Mar Biol (2011) 158:2515–2526
DOI 10.1007/s00227-011-1752-1

ORIGINAL PAPER

Photoinhibition of *Symbiodinium* spp. within the reef corals *Montastraea faveolata* and *Porites astreoides*: implications for coral bleaching

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Received: 10 January 2011 / Accepted: 7 July 2011 / Published online: 29 July 2011

Abstract It is speculated that differences in coral bleaching susceptibility may be influenced by the genotype of *in hospite Symbiodinium* and their differential responses to bleaching stressors. Photoinhibition of photosystem II (PSII), damage to the D1 (psbA) PSII reaction centre protein and production of reactive oxygen species by *in hospite Symbiodinium* are likely precursors of coral bleaching. In order to assess whether photorepair rates of *in hospite Symbiodinium* underlie the bleaching susceptibility of their hosts, photoinhibition (net and gross), photoprotection and photorepair rates were assessed in a bleaching-'tolerant' coral (*P. astreoides*) and a bleaching-'sensitive' coral (*M. faveolata*) using non-invasive fluorometric techniques and by blocking de novo synthesis of psbA. Previous studies using such techniques have demonstrated that in vitro *Symbiodinium* types 'sensitive' to bleaching stressors had reduced rates of photorepair relative to 'tolerant' *Symbiodinum* types. Our measurements demonstrated that *Symbiodinium* in the more bleaching tolerant *P. astreoides* had higher photorepair rates than *Symbiodinium* in *M. faveolata*. Higher repair rates in *P. astreoides* resulted in lower net photoinhibition relative to *M. faveolata*, where both corals exhibited similar susceptibility to photodamage (gross photoinhibition). Photoprotective mechanisms were observed in both corals; *M. faveolata* exhibited higher antennae-bed quenching than *P. astreoides* at low-light intensities, but at and above light-saturating intensities, which are different for each coral species, *P. astreoides* displayed more efficient non-photochemical quenching (Stern–Volmer quenching) of chlorophyll fluorescence than *M. faveolata*. Increased NPQ by *P. astreoides* at $E/E_k \geq 1$ was not driven by antennae-bed quenching. The ability of *in hospite Symbiodinium* in *P. astreoides* to mitigate the effects of photoinhibition under high light conditions compared with *Symbiodinium* in *M. faveolata*, and their high repair capacity following photoinhibition, may be a key factor to consider in future bleaching studies and may underlie the relative bleaching tolerance of *P. astreoides* compared to *M. faveolata.*

Communicated by P. Ralph.

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Introduction

Coral bleaching is a globally important phenomenon, which has a devastating impact upon (coral) reef community structure and function. While coral bleaching, which is broadly defined as a loss of coral colour through the loss of symbionts and/or pigments (i.e. Fitt et al. 2001; Smith et al. 2005), has been the subject of intense research in recent years, the mechanisms that underlie bleaching susceptibility remain largely unknown. Consequently, it is currently unknown which corals within a community are the most susceptible to future environmental stressors. Some work suggests that massive scleractinian corals are relatively tolerant of environmental disturbance compared with many non-massive species (Marshall and Baird 2000; Loya et al. 2001; West and Salm 2003; Kenyon et al. 2006).

However, this idea is largely developed from Pacific coral species and this pattern is not ubiquitous across all species (see Obura 2001), as considerable variability in bleaching tolerance exists within morphological types. Given the importance of massive and submassive coral species in the long-term stability of coral reef systems, particularly in terms of reef resilience (Hoegh-Guldberg and Salvat 1995; Loya et al. 2001; Kenyon et al. 2006), they represent an important group to study. Additionally, emerging evidence highlights the importance of understanding determinants of bleaching susceptibility in a range of coral species of all morphologies, as different bleaching characteristics can actually drive long-term coral community structure and function.

Many different mechanisms exist for coral bleaching, and both the host *and* the symbiotic microalgae that live within the host tissue (*Symbiodinium* spp.) can play a significant role (Fitt and Warner 1995; Warner et al. 1999; Fitt et al. 2001; Smith et al. 2005; Baird et al. 2009; Anthony et al. 2009). However, there is ample evidence that, in many cases, the responses of *Symbiodinium* spp. to bleaching stressors may act as a precursor to further effects exhibited throughout the host (Smith et al. 2005; Suggett et al. 2008; Weis 2008). One such precursor could be the increase in the production of reactive oxygen species (ROS) by the *Symbiodinium,* which can lead to a cascade of detrimental effects both within the host and the *Symbiodinium* (Lesser 1996, 1997; Smith et al. 2005; Suggett et al. 2008). Thermal- and light-induced stress can cause the production of these ROS by *Symbiodinium* as they become photoinhibited, which can damage several cellular targets, including photosystem II (PSII) (Warner et al. 1996, 1999; Ragni et al. 2010). The reaction centres within photosystem II (PSII), and in particular the D1 protein in PSII, are particularly susceptible to damage (Warner et al. 1999; Takahashi et al. 2004, 2009). Importantly, maintaining photosynthetic activity (and preventing or minimising net photoinhibition) is ultimately a careful balance between net rates of photoprotection, photodamage and photorepair (Ragni et al. 2010), which correlates with D1 content in cultured *Symbiodinium* and in intact corals (Warner et al. 1999; Hill et al. 2011).

There is substantial genetic diversity within the genus *Symbiodinium* (LaJeunesse 2001), as well as a broad array of strategies to adjusting to changing light intensity via photoacclimation. For in vitro *Symbiodinium* studied to date, the dominant pathway for photoacclimating to high light is a reduction in the number of photosynthetic units (Iglesias-Prieto and Trench 1997; Hennige et al. 2009). Many types of cultured *Symbiodinium* display different rates of photodamage and repair via chloroplast protein turnover (Warner et al. 1999; Takahashi et al. 2009; Ragni et al. 2010). This is important, since if the rate of photodamage occurs faster than photorepair, long-term photoinactivation and functional PSII reaction centre loss will result (Adir et al. 2003; Ragni et al. 2010). Photorepair is consequently extremely important for *Symbiodinium* and has been speculated to underlie differences in coral bleaching susceptibility (Warner et al. 1999; Takahashi et al. 2004, 2009; Ragni et al. 2010), but remains to be thoroughly tested in *Symbiodinium* while *in hospite*. Likewise, given recent evidence for the possibility of negligible D1 protein turnover in two isolates of cultured *Symbiodinium* (Takahashi et al. 2009) and the unique nature of the structure and transcription of the chloroplast genome in dinoflagellates (Howe et al. 2008; Dang and Green 2010), it would not be surprising if chloroplast protein repair pathways in *Symbiodinium* in general may deviate from model organisms such as green algae and cyanobacteria.

