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RESEARCH ARTICLE

Inter-polyp genetic and physiological characterisation of *Symbiodinium* in an *Acropora valida* colony

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Abstract Corals harbouring genetically mixed communities of endosymbiotic algae (*Symbiodinium*) often show distribution patterns in accordance with differences in light climate across an individual colony. However, the physiology of these genetically characterised communities is not well understood. Single stranded conformation polymorphism (SSCP) and real time quantitative polymerase chain reaction (qPCR) analyses were used to examine the genetic diversity of the *Symbiodinium* community in hospite across an individual colony of *Acropora valida* at the spatial scale of single polyps. The physiological characteristics of the polyps were examined prior to sampling with a combined O_2 microelectrode with a fibre-optic microprobe (combined sensor diameter 50–100 μm) enabling simultaneous measurements of O_2 concentration, gross photosynthesis rate and photosystem II (PSII) quantum yield at the coral surface as a function of increasing irradiances. Both sun- and shade-adapted polyps were found to harbour either *Symbiodinium* clade C types alone or clades A and C simultaneously. Polyps were grouped in two categories according to (1) their orientation towardps light, or (2) their symbiont community composition. Physiological differences were not detected between sun- and shade-adapted polyps, but O_2 concentration at 1,100 μmol photons m^{-2} s^{-1} was higher in polyps that harboured both clades A and C symbionts than in polyps that harboured clade C only. These results suggest that the acclimatisation of zooxanthellae of individual polyps of an *A. valida* colony to ambient light levels may not be the only determinant of the photosynthetic capacity of zooxanthellae. Here, we found that photosynthetic capacity is also likely to have a strong genetic basis and differs between genetically distinct *Symbiodinium* types.

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Introduction

An obligate symbiotic relationship exists between reef-building corals (Scleractinia) and endosymbiotic dinoflagellates of the genus *Symbiodinium*, known as zooxanthellae. The photosynthetic activity of zooxanthellae results in translocation of photosynthate, which represents a major energy source for the host, and enables corals to maintain high rates of $CaCO_3$ deposition contributing to the three-dimensional structure of coral reefs (Muscatine 1990). However, photo-physiological differences among members of *Symbiodinium* are not well characterised.

Symbiodinium is a diverse genus where six (A–D, F and G) out of eight known phylogenetic lineages or clades have been found in scleractinian corals (LaJeunesse 2001; Coffroth and Santos 2005; van Oppen et al. 2005). In most corals, these symbiotic communities are found to be genetically homogeneous (Goulet 2006), although additional strains, sometimes from different clades, and present at low abundances are likely to have been overlooked in most

studies (Ulstrup and van Oppen 2003; Mieog et al. 2007). Mixed communities comprising more than one *Symbiodinium* clade are known to occur within single colonies of some coral species, especially in the coral genus *Acropora* on the Great Barrier Reef (van Oppen et al. 2001; Ulstrup and van Oppen 2003; Berkelmans and van Oppen 2006) and in the *Montastraea annularis* complex in the Caribbean (Rowan et al. 1997; Toller et al. 2001; Thornhill et al. 2006). The ability of some corals to host a variety of *Symbiodinium* types simultaneously has been suggested to provide a means by which corals can withstand somewhat higher sea water temperatures by a process called symbiont shuffling (Baker 2003). Shuffling refers to a change in the relative abundance of genetically, and therefore potentially physiologically, different *Symbiodinium* types (Baker 2003) and has been documented for several coral species (Toller et al. 2001; Little et al. 2004; Thornhill et al. 2006).

Different physiological characteristics have been ascribed to different *Symbiodinium* clades in hospite as certain symbiont types exhibit distinctive distribution patterns that correlate with temperature and light microclimate (Rowan et al. 1997; Toller et al. 2001; Fabricius et al. 2004). Physiologically, zooxanthellae in hospite have been shown to perform differently between sun- and shade-adapted colonies (Falkowski and 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), between polyp and coenosarc tissue (Kühl et al. 1995; Ralph et al. 2002; Ulstrup et al. 2006b), between diseased and healthy tissue of a branch (Ulstrup et al. 2007), and along the length of coral branches (Gladfelter et al. 1989; Hill et al. 2004). Although accounting for physiological differences at a range of spatial scales, these studies were never accompanied by genetic characterisation of the zooxanthellae. Iglesias-Prieto et al. (2004) were the first to combine physiological in situ measurements using a pulse–amplitude–modulation (PAM) submersible fluorometer with genetic characterisation of zooxanthellae. They showed that the vertical distribution of genetically different zooxanthellae could be explained by their light utilisation capabilities. However, using a similar instrument, Warner et al. (2006) found no physiological differences among different symbionts occurring in the same species of coral at a particular depth.

