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Differential recovery of PSII function and electron transport rate in symbiotic dinoflagellates as a possible determinant of bleaching susceptibility of corals

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ABSTRACT: Differences in the time course of recovery of the photochemical efficiency (F_v/F_m) of PSII and the photosynthetic capacity (relative maximum electron transport rate; $rETR_{max}$), of symbiotic dinoflagellates from damage due to thermal and light stresses might have profound effects on the subsequent photodamage of photosynthetic apparatus. The reduction and recovery of F_v/F_m and $rETR_{max}$ in symbiotic dinoflagellates were investigated at different temperatures (25, 28 and 31°C) after 3 h exposure to high light (1100 µmol photons m^{-2} s^{-1} of photosynthetically active radiation [PAR]) in 2 corals with different bleaching susceptibility, *Pavona divaricata* and *Montipora digitata*. There was a marked difference in both the reduction in $rETR_{max}$ after high light exposure and the time course of its recovery between *P. divaricata* and *M. digitata*. In *P. divaricata*, the reduction in F_v/F_m and $rETR_{max}$ during high light exposure was independent of temperature, and their recovery was almost complete within 24 h after stress treatment at all temperatures examined. In *M. digitata*, while the reduction and recovery of F_v/F_m did not show temperature dependency, $rETR_{max}$ exhibited a greater decrease and lower recovery at 31°C than at 25 and 28°C. The recovery of $rETR_{max}$ was slower than that of F_v/F_m in symbiotic dinoflagellates of *M. digitata*, and this delay of $rETR_{max}$ recovery increased with increasing temperature during the recovery period. Significant decreases in dinoflagellate density and chlorophyll content were evident only in the 31°C-treated fragments of *M. digitata* kept at 31°C during the recovery period. These results indicate that it is the difference in the rate of recovery between the PSII function and the dark reaction of symbiotic dinoflagellates that determines whether the photosynthetic ability of algae recovers from the combined effect of thermal and irradiance stresses. The delayed recovery of $rETR_{max}$ at high temperature made fragments of *M. digitata* susceptible to photodamage even under moderate light conditions. *P. divaricata* showed a high ability to recover its $rETR_{max}$ and consequently was resistant to high light and thermal stresses. It is likely that high temperature affects the rate of recovery of the carbon-fixation cycle and that the ability of symbiotic dinoflagellates to repair damage determines susceptibility of corals to bleaching.

KEY WORDS: Bleaching · Coral · Symbiotic dinoflagellate · PAM · Photosystem II · ETR

INTRODUCTION

Coral bleaching, discoloration of corals due to loss of symbiotic algae (dinoflagellates) and/or algal pigments, can be caused by a variety of stresses, but large-scale bleaching events are generally ascribed to thermal stress and solar radiation (Brown 1997, Hoegh-Guldberg 1999, Fitt & Cook 2001). A slight increase in seawater temperature (1–2°C) above normal summer maxima can induce bleaching of corals (e.g. Glynn 1993, Brown 1997, Hoegh-Guldberg 1999). High temperature is considered to make algal symbionts more sensitive to photoinhibition of photosynthesis by solar radiation and resulting photodamage of algae leads to bleaching (Warner et al. 1996, 1999, Lesser 1997, Jones et al. 1998, 2000).

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Photoinhibition is classified as being either dynamic or chronic. Dynamic photoinhibition due to damage of the D1 protein, which is normally repaired within several hours, is considered as photoprotective, as the damaged reaction centers continue to trap excitation energy but dissipate it as heat (Krause 1988). Chronic photoinhibition involves irreversible photodamage to photosystem II (PSII) via degradation of the D1 protein and other PSII proteins (Krause 1988, Demmig-Adams & Adams 1992), where repair mechanisms may take many hours or days (Bose et al. 1988). Extended exposure of corals to high temperature and strong light causes long-term reduction in photochemical efficiency, F_v/F_m, resulting in its slow and incomplete recovery, and is associated with bleaching (Fitt & Warner 1995, Warner et al. 1996, 1999, Jones et al. 2000, Bhagooli & Hidaka 2002, Brown et al. 2002). A decline in the D1 reaction center protein in symbiotic dinoflagellates found in bleached corals has also been reported (Warner et al. 1999). These observations indicate that symbiotic dinoflagellates in naturally bleached corals are suffering from chronic photoinhibition. However, most laboratory studies on the effect of thermal stress in symbiotic dinoflagellates have typically been concentrated on rather short-term changes in the photochemical efficiency of PSII or light curve of photosynthesis (Iglesias-Prieto et al. 1992, Fitt & Warner 1995, Iglesias-Prieto 1997, Lesser 1997, Jones et al. 1998, Warner et al. 1999). There is a limited number of long-term studies on the recovery process of the photochemical efficiency of PSII (Jones et al. 2000, Saxby et al. 2003) in corals following combined thermal and light stress treatment, and the effects of temperature on the recovery of electron transport rate of symbiotic dinoflagellates or both parameters together are yet to be explored.

Thermal stress affects both light and dark reactions of photosynthesis in symbiotic algae (e.g. Warner et al. 1996, 1999, Igresias-Prieto 1997, Lesser 1997, Jones et al. 1998, 2000, Hoegh-Guldberg 1999). According to Warner et al. (1999), heat stress causes damage to PSII via degradation of the D1 protein because the rate of its degradation is greater than that of its re-synthesis at high temperature. On the other hand, based on Jones et al. (1998), Hoegh-Guldberg (1999) proposed a 'photoinhibition' model for coral bleaching. According to their model, the onset of coral bleaching begins by the impairment of carbon fixation in dinoflagellates, subsequent slowdown of the electron transport from PSII to the Calvin-Benson cycle, and hence, over-reduction of the light reactions under elevated temperature and high light. Thus, there are still debates on the primary site of action of thermal stress and whether damage of PSII or the carbon-fixation cycle is a determinant of coral bleaching (e.g. Hoegh-Guldberg 1999, Warner et al. 1999).

