

# Seasonal variation in the photo-physiology of homogeneous and heterogeneous *Symbiodinium* consortia in two scleractinian corals

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**ABSTRACT:** Seasonal variation in the composition of the algal endosymbiont community and photo-physiology was determined in the corals *Pocillopora damicornis*, which show high local fidelity to one symbiont type (*Symbiodinium* C1), and *Acropora valida*, with a mixed *Symbiodinium* symbiont community, comprising members of both clades A and C. The relative abundances of *Symbiodinium* types varied over time. A significant decline in symbiont densities in both coral species during the summer of 2005 coincided with a NOAA 'hotspot' warning for Heron Island. This also coincided with a relative increase in the presence and dominance of clade A in *A. valida*, particularly in sun-adapted surfaces. The effective quantum yield of Photosystem II ( $\Phi_{PSII}$ ) suggested that sun-adapted surfaces of *P. damicornis* are more sensitive than shade-adapted surfaces to combined effects of higher temperature and irradiance in summer. Xanthophyll cycling was greater in *P. damicornis* than *A. valida*, irrespective of branch position and sampling time; this may be a mechanism by which *P. damicornis* compensates for its fidelity to *Symbiodinium* C1. Furthermore, xanthophyll de-epoxidation in *P. damicornis* symbionts was greater in sun-adapted than shade-adapted surfaces, correlating with non-photochemical quenching (NPQ<sub>REL</sub>). No variation was found in *A. valida*, indicating that resident symbiont communities may not have been physiologically compromised, perhaps as a result of changes in the composition of the *Symbiodinium* community consortia.

**KEY WORDS:** Fast fluorescence kinetics · ITS1 rDNA · LSU rDNA · *Symbiodinium* · Symbiont density

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## INTRODUCTION

Symbiotic algae of the genus *Symbiodinium* (zooxanthellae) are genetically diverse and are thought to play an important role in the acclimatisation and/or adaptation of corals to changes in the environment (Baker 2003). The relative abundances of genetically different *Symbiodinium* types forming heterogeneous symbiont consortia in corals may change over time scales of months, seasons and years (e.g. Toller et al.

2001, Little et al. 2004, Berkelmans & van Oppen 2006, Thornhill et al. 2006) in response to changes or disturbances in the environment (Toller et al. 2001, Berkelmans & van Oppen 2006). Genetically different types of *Symbiodinium* also exhibit distinct physiologies, some of which may mitigate the effects of coral bleaching conditions (Rowan 2004).

Evidence of different physiologies was initially sought among *Symbiodinium ex symbio* (Chang et al. 1983, Iglesias-Prieto & Trench 1994). However, incon-

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sistencies between phylogenetic and physiological attributes (Savage et al. 2002b, Tchernov et al. 2004, Robison & Warner 2006), and between physiological responses of isolated versus *in hospite* *Symbiodinium* (Bhagooli & Hidaka 2003, Goulet et al. 2005) suggest that the coral host, at least in part, determines the fitness of the holobiont. The photo-physiological response of *Symbiodinium* is influenced by photo-protective agents such as antioxidant enzymes (e.g. Lesser & Shick 1989) and heat-dissipating pigments (Ambarsari et al. 1997) as well as host-associated fluorescent pigments that scatter and fluoresce light (Salih et al. 2000). Photosynthetic processes of *Symbiodinium* are also influenced by their local irradiance regime, which is controlled by water depth and light scattering properties of coral tissues (Enríquez et al. 2005). Thus, photo-physiological differences have been documented between sun- and shade-adapted colonies (Falkowski & Dubinsky 1981, Porter et al. 1984, Gorbunov et al. 2001), between sun- and shade-adapted surfaces of individual colonies (Jones et al. 1998, Ralph et al. 2005), and between polyp and coenosarc tissues (Kühl et al. 1995, Ralph et al. 2002, 2005, Hill et al. 2004b, Ulstrup et al. 2006b).

The photo-protective process of excess excitation energy dissipation away from the photosynthetic apparatus (non-photochemical quenching, NPQ) is usually enhanced by high irradiance exposure (Warner et al. 1996, Gorbunov et al. 2001, Warner & Berry-Lowe 2006). The formation of diatoxanthin (Dt) by depoxidation of diadinoxanthin (Dn) is one of the dissipation pathways that is also thought to be important for the scavenging of reactive oxygen species (Ambarsari et al. 1997, Lesser et al. 2000, Brown et al. 2002, Venn et al. 2006, Warner & Berry-Lowe 2006). We studied the effect of seasonal acclimatisation on the proportion of xanthophyll cycling ( $Dt/(Dn + Dt)$ ) in coral symbionts at low irradiances as a proxy for dissipation capacity.

Photo-acclimatisation of *Symbiodinium* operates on a variety of time scales and by different mechanisms. At high irradiances, coral polyps use behavioural mechanisms such as tissue retraction for shade (Brown et al. 2002), or tissue expansion to increase the surface area for solute exchange, which may alleviate potential carbon limitation and excess oxygen levels in the tissue (Levy et al. 2006). Diurnal (Brown et al. 1999a, Jones & Hoegh-Guldberg 2001, Levy et al. 2004, Hill & Ralph 2005) and seasonal changes in irradiance (Brown et al. 1999b, Fitt et al. 2000, Warner et al. 2002) as well as temperature cause modulation of symbiont photosynthesis that affects photochemical efficiency. Also, *Symbiodinium* cell density is inversely correlated to seasonal increases in light and temperature (Fagoonee et al. 1999, Fitt et al. 2000). Finally, tempo-

ral patterns of symbiont population density, pigment content and photochemical efficiency related to seasonal changes in environmental conditions may also be partially due to changes in the phylogenetic composition of symbiotic communities associated with corals (Fitt et al. 2000, Warner et al. 2002, Baker 2003).

There have been few long-term studies of genetic flexibility (Goulet & Coffroth 2003, Thornhill et al. 2006) and photo-physiological variation (Brown et al. 1999b, Fitt et al. 2000, Warner et al. 2002) of symbiont communities *in hospite*, and to our knowledge, never in combination. We report the intra-colony variability of physiological responses and *Symbiodinium* composition in *Pocillopora damicornis* and *Acropora valida* monitored twice yearly for 3 yr at Heron Island in the southern section of the Great Barrier Reef, Australia.

## MATERIALS AND METHODS

*Pocillopora damicornis* (Linnaeus) and *Acropora valida* (Dana) colonies were collected twice yearly over a 1 wk period in the austral summer (January) and winter (July) of 3 consecutive years (2004–2006) in the Heron Island lagoon (23° 26.6' S, 151° 54.7' E). Sea surface (1 to 3 m) temperature at Heron Island was obtained from the daily monitoring program at Heron Island Research Station between January 2004 and August 2006. A spirit thermometer was used to record the minimum and maximum daily temperature over the previous 24 h. The maximum temperature observed each day was used to map the thermal variation for the duration of this study (see Fig. 1).

Corals were collected haphazardly at <3 m depth in the afternoon in each season in a localised area of ~0.5 ha adjacent to Heron Island Research Station. *Pocillopora damicornis* is very abundant in the lagoon area in front of the station; *Acropora valida* is less abundant. Collections of *A. valida* were therefore limited to a small number of colonies (<15), most of which would have been re-sampled between years. An upward-facing branch (sun-adapted) and a basal (shade-adapted) branch of individual fragments of each of 10 colonies were sub-sampled for each of the following analyses: rapid light curve (RLC), fast induction curve (FIC), symbiont density, xanthophyll pigmentation and genetic identification of symbiont communities. All measurements were examined after dusk on the day of collection following a minimum of 1 to 2 h of acclimation to irradiances between 25 and 35  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  provided by a flood light calibrated to a quantum irradiance meter (LI-190 cosine corrected, Li-Cor). This low illumination was intended to keep the photosynthetic apparatus active and prevent any dark-induced reduction of the electron trans-

port chain via chlororespiration (Garab et al. 1989, Peltier & Cournac 2002, Beardall & Quigg 2003), which has been shown to be active in corals (Jones & Hoegh-Guldberg 2001, Ulstrup et al. 2005).