It is recognised that both high light and high temperature conditions can cause net photoinhibition (Jones and Hoegh-Guldberg 2001; Gorbunov et al. 2001; Ragni et al. 2010) and that both stressors can act cumulatively as well as independently. For shallow water corals, high light is a daily stressor at midday, whereas higher temperatures are usually seasonal, such that a certain percentage of PSII reaction centres remain inactive over a daily cycle, while this number may rise on a seasonal basis (Gorbunov et al. 2001; Warner et al. 2002). Consequently, *in hospite Symbiodinium* that show increased net photoinhibition under 'normal' conditions may be more susceptible to thermally induced coral bleaching. Conversely, down-regulation of PSII reaction centres may provide a means to quench excess light energy during enhanced stress. This study investigated the rate of photoinhibition and photorepair, and the efficiency of *preventative* mechanisms *Symbiodinium* can use to prevent net photoinhibition (such as non-photochemical quenching) in a coral that is historically sensitive to thermally induced bleaching (*M. faveolata*) and a coral that is known to be more tolerant to thermal stress (*P. astreoides*).

Materials and methods

Sample collection

Samples were collected near the Institute for Marine and Freshwater Science (UNAM) in Puerto Morelos, Mexico. Two coral species were used in this investigation: *M. faveolata* and *P. astreoides*. *M. faveolata* is a primary frame-work building massive coral in the Caribbean, while *P. astreoides* is represented by smaller encrusting to mounding colonies that do not contribute as largely to reef

topography overall, but are a common species in the back-reef lagoon in this area. Although not considered a reef framework builder, *P. astreoides* is increasing in ecological dominance in some parts of the Caribbean (Green et al. 2008; Edmunds 2010). While both species are noted to bleach (Brandt 2009; DeSalvo et al. 2008), *M. faveolata* typically has a lower 'bleaching threshold' in terms of temperature and exposure time and displays a greater bleaching prevalence as compared to *P. astreoides* on the reef (Brandt 2009). Similarly, the endosymbionts within *M. faveolata* tend to show higher levels of PSII photoinactivation during initial experimental thermal treatment (Warner and Berry-Lowe 2006; Warner and Grottoli, personal observation).

In August 2009, parent colonies of both species were identified at 3–4 m depth (approximately 800 µmols photons m^{-2} s^{-1} recorded at peak midday irradiance with underwater light loggers, Odyssey, NZ) off the coast of Puerto Morelos, approximately 2 km North of the Institute of Marine Sciences and Limnology of the University of Mexico. A fragment ca. 10 cm^2 was collected from 5 parent colonies and transported immediately back to the laboratory facility. Each colony sample was then split into seven smaller fragments. One fragment was immediately processed for *Symbiodinium* genetic analysis. The remaining six fragments from each colony (30 total of each species) were allowed to recover from sampling stress in a shaded holding tank for 5 days with flow through reef seawater at ambient temperature to aid any recovery from fragmentation stress. Water temperature matched that of the back-reef lagoon where the corals were collected (maximum 30.5 ± 0.01°C, with submersible temperature loggers, Onset USA), and the peak average light level was slightly lower relative to the collection site (589 ± 0.34 µmols photons m^{-2} s^{-1}, recorded with a 2π sensor LI-COR, USA).

Chlorophyll a fluorometry

PSII activity was assessed via single-turnover chlorophyll fluorescence as outlined by Hennige et al. (2009) recorded from a fluorescence induction and relaxation (FIRe) fluorometer (Satlantic) fitted with a Satlantic fibre optic probe attachment. The probe was placed against the coral in the water to avoid air–water signal dispersion on a surface parallel to the water surface. Fluorescence transients comprised of a 2-step sequence that included a 100 µs saturating single-turnover (ST) excitation light pulse, followed by a 60 µs relaxation phase from a series of weaker light pulses used to follow the reoxidation of PSII. Fifteen iterations of this sequence were averaged into a single fluorescence transient to increase the signal-to-noise ratio for each sample. Fluorescence parameters were calculated in *FIREPRO* software (Satlantic Inc.), based on the biophysical (KPF) model of Kolber et al. (1998) with specific gain excitation profiles for the fibre optic probe to curve fit several physiological components of PSII activity under dark-acclimated conditions, including minimum fluorescence, maximum fluorescence and absorption cross section of PSII (F_o, F_m and σ_{PSII}, respectively, Table 1). Under actinic light, these terms are F_o', F_m' and σ_{PSII}', respectively (Table 1; Kromkamp and Forster 2003; Baker and Oxborough 2005; Cosgrove and Borowitzka 2010). F_o' is problematic to measure directly (Kromkamp and Forster 2003) and was calculated according to Suggett et al. (2003) as $F_o/[(F_v/F_m) + (F_o/F_m')]$.

Dark-acclimated fluorescence indicates the physiological 'state' when it is assumed the majority of photoprotective mechanisms are relaxed, PSII reaction centres are 'open' and the electron transport chain is oxidised (Kromkamp and Forster 2003; Ralph and Gademann 2005; Hennige et al. 2009). Fluorescence transients collected under actinic light exposure indicate the capacity of cells to utilise absorbed excitation energy (Ralph and Gademann 2005; Falkowski and Raven 1997). The maximum PSII photochemical efficiency (also referred to as the maximal quantum yield of PSII) was determined as $(F_m - F_o)/F_m = F_v/F_m$ (dimensionless) following the terminology of Kromkamp and Forster (2003). Similarly, the effective photochemical efficiency of PSII photochemistry (or effective quantum yield) at any given actinic irradiance is determined as $(F_m' - F')/F_m'$(termed F_q'/F_m'). The photochemical quenching parameter (qP), calculated as $(F_m' - F')/(F_m' - F_o')$ or F_q'/F_v', can be used to describe the decrease in effective photochemical efficiency of PSII due to changes in F_q' which decreases with the increased closure of PSII reaction centres (Hennige et al. 2008a). The quantum yield of non-photochemical quenching at any actinic irradiance is $(F_m - F_m')/F_m'$, termed as NPQ hereafter (Table 1). NPQ describes the quenching of maximum fluorescence, F_m, through many possible pathways such as xanthophyll cycling or inactive reaction centres (see Kromkamp and Forster 2003). The proportion of non-photochemical quenching specifically through the antennae was estimated by normalising the light-acclimated functional absorption cross section of PSII, σ_{PSII}', to the dark-acclimated functional absorption cross section of PSII, as $1 - (\sigma_{PSII}'/\sigma_{PSII})$ (Gorbunov et al. 2001; Hennige et al. 2009).

