Limnol. Oceanogr., 56(1), 2011, 139–146

doi:10.4319/lo.2011.56.1.0139

Increased rate of D1 repair in coral symbionts during bleaching is insufficient to counter accelerated photo-inactivation

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Abstract

We dissect the primary photo-inactivation and the counteracting metabolic repair rates in fragments of the scleractinian coral, *Pocillopora damicornis*, subjected to a combined stress of a shift to elevated temperature (from 26°C to 32°C) and increased light (from 200 μmol photons m^{-2} s^{-1} to 400 μmol photons m^{-2} s^{-1}) to induce bleaching. During the bleaching treatment the dinoflagellate symbionts showed a 5.5-fold acceleration in their photosystem II (PSII) repair rate constant, demonstrating that they maintain strong metabolic capacity to clear and replace photo-damaged D1 protein at the elevated temperature and light conditions. Nevertheless, the symbionts concurrently suffered a seven-fold increase in the rate constant for PSII photo-inactivation. This rapid photo-inactivation exceeded the PSII repair capacity, therefore tipping the symbionts, and by implication the symbiosis, into net photo-inhibition. Increased photo-inactivation in hospite, rather than an inhibition of PSII repair, is the principle trigger for net photo-inhibition under bleaching conditions.

In scleractinian corals the dinoflagellate symbionts (genus *Symbiodinium*), found within the gastrodermal cells of the cnidarian host, are highly susceptible to photo-inhibition under elevated temperature and high light conditions (Hoegh-Guldberg and Jones 1999; Bhagooli and Hidaka 2004). Climate change is causing a rise in sea surface temperatures and increases as small as 1–2°C above summertime averages are enough to trigger mass coral bleaching events where symbionts are expelled from the host, often leading to widespread coral death (Hoegh-Guldberg 1999). The breakdown in the symbiosis has been attributed to photo-inactivation within the symbionts, with studies evaluating potential sites of damage within the photosynthetic machinery (Jones et al. 1998; Warner et al. 1999; Hill and Ralph 2008).

All oxygenic photosynthetic organisms acclimate to balance the rate of photo-inactivation, the primary event that leads to a photosystem II (PSII) reaction center losing activity (Sinclair et al. 1996; Nishiyama et al. 2006; Six et al. 2007), and their rate for metabolic clearance and replacement of damaged components of PSII (Aro et al. 1993; Silva et al. 2003; Murata et al. 2007). When photo-inactivation exceeds PSII repair the symbionts suffer a drop in content of active PSII, which can eventually lead to photo-inhibition of photosynthetic capacity.

Photo-protective mechanisms in the form of nonphotochemical quenching (NPQ) can be activated (Demmig-Adams and Adams 1992; Sarvikas et al. 2006) to dissipate excess energy before it leads to the production of toxic reactive oxygen species (Nishiyama et al. 2005). Energy-dependent quenching and activation of the xanthophyll cycle is an important mechanism for dissipating excess energy in coral symbionts (Brown et al. 1999), and the dissociation of light-harvesting complexes, similar to state transitions, has recently been suggested to function as a means of NPQ (Reynolds et al. 2008).

The D1 protein is an essential component of PSII, responsible for binding the primary electron donors and acceptors. It has an unusually high rate of light-dependent turnover (Mattoo et al. 1984; Edelman and Mattoo 2008) with photo-inactivation (Tyystjärvi 2008) occurring in the light with the release of damaged D1 from partially disassembled PSII complexes (Aro et al. 1993), through activity of a membrane-bound FtsH protease complex (Silva et al. 2003; Nixon et al. 2004, 2010). Replacement of the cleared D1 occurs via de novo synthesis of the protein, which is then incorporated during the reassembly of the PSII complex (Aro et al. 1993) to regenerate a functional PSII. The abundance of the D1 protein (encoded by the *psbA* gene) can be determined using immunodetection techniques (Bouchard et al. 2005; Six et al. 2007, 2009). The function of the PSII pool can be monitored through measures of chlorophyll *a* (Chl *a*) fluorescence, particularly maximum quantum yield monitored as $F_V : F_M$, to track the degree of net photo-inactivation. Addition of lincomycin, an inhibitor of chloroplast protein synthesis (Aro et al. 1993; Singleton and Sainsbury 1994), allows for the determination of the rate of PSII photo-inactivation, and the rate of clearance of the D1 protein from photo-inactivated PSII centers, in the absence of the counteracting repair processes. The balance between repair and photo-inactivation is modulated by the light acclimation state, in response to environmental variables, and indicates the capacity for photo-protection and short-term photo-acclimatory plasticity under different experimental treatments (Six et al. 2007).