**PAM fluorescence measurements of Photosystem II.** An RLC with 10 s intervals between 9 irradiance steps was performed by placing a 6 mm fibre-optic cable fitted to a Diving-PAM (Pulse-Amplitude-Modulation) (Walz; settings for the PAM: Gain = 2, Damping = 2) adjacent to the coral, approximately 3 cm from the tip of a branch. The Diving-PAM employs 3  $\mu$ s pulses from a red light-emitting diode (LED) with a peak emission at 650 nm as the measuring light (0.15  $\mu$ mol photons  $m^{-2} s^{-1}$ ). Saturation pulses were of >4500  $\mu$ mol photons  $m^{-2} s^{-1}$  for 0.8 s. Chl *a* fluorescence was detected at wavelengths >710 nm. The basic parameters measured were  $F_t$  (the fluorescence yield in the light at time *t*) and  $F_m'$  (the maximum fluorescence yield in the light induced by a saturating flash of light). From these parameters, the effective quantum yield of Photosystem II ( $\Phi_{PSII} = [F_m' - F_t]/F_m'$ ) and relative electron transport rate ( $rETR = [F_m' - F_t]/F_m' \times PAR$ ) were derived, where PAR is the photosynthetically active radiation in  $\mu$ mol photons  $m^{-2} s^{-1}$ . RLCs ( $rETR$  measured over a range of irradiances between 0 and 1800  $\mu$ mol  $m^{-2} s^{-1}$ ) were subsequently fitted to the formula of Platt et al. (1980), whereby quantitative parameters of maximum relative electron transport rate ( $rETR_{max}$ ),  $\alpha$  (light utilisation coefficient) and  $E_k$  (minimum saturating irradiance) were calculated. The highest  $\Phi_{PSII}$  ( $[F_m' - F_t]/F_m'$ ) was derived from the first measure of the RLCs in light (25 to 35  $\mu$ mol photons  $m^{-2} s^{-1}$ ). Finally, non-photochemical quenching ( $NPQ = [F_m - F_m']/F_m'$ ) was calculated for irradiances between 800 and 1000  $\mu$ mol photons  $m^{-2} s^{-1}$  for each RLC ( $NPQ_{RLC}$ ), depending on sampling season. The actinic light intensities of the Diving-PAM were calibrated before each sampling period. The irradiance settings for each season were chosen to approximate each other. The individual irradiance steps, however, could not be replicated between seasons due to possible decay of the battery, probe and LEDs of the Diving-PAM.

**Fast induction kinetics of Photosystem II.** Upon completion of an RLC, a FIC was measured using the Plant Efficiency Analyser (PEA) (Hansatech, Instruments) after 5 min of dark-acclimation in a purpose-built chamber (Hill et al. 2004b). Fast induction kinetics provide detailed information on the photochemical state of PSII, as well as the plastoquinone (PQ) pool (Govindjee 1995, Hill et al. 2004a). The array of 6 red LEDs (peak wavelength 650 nm) produced the saturating irradiance (3200  $\mu$ mol photons  $m^{-2} s^{-1}$ ) focusing on an area 4 mm in diameter approximately 3 cm from the tip of the branch. A PIN-photodiode (shielded by a long-pass filter >720 nm) detected the fluorescence

signal from the *in hospite* symbionts. The fluorescence signal was obtained over 2 s and recorded every 10  $\mu$ s for the first 2 ms, every 1 ms for the first 1 s of sampling, and every 100 ms thereafter. The base fluorescence ( $F_0$ ) was measured at 0.05 ms (O step), the J step at 1.5 ms, the I step at 50 ms and the P step ( $F_m$ ) was recorded as the maximum fluorescence was reached at the end of the 2000 ms sampling period. The amplitude of each step along the FICs was plotted for the 2 coral species for sun- and shade-adapted coral surfaces (see Fig. 5). Maximum quantum yield ( $F_v/F_m = [F_m - F_0]/F_m$ ) was calculated from the O step (at 50  $\mu$ s) and P step (maximum fluorescence), which are equivalent to  $F_0$  and  $F_m$ , respectively (see Fig. 4).  $F_v/F_m$  was also determined the following morning prior to sunrise.

The OJIP transient (nomenclature of Strasser et al. 1995) can be divided into 2 phases, the fast rise from the minimum fluorescence (O) to J and onwards to the intermediate I step, and a slower rise to the P step (Hill et al. 2004a). The rise in fluorescence from O to J corresponds to the reduction of the PSII primary electron acceptor  $Q_A$  to  $Q_A^-$  (Strasser et al. 1995, Ulstrup et al. 2005). Following the J step, further reduction of  $Q_A$  and, subsequently,  $Q_B$  (secondary electron acceptor of PSII) occurs up until the I step (Strasser et al. 1995, Srivastava et al. 1997). The final component of the fluorescence rise to the P step is ascribed to the filling of the PQ pool (Lavorel & Etienne 1977, Lazar 2006), which takes approximately 2 s in corals (Hill & Ralph 2005, Ulstrup et al. 2005).

**Molecular methods.** A ~30 mm length coral piece was fixed in 95 % ethanol for later sample processing to genetically identify the resident symbiont community. The DNA of preserved coral samples ( $N = 240$ ) was extracted using the DNeasy™ Tissue Kit (Qiagen). Single-stranded conformation polymorphism (SSCP) and sequence analysis of the rDNA ITS1 region were used as markers for identification (van Oppen et al. 2001); PCR and gel conditions are described in Ulstrup & van Oppen (2003). Where the ITS1 PCR protocol failed to amplify (~10% of samples), LSU (large subunit) was amplified using the protocol specified in van Oppen et al. (2001) with the assumption that the 2 primers have equal affinities for the different *Symbiodinium* types amplified. LSU resolves *Symbiodinium* types to sub-clade levels C1 and C2 (sensu van Oppen et al. 2001). Ten  $\mu$ l of PCR product were combined with 10  $\mu$ l of RFLP mix (0.3  $\mu$ l TaqI (10 units  $\mu$ l $^{-1}$ ), 2  $\mu$ l 10 $\times$  restriction buffer (Fermentas) and 7.7  $\mu$ l dH<sub>2</sub>O). Restriction digests were analysed by running an aliquot (5  $\mu$ l) on a 2% TAE-agarose gel (1 h, 80 V) stained with ethidium bromide, and representatives of disparate profiles were sequenced (Macrogen). Finally, sequences were compared to those stored in GenBank (available at: [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

**Symbiont cell determination.** The densities of symbionts were determined as number of cells  $\text{cm}^{-2}$ . Coral tissue was stripped from an approximately 3 cm long branch using an air-gun and submerged in 1  $\mu\text{m}$ -filtered seawater. Symbiont cell densities in 10 ml aliquots were determined from the average of 8 replicate haemocytometer counts, standardised to the liquid volume in which they were extracted and related to the coral surface area using the wax weight method (Stimson & Kinzie 1991) for triplicate samples. The regression relationship was determined empirically using acrylic cylinders with a range of known surface areas ( $r^2 = 0.998$ ).

**Symbiont xanthophyll determination.** The relative proportions of Dt to the total xanthophyll pool (Dt + Dn) were determined using reverse-phase high performance liquid chromatography (HPLC). Upon completion of chl a fluorescence measurements of PSII, approximately 3 cm long sun- and shade-adapted branches from each colony collected were snap frozen in liquid nitrogen and placed in a freezer at  $-80^\circ\text{C}$  until further analysis. Coral tissue (including symbionts) was removed from each branch using sonication and subsequently spun at  $5000 \times g$  for 10 min. Resulting pellets were extracted in 2 ml acetone and vortexed for 30 s. The extract was spun using high-speed centrifugation ( $10\,000 \times g$ ) for 5 min, and the clarified supernatant extract (150  $\mu\text{l}$ ) was loaded into the autosampler for immediate injection onto a Phenomenex C18 column (5  $\mu\text{m}$  particle size,  $250 \times 4.6$  mm) at room temperature ( $21^\circ\text{C}$ ). All samples were prepared under low light.