Experimental treatment and sampling regime

At the start of the experiment, 2 fragments from 5 colonies of each species were shifted from the holding tanks to each of three light levels: low light (LL), medium light (ML) and high light (HL), which were 100, 200 and 589 µmols

Table 1 Table of terms, their definitions and their units

Term	Synonyms	Definition	Units
RCII		Reaction centres in photosystem II	
PSII		Photosystem II	
ETR^{RCII}		RCII-specific electron transport rate	mol e^- mol $RCII^{-1}h^{-1}$
F_o, F_m		Minimum and maximum fluorescence measured in the dark	Instrument units
F_o', F_m'		Minimum and maximum fluorescence measured under actinic light	Instrument units
F'	F, F_T	Steady state fluorescence measured under actinic light	Instrument units
F_q'	ΔF	The difference between F_m' and F'	Instrument units
F_v, F_v'		Variable fluorescence in darkness ($F_m - F_o$), and under actinic light ($F_m' - F_o'$)	Instrument units
F_v/F_m		Maximum photochemical efficiency of PSII	Dimensionless
F_q', F_m'	$\Delta F/F_m'$	Operating efficiency of PSII in the light-acclimated state	Dimensionless
NPQ		Non-photochemical quenching ($(F_m - F_m')/F_m'$) also called Stern–Volmer quenching	Dimensionless
qP		Photochemical quenching (F_q'/F_v')	Dimensionless
PFD	PAR	Photon flux density	µmols photons m^{-2} s^{-1}
GPiR		Gross photoinhibition	min^{-1}
NPiR		Net photoinhibition	min^{-1}
RR		Repair Rate (GPiR − NPiR)	min^{-1}
$\sigma_{PSII}, \sigma_{PSII}'$		Effective absorption cross section of PSII in the dark and under actinic light	$Å^2 q^{-1}$
$\sigma_{PSII}'/\sigma_{PSII}$		Non-photochemical quenching in the antennae bed	Dimensionless

photons m^{-2} s^{-1}, respectively, and placed in individual plastic beakers (400 ml). One fragment from each colony, species, and light level was assigned to the experimental treatment of chloramphenicol addition (+CAP), and one was assigned to the control treatment (no CAP). All light levels were controlled with neutral density screening to modify ambient irradiance. Within each light level, chlorophyll fluorescence measurements of control and experimental fragments were taken immediately preceding the addition of CAP, and then 1 and 2 h following the addition. As the efficiency of CAP decreases over time, experiments were terminated after 2 h when changes in efficiency were no longer detectable. All fluorescence measurements first were taken on light acclimated samples, i.e., samples that were in the light (LL, ML or HL). At the end of the 2-h experiment, samples were dark acclimated for 30 min (using a black shade cloth over the tank), before an additional fluorescence measurement was taken from all coral fragments. During the course of the 2-h experiment, natural variations in sun light intensity resulted in HL tanks having an approximate variation of ±50 µmols photons m^{-2} s^{-1}, ML tanks ~20 µmols photons m^{-2} s^{-1} and LL tanks ~10 µmols photons m^{-2} s^{-1}. The exposure time was chosen for since it represents the point of peak irradiance with relatively little variation. Potential photodamage is therefore high over this time period.

At time 0 (11:30 h), chloramphenicol (CAP) dissolved in 99% ethanol, which blocks de novo translation of chloroplast encoded proteins (including the psbA or D1 protein) (Warner et al. 1999), was added to the 5 treatment (+CAP) beakers at each light level to a final concentration of 386 µM (1.17 ml from a stock of 0.13 M). Previous work has confirmed that CAP additions result in the loss of D1 protein content in *Symbiodinium* in vitro (Warner et al. 1999) and *in hospite* (personal observation). The CAP concentration required to illicit a significant decline in photochemical activity was determined by preliminary experiments, and inclusion of 1.17 ml of 99% ethanol alone did not significantly impact the photosynthetic activity of either coral species (data not shown).

Calculation of gross and net photoinhibition

Values of F_v/F_m from CAP-treated and CAP-untreated samples at the end of 2 h of incubation were used to calculate net photoinhibition (*NPiR*, Eq. 1), gross photoinhibition (*GPiR*, Eq. 2) and repair rate (RR, Eq. 3) following equations from Ragni et al. (2008).

$$NPiR(E) = -\frac{Ln\frac{F_v/F_m(E)}{F_v/F_m(DA)}}{\Delta T} \quad (1)$$

$$GPiR(E) = -\frac{Ln\frac{F_v/F_{mCAP}(E)}{F_v/F_m(DA)}}{\Delta T} \quad (2)$$

$$\mathrm{RR} = GPiR - NPiR \quad (3)$$

where E is the light level that samples were subject to, ΔT is the duration in minutes of the light incubation period (120 min), $F_v/F_m(DA)$ is the F_v/F_m measured on dark-acclimated samples at night, a few hours after sunset (to ensure full relaxation of NPQ), $F_v/F_m(E)$ are the values of F_v/F_m measured at the end of light exposure at different Photon Flux Densities (PFD) levels (either LL, ML or HL) and $F_v/F_{mCAP}(E)$ is the same parameter measured on chloramphenicol-treated samples (Ragni et al. 2010). These equations assume that the change in F_v/F_m occurs at a constant specific rate (e.g., $\mathrm{d}(F_v/F_m)/\mathrm{d}t = \mathrm{k}F_v/F_m$) (Six et al. 2008; Ragni et al. 2010). Two-sample T tests were used to compare fluorescence and photoinhibition (net and gross) results between both coral species in CAP-treated and CAP-untreated samples. Fluorescence parameters were compared with each other (to test for potential relationships) by using a Pearson's Correlation test.

Light curve experiment

In the days following outdoor incubation experiments, control fragments from HL (fragments not exposed to CAP) of each coral species were exposed to a series of light levels using an independent light source to calculate light response curves. A halogen light source was (Schott KL1500 LCD) fitted with a DT-cyan filter (to provide irradiance from 400 to 600 nm) in conjunction with a Schott annular ring light to expose the coral fragments to actinic light. The FIRe fibre optic probe was fitted into the centre of the ring light and enabled fluorescence readings to be taken on an illumination 'spot' approximately 1 cm in diameter on the coral. Eleven light levels were used: 0, 40, 60, 100, 160, 240, 400, 660, 1,100, 1,600 and 2,200 µmols photons m^{-2} s^{-1}, with each light step lasting 4 min. All light curves were carried out on dark-acclimated (30 min) samples at 08:00 h on sequential days to ensure that light curves accurately reported the kinetics of electron transport from samples wherein the majority of PSII was in the oxidised state, and was not influenced by 'midday depression' (Lesser and Gorbunov 2001; Warner et al. 2002), as dark acclimation at midday may not allow full reversal of NPQ (per obs).