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Previous studies on the function of PSII have shown a causal link between photo-inhibition and coral bleaching with a temperature-dependent decline in PSII quantum yield (Warner et al. 1996; Hill and Ralph 2006). Warner et al. (1999) and Robison and Warner (2006) correlated this photo-inhibition with a drop in D1 content, while Lesser and Farrell (2004) showed a drop in D1 content in corals exposed to high light when compared to those under low light at the same thermal stress. Recent work on cultured *Symbiodinium* isolated from clams indicated that thermal sensitivity is linked to the repair cycle of photo-damaged PSII (Takahashi et al. 2009). However, until now, no parallel analysis of D1 damage or repair under both optimal growth and bleaching conditions has been performed on in hospite coral symbionts. We therefore monitored PSII function and D1 dynamics in corals exposed to elevated light and temperature conditions. We evaluated the rates of photo-inactivation and counteracting repair along with the capacity to tolerate increases in irradiance and temperature for in hospite and expelled symbionts from the scleractinian coral *Pocillopora damicornis*.

Methods

Coral specimens—Colonies of *Pocillopora damicornis* (Linnaeus), originally obtained from Heron Island lagoon (< 2 m deep; 152°06′E, 20°29′S) and known to contain Clade C *Symbiodinium* (Ulstrup et al. 2006; Sampayo et al. 2007; Hill et al. 2009), were maintained in recirculating artificial seawater at 26 ± 0.5°C and 200 μmol photons m^{-2} s^{-1} (400-W, 14,000-K metal halide lamps) in a 500-liter aquarium at the University of Technology, Sydney, Australia. Twenty-eight nubbins (single branches 2–4 cm in length) were broken from each of four *P. damicornis* colonies and held in the aquarium to acclimatise for 14 d prior to experimentation ($n = 4$).

Experimental protocol—Nubbins were placed in aerated, 2-liter beakers maintained at 26°C and exposed to 0 μg mL^{-1} or 500 μg mL^{-1} of lincomycin. Eight beakers were maintained under control conditions of 26°C and 200 μmol photons m^{-2} s^{-1} (400-W, 14,000-K metal halide lamps), with four containing lincomycin (+ lincomycin) and another four without lincomycin (− lincomycin). Another eight beakers (including four treated with 500 μg mL^{-1} lincomycin) were exposed to 400 μmol photons m^{-2} s^{-1} (400-W, 14,000-K metal halide lamps), with the temperature ramped from 26°C to 32°C over 6 h, constituting bleaching conditions. After a further 8 h (time [t] = 14 h), the lights were switched off for both the control and bleaching treatments. Simultaneously, the temperature was ramped down to 26°C in the bleaching treatment over 3 h, the beginning of the recovery period. Following 12 h of darkness (t = 26 h), lights were switched back on at 200 μmol photons m^{-2} s^{-1} for both treatments for a further 12 h (t = 38 h).