If the above 'photoinhibition' hypothesis of bleaching is correct, the relative recovery rates of PSII function and the carbon-fixation cycle would have significant effects on the photodamage of symbiotic dinoflagellates in corals ('differential recovery' hypothesis). If the recovery of the carbon-fixation cycle is slower than that of PSII, high-energy electrons generated by photochemical reaction in PSII cannot be fully consumed in the Calvin-Benson cycle even under subsaturation irradiance. The reduced photosynthetic electron transport combined with continued absorption of excitation energy may lead to production of active oxygen molecules, which cause damage or inactivation of photosynthetic components (including PSII) of symbiotic dinoflagellates. If the damage is severe and long lasting, corals will eventually bleach.

To test the differential recovery hypothesis, we studied the effects of temperature on the reduction and the rate of recovery of photochemical efficiency, F_v/F_m, of PSII and of the relative photosynthetic electron transport rate (photosynthetic capacity), rETR, in symbiotic dinoflagellates associated with a bleaching-susceptible coral, *Montipora digitata* (Loya et al. 2001), and a resistant coral, *Pavona divaricata* (Sakai pers. comm.), after 3 h exposure to high light. At the same time, dinoflagellate density and chlorophyll (Chl) content were measured to detect signs of bleaching in the corals during the experiments.

MATERIALS AND METHODS

Collection and maintenance of corals. Colonies of *Pavona divaricata* (Lamarck 1816) and *Montipora digitata* (Dana 1846) were collected from a depth of 0.2 to 0.5 m during low tide at Bise and the eastern reef of Sesoko Island, Okinawa, Japan. Colonies were kept in an outdoor tank supplied with running seawater for a few weeks until use. During this period, seawater temperature in the tank was 21–22°C and incident photosynthetically active radiation (PAR) did not exceed 1100 µmol photons m^{-2} 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 during January–February 2002. Branches about 3 cm in length were taken from colonies of *Montipora digitata*, and fragments about 4 cm^2 of tissue surface area were removed from *Pavona divaricata*. Four replicate colonies were used for each species. Fifty-one fragments were prepared from each coral colony, and mounted on glass slides using rubber bands. Fragments from 4 replicate colonies were divided into 3 groups (36, 68, and 100 fragments for the first, second, and third groups, respectively). Fragments of each

group were placed into each of 3 aquaria containing aerated seawater. Temperature in the aquarium for the first group was maintained at 25°C. The seawater temperature in the aquaria for the second and third groups was increased gradually (0.5 to 1°C d^{-1}) from 25°C up to 28 and 31°C, respectively, over a 5 d period (Fig. 1). The temperature was regulated using automatic thermostats (±0.5°C, IC Thermostat, EX-003) and aquarium heaters. The coral fragments were illuminated at a light intensity of 70 µmol photons m^{-2} s^{-1} with 3 white fluorescent lamps (National, FL20SS-N/18) during day time (12 h). Half of the seawater in the aquaria was replaced every third day with fresh seawater adjusted to respective temperatures during the experimental period.

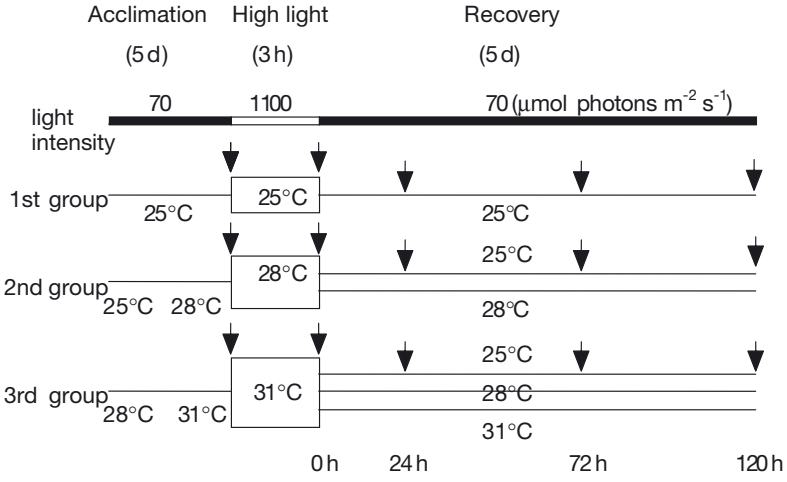

Fig. 1. Schematic of the experimental protocol. Three groups of coral fragments were acclimated to 25, 28 and 31°C during 5 d acclimation period. Then they were exposed to high light (1100 µmol photons m^{-2} s^{-1}) for 3 h. The fragments were allowed to recover at different temperatures. Chlorophyll fluorescence, photochemical efficiency (F_v/F_m) and the photosynthetic capacity ($rETR_{max}$), symbiotic dinoflagellate density and chlorophyll content were measured before and after high-light treatment and at 24, 72 and 120 h during the recovery period (arrows)

After a 5 d acclimation period, the initial measurements of chl *a* fluorescence parameters, Chl content and dinoflagellate density were performed with 4 fragments each from different colonies for both species. Half of the remaining fragments were placed in transparent plastic chambers (30 cm × 40 cm) containing aerated seawater and used as the experimental units. The other half of the fragments were left in the aquaria at the respective acclimation temperatures under low white light as the controls. The chambers with experimental fragments were put into one of the water baths whose temperature was regulated at 25, 28 and 31°C by temperature control units (EYELA, Thermister Tempet T-80). The experimental fragments were exposed to 1100 µmol photons m^{-2} s^{-1} of PAR (similar to those in the field in midday winter sunlight) for 3 h, as shown in Fig. 1. PAR treatment was carried out using two 500 W incandescent lamps (NIKKO). Visible irradiance was measured using a light meter (LICOR, LI-250). After 3 h exposure to high light, the samples were allowed to recover at different temperatures under low light (70 µmol photons m^{-2} s^{-1}). Fluorescence kinetics, Chl content and dinoflagellate density were measured before, immediately after light exposure and then after 24, 72 and 120 h of recovery approximately at the same time of the day (Fig. 1). Control fragments were exposed to the same temperature treatment as the experimental ones but were kept under low light (70 µmol photons m^{-2} s^{-1}) during the daytime throughout the experimental period.