The electron transport rate (ETR) specific to the functional absorption cross section and PSII reaction centres (RC) (ETR^{RCII}, mol e^- mol $RCII^{-1}$ h^{-1}, Table 1) was calculated as in Ragni et al. 2010 (Eq. 4):

$$ETR^{RCII} = PFD \times \sigma_{PSII} \times F_q'/F_m' \times 21.683 \quad (4)$$

where 21.683 converts seconds to hours, µmol e^- mol e^- and $Å^2$ $quanta^{-1}$ to m^2 mol $RCII^{-1}$ (Suggett et al. 2003, 2006). F_q'/F_m' was also used to calculate the light saturation coefficient of electron transport, E_k (µmols photons m^{-2} s^{-1}) for both coral species following methodology from Hennige et al. (2008a) using least-squares non-linear regression. E_k, which is also known as the minimum saturating irradiance (Hill et al. 2004), describes the transition between light-limited and light-saturated photochemical efficiency (Hennige et al. 2008a).

To account for changes in light quality and hence the amount of photosynthetically useable radiation (PUR) (Kirk 1994; Hennige et al. 2009; Ragni et al. 2010) between the halogen light source and sunlight, the spectrum and intensity of the halogen light source were measured using a fibre optic spectrometer (Ocean Optics) and weighted to sunlight using a conversion factor calculated according to Ragni et al. (2008). The PUR of the filtered halogen light source was 99% that of the sun at any given PAR.

Symbiodinium identification

Algal DNA extractions were performed on coral fragments using the Wizard® isolation (Promega) protocol adapted by LaJeunesse et al. (2003). For each sample, the ribosomal internal transcribed spacer 2 (ITS2) region was amplified using the 'ITSintfor2' and 'ITS2 clamp' primers under a touch-down PCR protocol (LaJeunesse et al. 2002). The resulting products were subjected to denaturing gradient gel electrophoresis (DGGE; 45–80% denaturing gradient) for 15–16 h using a CBS Scientific system (Del Mar, California). Fingerprints for each sample were compared with an existing database for corals sampled in this region (LaJeunesse 2001; Warner and Berry-Lowe 2006).

Results

Photoinhibition and photorepair

Both coral species exhibited at least a twofold decrease in the operating efficiency of PSII, F_q'/F_m', between control fragments and CAP-treated fragments after 2 h at all three light intensities (LL, ML and HL) (Fig. 1a, b). As expected, there was a progressive decline in F_q'/F_m' in CAP-treated fragments across LL, ML and HL. No significant change in F_q'/F_m' was observed at any time in HL or ML control (no CAP) fragments. However, LL control fragments, which were subjected to a fivefold decrease in light

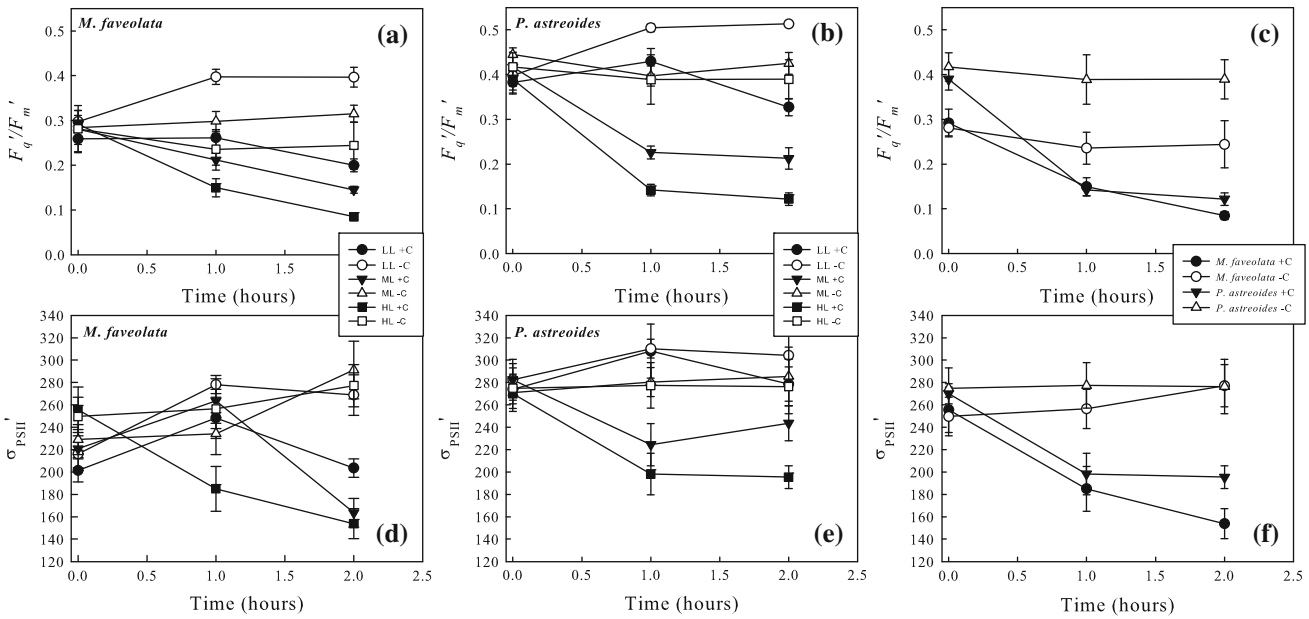

Fig. 1 (**a**–**c**) Operating efficiency of PSII, (F_q'/F_m') and (**d**–**f**) effective absorption cross section of PSII, (σ_{PSII}') in *M. faveolata* and *P. astreoides* coral fragments at LL, ML and HL with (+C) and without (−C) added chloramphenicol at Time 0, $T + 1$ and $T + 2$ h ($n = 5 \pm$ SE)

Table 2 Mean values ($n = 5 \pm$ SE) of fluorescence-derived parameters of high light (589 µmols photons m^{-2} s^{-1}) coral fragments with and without chloramphenicol (CAP)

All fluorescence parameters are from dark-acclimated samples at $T + 2$ h from experiment start, or from night time readings. *T* tests were carried out between species for each parameter. Bold results indicate significant differences

	Coral species		
	P. astreoides	*M. favoelata*	*T* test
CAP experiment			
F_v/F_m control	0.45 (0.02)	0.28 (0.02)	**$t_8 = 5.52$, $P = 0.00$**
F_v/F_m treatment	0.12 (0.01)	0.12 (0.01)	$t_8 = 0.26$, $P = 0.80$
σ_{PSII} Control	304 (3.47)	283 (10.6)	$t_8 = 1.92$, $P = 0.09$
σ_{PSII} Treatment	274 (10.2)	208 (55.3)	$t_8 = 1.20$, $P = 0.27$
GPiR (min^{-1}) (10^{-2})	1.20 (0.06)	1.08 (0.06)	$t_8 = 1.50$, $P = 0.17$
NPiR (min^{-1}) (10^{-3})	1.44 (0.45)	3.62 (0.54)	**$t_8 = 3.10$, $P = 0.02$**
RR (min^{-1}) (10^{-2})	1.06 (0.07)	0.72 (0.10)	**$t_8 = 2.72$, $P = 0.03$**
Night measurements			
F_v/F_m control	0.43 (0.02)	0.53 (0.01)	**$t_8 = 6.18$, $P = 0.00$**

intensity relative to pre-experiment conditions, had significantly higher F_q'/F_m' after 2 h than at *T*0 ($t_8 = 4.42$, $P = 0.01$; $t_8 = 16.88$, $P = 0.00$ for *M. faveolata* and *P. astreoides*, respectively).