Seven nubbins were placed in each beaker and progressively removed for destructive sampling throughout the experiment. At time 0 h and 14 h (end of bleaching conditions), one nubbin from each beaker was harvested for determination of in hospite zooxanthellae density (cm^{-2}) and Chl *a* and c_2 contents (μg cm^{-2}) using the techniques described in Hill and Ralph (2006). A nubbin from each beaker was collected for D1 and ribulose-1,5-bisphosphate carboxylase (Rubisco) protein detection at time 0 h, 6 h, 14 h, 26 h, and 38 h. Coral tissue was removed from the skeleton using the air-brush technique in 15 mL 0.45-μm-filtered seawater and the slurry centrifuged at 1000 revolutions min^{-1} for 5 mins. The supernatant was discarded and the pellet resuspended in 10 mL of 0.45-μm-filtered seawater. The solution was then filtered onto a 47-mm-diameter Whatman GF/F filter, placed into a 2-mL cryovial and frozen at −80°C (Petrou et al. 2010). In addition, expelled zooxanthellae were in adequate abundance for D1 protein detection in the bleaching treatments and were collected at 14 h on GF/F filters. D1 and Rubisco protein quantification was performed using the protein extraction and immunoblotting techniques detailed in Six et al. (2007), Brown et al. (2007), and Brown et al. (2008). The anti-PsbA antibody used for D1 detection was raised in chickens against a conserved peptide sequence that is present at the C-terminus of mature, PsbA proteins from eukaryotes and cyanobacteria. The RbcL antibody for Rubisco detection was raised in rabbits against a peptide sequence that is conserved across RbcL proteins from oxygenic photo-autotrophs. Protein extract samples were co-loaded with accompanying protein quantitation standards (www.agrisera.com) to immunodetect and quantify the content of the D1 and Rubisco proteins.

Chlorophyll fluorescence measurements—In addition to the destructive sampling procedures, Chl *a* fluorescence measurements were taken on the upper, light-exposed surface of nubbins (within 2 cm of the branch tip) using a Mini-PAM (Pulse Amplitude Modulated) Fluorometer (Walz, GmbH). Samples were temporarily transferred to individual dark-adaptation chambers for 10 min and the PSII photochemical yield ($F_V : F_M$) calculated from a multiple turnover flash using 1.5-mm-diameter acrylic fibre optic probes. Ten minutes of dark adaptation were found to be sufficient to relax most of the NPQ, with longer periods of dark adaptation not resulting in any significant increase in $F_V : F_M$.

During the first 6 h of treatment both temperature and light intensity were changing and initial fluctuations in $F_V : F_M$ could be attributed in part to slowly reversible NPQ induction, as opposed to full photo-inactivation of PSII. Photo-inactivation was estimated, starting from the 6-h time point, as an exponential decay rate constant (h^{-1}) in corals treated with lincomycin to block chloroplast ribosome function and, thus, stop PSII repair. To determine the pseudo–first-order rate constant for PSII repair (h^{-1}), starting from the 6-h time point, the difference in the rates of decay in $F_V : F_M$ plotted vs. time in the absence of lincomycin (repair active) and in the presence of lincomycin (repair blocked) was estimated. To withstand changes in irradiance (and temperature), the rate of PSII repair must equal or exceed the rate of photo-inactivation.

Table 1. Zooxanthellae density (cm^{-2}) and Chl *a* and c_2 content ($\mu g\ cm^{-2}$) at time 0 h and 14 h for the control and bleaching treatments. Averages ± standard error (SE) of mean for both lincomycin-free (−) and lincomycin-treated (+) samples are shown ($n = 4$). * indicates significant difference between time 0-h and 14-h measurements.

Treatment	Time (h)	Lincomycin	Cell density (cm^{-2})	Chl *a* ($\mu g\ cm^{-2}$)	Chl c_2 ($\mu g\ cm^{-2}$)
Control	0	−	$1.54\times10^6\pm0.16$	2.91±0.38	1.52±0.21
		+	$1.67\times10^6\pm0.25$	3.03±0.33	1.45±0.14
	14	−	$1.68\times10^6\pm0.40$	3.14±0.72	1.57±0.15
		+	$1.59\times10^6\pm0.38$	2.74±0.50	1.53±0.26
Bleaching	0	−	$1.85\times10^6\pm0.16$	3.21±0.56	1.44±0.06
		+	$1.82\times10^6\pm0.33$	3.16±0.71	1.45±0.33
	14	−	$0.44\times10^6\pm0.13$*	0.93±0.30*	0.37±0.12*
		+	$0.40\times10^6\pm0.08$*	0.92±0.24*	0.41±0.09*