Chl *a* fluorescence measurements. Chl fluorescence was measured using a pulse-amplitudemodulation fluorometer (MINI-PAM). Coral fragments were taken from the experimental chamber or aquarium and 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 µmol photons m^{-2} s^{-1}). Maximum fluorescence (F_m) was determined by applying a 0.8 s saturation pulse of intense white light (8000 µmol photons m^{-2} 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 PSII (Oquist et al. 1992). The F_v/F_m values of coral fragments before stress treatment ranged from 0.56 to 0.68 (Table 1), typical of those in corals experiencing no damage to PSII (e.g. Fitt et al. 2001). Immediately after F_v/F_m measurement, 5 min of 500 µmol photons m^{-2} s^{-1} (saturated light for photosynthesis of the species under investigation) from the Mini-PAM internal halogen lamp was applied to activate photosynthetic dark reactions followed by a 30 s dark period, and then rapid light curves were recorded: samples were irradiated with increasing irradiance (photon flux density, PFD = 100 to 1300 µmol photons m^{-2} s^{-1}). Every 20 s, a saturating pulse was applied to measure effective quantum yield of PSII ($\Delta F/F_m'$) before irradiation was further increased. Respective rETR were determined by multiplying the effective quantum yield with the respective photon irradiance (Schreiber et al. 1994). The rETR values given here are relative because we did not consider the fraction of light

Table 1. *Pavona divaricata* and *Montipora digitata*. Physiological parameters (means ± SD; n = 4) of symbiotic dinoflagellates after 5 d acclimation at 25, 28 and 31°C under 70 µmol photons m^{-2} s^{-1}. *Significantly different from 25°C-acclimated corals (Tukey-Kramer HSD test, $p < 0.05$)

	F_v/F_m	$rETR_{max}$ (µmol m^{-2} s^{-1})	Chl ($a+c_2$) (pg $cell^{-1}$)	Density (10^6 ind. cm^{-2})
Pavona divaricata				
31°C	0.62 ± 0.03	1.76 ± 0.19*	9.6 ± 1.1*	1.75 ± 0.13
28°C	0.65 ± 0.04	2.47 ± 0.19	16.7 ± 3.3	1.79 ± 0.12
25°C	0.68 ± 0.02	2.43 ± 0.36	16.0 ± 3.2	2.05 ± 0.15
Montipora digitata				
31°C	0.60 ± 0.03	1.82 ± 0.02*	13.0 ± 1.2*	1.01 ± 0.08*
28°C	0.62 ± 0.04	1.72 ± 0.06	9.8 ± 0.8	1.32 ± 0.11
25°C	0.58 ± 0.03	1.53 ± 0.09	9.9 ± 0.5	1.40 ± 0.07

absorbed by the photosynthetic pigments of the zooxanthellae within the coral tissue (see also Hoegh-Guldberg & Jones 1999, Winters et al. 2003) or the distribution of absorbed light between pigments associated with 2 photosystems. $rETR_{max}$ was calculated by regression analysis. Our preliminary experiments revealed that rETR and O_2 evolution showed a significantly linear correlation at irradiance up to 500–550 µmol photons m^{-2} s^{-1} of incident PAR in both coral species ($r^2 = 0.87$ and $r^2 = 0.90$ for *Montipora digitata* and *Pavona divaricata*, respectively). Thus, the $rETR_{max}$ values, which were usually attained at 470 µmol photons m^{-2} s^{-1} in the present study, represented a good measure of the maximal photosynthetic capacity of symbiotic dinoflagellates in the coral species investigated. The $rETR_{max}$ values were normalized to dinoflagellate density (10^6 cells) by dividing the ETR_{max} value by actual dinoflagellate cell number in each measured coral fragment, so that changes in photosynthetic capacity of an algal cell remaining in the host tissue could be compared among different stress treatments. The measurements of chlorophyll fluorescence were done at the treatment temperatures: 25, 28 and 31°C.

Determination of Chl content and symbiotic dinoflagellate density. Coral fragments were rinsed gently with filtered seawater (FSW), and then coral tissue was removed from the coral skeleton by water picking with FSW (Johannes & Wiebe 1970). The coral blastate (70 to 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 symbiotic algae were immersed in an aqueous solution of 90% acetone and placed in a refrigerator for 2 d. 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 chl *a* and c_2 were determined using the equations of Jeffrey & Humphrey (1975).

For measurements of dinoflagellate density, 30 ml of the homogenate was centrifuged at 1250 × *g* for 15 min. The pellet was re-suspended and the volume was adjusted to 1 ml. Counting was performed on eight 1 mm^2 squares on a hemocytometer for each sample. Only healthy-looking symbiotic dinoflagellates (Titlyanov et al. 1996) were counted. Microscopic observations were made under 400× magnification using a Nikon OPTIPHOT-2 microscope. The surface area of coral fragments was determined by the paraffin-wax technique (Stimson & Kinzie 1991). Symbiotic algal density was expressed as a number of algae per unit surface area. The symbiotic dinoflagellate density and Chl content were measured for 4 coral fragments from the 4 respective colonies in each treatment.

Statistical analyses. Differences between mean values of experimental and control fragments and the comparisons of F_v/F_m and $rETR_{max}$ recoveries at different temperatures or at different times after high light stress were analyzed using a *t*-test. The effects of temperature on F_v/F_m, $rETR_{max}$, dinoflagellate density and Chl content after high light exposure and during the recovery period were assessed by ANOVA. When ANOVA showed significant differences, the Tukey-Kramer HSD test was performed for multiple comparisons among means. Arcsine transformation was applied to proportional data prior to ANOVA. The amount of influence of temperature was analyzed with the Snedekor function (SF, Snedecor 1961), and the significance of values was evaluated by the Fisher test at the $p = 0.05$ level. The effects of temperature and high light on fluorescence kinetics were statistically analyzed using relative F_v/F_m and relative $rETR_{max}$, which were normalized to the values of controls that experienced the same temperature treatment without high light exposure.