In the presence of CAP and HL, *P. astreoides* displayed a more rapid decline in the light-acclimated PSII operating efficiency relative to *M. faveolata*. However, by the end of the HL incubation, F_v/F_m and F_q'/F_m' in fragments from both species did not significantly differ (Fig. 1a–c, Table 2), despite the initially higher F_q'/F_m' in *P. astreoides* (Fig. 1a, b) ($t_8 = 3.07$, $P = 0.02$) and higher F_v/F_m in dark-acclimated control fragments (Table 2).

In both species, addition of CAP elicited similar trends in the absorption cross section of PSII in the light (σ_{PSII}') as observed in F_q'/F_m' (Fig. 1d–f). At HL, both species exposed to CAP decreased σ_{PSII}' over the 2-h incubation period compared with the controls, which did not significantly differ from each other or over the course of the experiment. Similarly, dark-acclimated σ_{PSII} did not differ between species at the end of the experiment in either control or CAP-treated fragments (Table 2). The PSII absorption cross section (σ_{PSII}) was also compared with maximum photochemical efficiency (F_v/F_m) in both species following 2 h of incubation (both with and without CAP addition) to assess whether an inverse relationship was observed as noted in previous studies on low-light cultured *Symbiodinium* (Ragni et al. 2010). However, no correlation was observed in either in *P. astreoides*

or *M. faveolata* fragments (Pearson's correlation = 0.135, $r = 0018$ and 0.074, $r = 0.006$, respectively).

Both species were subject to similar gross photoinhibition (*GPiR*), at LL, ML and HL (Fig. 2a; Table 2). However, *M. faveolata* displayed significantly higher net photoinhibition (*NPiR*) than *P. astreoides* at the medium and high light levels (Fig. 2b; Table 2). This equated to a significantly higher repair rate (RR) in *P. astreoides* than *M. faveolata* (Fig. 2; Table 2). Differences between species in *NPiR* or RR were not evident at LL or ML (Fig. 2b; c).

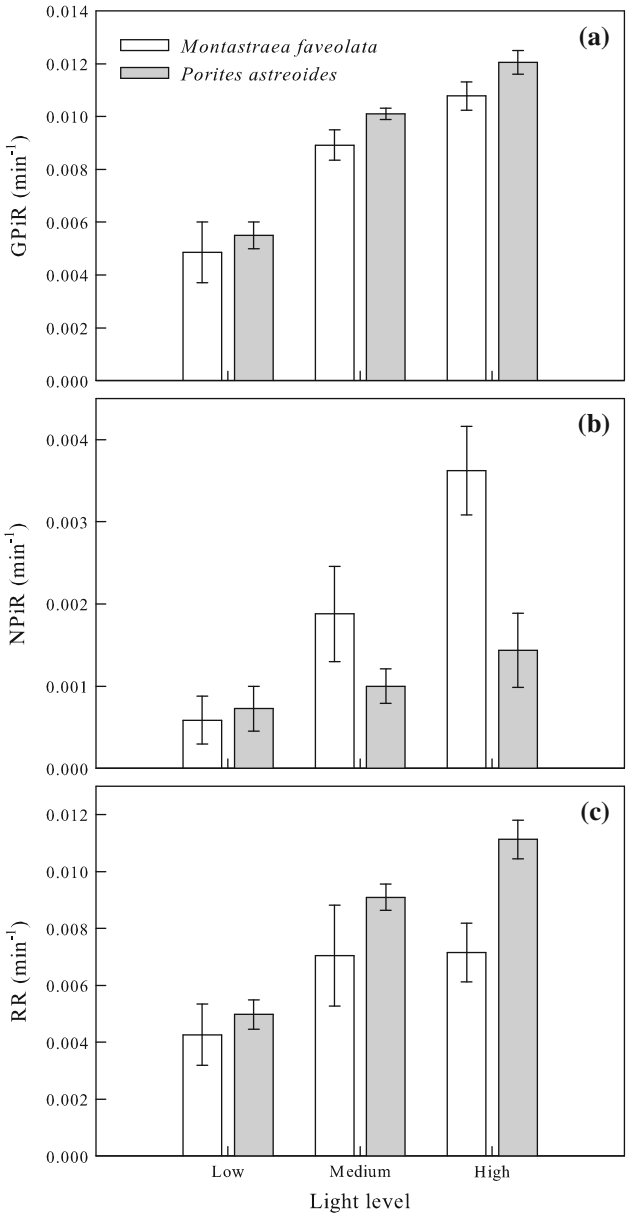

Fig. 2 Gross photoinhibition (*GPiR*), net photoinhibition (*NPiR*) and repair rates (*RR*) (min^{-1}) in *M. faveolata* and *P. astreoides* coral fragments under low (100) medium (200) and high light (589 µmols photons m^{-2} s^{-1}) ($n = 5 \pm$ SE)

Photophysiology

Both species exhibited typical responses when exposed to sequentially higher light levels, such that non-photochemical quenching (NPQ), photochemical quenching (1 − qP), antennae-bed quenching ($(1 - (\sigma_{PSII}'/\sigma_{PSII}))$ and Electron Transport Rate (ETR), all increased with increasing light until the highest light level, at which point, data began to asymptote or decline (Figs. 3, 4). However, differences were observed between species. NPQ was significantly higher in *P. astreoides* at the highest light curve intensity, 2,200 µmols photons m^{-2} s^{-1} ($t_8 = 4.01$, $P = 0.00$). At light levels comparable to growth irradiance (i.e. HL), there was no difference in NPQ between species (Fig. 3a). For antennae-bed non-photochemical quenching ($(1 - (\sigma_{PSII}'/\sigma_{PSII}))$, Fig. 3b), which contributes to NPQ, the only difference between species was at irradiances below 400 µmols photons m^{-2} s^{-1}, where *M. faveolata* had significantly higher quenching than *P. astreoides.*

