

Differential Bleaching Susceptibility and Mortality Patterns among Four Corals in Response to Thermal Stress

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Abstract

Photo-physiological responses of one massive, *Platygyra ryukyuensis*, and three branching corals, *Porites cylindrica*, *Montipora digitata* and *Seriatopora caliendrum*, with different bleaching susceptibilities, exposed to thermal stress over a period of 30 h and thereafter their bleaching and mortality over a period of four months were investigated. The changes in maximum quantum yield (F_V/F_M) and maximum electron transport rate (ETR_{max}) revealed three patterns during the 30 h treatment at 32°C under 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. *P. ryukyuensis* showed small but significant changes in F_V/F_M without changes in ETR_{max} and *S. caliendrum* showed a drastic decrease in both parameters, while *M. digitata* and *P. cylindrica* showed similar trend in decrease of the F_V/F_M values only. After 30 h exposure to thermal stress, the total chlorophyll content decreased significantly only in *S. caliendrum* and zooxanthellae density decreased significantly in all tested coral species except of *P. ryukyuensis*. Mortality data over four months after the thermal stress treatment showed three trends. *P. ryukyuensis* and *S. caliendrum* showed no mortality and 100% mortality, respectively. Mortality in *M. digitata* was greater than that in *P. cylindrica*.

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These results suggest that in *P. ryukyuensis* (resistant coral) both the algae and the host are strong while in *S. caliendrum* (susceptible coral) both partners are weak. Although intermediate coral species, *M. digitata* and *P. cylindrica* showed the same level of F_v/F_m reductions and percentage loss of zooxanthellae, the host component seemed to be stronger in *P. cylindrica* than in *M. digitata* as revealed by their different mortalities. Thus, we conclude that variations in bleaching susceptibility and mortality among scleractinian corals are a result of the combined physiological responses of both partners of the symbiotic system to thermal stress.

Keywords: Bleaching susceptibility, ETR_{max} , F_v/F_m , mortality, thermal stress, coral host role

1. Introduction

Coral bleaching refers to a change in coloration of coral tissue through the loss of endosymbiotic dinoflagellates (=zooxanthellae) (Hoegh-Guldberg and Smith, 1989), their photosynthetic pigments (Kleppel et al., 1989) or *in situ* degradation of the algae (Brown et al., 1995). This phenomenon has been reported to occur as part of a natural seasonal cycle superimposed by environmental fluctuations in tropical waters (Fagoonee et al., 1999; Brown et al., 1999; Fitt et al., 2000). However, mass bleaching events, due to 2–6°C rise above normal summer maxima related to global warming, are increasing in frequency and have been documented extensively over the last two decades (Wilkinson, 2000). The subsequent recovery or death of all or part of the colonies of hermatypic corals has also been subject of several studies (Brown and Suharsono, 1990; Williams and Bunkley-Williams, 1990; McField, 1999).

The underlying cause of bleaching is believed to involve the impairment of the algal symbiont photosynthesis (Iglesias-Prieto et al., 1992; Jones et al., 1998; Warner et al., 1999) (but see Ralph et al., 2001), although the importance of host tissue in the maintenance of the intact coral symbiosis under stress conditions has not been ignored (Brown et al., 2002; Bhagooi and Hidaka, 2002, 2003). The reduced chlorophyll fluorescence yield of zooxanthellae in thermally bleached corals has been interpreted as indicative of damage to components of the electron transport system of photosystem II (PSII), probably at the D1 protein level (Fitt and Warner, 1995; Warner et al., 1996; Warner et al., 1999). Possible damage to carboxylation mechanisms (Jones et al., 1998) of zooxanthellae has also been implicated in high temperature induced bleaching.

In many coral bleaching reports, there is noticeable variation, both intra- and inter-species, in the level of susceptibility and extent of bleaching on the

reef (Brown and Suharsono, 1990; Marshall and Baird, 2000; Loya et al., 2001). Within species variation in bleaching susceptibility has been attributed to either different genetic types of zooxanthellae (Rowan et al., 1997) or light history of corals (Brown et al., 2000). Variation among coral species so far has been associated with zooxanthellae possessing different capabilities to dissipate excess excitation energy through non-photochemical pathways (Warner et al., 1996), coral growth and metabolic rates (Gates and Edmunds, 1999), and colony morphology (branching versus massive) and tissue thickness (Loya et al., 2001). These observations also raise the question of which partner in the coral/algal association is determining the bleaching susceptibility and the subsequent mortality.