Analyses were performed on a PC-interfaced HPLC system (Shimadzu). System control, data collection and integration were performed using Class-VP v. 6 Shimadzu 2002 software. Pigments were separated using a binary mobile phase system (60/40 [w/v] methanol/1.0 M ammonium acetate: 95% methanol) over 10 min followed by an isocratic hold at 95% methanol for 30 min. Flow rate was constant at  $1 \text{ ml min}^{-1}$ . Dt and Dn were detected using a photodiode array (PDA) detector and pigments were identified by spectral recognition (Jeffrey et al. 1997). Absorbance chromatograms were extracted at 440 nm. The xanthophyll ratio was calculated as the ratio of area of the peak corresponding to Dt to the peak area of Dt + Dn.

**Statistical analyses.** Coral specimens ( $n = 10$ ) of *Pocillopora damicornis* and *Acropora valida* were collected in the summer and winter of 2004, 2005 and 2006. Changes in photo-physiological parameters and symbiont cell densities among sampling times (seasons, years) and positions (sun- and shade-adapted surfaces) were assessed by repeated measures ANOVAs. Each species was analysed separately, year was treated as a within-subject factor, and season and position as between-subject factors. Where ANOVA

determined a significant difference, Box's test of equality of covariance matrices was used to test the homogeneity of variances across groups. Where the assumption of homogeneity was violated, the data were  $\log_{10}$ -transformed. When this transformation failed to produce homoscedasticity, significant differences indicated by the test were accepted if  $p < 0.01$ . The assumption of sphericity was tested and, if violated, the Greenhouse-Geisser adjustment was performed (Quinn & Keough 2002). Post-hoc comparisons of means for significant factors and interactions were carried out using Tukey's HSD tests. Parameters analysed were  $F_v/F_m$ ,  $\Phi_{\text{PSII}}$ , descriptive parameters of fitted RLC curves ( $r\text{ETR}_{\text{max}}$ ,  $\alpha$  and  $E_k$ ), descriptive parameters of FICs (O, J, I, and P),  $\text{NPQ}_{\text{RLC}}$ , symbiont cell density and  $\text{Dt}/(\text{Dn} + \text{Dt})$ . In the case of  $F_v/F_m$  derived from FICs, each sun- and shade-adapted surface of each species was also analysed separately, year was treated as within-subject factor, and season and time (dusk and dawn) as between subject factors. Statistical significance of the ANOVAs was accepted at  $p < 0.05$ . All analyses were performed with STATISTICA v. 7.1 software (SysSoft).

## RESULTS

### Measurements of temperature

Fig. 1 shows the maximum temperature regime observed in the Heron Island lagoon where corals were collected between January 2004 and July 2006. Although coral paling was observed during the summer in all years of this study, Heron Island was considered a coral bleaching 'hotspot' (Goreau & Hayes 1994) only in the summer of 2005. In the lagoon, bleaching was manifested as greater-than-normal paling of branching corals, but no mortality was observed at the time of collection.

### Quantum yields

The  $\Phi_{\text{PSII}}$  (derived from the first measure of the RLCs in light at 25 to 35  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) and the  $F_v/F_m$  (measured with the PEA) were greater on shade-adapted surfaces than in sun-adapted surfaces of *Pocillopora damicornis* ( $\Phi_{\text{PSII}}$ :  $F_{1,36} = 65.91$ ,  $p < 0.001$ ;  $F_v/F_m$ :  $F_{1,36} = 88.10$ ,  $p < 0.001$ ; Figs. 2a & 3a). Significant interactions were observed between effects of season and position on  $\Phi_{\text{PSII}}$  ( $F_{1,36} = 20.97$ ,  $p < 0.001$ ) for *P. damicornis*, suggesting that the branch orientation influenced the variation between seasons.

Sun-adapted surfaces of *Acropora valida* had lower  $F_v/F_m$  than shade-adapted surfaces ( $F_{1,36} = 17.81$ ,  $p <$

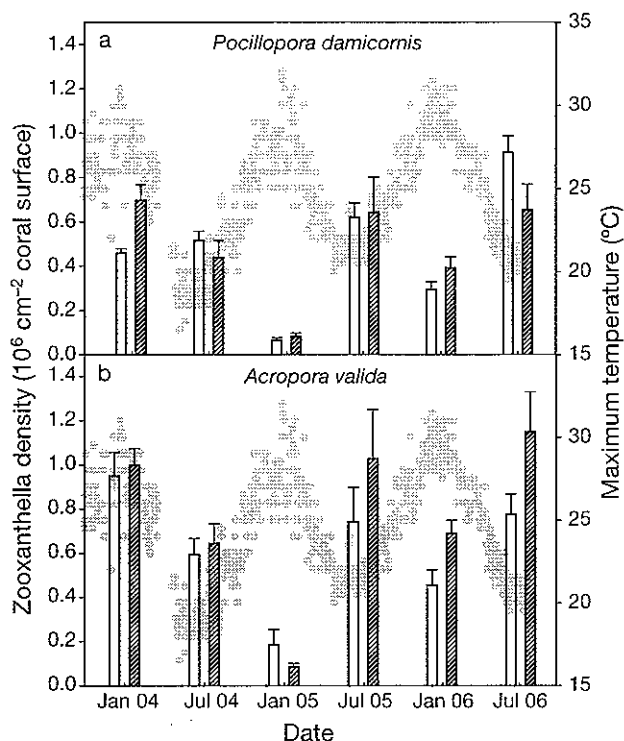


Fig. 1. (a) *Pocillopora damicornis* and (b) *Acropora valida*. Maximum daily seawater temperatures (°) at Heron Island and symbiont cell densities of corals in sun- (white bars) and shade-adapted surfaces (hatched bars) of colonies. Means ± SE, n = 10

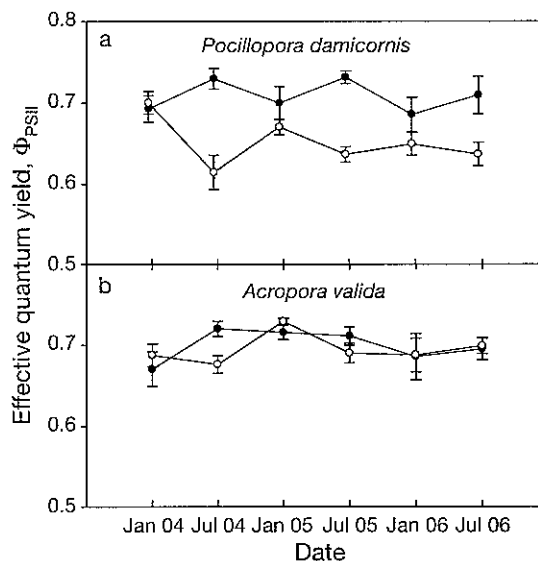


Fig. 2. (a) *Pocillopora damicornis* and (b) *Acropora valida*. Effective quantum yield (Φ<sub>PSII</sub>) measured for corals under quasi-darkness conditions in sun- (○) and shade-adapted (●) surfaces at dusk. Means ± SE, n = 10