However, it is important to note that the light curve data displayed here (Fig. 3a, b) do not account for differences in species-specific minimum saturating irradiances (E_k) calculated using least-squares non-linear regression (see Hennige et al. 2008a), which was significantly higher in *P. astreoides* than *M. faveolata*; 346 (±46.1) µmols photons m^{-2} s^{-1} and 222 (±37.2) µmols photons m^{-2} s^{-1}, respectively ($t_8 = 2.09$, $P = 0.03$). When the minimum saturating irradiance was taken into account by plotting light curves against irradiance relative to E_k (E/E_k) following Hennige et al. (2008a), NPQ differed to that plotted against irradiance alone (Fig. 3a, c). Importantly, NPQ at $E/E_k = 1$ and at $E/E_k > 1$ (where irradiance for photosynthesis is optimal and light saturating, respectively) was higher in *P. astreoides* than in *M. faveolata*, and the higher antennae-bed quenching observed in *M. faveolata* at low irradiances did not equate to higher NPQ than *P. astreoides*. It is worth noting that electron transport and antennae-bed quenching were not plotted relative to E/E_k, as the absorption cross section for PSII partially accounts for different absorption properties between both species. The trend of increased NPQ in *P. astreoides* was not matched in $1 - (\sigma_{PSII}'/\sigma_{PSII})$ data versus E (Fig. 4). This result was similar in the independent assessment of antennae-based non-photochemical quenching following CAP incubation under sunlight. Although antennae-bed quenching was higher by ca. 30% in *P. astreoides* in both data sets, results were not significant as variability was high.

Photochemical quenching, calculated as 1 − qP, was significantly higher in *M. faveolata* fragments than *P. astreoides* at irradiances below 1,200 µmols photons m^{-2} s^{-1} (Fig. 3b). The opposite was observed in ETR^{RCII}, with *P. astreoides* fragments having greater specific electron transport rates than *M. faveolata*, but only below

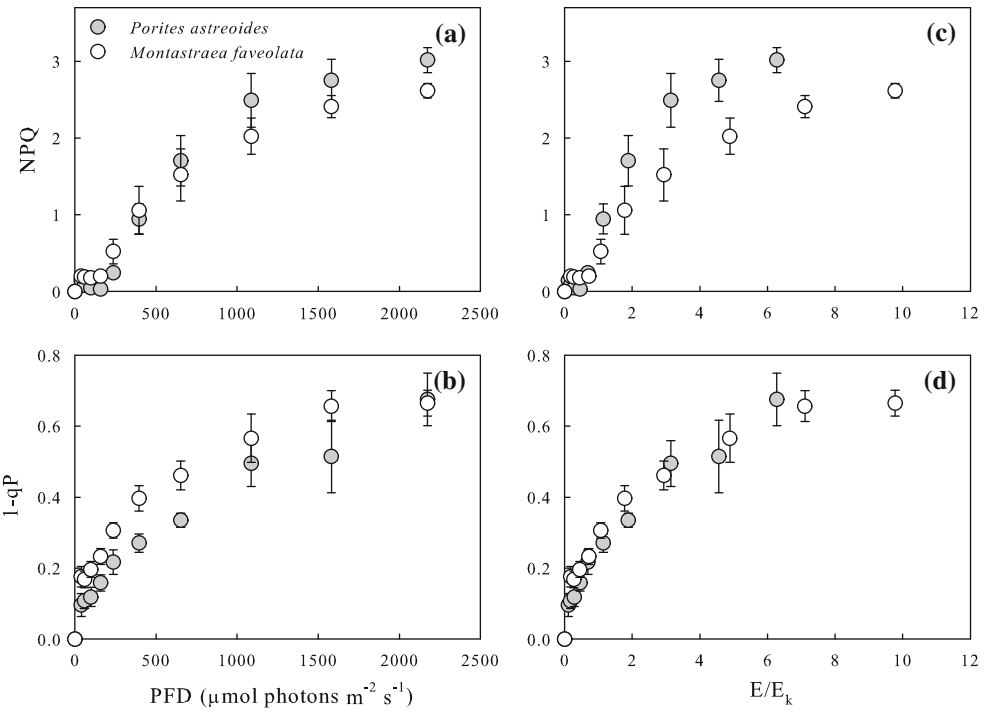

Fig. 3 Light response (PFD µmols photons m^{-2} s^{-1}) of *M. faveolata* and *P. astreoides* ($n = 5 \pm$ SE) of **a** Non-photochemical quenching [NPQ] ($(F_m - F_m')/F_m'$) and **b** photochemical quenching ($1 - qP(1 - F_q'/F_v')$). Panels (**c** and **d**) are light responses of *M. faveolata* and *P. astreoides* ($n = 5 \pm$ SE) standardised to E/E_k

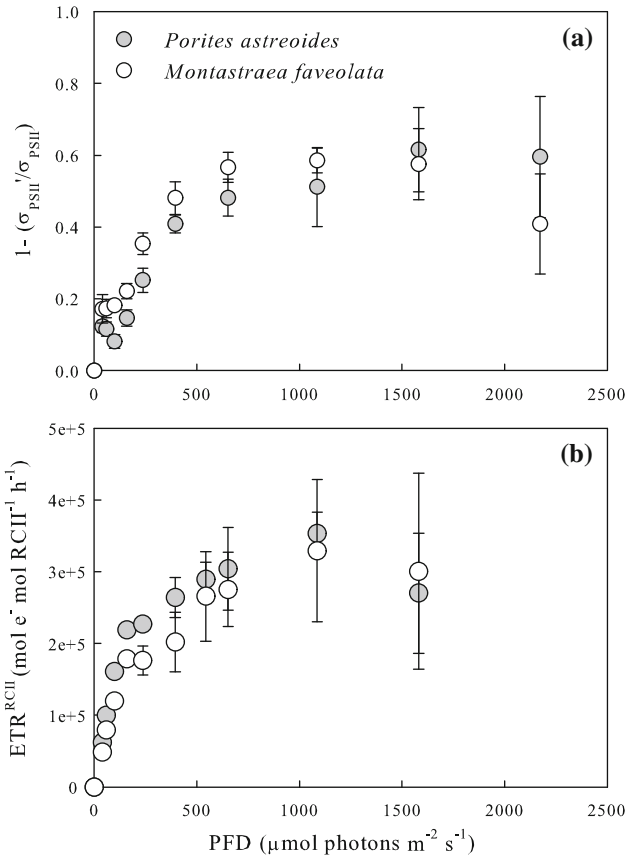

Fig. 4 Light response (PFD µmols photons m^{-2} s^{-1}) of *M. faveolata* and *P. astreoides* ($n = 5 \pm$ SE) of (**a**) antennae-based quenching ($1 - (\sigma_{PSII}'/\sigma_{PSII})$) and (**b**) RCII-specific electron transport rate (ETR^{RCII} mol e^- mol $RCII^{-1}$ h^{-1}). The last data point is omitted from (**b**) due to large variability

400 µmols photons m^{-2} s^{-1} (Fig. 4b). Higher ETR in *P. astreoides* was driven by a slower decrease in F_m and to a lesser degree F_o in response to increased light intensities (data not shown). In contrast to NPQ data, when expressed relative to E/E_k, 1 − qP plots from both species fell together (apart from values at very low E/E_k) rather than diverge from 10 to 600 µmols photons m^{-2} s^{-1} (Fig. 3b, d).