Most attention to date has been focused on species-specific responses to high temperature anomalies in the field. However, studies that have experimentally demonstrated the initiation and progression of thermally induced bleaching process and thereafter the mortality patterns are limited. This study examines the photo-physiological responses of branching and massive corals, with different field-observed bleaching susceptibilities, to thermal stress over a period of 30 h, bleaching responses in terms of zooxanthellae and chlorophyll content losses after 30 h thermal stress, and variations in mortality and bleaching over a ~4 months period in branching and massive corals after 30 h exposure to thermal stress.

2. Materials and Methods

Collection and maintenance of specimens

Colonies of the scleractinian corals, *Platygyra ryukyuensis* (Yabe and Sugiyama 1935), *Porites cylindrica* (Dana 1846) and *Montipora digitata* (Dana 1846) were collected from the eastern reef of Sesoko Island, Okinawa and those of *Seriatopora caliendrum* (Ehrenberg 1834) were collected from Aka Island, Japan. All collections were done at a depth of 1–2 m. Colonies were maintained in an outdoor tank supplied with running seawater and aeration till use. During this period, seawater temperature in the tank was 24–25°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 30% of incident PAR. After two months, branches of about 3 cm in length were taken from colonies of *S. caliendrum*, *P. cylindrica* and *M. digitata*, and fragments of about 6 cm² of tissue surface area were made from *P. ryukyuensis* colonies. Fragmented corals were mounted on ceramics tiles with cement and returned to the outdoor tank under same conditions for 5 days.

Experimental protocols

The experimental mounted fragments were exposed to high temperature (32°C) and light intensity of 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of PAR for 30 h. The temperature of the running seawater supply to experimental tanks was regulated using automatic thermostats ($\pm 0.5^\circ\text{C}$, IC Thermostat, EX-003) and aquaria heaters. 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). Chlorophyll fluorescence parameters, dark-adapted F_v/F_m and rapid light curves (RLCs), were measured initially (immediately before treatment) and every six hours during the experiment. Chlorophyll content and zooxanthellae density were measured before and after 30 h treatment.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using a pulse-amplitude-modulated fluorometer (Mini-PAM, Waltz, Germany). Before and after the stress treatments, coral fragments were dark-adapted for 20 min to allow the photosynthetic endosymbionts to relax all their PSII reaction centers. A constant weak light ($< 1 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) was used to measure the minimum fluorescence, F_o , while a saturating pulse (4,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was applied to measure the maximal fluorescence (F_m), when all the PSII centers are closed. The ratio of the variable ($F_v = F_m - F_o$) to maximal fluorescence (F_m) in a darkened sample (dark-adapted F_v/F_m) is correlated to the maximum quantum yield of PSII and thus represents the PSII functioning (Genty et al., 1989).

Immediately after F_v/F_m measurement, a five min of light-adaptation from the internal lamp of the PAM was applied to activate the photosynthetic dark reaction followed by a 30 s dark period just before the RLCs were recorded. The internal light source provided light intensities ranging from 0–1,280 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at intervals of 20 s to produce the RLC. When an illuminated sample is pulsed with weak and saturating light intensities, minimum (F_o') and maximum (F_m') fluorescence parameters can be recorded. The new ratio ($(F_m' - F_o')/F_m'$) is a measure of the effective quantum yield (Y), which is lower than F_v/F_m due to partial closure of the reaction centers and a relative increase in non-radiative energy dissipation.

Photosynthetic electron transport rate (ETR, relative units) was estimated by multiplying the effective quantum yield by photon flux density (400–700 nm) (Ralph et al., 1999). Maximum electron transport rate (ETR_{max}) indicates the "assimilatory power", which represents the initial products (NADPH and ATP) of the conversion of light energy into chemical energy (Hall and Rao,

1999). RLCs for ETR were fitted using Platt et al. (1980) equation and ETR_{max} was thus determined at light saturation. Chlorophyll fluorescence parameters F_v/F_m and ETR_{max} were used to monitor photosystem II functioning and "assimilatory power", respectively. Measurements at given intervals were taken at the same exact position on the coral surface as the initial one. All measurements were done at 32°C, except initial measurements, which were done at 25°C.

