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Long-term changes in the chlorophyll fluorescence of bleached and recovering corals from Hawaii

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SUMMARY

Chlorophyll fluorescence has been used to predict and monitor coral bleaching over short timescales (hours to days), but long-term changes during recovery remain largely unknown. To evaluate changes in fluorescence during long-term bleaching and recovery, *Porites compressa* and *Montipora capitata* corals were experimentally bleached in tanks at 30°C for 1 month, while control fragments were maintained at 27°C. A pulse amplitude modulated fluorometer measured the quantum yield of photosystem II fluorescence (F_v/F_m) of the zooxanthellae each week during bleaching, and after 0, 1.5, 4 and 8 months recovery. *M. capitata* appeared bleached 6 days sooner than *P. compressa*, yet their fluorescence patterns during bleaching did not significantly differ. Changes in minimum (F_o), maximum (F_m) and variable (F_v) fluorescence throughout bleaching and recovery indicated periods of initial photoprotection followed by photodamage in both species, with *P. compressa* requiring less time for photosystem II (PS II) repair than *M. capitata*. F_v/F_m fully recovered 6.5 months earlier in *P. compressa* than *M. capitata*, suggesting that the zooxanthellae of *P. compressa* were more resilient to bleaching stress.

Key words: coral bleaching, fluorescence, photodamage, photoinhibition, photobleaching recovery.

INTRODUCTION

Corals can obtain up to 100% of their daily carbon requirements from photosynthesis of their endosymbiotic zooxanthellae (e.g. Grottoli et al., 2006; Muscatine et al., 1981). Under normal conditions, light is absorbed by antenna pigments of the photosynthetic apparatus in zooxanthellae chloroplasts. Excitation energy is transferred to the reaction centers of photosystem II (PS II) and down the photosynthetic electron transport chain where the primary photochemical reactions of the cell produce reducing power and adenosine triphosphate (ATP) (Krause and Weis, 1991). Photoinhibition occurs when photosynthetic electron transport decreases and absorption of excitation energy increases (Osmond, 1994; Smith et al., 2005). In zooxanthellae, excess excitation energy can produce reactive oxygen species (ROS), ultimately affecting the quantum yield of PS II fluorescence (F_v/F_m; F_v, variable fluorescence; F_m, maximum fluorescence) (Lesser, 1996; Lesser, 2006). Therefore, F_v/F_m is a key indicator of the physiological status of zooxanthellae, particularly the status of chlorophyll *a* (Chl *a*) fluorescence and PS II reaction centers.

Zooxanthellae physiology varies seasonally because of changes in temperature, irradiance or a combination of both, with high F_v/F_m in mid-winter to early spring and low in the mid to late summer (Warner et al., 2002). F_v/F_m also fluctuates diurnally, decreasing from a maximum at night to a minimum at mid-day (Brown et al., 1999; Gorbunov et al., 2001; Hoegh-Guldberg and Jones, 1999; Jones and Hoegh-Guldberg, 2001; Lesser and Gorbunov, 2001; Ralph et al., 1999; Torregiani and Lesser, 2007; Warner et al., 2006; Winters et al., 2003). These light-dependent variations serve to protect the photosynthetic apparatus from excess excitation energy involved principally in non-photochemical quenching (Gorbunov et al., 2001). Over these seasonal and diurnal patterns, periods of environmental stress can also influence F_v/F_m. Coral bleaching is one stress response, often caused by elevated seawater temperatures that impacts both the zooxanthellae and host. During stressful conditions, zooxanthellae are expelled, photosynthetic pigments are lost, or some combination of both, resulting in a white coral colony (e.g. Brown, 1997; Rodrigues and Grottoli, 2007).

Most investigations of the effects of coral bleaching on fluorescence have been experimentally conducted over very short time periods (hours to days), or under extreme bleaching conditions (>4°C above ambient) to pinpoint locations of molecular damage (Bhagooli and Hidaka, 2003; Bhagooli and Hidaka, 2004; Brown et al., 1999; Brown et al., 2000; Hill et al., 2004; Hoegh-Guldberg and Jones, 1999; Jones et al., 1998; Jones et al., 2000; Lesser and Farrell, 2004; Torregiani and Lesser, 2007; Warner et al., 1996; Warner et al., 1999). One study over slightly longer periods measured fluorescence in bleached corals every 10 to 14 days over a total period of 7 weeks (Rodolfo-Metalpa et al., 2006), whereas long-term (months to years) studies have focused on natural variability of fluorescence (Warner et al., 2002; Winters et al., 2006). The long time span followed by Warner et al. (Warner et al., 2002) encompassed periods of visible bleaching, but the effect of bleaching on fluorescence was difficult to interpret because of the lack of control or non-bleached corals at the same time periods. No studies to our knowledge have experimentally followed bleaching effects on fluorescence over periods longer than weeks.