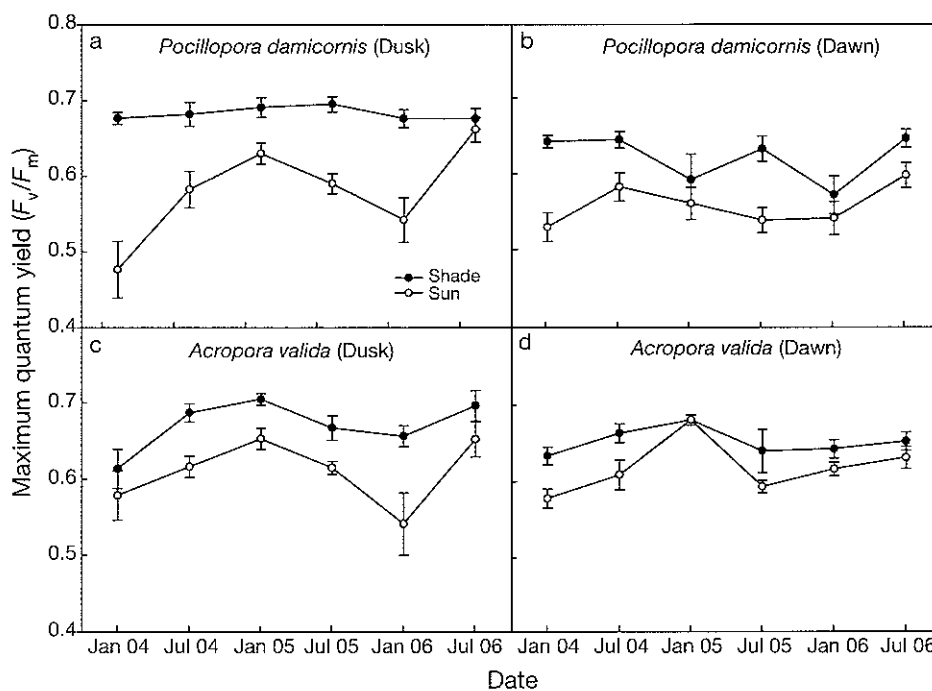


Fig. 3. (a,b) *Pocillopora damicornis* and (c,d) *Acropora valida*. Maximum quantum yield (F<sub>v</sub>/F<sub>m</sub>) of zooxanthellae in sun- (○) and shade-adapted (●) surfaces of corals at (a,c) dusk and (b,d) dawn. Means ± SE, n = 10

0.001, Fig. 3b), whereas there was no intra-colony differentiation in Φ<sub>PSII</sub> (Fig. 2b). F<sub>v</sub>/F<sub>m</sub> in summer fluctuated between years, with lower values in 2004 and 2006 than in 2005 (F<sub>2,72</sub> = 10.19, p < 0.001, Fig. 3c).

The differences between dusk and dawn measurements of  $F_v/F_m$  for sun- and shade-adapted surfaces, respectively, were also tested. Shade-adapted surfaces of *Pocillopora damicornis* yielded higher  $F_v/F_m$  values at dusk than at dawn ( $F_{1,36} = 26.92$ ,  $p < 0.001$ , Fig. 3).

#### Descriptive parameters of fitted rapid light curves

The descriptive parameters of fitted RLCs revealed lower  $rETR_{max}$  in shade-adapted than in sun-adapted surfaces of *Pocillopora damicornis* ( $F_{1,36} = 14.30$ ,  $p < 0.001$ ) and *Acropora valida* ( $F_{1,36} = 25.14$ ,  $p < 0.001$ )

Table 1. *Pocillopora damicornis* and *Acropora valida*. Quantitative parameters derived from fitted rapid light curves as a function of PAR. Variable chl *a* fluorescence:  $rETR_{max}$  (maximum relative electron transport rate, a.u. [arbitrary units]),  $\alpha$  (light utilisation coefficient),  $E_k$  (minimum saturating irradiance,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ); values are for sun- (Sun) and shade-adapted (Shade) corals in summer and winter over 3 consecutive years. Means  $\pm$  SE,  $n = 10$

		$rETR_{max}$	$\alpha$	$E_k$
<b><i>P. damicornis</i></b>				
2004				
Summer	Sun	114 $\pm$ 6	0.75 $\pm$ 0.03	155 $\pm$ 12
	Shade	87 $\pm$ 5	0.66 $\pm$ 0.03	134 $\pm$ 9
Winter	Sun	108 $\pm$ 7	0.62 $\pm$ 0.03	178 $\pm$ 14
	Shade	102 $\pm$ 7	0.75 $\pm$ 0.03	138 $\pm$ 13
2005				
Summer	Sun	168 $\pm$ 22	0.71 $\pm$ 0.02	243 $\pm$ 38
	Shade	152 $\pm$ 19	0.72 $\pm$ 0.01	208 $\pm$ 25
Winter	Sun	80 $\pm$ 4	0.56 $\pm$ 0.04	147 $\pm$ 10
	Shade	65 $\pm$ 4	0.66 $\pm$ 0.03	100 $\pm$ 7
2006				
Summer	Sun	145 $\pm$ 17	0.50 $\pm$ 0.03	291 $\pm$ 36
	Shade	121 $\pm$ 9	0.49 $\pm$ 0.03	252 $\pm$ 22
Winter	Sun	171 $\pm$ 5	0.61 $\pm$ 0.04	292 $\pm$ 24
	Shade	151 $\pm$ 13	0.64 $\pm$ 0.04	242 $\pm$ 26
<b><i>A. valida</i></b>				
2004				
Summer	Sun	79 $\pm$ 8	0.46 $\pm$ 0.04	172 $\pm$ 13
	Shade	56 $\pm$ 10	0.42 $\pm$ 0.06	132 $\pm$ 18
Winter	Sun	93 $\pm$ 8	0.55 $\pm$ 0.04	176 $\pm$ 16
	Shade	59 $\pm$ 4	0.73 $\pm$ 0.02	82 $\pm$ 6
2005				
Summer	Sun	138 $\pm$ 13	0.75 $\pm$ 0.03	187 $\pm$ 18
	Shade	133 $\pm$ 12	0.73 $\pm$ 0.02	186 $\pm$ 20
Winter	Sun	87 $\pm$ 5	0.56 $\pm$ 0.03	162 $\pm$ 14
	Shade	44 $\pm$ 4	0.67 $\pm$ 0.03	68 $\pm$ 7
2006				
Summer	Sun	142 $\pm$ 17	0.47 $\pm$ 0.03	297 $\pm$ 21
	Shade	92 $\pm$ 10	0.48 $\pm$ 0.05	199 $\pm$ 17
Winter	Sun	158 $\pm$ 10	0.63 $\pm$ 0.02	252 $\pm$ 14
	Shade	103 $\pm$ 10	0.58 $\pm$ 0.04	184 $\pm$ 17

(Table 1).  $E_k$  was also lower in shade-adapted than in sun-adapted surfaces of *P. damicornis* ( $F_{1,36} = 15.38$ ,  $p < 0.001$ ) and *A. valida* ( $F_{1,36} = 35.23$ ,  $p < 0.001$ ). For both species,  $E_k$  was lower in winter than in summer (*P. damicornis*:  $F_{1,36} = 8.21$ ,  $p = 0.007$ ; *A. valida*:  $F_{1,36} = 13.93$ ,  $p = 0.001$ ). A significant interaction ( $F_{1,36} = 10.33$ ,  $p = 0.003$ ) was observed for  $\alpha$  between season and position for *P. damicornis*, suggesting that branch orientation influenced the light utilisation efficiency between seasons.