Total chlorophyll (a + c₂) content and zooxanthellae density determination

Tissue was removed from the coral fragments using a Water-pik (Johannes and Wiebe, 1970). The coral blastate was then homogenized and 20–30 ml aliquots were filtered under vacuum through a Whatman GF/C glass fiber filter (4.7 cm in diameter). The glass fiber filters with adsorbed zooxanthellae were immersed in an aqueous solution of 90% cold acetone. The solution with sample was shaken daily and placed in a refrigerator for 48 h. The absorbance of the acetone extracts was read at 630, 633 and 750 nm using a Hitachi U-2000A spectrophotometer. The concentrations of chlorophyll (a + c₂) were determined using Jefferey and Humphrey (1975) equation and expressed as chlorophyll per cell.

To determine zooxanthellae density 10 ml of the homogenate was centrifuged at 3,000 rpm for 15 min and the supernatant was discarded. The pellet was resuspended in 1 ml of filtered seawater (0.45 µm). Zooxanthellae counts in each of the eight fields in the haemocytometer were recorded for each sample and expressed as million cells per surface area. The surface area of coral fragments was determined by Al-foil method (Marsh, 1970) for *P. ryukyuensis* and by paraffin-wax technique (Stimson and Kinzie, 1991) for *S. caliendrum*, *P. cylindrica* and *M. digitata*. Both zooxanthellae density and chlorophyll contents were determined before and after 30 h experimental period.

Mortality and visual bleaching estimation

Mortality, visual bleaching and partial bleaching/death were monitored at days 22, 36, 71 and 110 after the 30 h thermal exposures and expressed as percentage of initial fragments used for experimentation.

Statistical analyses

Relative F_v/F_m and ETR_{max} values normalized to their respective initial ones, were used as they provide reliable comparison among treatments to be made by avoiding large deviations in chlorophyll parameters due to coral

surface irregularities. Analysis of variance (ANOVA) analyses followed by post-hoc Tukey Honest significant difference comparison of means were employed to test significant differences in relative F_v/F_m and relative ETR_{max} among treatments and coral species. Relative data for both F_v/F_m and ETR_{max} were arcsine transformed prior to ANOVA analysis. After log transformation, zooxanthellae density and total chlorophyll content were tested using student t-test. Data plotted on figures represent mean \pm SE.

3. Results

Photo-physiological responses to thermal stress during 30 h treatment

The 30 h thermal stress at $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ induced significant reductions in PSII functioning (F_v/F_m) (Fig. 1A) and maximal electron transport rate (ETR_{max}) (Fig. 1B) in both the branching and massive coral species except in the ETR_{max} of the massive one. The reduction in both parameters differed significantly among the investigated coral species (Fig. 1, Table 1) and from each other within a given species (Table 2).

Three clear patterns emerged in F_v/F_m and ETR_{max} declines over the 30 h treatments when the coral species were compared. In *P. ryukyensis*, a significant decline (10–15%) in PSII functioning did not lead to any significant change in ETR_{max} at any given exposure time. *S. caliendrum* exhibited significant reductions in both F_v/F_m and ETR_{max} at all sampling points. *M. digitata* and *P. cylindrica* showed similar intermediate pattern in decrease of F_v/F_m but not in the reduction of ETR_{max} (Table 1). In *P. cylindrica*, significant changes in F_v/F_m and ETR_{max} were evident at 12 h and 30 h sampling points, respectively. In *M. digitata*, significant decreases in F_v/F_m and ETR_{max} were detected at 12 h and 6 h measurement times, respectively. Thus, more pronounced reduction in ETR_{max} was observed in *M. digitata* than in *P. cylindrica*.

Zooxanthellae density and total chlorophyll content after the 30 h thermal stress treatment

Zooxanthellae density decreased significantly ($P < 0.05$) in all the investigated species except in *P. ryukyensis* (Fig. 2A). The decline in zooxanthellae density was more pronounced in *S. caliendrum* ($98.9 \pm 2.3\%$) when compared to *M. digitata* and *P. cylindrica* ($\sim 65\text{--}80\%$). There were no significant differences ($P > 0.05$) in percentage loss of zooxanthellae between *M. digitata* ($68.0 \pm 1.8\%$) and *P. cylindrica* ($77.3 \pm 3.4\%$). After 30 h of thermal stress, the total chlorophyll content decreased significantly only in *S. caliendrum* (Fig.