Symbiodinium identification

Both coral species harboured different *in hospite Symbiodinium* types according to ITS2 identification. *M. faveolata* fragments harboured B1, B17 and C7, either singly or as a mixed assemblage. *P. astreoides* fragments were dominated by A4 symbionts, and only one fragment was identified with an additional background symbiont, A3.

Discussion

Photoinhibition and photorepair

The extent of gross photoinhibition was directly influenced by the light availability of the corals, consistent with previous studies (Takahashi et al. 2009; Ragni et al. 2010) (Fig. 2). However, gross photoinhibition did not differ between *P. astreoides* or *M. faveolata* corals treated with chloramphenicol at all light levels (Fig. 2a). Since gross

photoinhibition is calculated in the presence of an inhibitor of de novo protein synthesis, it represents the extent of damage that can occur in the absence of photorepair. Net photoinhibition data (Fig. 2b) demonstrated that under experimental conditions, *P. astreoides* exhibited substantially less photodamage than did *M. faveolata* at ML and HL. The increased variability in *M. faveolata* data compared with *P. astreoides* was possibly the result of the mixed *in hospite Symbiodinium* community, as opposed to the single symbiont identified in *P. astreoides.* The higher net photoinhibition in *M. faveolata* at ML and HL was driven by lower rates of PSII repair relative to *P. astreoides* and, to a lesser extent, lower NPQ than *P. astreoides* relative to E/E_k (Figs. 2c, 3c). This was also evident in the reduced photosynthetic efficiency of PSII, F_q'/F_m', in *M. faveolata* relative to *P. astreoides* at time zero (Fig. 1). Repair rates in both species increased from LL to HL. This implies that repair mechanisms were not damaged by increased light availability, but rather, that the increasing net photoinhibition at higher light intensities is due to increases in repair rate being insufficient relative to increased damage. This result corroborates recent evidence by Hill et al. (2011), who demonstrated that increases in gross photoinhibition, rather than decreases in repair rates, are a key trigger for net photoinhibition under bleaching conditions in *P. damicornis*. The high PSII repair rates observed in a *Symbiodinium* type considered 'thermally tolerant' by Ragni et al. (2010), complements work presented here, as *P. astreoides* (which had high PSII repair capacity relative to *M. faveolata*) is bleaching tolerant relative to *M. faveolata* (Warner and Berry-Lowe 2006).

Interestingly, the rate of gross photoinhibition in both coral species here was approximately 150% greater compared with the results by Ragni et al. (2010) for two different *Symbiodinium* clade A cultures (approximately 150% increase). Despite different types of *Symbiodinium* being used in previous studies, the current study provides preliminary evidence of substantially higher chloroplast protein turnover while the symbiont is *in hospite*.

Implications for coral bleaching

The high repair rate observed in *P. astreoides* relative to *M. faveolata* (Fig. 2c) corresponds well with speculation that photosynthetic repair rates may underlie bleaching susceptibility in corals in general (Warner et al. 1999; Takahashi et al. 2004; Yakovleva and Hidaka 2004; Ragni et al. 2010), and a central pathway to thermal tolerance in the *Symbiodinium* within *P. astreoides* in particular. This complements recent evidence which suggests that *P. astreoides* is increasing in dominance relative to other corals in the Caribbean and is poised to potentially undergo sustained population growth (Edmunds 2010). It is well established that high light can exacerbate the thermal stress response of *Symbiodinium* to induce coral bleaching (Lesser and Farrell 2004; Robison and Warner 2006), as both light and temperature act to inhibit or damage PSII, which can lead to an increase in ROS production (Smith et al. 2005; Suggett et al. 2008). Larger photosynthetic repair rates would potentially act to reduce (not eliminate) ROS production by *Symbiodinium* under bleaching conditions, which would ultimately confer a higher degree of resistance in coral holobionts to bleaching. This complements recent work where higher repair rates were observed in thermally tolerant *Symbiodinium* types than in thermally sensitive types in vitro under non-stressful conditions (Ragni et al. 2010). Although the thermal stability of repair mechanisms may remain an important factor to consider in any bleaching study (Takahashi et al. 2009), the different rates observed here between the bleaching tolerant *P. astreoides* and the bleaching sensitive *M. faveolata* under non-bleaching conditions indicate that initial repair rates and stimulated repair rates during stress, in addition to thermal tolerance and non-photochemical efficiency, may be key to mitigating the effects of bleaching events.

This raises interesting questions as to the host-*Symbiodinium* specificity in the corals studied here, and whether *M. faveolata*, may be constrained to harbouring *Symbiodinium* types that have lower repair rates than those in *P. astreoides*. Given that *M. faveolata* in this region may harbour as many as three different *Symbiodinium* spp. (Kemp et al. 2008) and the intraspecific and temporal variability in symbiont populations within this species is prevalent (Thornhill et al. 2006), rates of photodamage and repair may be quite different if other *Symbiodinium* species dominate in this coral.

Photophysiology

F_q'/F_m' values after 2 h of CAP incubation were not significantly different between *P. astreoides* and *M. favaolata* fragments (Fig. 1a, b). Dark-acclimated F_v/F_m of both species following incubation also matched this trend (Table 2). However, the *extent* of F_q'/F_m' decrease was greater in *P. astreoides* than in *M. faveolata.* This difference indicates that *Symbiodinium* in *M. faveolata* fragments were photoinactivated to some extent compared with *P. astreoides*. Since this difference persisted in samples recorded at night, this photoinhibition is likely chronic, i.e., does not recover in a matter or hours, and recovery may instead take days or longer (Krause 1988; Gorbunov et al. 2001). This is supported by the large difference between species F_v/F_m in dark-acclimated samples at midday, as there was very little difference between *M. faveolata*

F_q'/F_m' and F_v/F_m. This means that *M. faveolata* had greater damage within PSII reaction centres prior to and during this experiment, as photoprotective mechanisms and repair mechanisms have been inadequate to prevent PSII degradation (Gorbunov et al. 2001).