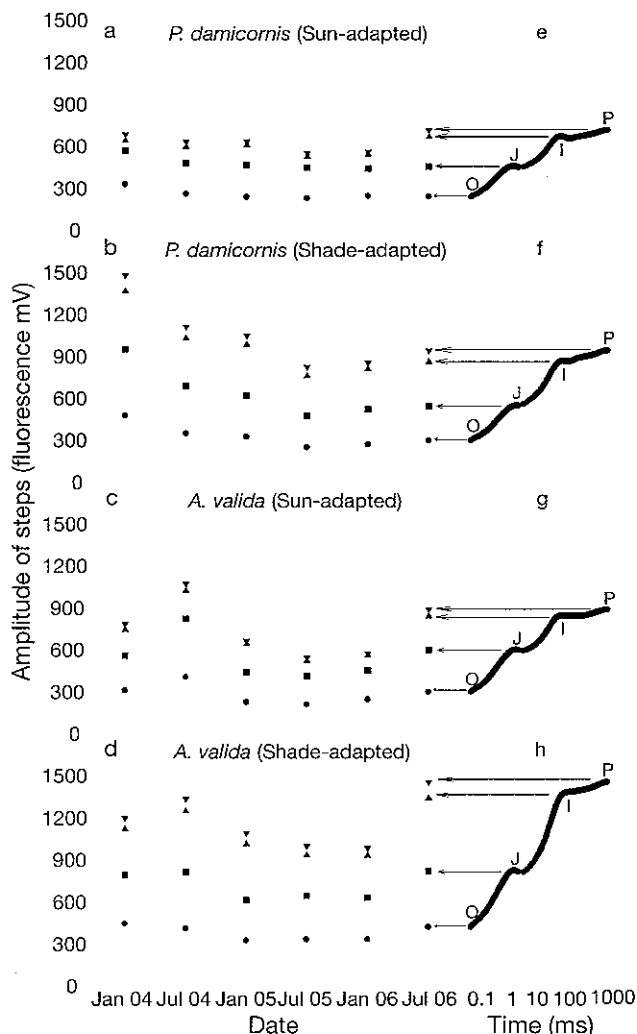


Fig. 4. *Pocillopora damicornis* and *Acropora valida*. Amplitude of the O, J, I and P steps (●, ■, ▲ and ▼, respectively) along fast induction curves (FICs) in *P. damicornis*: (a) sun- and (b) shade-adapted surfaces, and in *A. valida*: (c) sun- and (d) shade-adapted surfaces between January 2004 and July 2006 ( $n = 10$ , mean  $\pm$  SE). Average FICs ( $n = 10$ ) from *P. damicornis* on (e) sun- and (f) shade-adapted surfaces, and from *A. valida* on (g) sun- and (h) shade-adapted surfaces indicating how O, J, I and P step amplitudes were obtained. Arrows correlate to the amplitude of each of the steps for these particular FICs

**Fast induction kinetics**

The mean amplitudes of the O, J, I and P steps along the FICs measured on sun- and shade-adapted surfaces of *Pocillopora damicornis* and *Acropora valida* from January 2004 to July 2006 are shown in Fig. 4a–d. Fig. 4e,f display the average FICs of sun- and shade-adapted surfaces of *P. damicornis* in July 2006; Fig. 4g,h display equivalent data for *A. valida*. These provide an example of how the O, J, I and P step amplitudes were derived for Fig. 4a–d. In all cases, the O, J, I and P step amplitudes of the shade-adapted surfaces were higher than those of the sun-exposed surfaces (Fig. 4). The differences in the P step for both *P. damicornis* and *A. valida* indicate a reduction in  $F_m$  and are also reflected in the lower  $F_v/F_m$  values for *P. damicornis* of sun-adapted surfaces compared to shade-adapted surfaces (Fig. 3a,b).

In *Pocillopora damicornis*, all steps declined in the years following 2004 (O:  $F_{2,72} = 12.84$ ,  $p < 0.001$ ; J:  $F_{2,72} = 12.05$ ,  $p < 0.001$ ; I:  $F_{2,72} = 7.74$ ,  $p < 0.001$ ; P:  $F_{2,72} = 8.40$ ,  $p < 0.001$ ; Fig. 4a,b) and for the O and I steps alone in winter (O:  $F_{1,36} = 9.10$ ,  $p < 0.005$ ; I:  $F_{1,36} = 4.39$ ,  $p = 0.043$ ). A significant interaction was observed between season and position for the J step ( $F_{1,36} = 4.40$ ,  $p = 0.043$ ), suggesting that the seasonal variation in amplitude of this parameter depended on branch orientation (Fig. 4a,b).

In *Acropora valida*, the O step declined in the years following 2004 ( $F_{2,72} = 11.47$ ,  $p < 0.001$ ), while the J, I and P steps were lower only in 2005 (J:  $F_{2,72} = 9.18$ ,  $p < 0.001$ ; I:  $F_{2,72} = 5.47$ ,  $p = 0.006$ ; P:  $F_{2,72} = 5.24$ ,  $p = 0.008$ ) (Fig. 4c,d). For J, I and P, there were also rises in winter (O:  $F_{1,36} = 5.44$ ,  $p = 0.025$ ; J:  $F_{1,36} = 5.88$ ,  $p = 0.021$ ; P:  $F_{1,36} = 6.55$ ,  $p = 0.015$ ). For the I and P steps, there was a significant interaction between year and season (I:  $F_{2,72} = 4.27$ ,  $p = 0.018$ ; P:  $F_{2,72} = 4.46$ ,  $p = 0.015$ ) showing that the winter of 2005 was lower than in 2004 and 2006 (Fig. 4c,d).

**Variability in *Symbiodinium* types harboured**

Over the course of 3 yr, the population of *Pocillopora damicornis* experienced no variation in symbiont type (C1 sensu van Oppen et al. 2001, GenBank Accession # AY457958). *Acropora valida* colonies hosted a variety of clade C types, including C1 and C2 (sensu van Oppen et al. 2001, GenBank Accession # AY758477.1) as well as a member of clade A (GenBank Accession # AF380513) (Table 2). The strain belonging to clade A occurred simultaneously with *Symbiodinium* C2 and sometimes also with *Symbiodinium* C1. In the samples where LSU was used, clade A could not be resolved simultaneously to C1 as the restriction profile of clade

A overlaps that of C1. This may therefore have led to a slight underestimation of the presence of clade A in ~10% of samples.

*Symbiodinium* C2 was the dominant symbiont type in *Acropora valida* across sampling times and appeared in all specimens except for one sun-adapted surface sample in the summer of 2006. *Symbiodinium* C1 appeared in ~54% of all samples but was never dominant. In the summer of 2004 and winter of 2005, C1 was absent from all samples. Clade A was detected mostly in sun-adapted surfaces of corals and was particularly prevalent in the summer and winter of 2005 and in the winter of 2006 (Table 2).

Table 2. *Pocillopora damicornis* and *Acropora valida*. Frequencies of *Symbiodinium* type(s) within sun- and shade-adapted surfaces (Sun, Shade) and among colonies from Heron Island. n: sample number; f: observed SSCP (single-stranded conformation polymorphism) genotype frequencies (for clades A, C [C1, C2], D:  $f_A$ ,  $f_{C1}$ ,  $f_{C2}$ ,  $f_D$ ); %: proportions of clades. Where >1 *Symbiodinium* type was present, the dominant frequency is in bold; –: no data

		n	$f_A$	%	$f_{C1}$	%	$f_{C2}$	%
<b><i>P. damicornis</i></b>								
2004								
Summer	Sun	8	–	–	8	100	–	–
	Shade	3	–	–	3	100	–	–
Winter	Sun	10	–	–	10	100	–	–
	Shade	9	–	–	9	100	–	–
2005								
Summer	Sun	9	–	–	9	100	–	–
	Shade	8	–	–	8	100	–	–
Winter	Sun	10	–	–	10	100	–	–
	Shade	9	–	–	9	100	–	–
2006								
Summer	Sun	7	–	–	7	100	–	–
	Shade	10	–	–	10	100	–	–
Winter	Sun	2	–	–	2	100	–	–
	Shade	3	–	–	3	100	–	–
<b><i>A. valida</i></b>								
2004								
Summer	Sun	10	1 (1)	10	–	–	10 (9)	100
	Shade	8	–	–	–	–	8	100
Winter	Sun	9	3 (1)	33	4	44	9 (5)	100
	Shade	8	1	13	2	25	8 (7)	100
2005								
Summer	Sun	9	6 (3)	67	7	78	9 (5)	100
	Shade	9	1	11	7	78	9 (8)	100
Winter	Sun	7	5 (2)	71	–	–	7 (5)	100
	Shade	8	8	100	–	–	8 (8)	100
2006								
Summer	Sun	10	3 (2)	30	1	10	9 (2)	90
	Shade	10	–	–	–	–	10	100
Winter	Sun	9	4 (4)	44	7	78	9 (6)	100
	Shade	7	2 (1)	29	5	71	7 (7)	100

### Symbiont density

No evidence of intra-colony differentiation in symbiont cell density was detected in *Pocillopora damicornis* or *Acropora valida*. Seasonal changes in the density of symbionts were observed in both coral species with summer density being lower than winter density (*P. damicornis*:  $F_{1,36} = 132.8$ ,  $p < 0.001$ ; *A. valida*:  $F_{1,36} = 49.39$ ,  $p < 0.001$ ). In both species, there was a significant decline in symbiont density in summer 2005 (*P. damicornis*:  $F_{2,72} = 18.72$ ,  $p < 0.001$ ; *A. valida*:  $F_{2,72} = 57.45$ ,  $p < 0.001$ , Fig. 1). This correlated with the bleaching conditions in that year, resulting in a pronounced paling of corals in the lagoon.