2B). While no significant changes were recorded in *P. ryukyuensis* and *M. digitata*, the total chlorophyll content increased significantly in *P. cylindrica*.

Table 1. Summary of statistical results of comparisons of mean of the chlorophyll fluorescence parameters, F_v/F_m and ETR_{max} (bold), during the 30-h thermal stress period among species. Tukey HSD Post Hoc test was used to carry out multiple mean comparisons following ANOVA ($P < 0.0001$) with main effect being species. ** - $P < 0.01$, *** - $P < 0.001$, NS - no significant difference.

	<i>Platygyra ryukyuensis</i>	<i>Porites cylindrica</i>	<i>Montipora digitata</i>	<i>Seriatopora caliendrum</i>
<i>Platygyra ryukyuensis</i>		**	***	***
<i>Porites cylindrica</i>	NS		NS	***
<i>Montipora digitata</i>	***	***		***
<i>Seriatopora caliendrum</i>	***	***	***	

Table 2. Summary of statistical results of respective comparisons of F_v/F_m and ETR_{max} between the initial and after 6, 12, 24 and 30 h exposure measurements. Numbers in parentheses indicate average ($n=4$) percentage inactivation of the chlorophyll fluorescence parameters. Tukey HSD Post Hoc test was used to carry out multiple mean comparisons following ANOVA ($P < 0.0001$) with main effect being time of exposure. * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$, NS - no significant difference.

Species	Chlorophyll fluorescence parameters	Time (h)			
		6	12	24	30
Pr	F_v/F_m	*** (10)	*** (15)	*** (10)	*** (15)
	ETR_{max}	NS	NS	NS	NS
Pc	F_v/F_m	NS	*** (46)	** (24)	*** (59)
	ETR_{max}	NS	NS	NS	* (72)
Md	F_v/F_m	NS	* (39)	** (53)	*** (65)
	ETR_{max}	*** (54)	*** (70)	*** (83)	*** (86)
Sc	F_v/F_m	*** (90)	*** (98)	*** (98)	*** (99)
	ETR_{max}	*** (98)	*** (99)	*** (99)	*** (100)

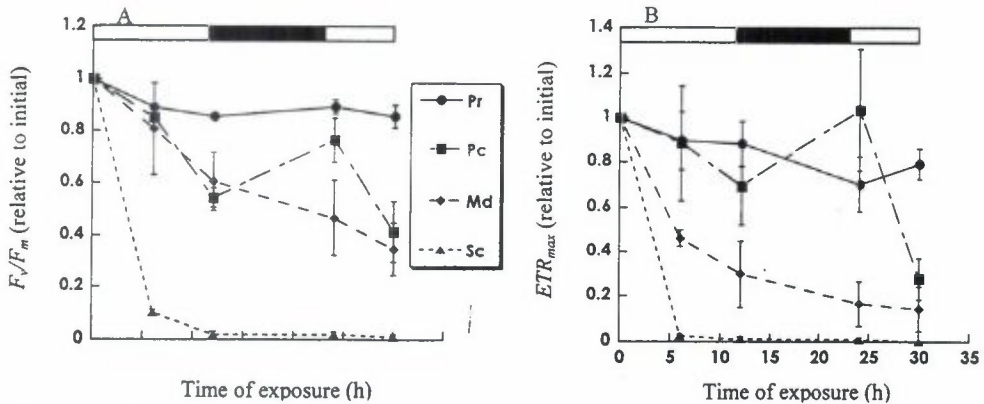


Figure 1. Photo-physiological responses, PSII functioning, F_v/F_m (A) and maximum electron transport rate, ETR_{max} (B), of zooxanthellae in corals during 30-h exposure to 32°C at 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The empty and filled bars at the top of each plot represent light and dark exposures. Data points represent mean \pm SE ($n = 4$). Pr (\bullet) - *Platygyra ryukyuensis*, Pc (\blacksquare) - *Porites cylindrica*, Md (\blacklozenge) - *Montipora digitata*, Sc (\blacktriangle) - *Seriopora caliendrum*.