Variability in fluorescence yields in different species may help predict long-term coral resilience following bleaching. However, understanding these changes in zooxanthellae physiology during bleaching and recovery requires both a comprehensive and long-

term study. The current study was designed to answer the following questions: (1) how long does zooxanthellae physiology take to recover from bleaching, (2) after bleaching, how do changes in zooxanthellae physiology vary between coral species, and (3) after bleaching, how do changes in zooxanthellae physiology coincide with known changes in host physiology of these same corals over the same time period (Grottoli et al., 2006; Rodrigues and Grottoli, 2006; Rodrigues and Grottoli, 2007). Active Chl *a* fluorescence was compared between experimentally bleached and non-bleached *Porites compressa* Dana 1846 and *Montipora capitata* Dana 1846 corals during 1 month of bleaching conditions and throughout 8 months of recovery following bleaching. Throughout bleaching and recovery, levels of photoprotection, photoinhibition and photodamage for each species of coral were assessed.

This design allowed for a quantitative assessment of the following hypotheses. (1) During 1 month of bleaching and throughout 8 months of recovery, F_v/F_m and its component variables [F_o (minimum fluorescence), F_m and F_v] are expected to undergo measurable changes in treated relative to control corals and relative to one another. Specifically, one or more of these outcomes may apply: (A) if F_v/F_m does not change while F_o of the treated group is greater than F_o of the control group and F_m of the treated group is greater than F_m of the control group, photodamage may be occurring (e.g. Jones and Hoegh-Guldberg, 2001); (B) if F_v/F_m of the treated group is greater than F_v/F_m of the control group prior to visible color loss in corals, photoprotection may be occurring (e.g. Brown et al., 2000); and/or (C) if F_v/F_m of the treated group is greater than F_v/F_m of the control group, while F_v of the treated group is greater than F_v of the control group or F_m of the treated group is less than F_m of the control group, photoinhibition may be occurring (e.g. Warner et al., 1999). (2) Measurable differences in zooxanthellae fluorescence of *P. compressa* and *M. capitata* are expected to reflect known differences in the bleaching response between species (Rodrigues and Grottoli, 2007).

MATERIALS AND METHODS

Study site and species

Corals were collected from Kaneohe Bay, Hawaii (21°26.18′N; 157°47.56′W). Seawater temperatures from June to October average 27±0.012°C (mean ± s.e.m.) and 24.5±0.015°C from November to May (data from Hawaii Institute of Marine Biology weather station).

Porites compressa is branching and yellow-brown to dark brown in color. *Montipora capitata* is plating to branching and medium to dark brown in color. All collected *M. capitata* used in this study were branching.

Experimental design

A detailed description of the experimental design is reported in Rodrigues and Grottoli (Rodrigues and Grottoli, 2006). Briefly, in August 2003, eight fragments were collected from each of twelve parent colonies from both species (totaling 192 fragments). All 24 parent colonies (12 per species) were located on the reef slope at the same depth (2 m) along a 100 m horizontal transect of the Point Reef off of Coconut Island. The temperature and irradiance levels were assumed to be constant over the small sampling area. The two species used in this study are the dominant coral species present on the fringing reef in this location and are sympatric along the reef slope. The fragments from each parent colony were randomly placed in one of eight tanks filled with filtered seawater that reduced zooplankton and coral heterotrophy. Beginning on 4 September 2003, the seawater temperature of four tanks was raised to 30.1±0.05°C (treated group), while the seawater of the other tanks remained at the ambient temperature of 26.8±0.04°C (control group) for 4 weeks. Within each temperature group, one fragment from each parent colony was randomly assigned to 0, 1.5, 4 or 8 months recovery on the reef. All tanks contained 24 fragments (i.e. 12 fragments from each species), with each parent colony represented once. Corals were rotated within and among tanks of the same treatment to minimize any positional and tank effects. The experiment mimicked the timing, duration and temperature of a 1996 natural bleaching event in Kaneohe Bay (Jokiel and Brown, 2004). All fragments were exposed to the same conditions except temperature during the 4 weeks in the tanks to accurately assess the physiological consequences of bleaching and recovery on each species.

Only dark-acclimated fluorescence was measured to assess the physiological status of the zooxanthellae, using a diving pulse amplitude modulated (PAM) fluorometer (Walz Inc., Effeltrich, Germany). The fluorometer first exposed the corals to a weak pulsed red light (<1 μmol quanta m^{-2} s^{-1}) to determine F_o (minimal Chl *a* fluorescence yield), followed by a saturating pulse of light (3000 μmol quanta m^{-2} s^{-1}) to determine F_m (maximal Chl *a* fluorescence yield) (Schreiber et al., 1986). Variable Chl *a* fluorescence yield (F_v=F_m–F_o) and the quantum yield of PS II fluorescence (F_v/F_m) were then calculated and reported. Corals were measured at least 2 h after sunset to allow for dark acclimation. The diving PAM fluorometer measured Chl *a* fluorescence at excitation and emission wavelengths of 470 nm and 685 nm, respectively. These do not interfere with the wavelength lifetimes of other known fluorescent proteins in coral species (Gilmore et al., 2003). During the period in the tanks, all fragments were repeatedly analyzed at the end of each week for three consecutive weeks on the evenings of 11, 18 and 25 September 2003. On 2 October 2003 corals were returned to the reef for recovery. Then, only those treated and control corals pre-assigned to 0 months recovery were analyzed that same evening. At 1.5 months (16 November 2003), 4 months (2 February 2004) and 8 months (4 June 2004) recovery, the respective pre-assigned treated and control corals were collected, returned to the outdoor, flow-through seawater tanks, and analyzed each evening.

