent with observations made on higher plants (Osmond, 1981) where utilization of trapped light energy was temperature dependent in such process as electron transport and carbon metabolism, while the harvest and trapping of light energy was essentially temperature independent. Clearly, dramatic adjustments must occur within the photosynthetic apparatus if it is to maintain a balance in the flux of light energy through the system. A critical problem for corals at elevated temperatures is the resultant increased susceptibility to damage by high irradiance (Brown, 1997; Hoegh-Guldberg, 1999). Any acclimation mechanism which would reduce light trapping capacity (decrease in antenna size = chlorophyll content) and/or diminish energy consuming carbon turnover would appear to provide greater protection of the photosynthetic apparatus of zooxanthellae during sudden changes in the light and/or temperature environment and obviously reduce potential damage to symbiosis.

The results presented here show that both types of adjustment occur in zooxanthellae of bleaching-tolerant species, *Pachyseris rugosa* and *Pavona divaricata*, during the pre-exposure to high temperature. Both species showed a substantial decrease in the chlorophyll content (light harvesting efficiency) and demonstrated a marked decrease in the photosynthetic activity of their zooxanthellae. Moreover, photosynthesis of fragments of *P. rugosa* and *P. divaricata* acclimated at 31°C became saturated at higher irradiance levels than those acclimated at 24°C (Table 1). Although high light exposure at 31°C resulted in a significant decline in both the P_{max} and chlorophyll content values of *P. rugosa* and *P. divaricata*, there were no significant differences in the high light reduction of these parameters between fragments acclimated at 24 and 31°C. The threshold at which massive inhibition of photosynthetic processes occurs has not yet been reached in the tissue of *P. rugosa* and *P. divaricata* with the stress temperature treatment used here.

In contrast, the increase in the total chlorophyll content in bleaching-susceptible species, *Stylophora pistillata* and *Montipora digitata*, after pre-exposure to high temperature, would enhance light trapping capacity of these species. This acclimatory response together with the decreased threshold for irradiance saturated photosynthesis (I_k) and enhanced photosynthetic activity increases a vulnerability of these corals to photoinhibition during exposure to high light. Our results clearly show that after high light treatment at both 24 and 31°C, the extent of reduction in P_{max} and chlorophyll content was significantly (ANOVA, $p < 0.05$) higher in fragments of *S. pistillata* and *M. digitata* acclimated at 31°C compared to those acclimated at 24°C. Thus, we conclude that acclimation at high temperature made fragments of *S. pistillata* and *M. digitata* more susceptible to photoinhibition as compared with fragments acclimated at low temperature.

Furthermore, the decline in both the P_{max} and chlorophyll content values of *P. rugosa* and *P. divaricata* after exposure to high light at 31°C was significantly less pronounced when compared to *S. pistillata* and *M. digitata*, irrespective of temperature pre-exposure. These results indicate not only acclimatory ability but also the remarkable tolerance of fragments of *P. rugosa* and *P. divaricata* acclimated at 31°C compared to similar fragments of *S. pistillata* and *M. digitata* to a fluctuating temperature regime. Thus, mechanisms underlying the thermal acclimation of photosynthesis vary with coral species, and a high photosynthetic acclimation potential to temperature is associated with a response of photosynthetic activity, P_{max} , of in hospite zooxanthellae to temperature.

The present results also suggest that zooxanthellae of *Stylophora pistillata* and *Montipora digitata* are less well adaptive to increase in temperature than those of *Pachyseris rugosa* and *Pavona divaricata*. Symbiotic dinoflagellates are known to photo-acclimate to changes in photon flux density, with respect to PAR using different mechanisms (Chang et al., 1983; Iglesias-Prieto and Trench, 1994; Brown et al., 2002) despite being cultured under the same conditions. An important conclusion from these works was that the different photo-acclimatory capabilities of the algae might be under genetic constraints. We also do not exclude that differences in acclimatory responses to high temperature between zooxanthellae of bleaching-susceptible and tolerant coral species may well be reflection of the differences in the genetically based adaptive capabilities of symbionts, which in turn may explain why under impact of the same stress, some associations are less tolerant than others.

The role of host tissue in the maintenance of the intact coral symbiosis under temperature stress also cannot be ruled out. All available direct evidence indicates that coral bleaching at elevated temperatures is correlated with the changes in dark respiration (R) rate (Jokiel and Coles, 1990). In the present study, bleaching-tolerant and susceptible coral species showed different

response to high light exposure at high temperature in terms of this parameter. *Pachyseris rugosa* and *Pavona divaricata* did not show any significant (t -test, $p < 0.05$) deviations in R rate before and after exposure to high light at 31°C irrespective of acclimation temperature, whereas *Stylophora pistillata* and *Montipora digitata*, maintained at the same conditions, demonstrated significant changes in the R values. The lack of any effects on respiration rates in bleaching-tolerant species suggests that the respiratory processes responsible for oxygen consumption did not sustain any appreciable impairment during the two hours of high light exposure.

In contrast, significant increase in the R rate of fragments of bleaching-susceptible species acclimated at 24°C is a well known response of corals to increasing temperature (Jokiel and Coles, 1990) and may be due to changes in the activity of rate-limiting respiratory enzymes (Davison and Davison, 1987), while the reduction in the R rate of fragments acclimated at 31°C points out the beginning of injury which is generally attributed to the heat sensitivity of enzymes (Kanwisher, 1966). This reduction in the R rate might suggest that host metabolism was also affected and influenced the susceptibility of corals to combined effect of temperature and light stress.

In summary, three major points arise from this study. First, it has been shown that zooxanthellae of bleaching-susceptible and tolerant corals employ different processes in acclimation to high temperature. Secondly, pre-exposure to high temperature affects the maximum rate of photosynthetic activity (P_{max}) of symbionts but not the photochemical efficiency of PSII (F_v/F_m). Lastly, it is suggested that susceptibility of *Stylophora pistillata* and *Montipora digitata* to synergistic effects of high light and elevated temperature depends on a decreased capacity of their zooxanthellae to cope with photodamage, although the role of the host can not be excluded.

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