The greatest decrease in F_q'/F_m' for both species during CAP incubation was at HL levels (Fig. 1c), which is a result of light exposure and damage to D1 protein within PSII. LL fragments would not have experienced as much damage to D1 proteins as HL fragments and hence the addition of CAP to LL fragments elicited less of a response. With regard to the light acclimation state, LL control fragments acclimatised during the 2-h experiment time to lower than ambient (i.e., the light level that fragments had been previously acclimatised to) light levels and hence can be considered 'low-light' acclimatised relative to time zero, which explains increased F_q'/F_m' values.

Both light-acclimated and dark-acclimated values of σ_{PSII} decreased for CAP-treated fragments of both species, whereas in control, HL fragments σ_{PSII} did not differ significantly between species (Table 2), due in part to the relatively large variability observed in *M. faveolata* data, and σ_{PSII}' did not differ significantly over the 2-h experimental time period. The lack of any change temporally and between species at a time that arguably should elicit the largest change (midday) indicates that rapid changes in absorption cross section are not the dominant acclimatisation strategy of *Symbiodinium* in both coral species. This is consistent with previous research that concluded that changes in reaction centre content and not antennae bed changes are the dominant acclimation strategy for cultured *Symbiodinium* (Iglesias-Prieto and Trench 1997; Hennige et al. 2009). The decrease in σ_{PSII} in CAP-treated samples is opposite to the trend observed in a recently published study that used an experimental design similar to this one with cultured *Symbiodinium* (Ragni et al. 2010). This difference likely results from the specific photosynthetic pigment/protein architecture used in *Symbiodinium* spp. and the possible differences therein between algal populations in vitro versus in vivo. Ragni et al. (2010) hypothesised that the cultured *Symbiodinium* exhibited a 'lake' model of light-harvesting complex organisation (i.e. light harvesting and energy transfer is shared among several PSII reaction centres, see Bernhardt and Trissl 1999). This would explain an increase in σ_{PSII} when PSII translational repair was chemically blocked, and inactive or photoinhibited Reaction Centres (RC's) transferred absorbed exciton energy to remaining functional RC's. However, the data here provide better support for a 'puddle' model of light harvesting, wherein there may have been greater isolation and lower connectivity (ρ) between PSII reaction centres (Bernhardt and Trissl 1999) in the *Symbiodinium* investigated *in hospite* here. Thus, once RC's became inactivated, the energy absorbed through the attached antennae beds was no longer available to neighbouring active RC's. Considering that different types of *Symbiodinium* exhibit different biophysical and bio-optical characteristics when photoacclimated to various light levels in culture (Hennige et al. 2009), it is not yet known whether this is a ubiquitous response of all *Symbiodinium* spp. Nevertheless, the assumption of limited photosynthetic unit connectivity is in agreement with earlier work that also noted substantially lower ρ in *Symbiodinium in hospite* as compared to other aquatic photosynthetic organisms (Gorbunov et al. 2001).

Light response curves (Figs. 3, 4) were broadly similar for both species when plotted against applied E with slight differences in photosynthetic parameters between species as may be expected (Hennige et al. 2009). However, typical light curves do not account for 'light' differences between coral species such as host absorption (Hennige et al. 2008b), skeleton scattering (Enriquez et al. 2005), *Symbiodinium* density or long-term light history of the coral. Likewise, the light received by the *in hospite Symbiodinium*, i.e., the photosynthetically useable light [PUR (Kirk 1994; Hennige et al. 2008a)] may differ dramatically in different coral species. To circumvent such limitations, the minimum saturating irradiance of PSII electron transport, E_k, was calculated for each coral. The significantly higher E_k in *P. astreoides* indicates that *P. astreoides Symbiodinium* were able to process more excitation energy through PSII before saturation than *Symbiodinium* in *M. faveolata*. When applied light (E) was expressed relative to E_k (which gives a more accurate representation of the efficiency of *Symbiodinium* photosynthesis relative to its acclimatisation state), NPQ was higher in *P. astreoides* than in *M. faveolata*, and photochemical quenching, qP, did not differ between species (Fig. 3c, d). This is consistent with work by Hennige et al. (2008a), where different coral species were found to modify NPQ to optimise photosynthesis towards E_k. The slightly higher NPQ in *P. astreoides* from $E/E_k = 1$ (where applied light is equal to the minimum saturating irradiance) to $E/E_k = 6$ (at saturating light intensities) indicates that *P. astreoides* has more capacity than *M. faveolata at that acclimatisation* state to dissipate light energy through certain pathways. However, while NPQ was higher in *P. astreoides* than in *M. faveolata* above growth irradiances (Fig. 3), it is likely that antennae-bed quenching was not the driving factor. Another possible pathway for NPQ is quenching through thermal dissipation in the reaction centres themselves, which would be recorded through NPQ but not through changes in σ_{PSII} (Gorbunov et al. 2001). Increased NPQ in *P. astreoides* where $E/E_k \sim 1$–2, contributed to the increased electron transport rate of PSII,

ETR^{RCII} at ca. 200–400 μmols photons m^{-2} s^{-1}, which indicates *potentially* increased productivity through higher electron flow at optimal light utilisation. Increased electron flow through functional reaction centres in these two species may be interesting for future studies, as the 'compensatory effect' (Behrenfeld et al. 1998; Kana et al. 2002), where electron turnover rates are increased and is potentially beneficial under conditions likely to cause net photoinhibition (Hennige et al. 2009), would complement increased electron transport rates in *P. astreoides*.

Conclusions

Under high light conditions and ambient temperature, *P. astreoides* exhibited less net photoinhibition than *M. faveolata*. Since gross photoinhibition was comparable in both species, the reduced net photoinhibition in *P. astreoides* was enabled through higher PSII repair rates relative to *M. faveolata.* NPQ at and above light-saturating intensities may confer some additional advantage to *P. astreoides* with regard to mitigating photodamage, but further study is required to expand upon this. The increased repair rate observed here in the bleaching tolerant *P. astreoides* coral highlights that repair rates are an interesting area for future study with regard to the bleaching susceptibility of coral, as *Symbiodinium* types with relatively high repair rates could potentially act to minimise net photoinhibition under thermal bleaching conditions. Further study will determine to what extent PSII repair capacity underlies bleaching susceptibility and whether repair capacity under non-bleaching conditions can be used as an indicator of coral bleaching susceptibility.

Acknowledgments The authors wish to thank Roberto Iglesias-Prieto, Susana Enríquez, Robin Smith and the staff of the Instituto de Ciencias del Mar y Limnologia, Universidad Nacional Autónoma de México for their generous time and logistical support. This work was supported by funding from the National Science Foundation (award 0825490 to A. Grottoli, and award 0825413 to M. Warner). All work undertaken in this study complied with the current laws of Mexico and the United States of America.

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