In addition, using published chlorophyll *a* (Chl *a*) and zooxanthellae concentration data for these same coral fragments measured at 0, 1.5, 4 and 8 months recovery (Rodrigues and Grottoli, 2007), Chl *a* per zooxanthella was calculated. This calculation provides a direct assessment of zooxanthellae status that can complement the fluorescence measurements from the same time periods.

Statistical analyses

A repeated measures two-way analysis of variance (ANOVA) compared the effects of species and temperature on F_v/F_m, F_o, F_m and F_v during the weeks of bleaching only. Sphericity or the assumption that the repeated samples have similar variances, must be tested and corrected for as it affects the power of a repeated measures ANOVA. Mauchly's test assessed sphericity; if the assumption of sphericity was violated, resulting in a loss of test power, the Huynh–Feldt (H-F) correction factor was used to adjust the *P*-values of the univariate tests. *A posteriori* slice tests [e.g. tests of simple effects (Winer, 1971)] directly compared the effect of temperature between the treated and control groups at each bleaching week within each species.

During the eight months of recovery, samples were independent of one another (i.e. pre-assigned to a recovery group and sampled only once). Therefore, ANOVA was used to compare the effects of species, genotype, temperature and recovery interval on F_v/F_m, F_o,

F_m, F_v and Chl *a* per zooxanthella. *A posteriori* slice tests directly compared the effect of temperature between the treated and control groups at each recovery interval and within each species. Since treated and control corals were exposed to identical conditions except temperature during the first 4 weeks of the experiment, observed differences throughout the entire study were independent of season and could be attributed to bleaching alone. The use of replicate genotypes across temperature treatments and recovery times reduced the overall variation between treatments. When treatment values were not statistically different from control values at a single time interval, they are referred to as 'fully recovered' throughout the text.

All data were normally distributed according to plots of residuals *versus* predicted values for each variable. Bonferroni corrections were not used (Quinn and Keough, 2002). Statistical analyses were conducted using SAS software, version 9.1.3 of the SAS System for Windows. (Copyright © 2000-2004 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.) *P*-values ≤0.05 were considered significant.

RESULTS

Zooxanthellae status during bleaching

Mauchly's tests revealed that the assumption of sphericity was violated for F_v/F_m during bleaching only, since the variances and covariances between the repeated samples were significantly different from each other (Table 1). As such, *P*-values adjusted with the H-F correction factor were used for F_v/F_m during bleaching only (Table 2).

For *P. compressa*, F_v/F_m and F_v were not significantly different in treated and control fragments after the first week of bleaching, whereas F_o and F_m were both 113% and 111% higher in treated than control corals, respectively (Fig. 1A–D). F_v/F_m, F_m and F_v of the treated group decreased relative to control values at the end of the second week to 90%, 84% and 77% of control values, respectively, whereas there was no difference in F_o between the treated and control groups (Fig. 1A–D). By the end of the third week, further decreases occurred and F_v/F_m, F_o, F_m and F_v of the treated group were at 84%, 82%, 57% and 49% of control values, respectively (Fig. 1A–D).

M. capitata followed a similar pattern to *P. compressa* for each week during bleaching. All fluorescence treatment and control variables in *M. capitata* were not significantly different after the first week of bleaching (Fig. 1E–H). At the end of the second week, F_v/F_m, F_m and F_v of the treated group decreased to 90%, 87% and 83% of control values, respectively, with no difference between treated and control F_o (Fig. 1E–H). By the end of the third week, further decreases occurred and F_v/F_m, F_o, F_m and F_v of the treated group were 87%, 75%, 62% and 58% of the control values, respectively (Fig. 1E–H).

Table 1. Mauchly's tests for the assumption of sphericity for F_v/F_m, F_o, F_m and F_v during the 3 weeks analyzed in the bleaching period

Variable	d.f.	χ^2-statistic	*P*-value
F_v/F_m	2	13.2873	0.0013
F_o	2	5.1821	0.0749
F_m	2	1.6615	0.4357
F_v	2	1.1539	0.5616

Sphericity is an assumption of a repeated measures ANOVA that the repeated groups have similar variances. When the assumption of sphericity was violated (i.e. $P<0.05$), *P*-values for within-subject tests were corrected using the Huynh–Feldt (H-F) correction factor (see Table 2).
d.f., degrees of freedom.

Zooxanthellae status during recovery

One week later, at the end of bleaching and the start of recovery (i.e. 0 months recovery), in *P. compressa* F_v/F_m, F_o, F_m and F_v of the treated group reached their lowest point at 88%, 42%, 33% and 29% of control values, respectively (Fig. 1A–D). At that same time, Chl *a* per zooxanthella in treated *P. compressa* was not significantly different from control values (Fig. 2A). F_v/F_m and F_o of the treated group fully recovered at 1.5 months recovery (Fig. 1A,B), whereas F_o surpassed control values at 4 and 8 months to 137% and 127%, respectively (Fig. 1B). F_m and F_v were 66% and 62% of controls at 1.5 months, respectively, and were fully recovered at 4 and 8 months (Fig. 1C,D). During that time, Chl *a* per zooxanthella of the treated group dramatically increased compared with control values by more than nine- and eightfold at 1.5 and 4 months, respectively, and was not significantly different from control values at 8 months (Fig. 2A).