RESULTS

Effects of acclimation temperatures on physiological parameters

Coral fragments of *Pavona divaricata* and *Montipora digitata* acclimated at 25 and 28°C under low light for 5 d did not show any significant change in either Chl content, symbiotic dinoflagellate density, F_v/F_m, or $rETR_{max}$. However, those acclimated at 31°C showed significant changes in some parameters (Table 1). Fragments of *M. digitata* acclimated at 31°C had slightly but significantly higher $rETR_{max}$ and total Chl

content than those acclimated at 25°C, while *P. divaricata* fragments acclimated at 31°C had significantly lower $rETR_{max}$ and total Chl content than those acclimated at 25°C. The dinoflagellate density was significantly lower only in *M. digitata* samples acclimated at 31°C. The F_v/F_m values did not differ in either corals among the thermal acclimation conditions. Hereafter values of F_v/F_m, $rETR_{max}$, symbiotic dinoflagellate density and Chl content after stress or after recovery are compared with those of non-exposed controls that were exposed to the same temperature treatment but without 3 h high light exposure.

Changes in dark-adapted F_v/F_m and $rETR_{max}$ after high light exposure

Photoinhibition

The 3 h high light (1100 µmol photons m^{-2} s^{-1}) exposure induced a significant reduction in F_v/F_m in both *Pavona divaricata* and *Montipora digitata*. Temperature during high light exposure did not affect the extent of reduction in F_v/F_m in both species (Fig. 2). $rETR_{max}$ also decreased significantly after 3 h high light exposure in both species except *P. divaricata* exposed to high light at 25°C (Fig. 2). The reduction in $rETR_{max}$ differed significantly (ANOVA, $p < 0.01$) between temperature treatments in *M. digitata*, while it was not affected by temperature during high light exposure in *P. divaricata* (Fig. 2). In symbiotic dinoflagellates of *M. digitata*, the $rETR_{max}$ decreased only to 70% of non-exposed control at 25 and 28°C, while it decreased to 40% at 31°C.

Recovery under different temperature conditions

The time course of recovery of F_v/F_m and $rETR_{max}$ after 3 h exposure to high light was investigated by comparing the F_v/F_m and $rETR_{max}$ values with those of non-exposed controls. If the F_v/F_m and $rETR_{max}$ values returned to the level that was not significantly different from those of controls, the recovery was considered to be complete. Control coral fragments, which were maintained at the respective temperatures but without exposure to high light, showed no change (*t*-test, $p < 0.05$) in F_v/F_m or $rETR_{max}$ during the experimental period.

In *Pavona divaricata*, no significant difference between the extent of recovery of F_v/F_m and that of $rETR_{max}$ was detected throughout the recovery period (Table 2). Both F_v/F_m and $rETR_{max}$ recovered completely in 24 h after high light exposure in all cases but one (Fig. 3). Only in fragments that were kept at 31°C

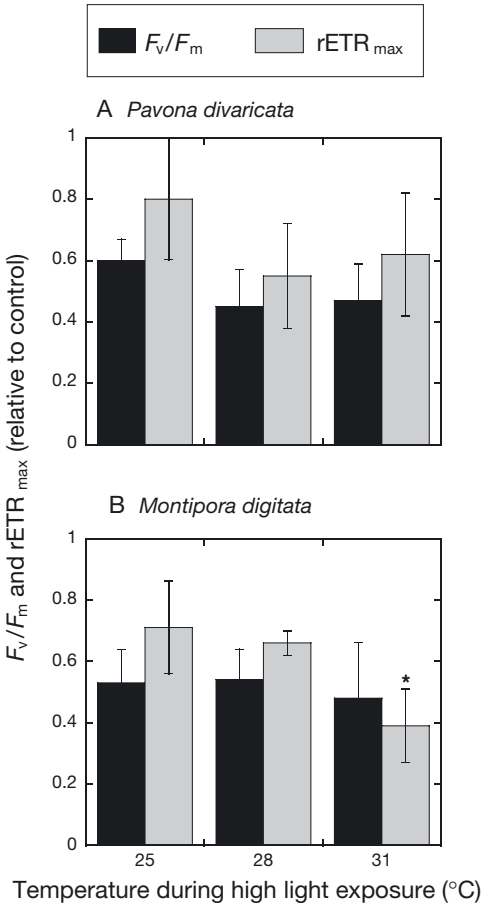

Fig. 2. Effects of temperature during high light exposure on F_v/F_m and $rETR_{max}$ in symbiotic dinoflagellates of (A) *Pavona divaricata* and (B) *Montipora digitata*. $rETR_{max}$ values were normalized to 10^6 dinoflagellate cells. Relative F_v/F_m and relative $rETR_{max}$, both of which were normalized to values of control fragments that experienced the same temperature treatment without high light exposure, are shown. Means ± SD (n = 4). *Value significantly different from those at other temperatures (Tukey multiple comparison test, $p < 0.05$)

during both high light exposure and recovery period were F_v/F_m values slightly but significantly ($p < 0.05$) less than those in the non-exposed control 24 h after the stress treatment. ANOVA showed that temperature during the recovery period did not have significant effect (ANOVA, $F_{F_v/F_m} = 0.11$, $F_{rETRmax} = 2.16$, $p > 0.05$) on both F_v/F_m and $rETR_{max}$ in *P. divaricata*.

In *Montipora digitata*, significant difference in the extent of recovery was observed between F_v/F_m and $rETR_{max}$ at least at one sampling time during the recovery period except fragments exposed to high light at 25°C (Table 2). The F_v/F_m recovered fully within 24 h after the stress treatment if the fragments exposed to high light were allowed to recover at 25 or 28°C (Fig. 4A,C). When the fragments exposed to high light at 31°C were allowed to recover at 31°C, complete