### Xanthophyll cycling and NPQ<sub>RLC</sub>

*Pocillopora damicornis* showed a greater proportion of Dt to the total pool of Dn + Dt than *Acropora valida* irrespective of orientation of the coral surface (Fig. 5). Sun-adapted surfaces of *P. damicornis* colonies had a greater proportion of Dt to the total pool of Dn + Dt

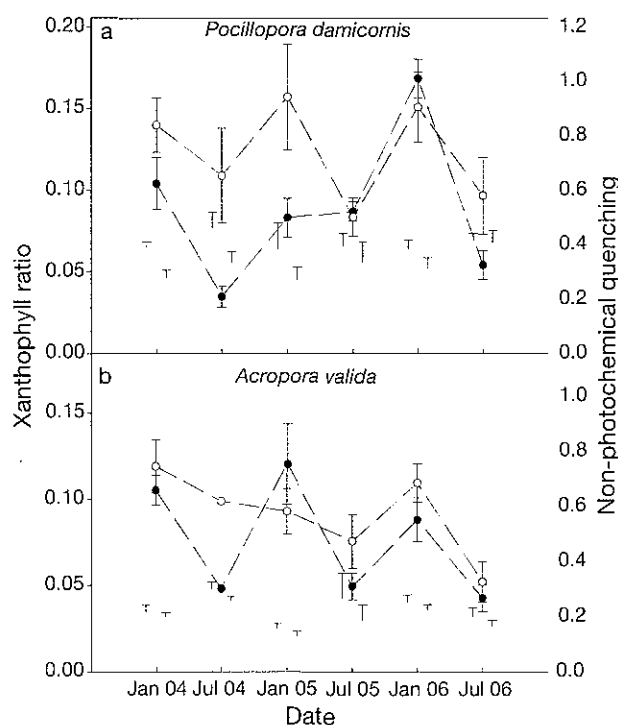


Fig. 5. (a) *Pocillopora damicornis* and (b) *Acropora valida*. Xanthophyll ratios (ratio of diatoxanthin to the sum of diatoxanthin + diadinoxanthin) on sun- (white bars) and shade-adapted surfaces (hatched bars) and non-photochemical quenching in sun- (O) and shade-adapted surfaces (●) of individual colonies. Xanthophyll ratio values are means + SE; non-photochemical quenching values are means  $\pm$  SE,  $n = 10$

than shade-adapted surfaces ( $F_{1,36} = 7.40$ ,  $p = 0.01$ ), whereas *A. valida* had low levels of Dt to the total pool of Dn + Dt for both orientations of the branches (Fig. 5). Furthermore, *P. damicornis* colonies in summer had lower de-epoxidation than in winter ( $F_{1,36} = 5.84$ ,  $p = 0.021$ ). NPQ<sub>RLC</sub> was highest in summer ( $F_{1,36} = 29.71$ ,  $p < 0.001$ ) and higher in sun-adapted than in shade-adapted surfaces of *P. damicornis* ( $F_{1,36} = 10.96$ ,  $p = 0.002$ , Fig. 5a).

## DISCUSSION

### Photochemical efficiency and photo-acclimatisation

As expected, shade-adapted surfaces generally exhibited higher photochemical efficiency than sun-adapted surfaces as indicated by the  $\Phi_{PSII}$  (*Pocillopora damicornis* only) and  $F_v/F_m$  values. In shade-adapted surfaces,  $F_v/F_m$  decreased between dusk and dawn measurements (Fig. 3). This may be due to chlororespiration, which occurs in corals (Jones & Hoegh-Guldberg 2001, Ulstrup et al. 2005) and is known to lower  $F_v/F_m$  values unless far-red light treatment is applied.

There was an inverse relationship between  $\Phi_{PSII}$  measured in sun- and shade-adapted surfaces among seasons in *Pocillopora damicornis*, whereby sun-adapted surfaces yielded lower  $\Phi_{PSII}$  in summer than in winter, and shade-adapted surfaces yielded higher  $\Phi_{PSII}$  in summer than in winter (Fig. 2a). This suggests that sun-adapted surfaces are more sensitive to combined effects of higher temperature and irradiance in summer, as would be expected. In *Acropora valida*, the lack of significant variation between sun- and shade-adapted surfaces and the lack of a significant interaction between branch orientation and season may be partly due to seasonal changes in the relative abundance of different *Symbiodinium* types (Fitt et al. 2000, Thornhill et al. 2006). The symbiont consortia may be continually modified to allow acclimatisation to changes in the environment (Thornhill et al. 2006), promoting well-suited *Symbiodinium* types that may compensate physiologically for other types that do not perform as well. Furthermore, sun-adapted branches of *A. valida* at Heron Island are distinctly purple-pigmented, suggesting that host pigments may provide some photo-protection for symbionts (Salih et al. 2000, Dove 2004), thus avoiding significant decrease of  $\Phi_{PSII}$ .

$F_v/F_m$  values were significantly lower in January 2004 and 2006 for both coral species, irrespective of branch orientation. A summer decline in  $F_v/F_m$  is likely due to a combination of photo-damage and photo-protective processes (cf. Warner et al. 2002) as well as to coral bleaching, which occurred in January 2005.



Interestingly, in January 2005,  $F_v/F_m$  remained similar to winter levels (Fig. 3) even though symbiont cell density declined significantly (Fig. 1) due to prolonged elevated seawater temperatures at Heron Island (available at: [www.noaa.gov](http://www.noaa.gov)). This suggests that remnant *Symbiodinium* are not physiologically compromised, possibly through effective dissipation of excess excitation energy and, in the case of *Acropora valida*, also through changes in the genetic composition of the symbiotic communities (Fitt et al. 2000, Warner et al. 2002, Baker 2003).

$\Phi_{PSII}$  and  $F_v/F_m$  measurements were collected with a Diving-PAM and a PEA instrument, respectively. Thus, direct comparison of these 2 parameters should be avoided when interpreting the data (due to differences in optics and intensity of the saturating flash). Furthermore, for corals, chlororespiration may lower  $F_v/F_m$  relative to  $\Phi_{PSII}$  (Jones & Hoegh-Guldberg 2001, Hill & Ralph 2005).

The FICs revealed physiological differences between sun- and shade-adapted surfaces of both coral species. The amplitudes of all 4 inflections along the transients were reduced in sun-adapted surfaces compared to shade-adapted surfaces (Fig. 4a–d), indicating inhibitory effects on PSII at each of these steps. The lower O step of sun-adapted surfaces can be attributed to a lower chlorophyll concentration within the tissue compared to the shade-adapted regions. As would be expected for the P step (indicating  $F_m$ ), the reduction was also reflected in lower  $F_v/F_m$  values in sun-adapted than shade-adapted surfaces. The decline in the O and J steps in July 2005 following the strong bleaching of *Pocillopora damicornis* in January 2005 suggests that damage occurred in PSII, which reduced electron flow to the primary electron acceptor  $Q_A$  (Strasser et al. 1995, Ulstrup et al. 2005); elevated temperature during the previous summer may have been the causal agent. There was also a decline in the I and P steps at this time, which suggests some blockage of electron flow beyond  $Q_A$  and reduced maximum capacity for electron flow, perhaps as a result of the prolonged exposure to elevated temperatures. In *Acropora valida*, the I and P steps recovered somewhat by July 2006, whereas the O and J steps remained low after January 2005, suggesting that PSII was intact and functioning, albeit photo-inhibited.

#### Genetic diversity of *Symbiodinium*

Many coral species are dominated by only one symbiont type (Goulet 2006, but see Baker & Romanski 2007). Therefore, it is likely that the physiological flexibility of symbiont communities residing in corals must, in many instances, be large to cope with not

only natural variation of the environment (Ulstrup et al. 2006b), but also rising seawater temperatures. This is the case for *Pocillopora damicornis*, which has high fidelity to symbiont type C1 (no other type was detected at any sampling time). This result supports previous findings by Ulstrup et al. (2006a) which show that *P. damicornis* has fidelity to C1 across the entire length of the Great Barrier Reef (GBR), with few exceptions. Coral colonies with such consistent symbioses are unlikely to respond to environmental shifts by changing to other *Symbiodinium* types (Thornhill et al. 2006).