For *M. capitata*, F_v/F_m, F_o, F_m and F_v of the treated group, similar to *P. compressa*, reached their lowest point at the start of recovery (i.e. 0 months) at 75%, 47%, 31% and 25% of controls, respectively. However, for the remainder of recovery, fluorescence variables and Chl *a* per zooxanthella in *M. capitata* followed a different pattern

Table 2. Results of two-way repeated measures ANOVAs for F_v/F_m, F_o, F_m and F_v comparing two species (*Porites compressa* and *Montipora capitata*) maintained at two temperatures (ambient and 30°C) and measured repeatedly over 3 weeks during the experimental bleaching period

Variable	Factor	Effect	d.f.	SS	*F*-statistic	*P*-value
F_v/F_m	Between	S	1	0.0762	10.42	0.0015
		T	1	0.5011	68.57	<0.0001
		S×T	1	0.0010	0.14	0.7053
	Within	W	2	0.2655	18.44	<0.0001*
		W×S	2	0.0165	1.15	0.3173*
		W×T	2	0.2603	18.07	<0.0001*
		W×S×T	2	0.0025	0.17	0.8344*
F_o	Between	S	1	395389	102.31	<0.0001
		T	1	5598	1.45	0.2303
		S×T	1	3514	0.91	0.3416
	Within	W	2	125278	19.88	<0.0001
		W×S	2	12300	1.95	0.1434
		W×T	2	112773	17.90	<0.0001
		W×S×T	2	611	0.10	0.9076
F_m	Between	S	1	1522223	30.90	<0.0001
		T	1	1979327	40.17	<0.0001
		S×T	1	51172	1.04	0.3095
	Within	W	2	3576179	56.30	<0.0001
		W×S	2	35194	0.55	0.5751
		W×T	2	3619162	56.98	<0.0001
		W×S×T	2	70785	1.11	0.3292
F_v	Between	S	1	366006	11.20	0.0010
		T	1	1774400	54.29	<0.0001
		S×T	1	81505	2.49	0.1160
	Within	W	2	2399434	54.15	<0.0001
		W×S	2	6348	0.14	0.8666
		W×T	2	2476744	55.89	<0.0001
		W×S×T	2	58687	1.32	0.2672

Effects of species (S) and temperature (T) were between-subject factors and were fixed and fully crossed. The effect of week (W) was the within-subject factor (repeated over 3 weeks) and was fixed and fully crossed with the between-subject factors.
d.f., degrees of freedom; SS, sum of squares of the effects; **P*-values corrected with the H-F correction factor because the assumption of sphericity was violated (see Table 1).