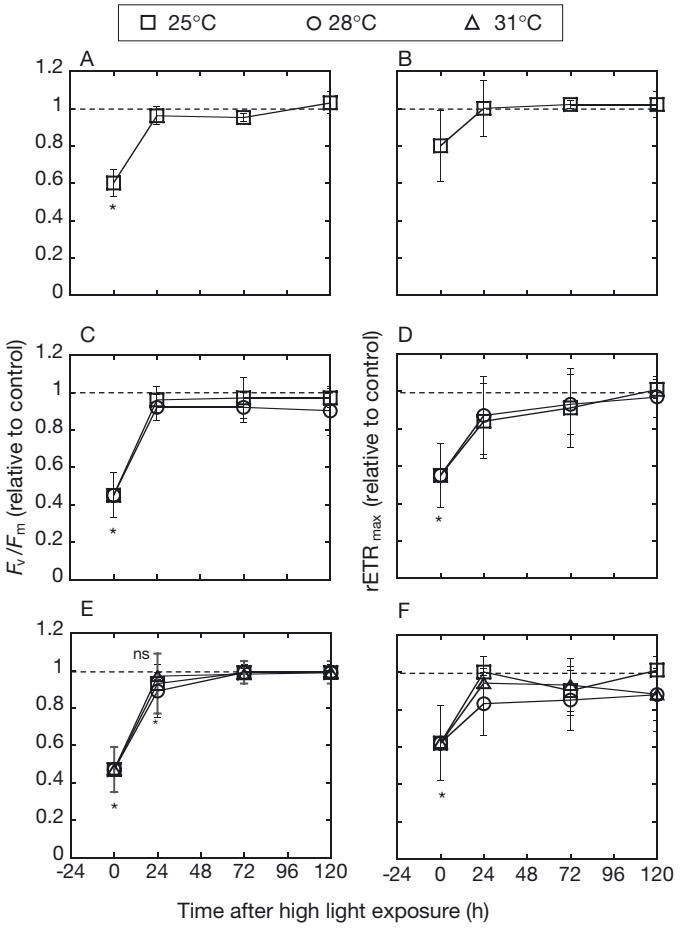

Fig. 3. Time course of recovery in F_v/F_m and $rETR_{max}$ in symbiotic dinoflagellates of *Pavona divaricata* exposed to high light (1100 µmol photons m^{-2} s^{-1}) for 3 h. The $rETR_{max}$ values were normalized to 10^6 dinoflagellate cells. The relative F_v/F_m and relative $rETR_{max}$, both of which were normalized to values of control fragments that experienced the same temperature treatment without high light exposure, are shown. Coral fragments were exposed to high light at (A,B) 25, (C,D) 28, and (E,F) 31°C. Fragments were allowed to recover at 25, 28, and 31°C. Means ± SD (n = 4). *Values significantly different from controls (*t*-test, $p < 0.05$). ns: not significant

recovery of F_v/F_m was observed only in 72 h after the stress treatment (Fig. 4E). In contrast, recovery of $rETR_{max}$ was incomplete in 24 h in fragments exposed to high light at 28 or 31°C (Fig. 4D,F). Complete recovery of $rETR_{max}$ was attained after 72 h in corals exposed to high light at 28°C. In the 31°C-treated fragments, it took 72 and 120 h for $rETR_{max}$ to recover completely at 25 and 28°C, respectively, whereas recovery was incomplete at 31°C recovery temperature even after 120 h in low light. Thus, the delay in the recovery of $rETR_{max}$ increased with increasing temperature during the recovery period. Temperature during the recovery period had a significant effect on the recovery of $rETR_{max}$ only in the 31°C-treated fragments of *M. digitata* (ANOVA, $F = 35.97$, $p < 0.01$), providing 59.9% (SF: $p = 0.0039$) of its variation, while it produced no significant effect on the recovery of F_v/F_m.

Table 2. Comparison between percentage of recovery of F_v/F_m and that of $rETR_{max}$ in symbiotic dinoflagellates of *Montipora digitata* and *Pavona divaricata* at different times after high light exposure. *Significant difference (*t*-test, $p < 0.05$). ns: not significant

Temperature during high light exposure (°C)	Temperature during recovery (°C)	F_v/F_m–$rETR_{max}$ 24 h	72 h	120 h
Montipora digitata				
31	31	ns	*	*
	28	*	*	ns
	25	*	ns	ns
28	28	*	ns	ns
	25	*	ns	ns
25	25	ns	ns	ns
Pavona divaricata				
31	31	ns	ns	ns
	28	ns	ns	ns
	25	ns	ns	ns
28	28	ns	ns	ns
	25	ns	ns	ns
25	25	ns	ns	ns

Changes in Chl content and symbiotic dinoflagellate density in response to high light exposure

Changes immediately after high light exposure

After 3 h high light exposure, the total Chl content in symbiotic dinoflagellates of *Montipora digitata* decreased significantly ($p < 0.05$) at all temperature conditions (Fig. 5). The reduction in the total Chl content was smaller at 25°C than at 28 and 31°C, representing a decrease up to 15 and 40% of non-exposed control, respectively. In symbiotic dinoflagellates of *Pavona divaricata*, exposure to high light at 28 and 31°C caused significant ($p < 0.05$) decrease in the total Chl content by 30 to 35%, though no significant changes in the total Chl content were detected in fragments exposed to high light at 25°C (Fig. 5). Temperature during high light exposure significantly affected the total Chl content in symbiotic dinoflagellates of *M. digitata* and *P. divaricata* (ANOVA: $F = 14.68$, $p < 0.01$, and $F = 16.11$, $p < 0.01$, respectively).

There were no significant (*t*-test, $p < 0.05$) differences in symbiotic dinoflagellate density between fragments exposed to high light exposure for 3 h and