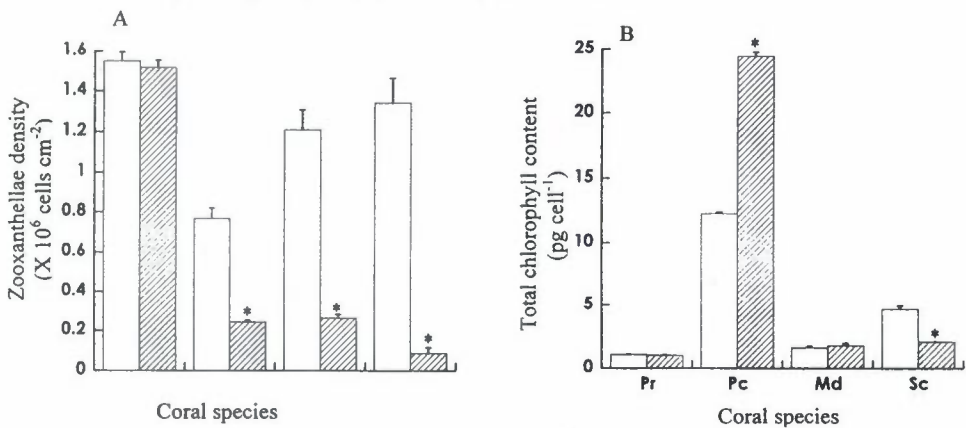


Figure 2. Zooxanthellae density (A) and their total chlorophyll content (B) in corals before (empty bars) and after (hatched bars) 30-h exposure to thermal stress. Error bars represent SE ($n = 4$). Pr - *Platygyra ryukyuensis*, Pc - *Porites cylindrica*, Md - *Montipora digitata*, Sc - *Seriopora caliendrum*. * - $P < 0.05$ (Student t-test).

Visual bleaching and mortality patterns among corals during 110 days after thermal stress

Both bleaching and mortality patterns differed among the four coral species studied over the 110 days recovery conditions (Fig. 3). Very low (<10%), high (80–100%) and moderate levels (30–60%) of bleaching were observed in *P.*

ryukyuensis (Fig. 3A), *Porites cylindrica* (Fig. 3B) and *M. digitata* (Fig. 3C), respectively. *S. caliendrum* treated samples did bleach during the first 5 d of recovery (data not shown). *P. ryukyuensis* showed no mortality while *S. caliendrum* showed 100% mortality (Fig. 3D). After 110 d of recovery, mortality in *M. digitata* (~70%) was greater than that in *P. cylindrica* (~3%).

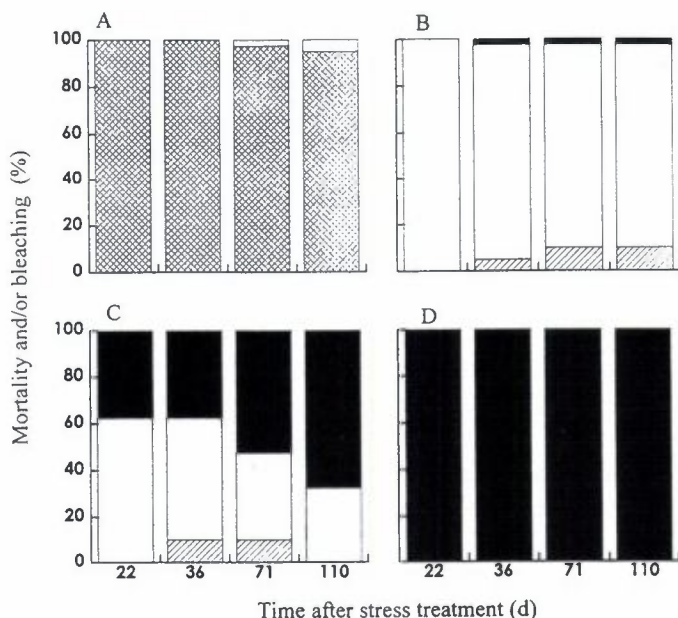


Figure 3. Percentage mortality and/or bleaching monitored in *Platygyra ryukyuensis* (A), *Porites cylindrica* (B), *Montipora digitata* (C) and *Seriatopora caliendrum* (D) over 110 d after 30-h thermal stress. Empty, filled, hatched and crosshatched bars represent bleaching, dead, partially bleached/dead and non-bleached data, respectively.