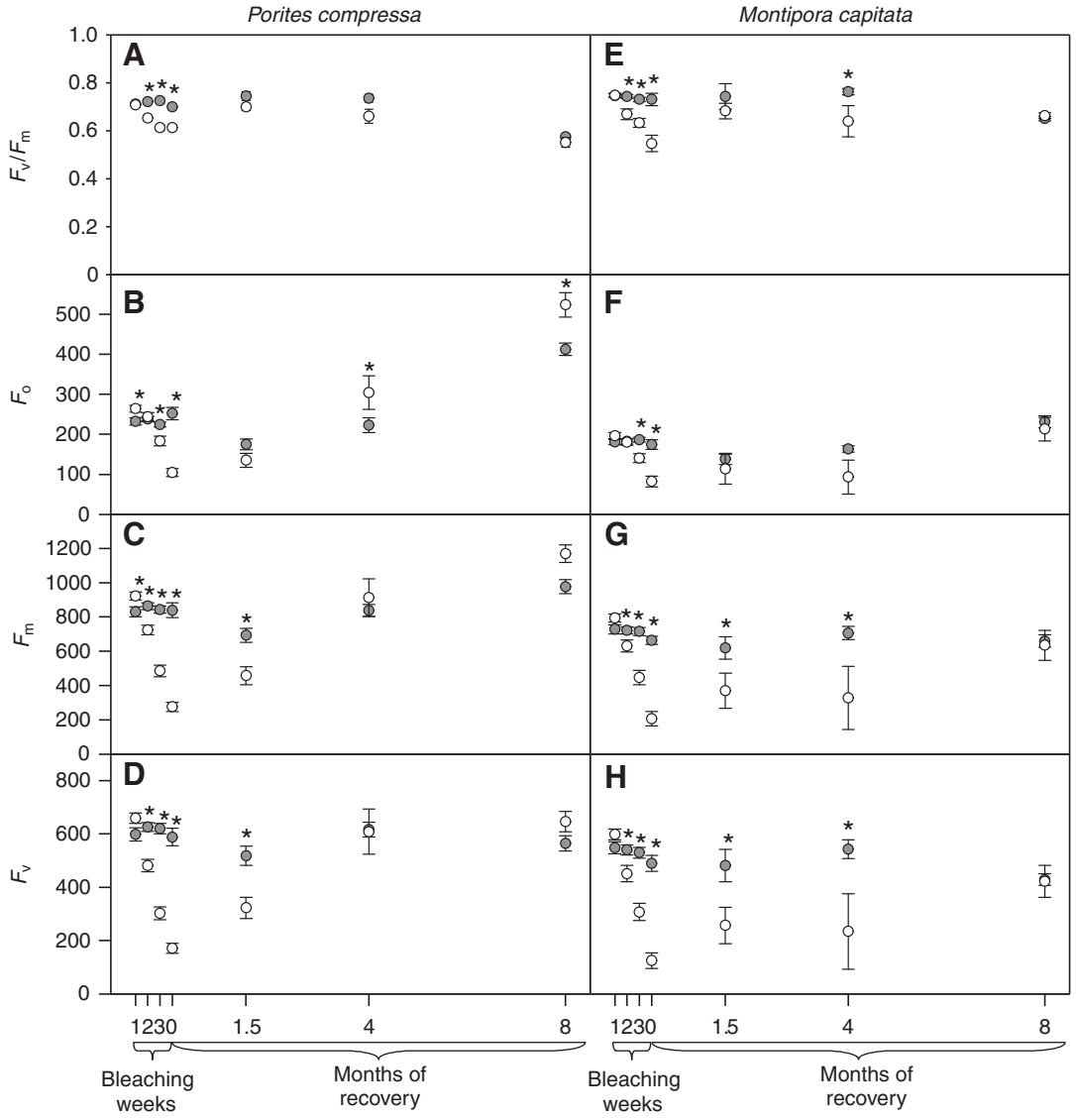

Fig. 1. Average (A,E) quantum yield of photosystem II fluorescence (F_v/F_m), (B,F) minimal chlorophyll *a* fluorescence (F_o), (C,G) maximal chlorophyll *a* fluorescence (F_m) and (D,H) variable chlorophyll *a* fluorescence (F_v) of *Porites compressa* and *Montipora capitata* at weekly intervals during 1 month of bleaching and then at 0, 1.5, 4 and 8 months recovery. Values are means ± s.e.m. *Significant differences at $P \leq 0.05$ between control (grey) and treated (white) samples within a single time interval, by *a posteriori* slice tests. Sample size during bleaching was 48 for each species. Sample sizes during recovery ranged from 4 to 12. Statistical analyses are provided in Tables 1–3. All fluorescence variables are dimensionless.

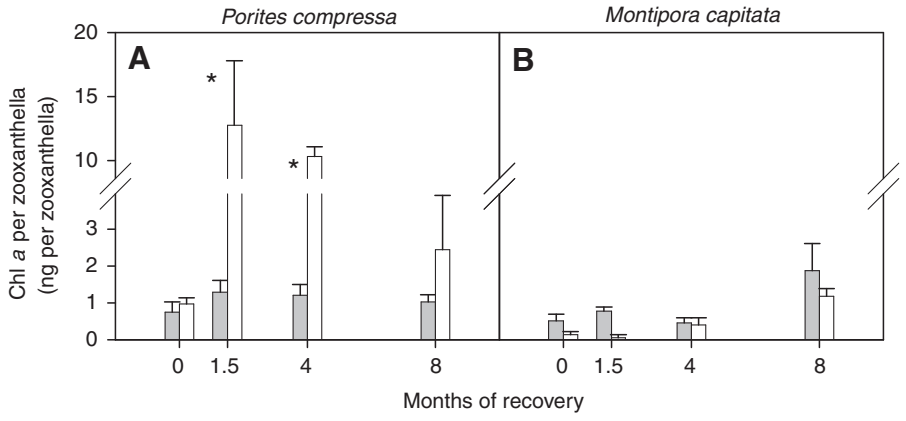

Fig. 2. Chlorophyll *a* (Chl *a*) per zooxanthella during 0, 1.5, 4 and 8 months of recovery for (A) *Porites compressa* and (B) *Montipora capitata*. Values are means ± s.e.m. *Significant differences at $P \leq 0.05$ between control (grey) and treated (white) samples within a single time interval, by *a posteriori* slice tests. Sample sizes ranged from 4 to 6. Statistical analyses are provided in Table 3. Note, the scale of the *y*-axis changes at 10 ng of Chl *a* per zooxanthella.

than that of *P. compressa*. In *M. capitata*, F_v/F_m of the treated group appeared to recover at 1.5 months, but decreased again to 84% at 4 months, before fully recovering at 8 months (Fig. 1E). F_o was fully recovered by 1.5 months and remained so until the end of the analysis at 8 months (Fig. 1F). Both F_m and F_v of the treated group were 60% and 53% of control values at 1.5 months, respectively, and 46% and 43% of controls at 4 months, respectively, before fully recovering at 8 months (Fig. 1G–H). In contrast to *P. compressa*, the Chl *a* per zooxanthella in treated *M. capitata* was not significantly different from control values at 0, 1.5, 4 and 8 months (Fig. 2B).