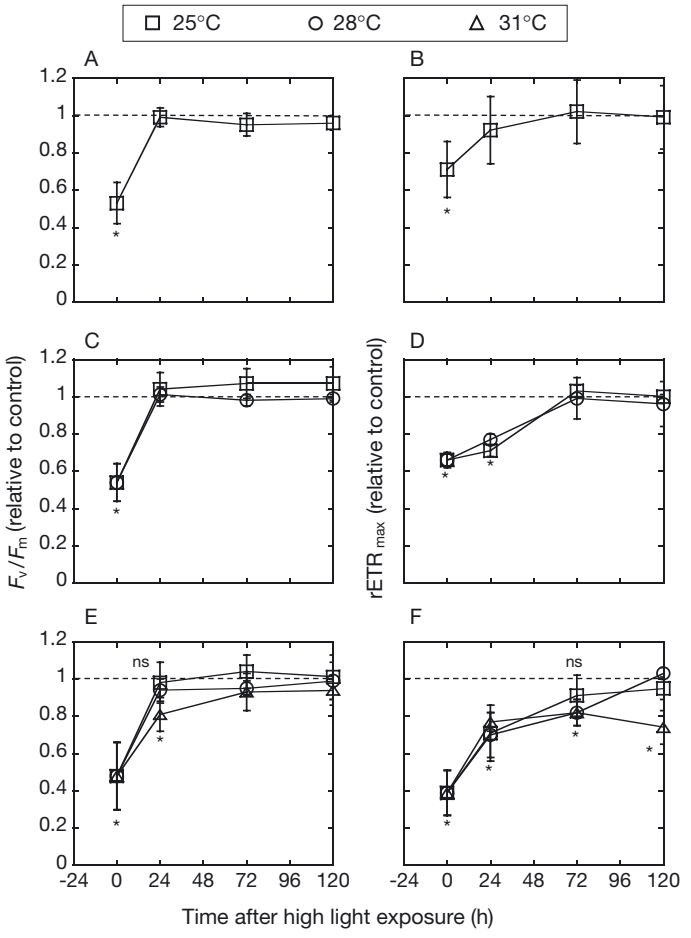

Fig. 4. Time course of recovery in F_v/F_m and $rETR_{max}$ in symbiotic dinoflagellates of *Montipora digitata* exposed to high light (1100 µmol photons m^{-2} s^{-1}) for 3 h. Details as in Fig. 3

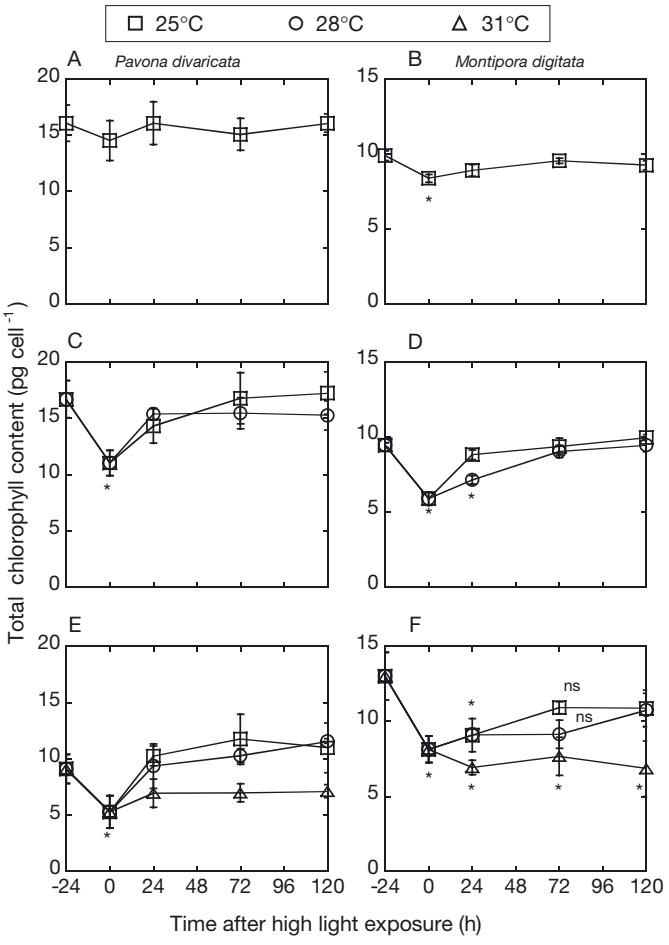

Fig. 5. Time course of recovery in the total Chl content in symbiotic dinoflagellates of *Pavona divaricata* and *Montipora digitata* exposed to high light (1100 µmol photons m^{-2} s^{-1}) for 3 h. Coral fragments were exposed to high light at (A,B) 25, (C,D) 28, and (E,F) 31°C. Fragments were allowed to recover at 25, 28, and 31°C. Means ± SD (n = 4). *Values significantly different from controls (*t*-test, $p < 0.05$). ns: not significant

non-exposed controls in both coral species investigated (Fig. 6 for *Montipora digitata*; data not shown for *Pavona divaricata*).

Recovery under different temperature conditions

The time course of recovery in the total Chl content in symbiotic dinoflagellates after high light exposure differed between *Pavona divaricata* and *Montipora digitata* (Fig. 5). In *P. divaricata*, the total Chl content returned to the control level within 24 h in all cases regardless of temperature during recovery (Fig. 5A,C,E). When the fragments of *M. digitata* exposed to high light at 25 and 28°C were allowed to recover at 25°C, the total Chl content returned to the control level within 24 h (Fig. 5B,D). However, when the 28°C-treated fragments were allowed to recover at 28°C, it took longer, 72 h, for the total Chl content to return to the control level (Fig. 5D). Transfer of the 31°C-treated fragments of *M. digitata* after high light exposure to 25 and 28°C resulted in a complete recovery of the total Chl content within 72 h (Fig. 5F). The Chl content remained at a reduced level even 120 h after high light exposure in the 31°C-treated fragments of *M. digitata* when they were kept at 31°C during the recovery period (Fig. 5F). Temperature during the recovery period had a significant effect (ANOVA, $F = 8.99$, $p < 0.01$) on Chl content in the 31°C-treated fragments of *M. digitata* and provided 17.0% (SF: $p < 0.01$) of Chl variation. There was no significant ($p > 0.05$) change in the total Chl content of non-exposed control coral fragments during the experimental period for both coral species.