Statistical analyses—Immunoblot quantification of D1 and Rubisco contents was performed with a Kodak 4000MMPro charge-coupled device imager and associated software (Carestream Molecular Imaging). Repeated-measures analysis of variance (rmANOVA) was used to identify changes in protein contents and $F_V:F_M$ over the time period of the experiment ($\alpha = 0.05$). Tukey's post hoc comparisons were used to detect the location of any significant changes over time ($\alpha = 0.05$). One-way ANOVA tests were employed to detect: (1) any changes in zooxanthellae density and Chl *a* and c_2 contents from time 0 h to 14 h, (2) differences between lincomycin treatments (+ or − lincomycin), and (3) differences in protein contents between expelled and extracted zooxanthellae at 14 h ($\alpha = 0.05$). To determine whether the assumptions of the parametric ANOVA tests were satisfied, the Kolmogorov–Smirnov normality test and Levene's homogeneity of variance test were applied. The SPSS statistical software (version 11.0.0, 2001) was used to perform these analyses.

Results

Symbiont density and chlorophyll content (*a* and c_2) within the host tissue were steady during the 14 h of control conditions, but declined significantly during the bleaching treatment, with both − and + lincomycin (Table 1). The lincomycin treatment had no significant effect on the extent of the decrease in symbiont density, or on chlorophyll levels. Furthermore, during bleaching, symbiont density and chlorophyll declined by similar extents, so the drop in chlorophyll was explicable through losses of symbionts from the coral nubbin, rather than bleaching of pigment in the remaining symbionts.

In the absence of lincomycin, $F_V:F_M$ remained unchanged at around 0.7 during the experimental period in the controls ($p = 0.356$; Fig. 1a), but significantly declined to 0.32 after 38 h in nubbins under control conditions but exposed to lincomycin ($p = 0.004$). Under bleaching conditions, a decline in $F_V:F_M$ from 0.7 at 0 h was observed in the presence ($p = 0.001$) and absence of lincomycin ($p < 0.001$), with a greater decline by the end of the exposure period (14 h) in the + lincomycin samples (0.02) compared to the − lincomycin samples (0.32; Fig. 1b). Lincomycin had a significant effect on $F_V:F_M$ following 8 h of exposure and this difference was maintained up to 38 h. During the recovery period, a significant increase in $F_V:F_M$ was found in the − lincomycin treatment by 38 h where $F_V:F_M$ rose to 0.59.

Immunoblots supported the chlorophyll fluorescence data, showing changes in D1 protein content over time (Fig. 1c,d) in close parallel to the changes in PSII function. In the presence of lincomycin, resynthesis of D1 was inhibited, while photo-inactivation and D1 clearance continued, leading to greater drop in $F_V:F_M$ than in samples in the absence of lincomycin. Initial levels of D1 were 0.017 pmol μg total protein^{-1} and subsequent changes generally tracked changes in $F_V:F_M$ (Fig. 1c,d). The only statistically significant change was, however, a decrease in D1 content in the bleaching treatment in the presence of lincomycin where contents declined to 0.004 pmol μg total protein^{-1} ($p = 0.049$). No statistically significant change was detected in the absence of lincomycin under bleaching conditions ($p = 0.117$). Similarly, there was no significant change in D1 content in the presence ($p = 0.239$) or absence ($p = 0.995$) of lincomycin in the nubbins under control treatment conditions. Figure 2 provides a representative example of D1 protein immunodetection in a single replicate of the bleaching treatment in the presence and absence of lincomycin. In Fig. 2 the main D1 protein band is observed at an apparent molecular weight of ~ 30 kDa. Near the bottom of the image multiple smaller fragments derived from the C-terminus of the D1 protein are apparent.

At the 14-h time point in the expelled symbiont population, the D1 content was 0.0042 ± 0.0002 pmol μg total protein^{-1} in the absence of lincomycin and 0.0050 ± 0.0012 pmol μg total protein^{-1} in the presence of lincomycin (averages ± SEM). Although no difference was detected between + and − lincomycin ($p = 0.266$), these contents were significantly lower ($p = 0.036$) than the content in zooxanthellae extracted from nubbins undergoing bleaching (*see* Fig. 1d), suggesting that the expelled zooxanthellae subpopulation is more inhibited than the zooxanthellae subpopulation still in the nubbins.