Interaction effects

Additional patterns were detectable from interaction effects [of species (S), temperature (T), recovery (R) and week (W)] during bleaching and recovery. During bleaching, on average, all fluorescence variables (significant W×T effects) of treated corals decreased more than those of control corals, with similar patterns in both species (non-significant S×T and W×S×T effects; Table 2). All fluorescence variables in treated corals increased more than control corals during recovery (significant T×R effects) and recovery occurred at different months for each species (significant R×S effects; Table 3). Different recovery patterns of F_o and F_m (significant T×S and T×R×S effects) occurred in the two species, but not of F_v/F_m and F_v, where patterns were similar for both species (non-significant T×S and T×R×S effects; Table 3). Chl *a* per zooxanthella increased in treated compared with control *P. compressa*, but no change was detected between treated and control *M. capitata* (significant T×S effect; Table 3).

Table 3. Results of ANOVAs for F_v/F_m ($F_{37,121}$=4.01, P<0.0001), F_o ($F_{37,121}$=13.63, P<0.0001), F_m ($F_{37,121}$=8.74, P<0.0001), F_v ($F_{37,121}$=6.49, P<0.0001) and chlorophyll *a* per zooxanthella ($F_{33,45}$=2.84, P=0.0006), comparing *Porites compressa* and *Montipora capitata* from 12 colonies or genotypes, at ambient temperature and 30°C, and four recovery intervals

Variable	Effect	d.f.	SS	*F*-statistic	*P*-value
F_v/F_m	T	1	0.1786	28.28	<0.0001
	R	3	0.2106	11.11	<0.0001
	S	1	0.0095	1.50	0.2225
	T×S	1	0.0088	1.39	0.2399
	R×S	3	0.0707	3.73	0.0132
	T×R	3	0.0905	4.78	0.0035
	T×R×S	3	0.0283	1.49	0.2195
	G in S	22	0.1827	1.32	0.1751
F_o	T	1	24 094	6.37	0.0129
	R	3	739 324	65.11	<0.0001
	S	1	438 308	115.80	<0.0001
	T×S	1	21 691	5.73	0.0182
	R×S	3	228 666	20.14	<0.0001
	T×R	3	128 470	11.31	<0.0001
	T×R×S	3	67 316	5.93	0.0008
	G in S	22	100 086	1.20	0.2591
F_m	T	1	1 503 842	45.87	<0.0001
	R	3	2 525 727	25.68	<0.0001
	S	1	1 996 066	60.89	<0.0001
	T×S	1	161 417	4.92	0.0284
	R×S	3	707 422	7.19	0.0002
	T×R	3	1 479 416	15.04	<0.0001
	T×R×S	3	375 665	3.82	0.0118
	G in S	22	626 541	0.87	0.6347
F_v	T	1	1 147 237	59.70	<0.0001
	R	3	673 296	11.68	<0.0001
	S	1	563 660	29.33	<0.0001
	T×S	1	64 765	3.37	0.0688
	R×S	3	174 158	3.02	0.0324
	T×R	3	753 576	13.07	<0.0001
	T×R×S	3	144 204	2.50	0.0626
	G in S	22	364 883	0.86	0.6419
Chl *a* per zoox	T	1	133.46	9.19	0.0040
	R	3	86.88	1.99	0.1283
	S	1	175.05	12.06	0.0012
	T×S	1	184.33	12.69	0.0009
	R×S	3	106.85	2.45	0.0755
	T×R	3	108.58	2.49	0.0721
	T×R×S	3	106.99	2.49	0.0721
	G in S	18	168.54	0.64	0.8436

Effects of species (S), temperature (T), and recovery (R) were fixed and were fully crossed. Genotype was a random effect and nested within species (G in S); its interactions were combined with the residual.
Chl *a* per zoox, chlorophyll *a* per zooxanthella; d.f., degrees of freedom; SS, sum of squares of the effects.

DISCUSSION

Zooxanthellae status during bleaching

Most previous studies that measured F_v/F_m of both isolated symbionts and whole corals reported decreased values within hours of temperature stress associated with diminished photochemical efficiency of PS II and dynamic photoinhibition (Bhagooli and Hidaka, 2003; Hill et al., 2004; Jones et al., 1998; Jones et al., 1999; Jones et al., 2000; Lesser, 1996; Lesser and Farrell, 2004; Warner et al., 1996; Warner et al., 1999). However, in the present study, there was no change in F_v/F_m for either species 1 week after the start of bleaching (Fig. 1A,E). Rate of seawater temperature increase occurred rapidly over several hours prior to fluorescence measurements in previous studies (Bhagooli and Hidaka, 2003; Bhagooli and Hidaka, 2004; Dove et al., 2006; Hill et al., 2004; Jones et al., 1998; Jones et al., 2000; Rodolfo-Metalpa et al., 2006; Warner et al., 1996; Warner et al., 1999) compared to a slower increase over several days in the present study, possibly contributing to the different initial outcomes. Although quantum yield remained the same in *P. compressa*, its component variables, F_o and F_m, both significantly increased (Fig. 1B,C) with a similar, but non-significant pattern in *M. capitata* (Fig. 1F,G). Increased F_o in higher plants and algae (Krause, 1988) and corals (Jones and Hoegh-Guldberg, 2001) has been associated with photodamage, but in all of those cases F_m either remained the same or decreased. There are no reports of F_o and F_m both increasing, as observed here. The initial fluorescence response in *P. compressa* may simply be associated with zooxanthellae death and/or expulsion occurring during the first week of bleaching; however, further experimentation is required to determine the onset of zooxanthellae loss in this species. Alternatively, measurement of excess excitation energy dissipated as heat (i.e. nonphotochemical quenching) should also be conducted to establish whether the overall upward shift in initial and maximal fluorescence is a form of photoprotection (Gorbunov et al., 2001).