Temperature during recovery had a significant (ANOVA, $F = 56.24$, $p < 0.05$) effect on algal density in *Montipora digitata* fragments, and provided 81.0%

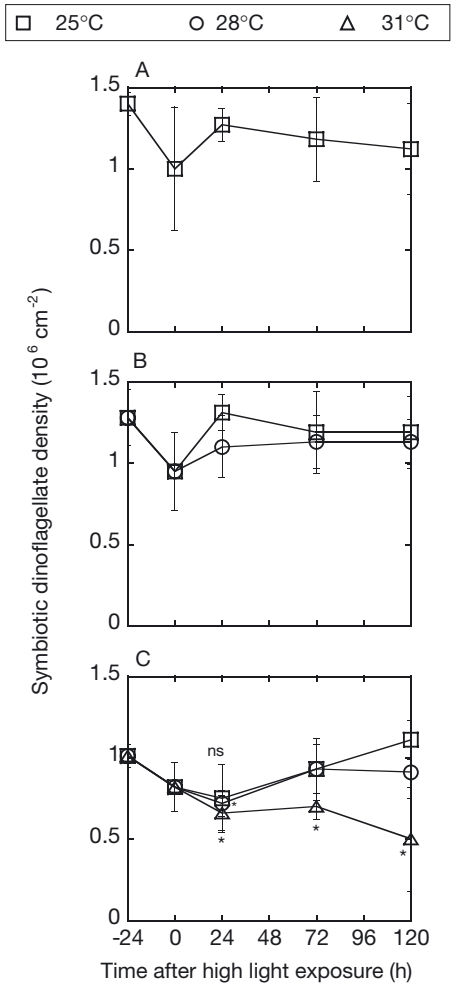

Fig. 6. Time course of recovery in the symbiotic dinoflagellate density of *Montipora digitata* exposed to high light (1100 µmol photons m^{-2} s^{-1}) for 3 h. Coral fragments were exposed to high light at (A) 25, (B) 28, and (C) 31°C. Fragments were then allowed to recover at 25, 28, and 31°C. Means ± SD (n = 4). *Values significantly different from controls (*t*-test, $p < 0.05$)

(SF: $p < 0.05$) of its variation. When the fragments of *M. digitata* exposed to high light at 31°C were allowed to recover at 28 or 31°C, significant ($p < 0.05$) decline in the symbiotic dinoflagellate density was observed after 24 h (Fig. 6). The dinoflagellate density returned to the control level after 72 h at 28°C, while it remained at a significantly lower level over the recovery period of 120 h at 31°C (Fig. 6C). No significant decrease in symbiotic dinoflagellate density was observed during the recovery period either in the fragments exposed to high light at 25 and 28°C (Fig. 6A,B) or in fragments that were exposed to high light at 31°C and allowed to recover at 25°C (Fig. 6C).

On the other hand, no change in symbiotic dinoflagellate density was detected in *Pavona divaricata* regardless of temperature during the recovery period (data not shown). Non-exposed controls showed no significant changes in dinoflagellate density at the respective temperatures during the course of the experiment for both coral species.

DISCUSSION

Recent studies have shown that elevated temperatures affect both the dark and light reactions of photosynthesis in symbiotic dinoflagellates of reef-building corals (Lesser 1996, Jones et al. 1998, Warner et al. 1999). If the recovery of dark reaction is slower than that of light reaction, limited availability of electron acceptors could result in an excess of electrons in the photosynthetic electron transport chain even under sub-saturating light levels. This may lead to production of active oxygen molecules, which are harmful to the photosynthetic apparatus of the algae as well as the host corals. Thus, differential recovery rates of dark and light reactions from damage due to temperature and light stresses might influence the susceptibility of corals to bleaching. This study tested the 'differential recovery' hypothesis and documented for the first time the difference in the rate of recovery between PSII function (photochemical efficiency, F_v/F_m) and photosynthetic capacity, (relative maximum electron transport rate, $rETR_{max}$) of symbiotic dinoflagellates in a bleaching susceptible coral, *Montipora digitata*, exposed to high temperature and strong light.

Exposing *Pavona divaricata* and *Montipora digitata* to high light for 3 h caused a significant reduction in photochemical efficiency, F_v/F_m, of *in hospite* dinoflagellates. Such a reduction during stress is considered to be associated with an imbalance between degradation and replacement of D1 reaction center protein causing accumulation of non-functional PSII, and resulting in a lower photosynthetic efficiency (Vasilikiotis & Melis 1994). The degree of reduction in F_v/F_m was independent of temperature during high light exposure in both species. However, the subsequent restoration of F_v/F_m showed temperature dependency during the recovery period in both species. The F_v/F_m values recovered completely within 24 h when the corals were returned to 25 or 28°C after high light exposure, while it took longer when *P. divaricata* and *M. digitata* were allowed to recover at 31°C. Thus, the damage of PSII function and its recovery process appeared to be similar in the 2 corals. The slow recovery of F_v/F_m at high temperature is consistent with the suggestion that re-synthesis of D1 reaction center protein was inhibited at high temperature (Warner et al. 1999). However, transient damage to D1 protein may serve for photoprotection as damaged reaction centers

continue to trap light energy and to dissipate it as heat (Krause 1988), though Gorbunov et al. (2001) reported that the dynamic photoinhibition through D1 protein damage plays a minor role in photoprotection, when compared with non-photochemical quenching via down-regulation of PSII reaction centers and through xanthophyll cycle in light harvesting complexes.

On the other hand, there were apparent differences in sensitivity of photosynthetic capacity, $rETR_{max}$, in symbiotic dinoflagellates to temperature between the 2 coral species. The reduction in $rETR_{max}$ of *Pavona divaricata* was independent of temperature, and its recovery was almost complete 24 h after the stress treatment at all temperatures examined. It is likely that the high temperature level used in the present experiment is not stressful to *P. divaricata*, and increases in temperature of 1 to 2°C above normal do not permanently damage the dark reactions of symbiotic dinoflagellates in this species. In contrast, the extent of reduction in $rETR_{max}$ of *Montipora digitata* increased with increasing temperature during high light exposure, and recovery was much slower at 28 and 31°C than at 25°C. This is in agreement with the previous experiments where increased temperature hampers photosynthetic performance of symbiotic dinoflagellates in corals, probably as a consequence of heat damage in the Calvin-Benson cycle reactions (Lesser 1996, Jones et al. 1998).