The quantitative immunoblots of RbcL protein did not detect significant differences in Rubisco levels between samples treated with or without lincomycin, nor did they show significant changes over the length of the experiment.

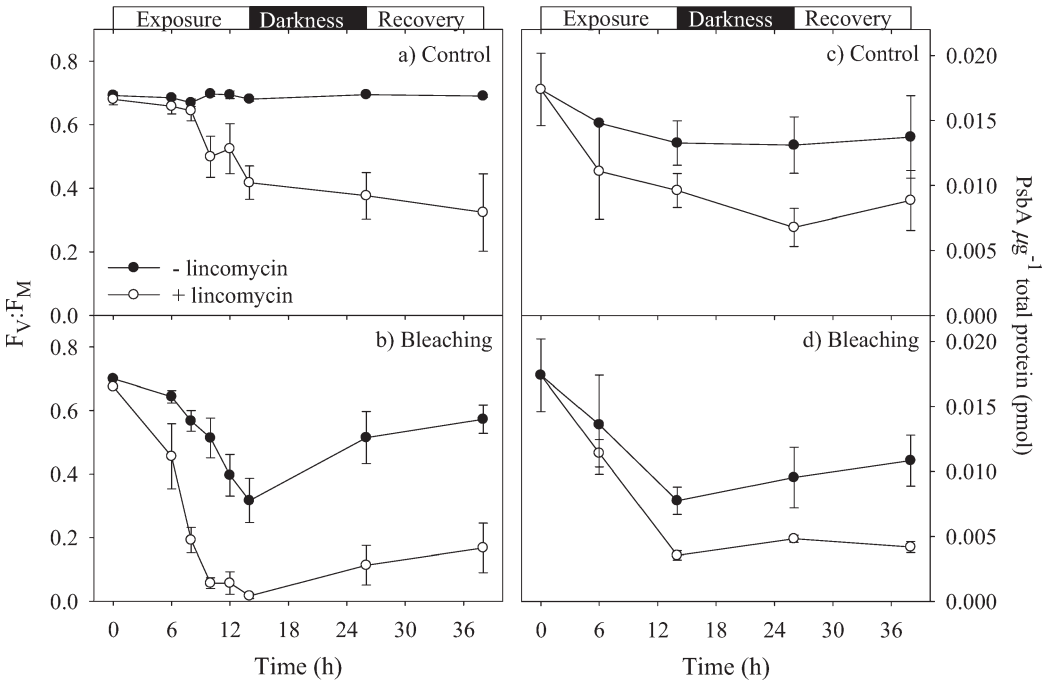

Fig. 1. (a, b) Maximum quantum yield of PSII ($F_V:F_M$) and (c, d) pmol PsbA μg^{-1} total protein of *P. damicornis* exposed to ± lincomycin under control conditions (200 μmol photon m^{-2} s^{-1} and 26°C) and bleaching conditions (400 μmol photon m^{-2} s^{-1} and 32°C). Samples were exposed to experimental conditions during the first 14 h, followed by 12 h of darkness and 26°C and a further 12 h recovery under 200 μmol photon m^{-2} s^{-1} (as indicated by the shaded bars). Data represent means (n = 4, SEM).