F_v/F_m did not decrease until *P. compressa* and *M. capitata* had experienced increased temperatures for 2 weeks – which is similar to the first indication of decreased F_v/F_m in the favid coral, *Cladocora caespitosa* after 10 days (Rodolfo-Metalpa et al., 2006). For *P. compressa* this first recorded decrease in F_v/F_m preceded the first visual indication of bleaching (i.e. decreased coloration), whereas some *M. capitata* colonies had visibly begun to bleach a few days before the first recorded decrease of F_v/F_m. Photoprotection

precedes loss of coloration and is typically reversible (e.g. Bhagooli and Hidaka, 2003; Brown et al., 2000; Hill et al., 2004; Jones et al., 2000; Lesser and Gorbunov, 2001). This type of photoprotection probably occurred at week 2 in *P. compressa.* In *M. capitata*, photoprotection may have occurred earlier in the experiment or may not normally occur in this species, suggesting an alternative strategy for dealing with the onset of elevated seawater temperatures. In either case, the decrease in quantum yield resulted from decreased F_m and no change in F_o, so that F_v also decreased in both species. Decreased F_v often occurs when heat dissipation from reaction centers increase (Jones and Hoegh-Guldberg, 2001). This probably occurred in both species in this study and may have been responsible for the decreased F_v that continued throughout the remainder of the bleaching period.

Zooxanthellae status during recovery

Zooxanthellae fluorescence recovery varied between coral species by several months with the return of normal quantum yield of PS II occurring 6.5 months earlier in *P. compressa* than *M. capitata* (Fig. 1A,E). This indicates considerable differences in the photochemical efficiency of zooxanthellae from sympatric coral species following the same bleaching event. Furthermore, when experimental conditions are comparable to a natural bleaching event, as they are here, recovery of normal quantum yield seems to take much longer than previous studies have suggested. The length of time corals were exposed to temperature stress in this study (one month) was much longer than the hours to days of previous studies (Bhagooli and Hidaka, 2003; Bhagooli and Hidaka, 2004; Hill et al., 2004; Jones et al., 1998; Jones et al., 2000; Warner et al., 1996; Warner et al., 1999), possibly contributing to the differences in recovery. In addition, the long time period required for recovery probably increases the susceptibility of corals to consecutive bleaching events. Both species incurred disruptions to photochemical efficiency during recovery (as indicated from statistically similar F_v/F_m), yet the underlying physiological causes were different for each species (as indicated from statistically different F_o and F_m; Table 3).

Although the quantum yield of treated *P. compressa* colonies was fully recovered by 1.5 to 8 months, both F_m and F_v remained low at 1.5 months. This may reflect the period of time required for complete zooxanthellae recovery [at 4 months (Rodrigues and Grottoli, 2007)]. This also suggests chronic photoinhibition, since both F_m and F_v recovered at 4 months. F_o increased and was over-compensating at 4 and 8 months, suggesting that photodamage had affected the structures and functions of PS II (Jones and Hoegh-Guldberg, 2001). The extensive period of photodamage further suggests that the D1 protein was most likely affected (e.g. Warner et al., 1999). At the same time, these small changes in fluorescence were marked by an eight- to tenfold increase in Chl *a* per zooxanthella in the treated group compared with that of control corals (Fig. 2A). Likewise, in several groups of marine phytoplankton, including dinoflagellates, significant increases in Chl *a* concentration did not result in significant and corresponding changes in fluorescence (Rochelle-Newall and Fisher, 2002), suggesting the importance of non-fluorescent pigments. For *P. compressa*, elevated Chl *a* per zooxanthella values may be due to a greater increase in the peridinin-chlorophyll-protein complex, which has been shown to have light-shielding properties (Dove et al., 2001). Further research is needed to elucidate the type of non-fluorescent chlorophyll molecule responsible for the dramatic increase in Chl *a* per zooxanthella concentration in *P. compressa.*

Unlike *P. compressa*, there was little evidence of the same type of photodamage in *M. capitata*, as F_o was fully recovered between 1.5 and 8 months and there were no changes in Chl *a* per zooxanthella throughout recovery. However, low F_m and F_v values, lasting for at least the first 4 months of recovery, indicated long-term photoinhibition that was probably chronic (Krause and Weis, 1984) and characteristic of photoinhibition on the donor side of PS II in higher plants (Bertamini and Nedunchezhian, 2004). Although chlorophyll pigments were lost, the total number of zooxanthellae was retained when *M. capitata* was stressed with increased ultraviolet radiation (Grottoli-Everett and Kuffner, 1995) or elevated seawater temperature (Rodrigues and Grottoli, 2007). Therefore, repair of the donor side of PS II took at least 4 months, since expulsion and acquisition of new zooxanthellae did not occur in *M. capitata*.