4. Discussion

During the last two decades, while changes in zooxanthellar photosynthesis (Iglesias-Prieto et al., 1992; Fitt and Warner, 1995; Jones et al., 1998; Warner et al., 1996, 1999), stress susceptibilities (Bhagooli and Hidaka, 2003), and mortality in several coral taxa observed in the field (Brown and Suharsono, 1990; Marshall and Baird, 2000; Loya et al., 2001) and under laboratory-based experimental conditions (Hueerkamp et al., 2001) due to elevated temperature have been intensively reported, these bleaching related features and mortality investigations were undertaken separately in different studies.

This study attempts to bridge these gaps by documenting both alteration in photosynthetic performance of the *in hospite* zooxanthellae during and after thermal stress, and bleaching and mortality patterns under recovery conditions following thermal stress in four scleractinian coral species with different field-observed bleaching susceptibilities.

Differential susceptibility of the four investigated coral species to bleaching and mortality was clearly linked to the photo-physiological responses of the *in hospite* zooxanthellae under thermal stress. Reduction in F_v/F_m (PSII functioning), as an indication of damage to photosynthetic machinery of zooxanthellae has been used in several coral bleaching studies (Jones et al., 1998, 2000; Brown et al., 2000; Jones and Hoegh-Guldberg, 2001; Warner et al., 1996, 1999; Bhagooli and Hidaka, 2003). However, very few studies have focused on changes in ETR (Ralph et al., 2001). In the present investigation, a 30 h thermal stress showed significant reductions in F_v/F_m but to different extents (Table 1) in zooxanthellae of all the tested species. In contrast, reductions in ETR_{max} were evident mostly in the bleaching susceptible branching corals, *M. digitata* and *S. caliendrum*, while the bleaching resistant massive, *P. ryukyuensis*, and branching, *P. cylindrica* (except for 30 h), corals showed no sign of reductions in ETR_{max} . It is well established in higher plants (Behrenfeld et al., 1998; Lee et al., 1999) and in zooxanthellae (Bhagooli and Hidaka, submitted) that a ~40% inactivation of the PSII functioning (F_v/F_m) is required to significantly reduce maximum photosynthesis or ETR_{max} . This might explain the fact that reductions in F_v/F_m in *P. ryukyuensis* and *P. cylindrica* were not generally large enough to lead to a significant reduction in ETR_{max} .

On the other hand, in *M. digitata*, although the F_v/F_m was not reduced significantly after 6 h thermal stress the ETR_{max} decreased significantly. This might suggest that ETR_{max} in *M. digitata* is more sensitive to thermal stress than F_v/F_m . In *S. caliendrum*, the most susceptible bleaching species tested here, both F_v/F_m and ETR_{max} were severely damaged.

These observations indicate that the sensitivity of assimilatory electron flow (ETR_{max}) in symbiotic algae of branching coral species to high temperature affected their susceptibility to bleaching compared to zooxanthellae in the massive coral. This is supported by the fact that almost no bleaching in terms of loss of zooxanthellae or total chlorophyll content was recorded in *P. ryukyuensis* while all the branching corals did exhibit bleaching.

The differences in the thermally induced stress susceptibilities among the *in hospite* zooxanthellae might be due to the coral species harboring different genotypes of zooxanthellae with differing photo-physiological responses to elevated temperature. Fitt and Warner (1995) and Warner et al. (1996) claimed that zooxanthellae from Caribbean corals with higher capabilities to dissipate excess excitation energy through non-photochemical pathways

protected their photosystems from damage during heat stress. Perez et al. (2001) demonstrated the differential release of different clades of zooxanthellae, due to decreased photosynthesis, from the same host species under thermal stress. Bhagooli and Hidaka (2003) also clearly demonstrated that isolated zooxanthellae from different coral species from Okinawan waters exhibited differential responses to thermal stress. Although the types of zooxanthellae were not characterized in this study, Loh et al. (2002) and Lajeunesse (pers. com.) have reported high genetic diversity of zooxanthellae in Okinawan corals.