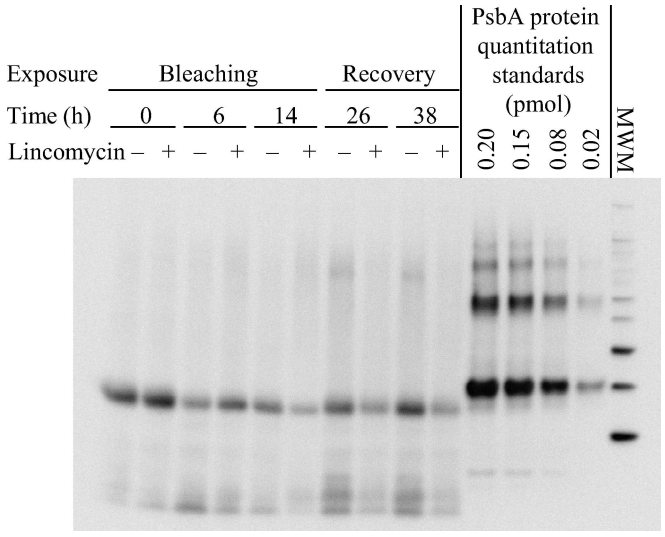

Fig. 2. Representative example of D1 protein detection by immunochemistry for a single replicate of the bleaching treatment (and subsequent recovery) showing band intensity for both − and + lincomycin at 0 h, 6 h, 14 h, 26 h, and 38 h. Quantitation standards of 0.20 pmol PsbA, 0.15 pmol PsbA, 0.08 pmol PsbA, and 0.02 pmol PsbA protein are shown, along with the molecular weight marker (MWM). The native D1 protein from the symbionts migrates at a slightly lower MW than the D1 quantitation standard, which is derived from a cloned gene that encodes an engineered affinity tag that slightly increases the MW of the standard protein. Low MW fragments represent C-terminal cleavage products derived from the D1 protein.

Rubisco (RbcL) contents were 0.014 ± 0.010 (SD) pmol μg total protein^{-1} in > 60 determinations from three experimental replicates.

The PSII photo-inactivation rate constant was determined from the decay in PSII maximum photochemical yield when plotted against time, in the presence of lincomycin (Fig. 3a,b), with the exponential decay curve fit starting after the initial 6-h induction period. This was applied to both the control and bleaching treatments. In the control treatment, the coral symbionts showed a rate constant of -0.056 h^{-1} (Fig. 3a), whereas in the bleaching treatment, the rate constant increased sharply to -0.392 h^{-1} (Fig. 3b; *see* Table 2). A pseudo–first-order rate constant for PSII repair was determined by estimating the difference between the exponential decay rate for $F_V:F_M$ plotted vs. elapsed time with repair present (− lincomycin) and repair blocked (+ lincomycin; Fig. 3a,b). In the control treatment, the achieved repair rate constant was 0.055 h^{-1}. Under the elevated light and temperature of the bleaching treatment, the repair rate constant increased to 0.303 h^{-1} (Table 2).

To avoid net photo-inhibition the repair rate of PSII must equal or exceed the photo-inactivation rate. Under control conditions of 26°C and 200 μmol photons m^{-2} s^{-1} the repair rate matched photo-inactivation, while under bleaching conditions, during exposure to 32°C and 400 μmol photons m^{-2} s^{-1}, the PSII repair rate was insufficient to counteract photo-inactivation (Table 2). Therefore, although the repair rate of PSII was 5.5 times