The present results showed that high light exposure produced significant reduction in both F_v/F_m and $rETR_{max}$ of *Pavona divaricata* and *Montipora digitata*, while the time course of recovery of these parameters was different between corals studied. The recovery of $rETR_{max}$ was slower than that of F_v/F_m in *M. digitata* at 28 and 31°C, while $rETR_{max}$ and F_v/F_m recovered almost in parallel in *P. divaricata*. A marked decrease in $rETR_{max}$ at high temperature and its slow recovery as compared with the recovery of PSII function (Fig. 4) are characteristic of *M. digitata*, which is more susceptible to bleaching than *P. divaricata* (Loya et al. 2001, Sakai pers. comm.). The reduced level of $rETR_{max}$ after full recovery of PSII function might indicate that electron transport beyond PSII was blocked probably by slowdown of the Calvin-Benson cycle. It is likely that the primary target of heat stress is enzymes catalyzing the operation of the Calvin-Benson cycle, as suggested in higher plants (Feller et al. 1998). If this is the case, high-energy electrons generated at PSII cannot be fully consumed in the carbon-fixation cycle. Extra high-energy electrons may react with adjacent oxygen molecules to produce active oxygen, which in turn cause damage or inactivation of photosynthetic components of symbiotic dinoflagellates (Lesser 1997). This typically results in a decrease in the overall photosynthetic rate (Richter et al. 1990), indicated by decreased yield (F_v/F_m); a phenomenon also observed in the present study (Fig. 4E). Thus, the sensitivity of enzymes in the Calvin-Benson cycle to high temperature and the capacity to repair their damage may play an important role in determining the susceptibility of corals to bleaching. Corals sampled during bleaching events have been reported to exhibit decreased Chl content per algal cell and/or loss of symbiotic dinoflagellates (Porter et al. 1989, Fitt & Warner 1995, Warner et al. 1996, Jones et al. 1998, 2000, Brown et al. 2002). Our results follow both trends, showing a marked decrease in symbiotic dinoflagellate density and Chl content when fragments of *M. digitata* exposed to high light were allowed to recover at 31°C (Figs. 5 & 6). The F_v/F_m values showed significant but incomplete recovery throughout the first 24 h after stressed corals were transferred to 31°C (Fig. 4E). This result is consistent with observations made by Jones et al. (2000) on *Plesiastrea versipora*, in which, despite the significant recovery of F_v/F_m after heat stress, the loss of symbiotic algae occurred during the first few days of the recovery period. The explanation for this phenomenon might be incomplete recovery of maximal electron transport rate for a prolonged time following exposure of corals to stress. In the present experiment, signs of bleaching were observed when $rETR_{max}$ remained substantially lower than initial levels throughout the 120 h monitoring period and, thus, the difference in the recovery rate between $rETR_{max}$ and F_v/F_m was largest. The present results support the differential recovery hypothesis described above. This hypothesis is not only consistent with the 'photoinhibition' hypothesis proposed by Hoegh-Guldberg (1999) but also extends their suggestion to the recovery phase. Temperature dependency of repair process of enzymes involved in carbon fixation might actually influence bleaching susceptibility of shallow-water corals, which can be exposed to sea-surface temperatures higher than normal summer maxima from a few days to weeks (e.g. Hoegh-Guldberg 1999).

Gradual temperature increase to 31°C during the 5 d acclimation period resulted in lower Chl content per algal cell and $rETR_{max}$ values in *Pavona divaricata*, while it increased pigments and $rETR_{max}$ slightly but significantly in *Montipora digitata*. It is not clear why the $rETR_{max}$ values increased in *M. digitata* after acclimation to 31°C, as the 3 h treatment at 31°C under high light decreased $rETR_{max}$ to a greater extent than at other temperatures. It is, however, likely that physiological parameters measured after 5d acclimation reflected changes due to acclimation to high temperature as the possible effect of photoacclimation to low light was avoided by maintenance of shallow-water-collected corals under the shade a few weeks before the experiment. An interesting aspect of temperature

acclimation of algal photosynthesis is the general similarity between temperature acclimation and photoacclimation (Davison 1991). Earlier, it was shown that high-temperature-acclimated algae had a high content of photosynthetic pigments and increased photosynthetic capacity (Zupan & West 1990, Machalek et al. 1996, Schofield et al. 1998), resembling those characteristics of photoacclimation to low light. It seems that the high temperature acclimation response of *M. digitata* (increased $rETR_{max}$ and algal Chl content) followed the same trend, and as a result this increased its susceptibility to further high light exposure, the general reaction for shade-adapted plants exposed to natural solar radiation (Powles 1984).

The 2 investigated corals harbor physiologically different symbiotic algae, though both corals live in shallow-water areas, which are well exposed to solar radiation. There is evidence for a high diversity of endosymbiotic dinoflagellates in coral species (LaJeunesse 2001). Previous studies have shown that physiological (Brown et al. 1994, 2002, Warner at al. 1999) and genetic differences (Rowan et al. 1997, Glynn et al. 2001) in algal symbionts play an important role in determining bleaching susceptibility of corals. *Pavona divaricata* might have adopted the strategy to harbor symbiotic dinoflagellates that possess a strong ability to repair photosynthetic capacity to cope with high irradiance at low depth during thermal stress. On the other hand, *Montipora digitata* might have adopted the strategy to expel the dinoflagellates from the host tissue when they become harmful by producing active oxygen species (Lesser 1997). Although *M. digitata* is one of the survivors of 1998 mass bleaching event in Okinawa, Sugihara et al. (1999) reported that colonies of *M. digitata* showed partial and variable bleaching in Kikai-jima, Ryukyus Islands, in 1998. It is likely that symbiotic algae remained in shaded part of colonies of *M. digitata* and proliferate quickly to repopulate the bleached colonies to enable this species to survive.

CONCLUSIONS

Our results clearly show that the process of recovery from thermal stress is different among corals species. In *Pavona divaricata*, where PSII function, F_v/F_m, and photosynthetic capacity, $rETR_{max}$, recovered in parallel, no sign of bleaching was detected. In *Montipora digitata*, bleaching was more or less correlated with significant delay of recovery of $rETR_{max}$ as compared with F_v/F_m. The delay in the recovery of $rETR_{max}$ after full restoration of F_v/F_m indicates incomplete recovery of damage in the Calvin-Benson cycle. The present findings strongly suggest that high temperature affects the rate of recovery of the carbon fixation cycle and that the ability of symbiotic dinoflagellates to repair damage determines susceptibility of corals to bleaching. Hence, the optimization of regulatory mechanisms to reduce the required time for recovery from high light stress might be an important strategy for bleaching tolerant coral species to cope with high irradiance at low depth during thermal stress.

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