However, the role of the host cannot be ignored as all the treatments involved only *in hospite* zooxanthellae exposures. Brown et al. (2002) provided convincing evidence that colonies of *Goniastrea aspera* with higher levels of host antioxidants and heat shock proteins did not bleach when exposed to high sea temperatures and thus demonstrated the significance of the host in maintaining the intact symbiosis under thermal stress. Moreover, Bhagooli and Hidaka (2003), by comparing the stress susceptibility of *in hospite* and isolated zooxanthellae among five coral species, suggested a significant role of host in determining bleaching susceptibility of corals. In the present study, it is noteworthy that *M. digitata* and *P. cylindrica* exhibited same percentage of zooxanthellae loss but higher level of mortality was observed in the case of *M. digitata*. This might further suggest that the host in *M. digitata* was more severely affected than in *P. cylindrica* and thus highlight the role of the host in mortality determination after thermal stress.

Several reports on the Great Barrier Reef (Marshall and Baird, 2000) and in Okinawan waters (Loya et al., 2001; Yamazato, 1981, 1999) pointed out that massive corals are more resistant to bleaching than branching corals. Gates and Edmunds (1999) made a tentative suggestion that corals with low growth rates and high metabolic rates such as massive species, acclimatize more effectively than those with high growth rates and low metabolic activities, characteristics of branching species.

Moreover, Loya et al. (2001) hypothesized that preferential survival of thick-tissued species and shape-dependent differences in colony mass-transfer efficiency could partially explain the field-observed mortality patterns of massive and branching corals due to warmer temperature throughout 1998 at Sesoko Island, Japan. Though Loya et al. (2001) reported the disappearance of *P. cylindrica* at Sesoko station in Japan, this species could be easily found at Sesoko Island in 1999 (Ranjeet Bhagooli, pers. observation) suggesting that *P. cylindrica* was not an absolute loser after the 1998-bleaching event in Okinawa. Loya et al. (2001) suggested that *P. ryukyuensis* has thicker tissues (4.0 ± 0.096 mm) than either *P. cylindrica* (1.3 ± 0.180) or *M. digitata* (0.9 ± 0.006). Our results corroborate with their findings in that the thick-tissued coral was more tolerant to bleaching and did not die, while the thin tissue corals suffered from

bleaching and mortality. However, this study also revealed different mortality between *P. cylindrica* and *M. digitata* although they have almost similar tissue thickness and shape-dependent mass-transfer efficiency, due to their branching morphology. This implies that the physiological characteristics of the host might be the determinant of mortality.

Comparison of the orders of mortality but not chlorophyll fluorescence parameters and total chlorophyll contents among the coral species studied in this work (Table 3) agree with the order of bleaching/mortality pattern observed in Sugihara et al. (1999) and Yamazato (1981, 1999). This is suggestive of the probable host role being important in determining field-observed bleaching/mortality patterns.

Table 3. Summary of species order, from least to highly damaged/affected ones, in terms of photo-physiological responses during stress, zooxanthellae biomass parameters and mortality patterns following stress treatments. Species order is based on results from Tukey HSD Post Hoc test used for multiple mean comparisons of each parameter following ANOVA ($P < 0.0001$), except for the field data from Sugihara et al. (1999) and Yamazato (1981, 1999). Pr = *Platygyra ryukyuensis*, Pc = *Porites cylindrica*, Md = *Montipora digitata*, Sc = *Seriatopora caliendrum*.

Observations	Parameters	Species order (from least to highly damaged/affected)
Experimental (this study)	Chlorophyll fluorescence	F_v/F_m (PSII functioning)
		ETR_{max} (maximum electron transport rate)
	Bleaching quantification	% Zooxanthellae loss
	Mortality	% Total chlorophyll loss
Field observation	Bleaching/Mortality	% Dead

5. Conclusions

The massive coral *P. ryukyuensis* showed the highest tolerance to the 30-h thermal stress and no mortality during the recovery period compared with the branching coral species: *S. caliendrum*, *M. digitata* and *P. cylindrica*. Variations in bleaching and mortality did occur among the branching coral species. In the tolerant coral *P. ryukyuensis* both the algae and the host components seem to be strong in response to the thermal stress, while in the most

susceptible coral *S. caliendrum* both partners were weak. Although intermediate coral species, *M. digitata* and *P. cylindrica* showed damage of photosynthesis in their endosymbionts, the host component was stronger in *P. cylindrica* than in *M. digitata* as revealed by their equal percentage of zooxanthellae loss followed by different mortality patterns. Taken together, variations in bleaching susceptibility and mortality among the studied scleractinian corals are a result of the combined physiological responses of both partners of the symbiotic system to thermal stress.

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