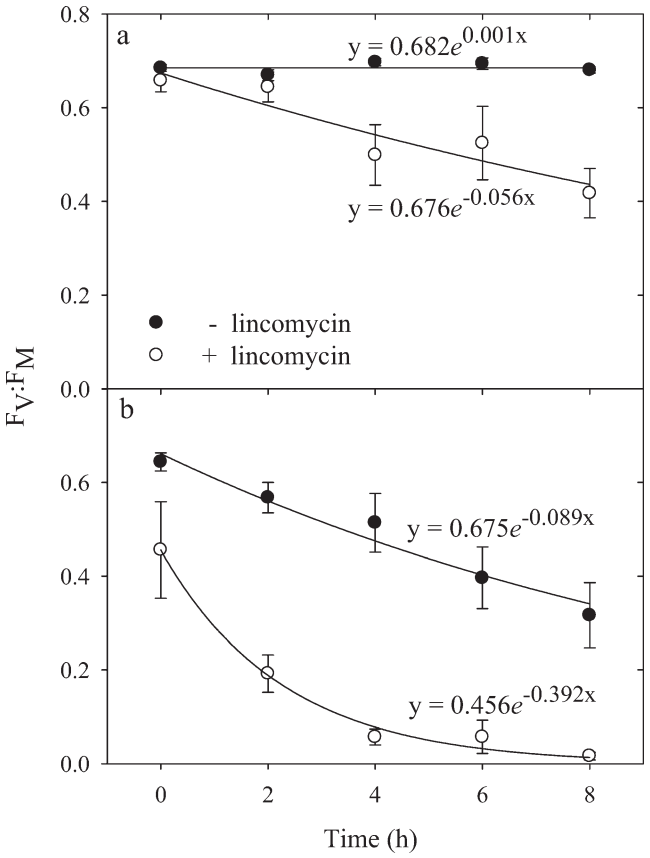

Fig. 3. Maximum quantum yield of PSII ($F_V:F_M$) of *P. damicornis* exposed to ± lincomycin under (a) control conditions (200 μmol photon m^{-2} s^{-1} and 26°C) and (b) bleaching conditions (400 μmol photon m^{-2} s^{-1} and 32°C), expressed as a function of time (h). $F_V:F_M$ values from 6 h onwards (once light and temperature became constant) are plotted starting at 0 h. Data are fitted to exponential decay functions. Data represent means (n = 4, SEM).

faster under bleaching conditions compared to optimal control growth conditions, it was well below the rate necessary to counteract the accelerated photo-inactivation, thus leading to net photo-inhibition of PSII activity (*see* Table 2). This imbalance under bleaching conditions is a result of both the increased incident light, but more importantly the large increase in the susceptibility of the symbionts to photo-inactivation of their PSII.

Discussion

Until now, a detailed study on the breakdown and repair of the D1 protein of PSII in the in hospite symbionts of scleractinian corals was lacking. Here, we dissected the repair rate of PSII and the susceptibility of the symbionts to photo-inactivation of their PSII for *Pocillopora damicornis* exposed to optimal and bleaching conditions.

The symbionts of corals maintained under control conditions at 26°C showed a rate constant for photo-inactivation of only 0.056 h^{-1} (Table 2). The primary photo-inactivation rate provoked by an incident irradiance of 200 μmol photons m^{-2} s^{-1} can be fully counteracted by the measured PSII repair rate (Table 2). This repair rate is slightly slower than the rates we have measured for large, free-living diatoms from temperate waters (Key et al. 2010), but is also reasonable for a symbiont dinoflagellate living under conditions of restricted metabolism.

Table 2. Photo-inactivation and repair rate constants of *in hospite* symbionts in the coral *Pocillopora damicornis* under control and bleaching conditions.

	Control	Bleaching
Temperature (°C)	26	32
Light intensity (μmol photons m^{-2} s^{-1})	200	400
Photoinactivation rate constant (h^{-1})	0.056	0.392
Repair rate constant (h^{-1})	0.055	0.303
Repair vs. photoinactivation	Repair= photoinactivation	Repair< photoinactivation

Bleaching stress led to a large increase in the susceptibility of the coral symbionts to photo-inactivation of their PSII, with the rate constant for photo-inactivation increasing 7 fold, far more than the doubling predicted by the simple doubling in incident irradiance (Table 2). Symbionts exposed to bleaching conditions also had accelerated PSII repair rates (Table 2), demonstrating they retained strong metabolic capacity for protein synthesis and clearance of photo-damaged D1 protein. Compared to other taxa where the clearance of photo-inactivated D1 appears to be the rate limiting step on repair (Six et al. 2007), these coral symbionts retained their capacity to remove photo-inactivated PSII as D1 protein content (Fig. 1d) declined approximately in parallel with the decline in PSII function (Fig. 1c), with no evidence for retention of intact but photo-inactivated D1 proteins. In contrast, we did detect significant levels of D1 C-terminal fragments (lower molecular weight fragments that have lost amino acids from the N-terminal end), consistent with breakdown products (Fig. 2). The RbcL immunoblots showed no detectable evidence of RbcL cleavage products, or larger cross-linked products, which have been found in other organisms under ultraviolet light or reactive oxygen stress (Wilson et al. 1995; Marcus et al. 2003; Nakano et al. 2006). Nishiyama et al. (2006) found that stress from reactive oxygen species (ROS) manifests as inhibition to PSII repair, whereas optical changes or changes in PSII organisation would manifest as altered susceptibility to primary photo-inactivation. Our data thus show that increased susceptibility to photo-inactivation, rather than an inhibition of PSII repair (Nishiyama et al. 2006), is the principal trigger for net photo-inhibition of PSII activity under these bleaching conditions in hospite, in contrast to the recent findings of Takahashi et al. (2009) on cultured, Clade A *Symbiodinium*. Despite their elevated PSII repair rates during bleaching, the symbionts were unable to counteract the increased rate of photo-inactivation provoked by the combination of increased temperature and an

increased irradiance of 400 μmol photons m^{-2} s^{-1} impinging on the coral surface, and so the zooxanthellae suffered net photo-inhibition of PSII.