In contrast, the genetically polymorphic zooxanthellar communities of *Acropora valida* varied over time and between sun- and shade-adapted surfaces. Although symbiont C2 was the predominant type, clade A appeared regularly, with a higher proportion of colonies hosting clade A in sun-adapted (10 to 67%) than in shade-adapted surfaces (0 to 29%) except for in the winter of 2005 (Table 2). The higher proportion of clade A in sun-adapted surfaces suggests that this *Symbiodinium* type may be adapted to high light. In the winter following the summer bleaching of 2005, more colonies contained clade A (71 to 100%), possibly as a response to the past bleaching conditions (Table 2).

Several studies have found relatively high frequencies of clade A in a variety of invertebrate hosts in cooler climates (Loh et al. 2001, 2006, Savage et al. 2002a), whereas rare observations (<3% of samples) of clade A have been reported from other species of *Acropora* in tropical waters (van Oppen et al. 2001, 2005). Overall, the cooler climate at Heron Island than in other parts of the GBR (Ulstrup et al. 2006a) may favour the presence of clade A over other types that have been found in warmer parts of the GBR (Ulstrup & van Oppen 2003).

#### Symbiont cell density

Corals sampled seasonally have variable symbiont cell density that is usually inversely correlated with increases in temperature and light (Stimson 1997, Fagoonee et al. 1999, Fitt et al. 2000). Both species we studied showed a significant decline in symbiont cell density in summer 2005 coincident with the bleaching hotspot at Heron Island. The reduction in symbiont density observed in summer 2005 in *Acropora valida* also coincided with an increase in the dominance of *Symbiodinium* clades A and C1 (Table 2). Although *Symbiodinium* C2 was still present in January 2005, a decline in dominance suggests that this type may be compromised during prolonged, elevated temperatures.

## Photo-protection

The de-epoxidation of Dn to form Dt is one of the non-photochemical pathways by which excess excitation energy can be dissipated by PSII, and is elevated in corals undergoing bleaching (Lesser et al. 2000, Venn et al. 2006). This process is induced by light, and intensifies over the course of the morning (Brown et al. 1999a, Warner & Berry-Lowe 2006). In the present study, we did not have the resources to follow the time course of the xanthophyll de-epoxidation and thus we measured the ratio of Dt to the sum of Dn + Dt concentration at dusk (25 to 35  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Under these sub-saturating irradiance conditions, <10% of Dn was de-epoxidised. *Pocillopora damicornis* showed greater degree of de-epoxidation than *Acropora valida* (Fig. 5). This suggests that the 2 species of corals may use different mechanisms for acclimatisation. While *A. valida* colonies may change the relative abundance of different *Symbiodinium* types hosted, *P. damicornis* colonies may compensate for their inability to change their symbiont consortia by exhibiting greater capacity for xanthophyll cycling.

Corals may also differ in baseline de-epoxidation capacity depending on their irradiance history. Shade-exposed surfaces of *Pocillopora damicornis* had a lower degree of de-epoxidation than sun-exposed surfaces, probably as a result of greater requirement for excess energy dissipation in sun-exposed surfaces.

## SUMMARY

The corals *Pocillopora damicornis* and *Acropora valida* were studied over 3 yr to investigate photo-physiological responses and genotypic character of *Symbiodinium*. *P. damicornis* harboured a single *Symbiodinium* type (C1). *A. valida* possessed *Symbiodinium* types C1, C2 and a member of clade A, and the proportions of these changed seasonally. Marked changes in the photo-physiological characteristics were recorded in both corals. In *P. damicornis*, these were not attributable to changes in the *Symbiodinium* community but were brought about by changes in symbiont cell density, photochemical efficiency and xanthophyll cycle activity. In *A. valida*, these same changes probably operated in combination with genotypic changes in the *Symbiodinium* community.

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## LITERATURE CITED

- ▶ Ambarsari I, Brown BE, Barlow RG, Britton G, Cummings D (1997) Fluctuations in algal chlorophyll and carotenoid pigments during solar bleaching in the coral *Goniastrea aspera* at Phuket, Thailand. Mar Ecol Prog Ser 159: 303–307
- ▶ Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annu Rev Ecol Syst 34:661–689
- ▶ Baker AC, Romanski AM (2007) Multiple symbiotic partnerships are common in scleractinian corals, but not in octocorals: Comment on Goulet (2006). Mar Ecol Prog Ser 335: 237–242
- ▶ Beardall J, Quigg A (2003) Oxygen consumption: photorespiration and chlororespiration. In: Larkum AWD, Douglas SE, Raven JA (eds) Photosynthesis in algae. Kluwer, Dordrecht, p 157–181
- ▶ Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. Proc R Soc Lond B Biol Sci 273:2305–2312
- ▶ Bhagooli R, Hidaka M (2003) Comparison of stress susceptibility of *in hospite* and isolated zooxanthellae among five coral species. J Exp Mar Biol Ecol 291:181–197
- ▶ Brown BE, Ambarsari I, Warner ME, Fitt WK, Dunne RP, Gibb SW, Cummings DG (1999a) Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. Coral Reefs 18:99–105
- ▶ Brown BE, Ambarsari I, Le Tissier MDA, Satopoomin U (1999b) Seasonal fluctuations in environmental factors and variation in symbiotic algae and chlorophyll pigments in four Indo-Pacific coral species. Mar Ecol Prog Ser 191:53–69
- ▶ Brown BE, Downs CA, Dunne RP, Gibb SW (2002) Preliminary evidence for tissue retraction as a factor in photoprotection of corals incapable of xanthophyll cycling. J Exp Mar Biol Ecol 277:129–144
- ▶ Chang SS, Prezelin BB, Trench RK (1983) Mechanisms of photoadaptation in three strains of the symbiotic dinoflagellate *Symbiodinium microadriaticum*. Mar Biol 76:219–229
- ▶ Dove S (2004) Scleractinian corals with photoprotective host pigments are hypersensitive to thermal bleaching. Mar Ecol Prog Ser 272:99–116
- ▶ Enriquez S, Mendez ER, Iglesias-Prieto R (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. Limnol Oceanogr 50:1025–1032
- ▶ Fagoonee I, Wilson HB, Hassell MP, Turner JR (1999) The dynamics of zooxanthellae populations: a long-term study in the field. Science 283:843–845
- ▶ Falkowski PG, Dubinsky Z (1981) Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature 289:172–174
- ▶ Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol Oceanogr 45:677–685
- ▶ Garab G, Lajko F, Mustardy L, Marton L (1989) Respiratory control over photosynthetic electron transport electron in chloroplasts of higher-plant cells: evidence of chlororespiration. Planta 179:349–358
- ▶ Gorbunov MY, Kolber ZS, Lesser MP, Falkowski PG (2001) Photosynthesis and photoprotection in symbiotic corals. Limnol Oceanogr 46:75–85
- ▶ Goreau TJ, Hayes RL (1994) Coral bleaching and ocean 'hot spots'. Ambio 23:176–180