Although several parameters of dark-acclimated fluorescence were affected throughout recovery in both species (Fig. 1), mid-day photosynthesis had recovered by 1.5 and 4 months in *P. compressa* and *M. capitata*, respectively (Rodrigues and Grottoli, 2007). Similarly, Hoogenboom et al. (Hoogenboom et al., 2006) found no measurable difference in coral photosynthesis in high light, although the electron transport rate from PS II declined by more than half. Dynamic photoinhibition during the day protected the photosynthetic apparatus and maintained normal photosynthesis rates (Lesser and Gorbunov, 2001). However, reduced metabolic rates (decreased net photosynthesis and coral plus zooxanthellae respiration) did occur after bleaching in both species (Rodrigues and Grottoli, 2007), preceding the period of photodamage in *P. compressa* and chronic photoinhibition in *M. capitata*. Together this suggests that reduced coral metabolism may be an indication of severe stress in the remaining zooxanthellae.

Zooxanthellae and host strategies

P. compressa and *M. capitata* have previously been found to contain zooxanthellae types C15 and C31, respectively (LaJeunesse et al., 2004); zooxanthellae type was not confirmed in the present study. Although *P. compressa* took 6 days longer (this study) and 36 days longer after a natural event than *M. capitata* to visibly bleach (Grottoli et al., 2004), PS II fluorescence was not different between species during the bleaching period. The mechanisms underlying the different bleaching rates in both species are difficult to identify, since at the level of PS II both zooxanthellae types were similarly affected. Activity at one or more other levels within the chloroplast (i.e. electron transport, photosystem I, ATP synthase, or carbon fixation) may differ between zooxanthellae types and coral species and account for the visible differences in bleaching (e.g. Smith et al., 2005; Tchernov et al., 2004). Despite their similarities during bleaching, zooxanthellae from each coral species differed in how they recovered. Type C15 in *P. compressa* was more prone to long-term photodamage (i.e. increased F_o; Fig. 1B), significant increases in Chl *a* per zooxanthella (Fig. 2A) and recovery of Chl *a* and zooxanthellae concentrations at 4 and 8 months, respectively (Rodrigues and Grottoli, 2007). By contrast, type C31 in *M. capitata* experienced chronic photoinhibition of the donor side of PS II (i.e. decreased F_m and F_v; Fig. 1G–H) and recovery of Chl *a* concentration at 8 months (Rodrigues and Grottoli, 2007), with no significant change to Chl *a* per zooxanthella (Fig. 2B) or zooxanthellae concentrations (Rodrigues and Grottoli, 2007). Quantum yield of PS II fluorescence (F_v/F_m) also recovered faster in *P. compressa*, indicating that its zooxanthellae type may be more resilient than that of *M. capitata*. Together these differences may account for previously observed differences in Chl *a* recovery, since

P. compressa recovered Chl *a* at least 4 months sooner than *M. capitata* (Rodrigues and Grottoli, 2007).

The two coral species used in this study also exhibited contrasting host strategies during recovery from bleaching: *P. compressa* relies on stored energy reserves and photosynthetically acquired carbon and *M. capitata* relies on heterotrophically acquired carbon when photosynthesis was not available (Grottoli et al., 2006; Rodrigues and Grottoli, 2006; Rodrigues and Grottoli, 2007). Changes to zooxanthellae fluorescence and differences in Chl *a* per zooxanthella further support these two distinct strategies. The more resilient zooxanthellae of *P. compressa* play an important role in colony recovery, since increases in Chl *a* per zooxanthella are required before energy reserves (Rodrigues and Grottoli, 2007) and calcification (Rodrigues and Grottoli, 2006) can recover. By contrast, the zooxanthellae of *M. capitata* are probably not as essential to colony recovery, since heterotrophy can be relied upon during the recovery period (Grottoli et al., 2006). Altogether our data highlight two strategies utilized by these two coral species to survive and recover from bleaching events, with *P. compressa* relying primarily on more resilient zooxanthellae and *M. capitata* relying on a more resilient host. Ultimately, coral bleaching and recovery appear to involve the combined effects of zooxanthellae and host physiology.

LIST OF ABBREVIATIONS

Chl *a*	chlorophyll *a*
F_m	maximal Chl *a* fluorescence yield measured after dark acclimation
F_o	minimal Chl *a* fluorescence yield measured after dark acclimation
F_v	variable Chl *a* fluorescence yield measured after dark acclimation (F_v=F_m–F_o)
F_v/F_m	quantum yield of photosystem II fluorescence measured after dark acclimation
H-F	Huynh–Feldt correction
PAM	pulse amplitude modulated
PS II	photosystem II

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