An alternate bleaching hypothesis has attributed the initial weakness to damage to the dark reaction of the zooxanthellae (Jones et al. 1998). The lack of change in Rubisco protein content found during thermal stress of these experiments does not support the results of Jones et al. (1998). However, the simultaneous decline in PSII photochemical efficiency and D1 protein content detected in our study during exposure to bleaching conditions, closely matched the study of Warner et al. (1999). Therefore, these results support the model of increased PSII photo-inactivation as the initial step in damage to the photosynthetic apparatus of zooxanthellae during bleaching events (Warner et al. 1999; Hill and Ralph 2006; Robison and Warner 2006).

In this study on in hospite symbionts, contrary to a study on cultured *Symbiodinium* (Takahashi et al. 2009), we show that coral symbionts not only retain their full capacity for the repair of PSII when exposed to bleaching conditions (400 μmol photons m^{-2} s^{-1} and 32°C), but were also able to significantly increase their achieved rate of PSII repair. The applied bleaching treatment does not, therefore, remove the symbionts' metabolic capacity for photoprotection. The combination of thermal stress, which increases the susceptibility of the symbionts to photoinactivation, and increased light, nevertheless overwhelms the accelerated PSII repair rate, which ultimately leads to photo-inhibition of PSII. However, our analyses do not reveal the mechanism for the increase in symbiont susceptibility to photo-inactivation. We do find that even under bleaching conditions, upon photo-inactivation, damaged D1 proteins are removed, because D1 protein content declined approximately in parallel with PSII photoinactivation, with no evidence for retention of photoinactivated PSII centers.

The achieved PSII repair rate of the control samples under 200 μmol photons m^{-2} s^{-1} is slow, but sufficient to keep pace with the slow photo-inactivation rate. When, however the coral was exposed to bleaching conditions, the photo-physiology changes sharply. The photo-inactivation rate accelerates. The repair rate also accelerates under the elevated temperature of the bleaching treatment, but PSII photo-inactivation occurs at a much higher rate than PSII repair, resulting in net photo-inhibition of PSII, and ultimately coral bleaching through symbiont expulsion. As supporting evidence, the sub-population of expelled symbionts showed lower contents of the D1 protein than the symbiont sub-population remaining in the nubbins during bleaching.

In our results, it is the combination of increased irradiance and increased susceptibility to photo-inactivation, presumably as a result of thermal stress, that causes photo-inactivation to exceed repair even though repair rate also increases. This supports previous studies that show coral bleaching only occurs during a combination of elevated light and temperature (Brown 1997). The application of our bleaching treatment (a concomitant increase in irradiance and temperature), does not allow for distinction between independent effects of light and temperature, but thermal stress is well-known to increase the susceptibility of coral symbionts to light-induced PSII photo-inactivation (Bhagooli and Hidaka 2004; Takahashi et al. 2008; Hill et al. 2009).

In conclusion, we find that increased thermal stress renders the symbionts more susceptible to primary photoinactivation of PSII, possibly due to an increase in irradiance scattered through the coral skeleton due to reduced pigment density (Enríquez et al. 2005). The symbionts also accelerate their repair of damaged PSII, but their increased repair rate is insufficient to counter the increased photo-inactivation of PSII, leading to net photoinhibition.

Acknowledgments

We would like to thank the two anonymous reviewers for their detailed comments on earlier versions of this paper.

This project was supported by the Chancellor's Post Doctoral research fund at the University of Technology, Sydney (RH), the New Brunswick Innovation Foundation (KD), the Natural Sciences and Engineering Research Council of Canada Discovery and Canada Research Chair funding (DC), and the Australian Research Council (PR).

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Associate editor: Heidi M. Sosik

Received: 30 April 2010
Amended: 12 October 2010
Accepted: 13 October 2010