- ▶ Goulet TL (2006) Most corals may not change their symbionts. *Mar Ecol Prog Ser* 321:1–7
- ▶ Goulet TL, Coffroth MA (2003) Stability of an octocoral-algal symbiosis over time and space. *Mar Ecol Prog Ser* 250: 117–124
- Goulet TL, Cook CB, Goulet D (2005) Effects of short-term exposure to elevated temperatures and light levels on photosynthesis of different host-symbiont combinations in the *Aiptasia pallida/Symbiodinium* symbiosis. *Limnol Oceanogr* 50:1490–1498
- Govindjee (1995) Sixty-three years since Kautsky: chlorophyll a fluorescence. *Aust J Plant Physiol* 22:131–160
- ▶ Hill R, Ralph PJ (2005) Diel and seasonal changes in fluorescence rise kinetics of three scleractinian corals. *Funct Plant Biol* 32:549–559
- ▶ Hill R, Frankart C, Larkum AWD, Kühl M, Ralph PJ (2004a) Loss of functional photosystem II reaction centres in zooxanthellae of corals exposed to bleaching conditions: using fluorescence rise kinetics. *Photosynth Res* 82:59–72
- ▶ Hill R, Schreiber U, Gademann R, Larkum AWD, Kühl M, Ralph PJ (2004b) Spatial heterogeneity of photosynthesis and the effect of temperature-induced bleaching conditions in three species of coral. *Mar Biol* 144:633–640
- ▶ Iglesias-Prieto R, Trench RK (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Mar Ecol Prog Ser* 113:163–175
- Jeffrey SW, Mantoura RFC, Bjørnland T (1997) Data for the identification of 47 key phytoplankton pigments. In: Jeffrey SW, Mantoura RFC, Wright SW (eds) *Phytoplankton pigments in oceanography: guidelines to modern methods*. UNESCO Publishing, Paris, p 447–559
- ▶ Jones RJ, Hoegh-Guldberg O (2001) Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: photoprotection, photoinactivation and the relationship to coral bleaching. *Plant Cell Environ* 24:89–99
- ▶ Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO<sub>2</sub> mechanism in zooxanthellae. *Plant Cell Environ* 21:1219–1230
- ▶ Kühl M, Cohen Y, Dalsgaard T, Jørgensen BB, Revsbech NP (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with micro-sensors for O<sub>2</sub>, pH and light. *Mar Ecol Prog Ser* 117: 159–172
- Lavorel J, Etienne AL (1977) *In vivo* chlorophyll fluorescence. In: Barber J (ed) *Primary processes of photosynthesis*. Elsevier, Amsterdam, p 203–268
- ▶ Lazar D (2006) The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. *Funct Plant Biol* 33:9–30
- ▶ Lesser MP, Shick JM (1989) Photoadaptation and defenses against oxygen toxicity in zooxanthellae from natural populations of symbiotic cnidarians. *J Exp Mar Biol Ecol* 134:129–141
- Lesser MP, Mazel C, Phinney D, Yentsch CS (2000) Light absorption and utilization by colonies of the congeneric hermatypic corals *Montastraea faveolata* and *Montastraea cavernosa*. *Limnol Oceanogr* 45:76–86
- Levy O, Dubinsky Z, Schneider K, Achituv Y, Zakai D, Gorbunov MY (2004) Diurnal hysteresis in coral photosynthesis. *Mar Ecol Prog Ser* 268:105–117
- ▶ Levy O, Dubinsky Z, Achituv Y, Erez J (2006) Diurnal polyp expansion behavior in stony corals may enhance carbon availability for symbionts photosynthesis. *J Exp Mar Biol Ecol* 333:1–11
- ▶ Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science* 304: 1492–1494
- Loh WKW, Loi T, Carter D, Hoegh-Guldberg O (2001) Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. *Mar Ecol Prog Ser* 222:97–107
- Loh WKW, Cowlishaw M, Wilson NG (2006) Diversity of *Symbiodinium* dinoflagellate symbionts from the Indo-Pacific sea slug *Pteraeolidia ianthina* (Gastropoda: Mollusca). *Mar Ecol Prog Ser* 320:177–184
- ▶ Peltier G, Cournac L (2002) Chlororespiration. *Annu Rev Plant Biol* 53:523–550
- Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J Mar Res* 38:687–701
- Porter JW, Muscatine L, Dubinsky Z, Falkowski PG (1984) Primary production and photoadaptation in light and shade-adapted colonies of the symbiotic coral, *Stylophora pistillata*. *Proc R Soc Lond B* 222:161–180
- Quinn GP, Keough MJ (2002) *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge
- ▶ Ralph PJ, Gademann R, Larkum AWD, Kühl M (2002) Spatial heterogeneity in active chlorophyll fluorescence and PSII activity of coral tissues. *Mar Biol* 141:639–646
- ▶ Ralph PJ, Schreiber U, Gademann R, Kühl M, Larkum AWD (2005) Coral photobiology studied with a new imaging pulse amplitude modulated fluorometer. *J Phycol* 41: 335–342
- ▶ Robison JD, Warner ME (2006) Differential impacts of photo-acclimation and thermal stress on the photobiology of four different phylotypes of *Symbiodinium* (Pyrrhophyta). *J Phycol* 42:568–579
- ▶ Rowan R (2004) Thermal adaptation in reef coral symbionts. *Nature* 430:742
- ▶ Salih A, Larkum AWD, Cox G, Kühl M, Hoegh-Guldberg O (2000) Fluorescent pigments in corals are photoprotective. *Nature* 408:850–853
- Savage AM, Goodson MS, Visram S, Trapido-Rosenthal H, Wiedenmann J, Douglas AE (2002a) Molecular diversity of symbiotic algae at the latitudinal margins of their distributions: dinoflagellates of the genus *Symbiodinium* in corals and sea anemones. *Mar Ecol Prog Ser* 244:17–26
- Savage AM, Trapido-Rosenthal H, Douglas AE (2002b) On the functional significance of molecular variation in *Symbiodinium*, the symbiotic algae of Cnidaria: photosynthetic response to irradiance. *Mar Ecol Prog Ser* 244:27–37
- ▶ Srivastava A, Guissé B, Greppin H, Strasser RJ (1997) Regulation of antenna structure and electron transport in PSII of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll a fluorescence transient: OKJIP. *Biochim Biophys Acta* 1320:95–106
- ▶ Stimson J (1997) The annual cycle of density of zooxanthellae in the tissues of field and laboratory-held *Pocillopora damicornis* (Linnaeus). *J Exp Mar Biol Ecol* 214:35–48
- ▶ Stimson J, Kinzie RA III (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen enrichment and control conditions. *J Exp Mar Biol Ecol* 153:63–74
- Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochem Photobiol* 61:32–42
- ▶ Tchernov D, Gorbunov MV, de Vargas C, Yadav SN, Milligan AJ, Häggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to

- thermal bleaching in corals. Proc Natl Acad Sci USA 101: 13531–13535
- ▶ Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK (2006) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Mar Biol 148:711–722
- Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. Biol Bull 201:348–359
- ▶ Ulstrup KE, van Oppen MJH (2003) Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. Mol Ecol 12:3477–3484
- ▶ Ulstrup KE, Hill R, Ralph PJ (2005) Photosynthetic impact of hypoxia on *in hospite* zooxanthellae in the scleractinian coral *Pocillopora damicornis*. Mar Ecol Prog Ser 286: 125–132
- ▶ Ulstrup KE, Berkelmans R, Ralph PJ, van Oppen MJH (2006a) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. Mar Ecol Prog Ser 314:135–148
- ▶ Ulstrup KE, Ralph PJ, Larkum AWD, Kühl M (2006b) Intra-colonial variability in light acclimation of zooxanthellae in coral tissues of *Pocillopora damicornis*. Mar Biol 149: 1325–1335
- ▶ van Oppen MJH, Palstra FP, Piqueet AMT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. Proc R Soc Lond B Biol Sci 268:1759–1767
- van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. Coral Reefs 24:482–487
- ▶ Venn AA, Wilson MA, Trapido-Rosenthal HG, Keely BJ, Douglas A (2006) The impact of coral bleaching on the pigment profile of the symbiotic alga, *Symbiodinium*. Plant Cell Environ 29:2133–2142
- ▶ Warner ME, Berry-Lowe (2006) Differential xanthophyll cycling and photochemical activity in symbiotic dinoflagellates in multiple locations of three species of Caribbean coral. J Exp Mar Biol Ecol 339:86–95
- ▶ Warner ME, Fitt WK, Schmidt GW (1996) The effect of elevated temperature on the photosynthetic efficiency of zooxanthellae *in hospite* from four different species of reef coral: a novel approach. Plant Cell Environ 19: 291–299
- ▶ Warner ME, Chilcoat GC, McFarland FK, Fitt WK (2002) Seasonal fluctuation in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Montastraea*. Mar Biol 141:31–38

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