Studies on cultured (Chang et al. 1983; Iglesias-Prieto and Trench 1994) as well as freshly isolated zooxanthellae (Savage et al. 2002; Bhagooli and Hidaka 2003; Robison and Warner 2006) also indicated that photosynthetic responses may differ between *Symbiodinium* types. In some instances, however, more variation was documented within, than between, individual *Symbiodinium* clades (Savage et al. 2002; Robison and Warner 2006). Moreover, Bhagooli and Hidaka (2003) showed little congruence in physiological responses between freshly isolated and in hospite counterparts, supporting the notion that the host is an important determinant of the symbiont physiology. For example, it has been demonstrated that *Symbiodinium* incubated in homogenised host tissue releases more photosynthate (Grant et al. 1997; Gates et al. 1999) and exhibits higher photosynthetic carbon fixation and oxygen production as compared to in vitro incubations in sea water (Gates et al. 1995, 1999).

Physico-chemical microenvironmental conditions strongly influence the photosynthetic condition of the symbionts (Kühl et al. 1995; Ulstrup et al. 2006b). Microchemical conditions as well as light attenuation and amplification are highly variable at a very small scale <50–100 µm (Kühl et al. 1995; De Beer et al. 2000; Ulstrup et al. 2006b). The irradiance that corals have experienced prior to measurement may also influence the photosynthetic performance of zooxanthellae (Falkowski and Dubinsky 1981; Porter et al. 1984; Jones et al. 1998) and the duration of light exposure may affect chlorophyll *a* fluorescence-derived measurements (Kühl et al. 2001; Ralph et al. 2005; Ulstrup et al. 2007).

The physiological plasticity and genetic diversity of zooxanthellae harboured by corals have previously been correlated with depth (Rowan et al. 1997), geographic location (Ulstrup and van Oppen 2003; Ulstrup et al. 2006a) as well as temperature (Glynn et al. 2001; Fabricius et al. 2004; Ulstrup et al. 2006a). However, the physiological attributes of zooxanthella communities in hospite have never before been correlated with their intra-colony genetic diversity. In this study, the mixed community of *Symbiodinium* and their physiological characteristics in hospite were examined across an individual colony of *Acropora valida* at the spatial scale of single polyps (<2 mm in diameter). By determining the diversity as well as the relative abundance of individual strains of *Symbiodinium* in *A. valida* in combination with estimates of photosynthesis capacity at the scale of single polyps, we were able to show a level of physiological specialisation of genetically distinct *Symbiodinium* communities in hospite.

Materials and methods

One *A. valida* colony of ~20 cm in diameter with branch lengths of up to ~10 cm was collected on the inner reef flat of Heron Island at less than 2 m depth adjacent to Heron Island Research Station (23°26′S, 152°06′E) in July 2004. The colony was maintained in natural seawater at an ambient temperature of 20 ± 0.5°C in a flow-through aquarium under shaded conditions at <100 µmol photons m^{-2} s^{-1} for 3 days prior to measurements. Before each measuring sequence (n = 17), a ~5 cm branch either in sun- or shade-adapted orientation was detached from the colony. The

branch was held securely in plasticine within a custom-built flow chamber (25 × 10 × 10 cm) at a constant mean flow velocity of ~1 cm s^{-1}. A combined O_2 microelectrode and fibre-optic microsensor (Kühl 2005) was placed on sun-adapted surfaces 4–10 polyps away from the tip and on shade-adapted surfaces 2–8 polyps from the tip. After microsensor measurements the measured polyp of ~2 mm in diameter was dislodged from the branch using a scalpel, carefully isolating only the tissue on that corallite and not adjacent polyps. The polyp was transferred to an Eppendorf tube containing 1 ml of 95% ethanol. This allowed preservation of DNA for genetic analysis. The branch and polyp dislodgement scar was then photographed.

DNA extraction and assessment of zooxanthella diversity

DNA extractions from 17 individual polyps were carried out using the DNeasy™ tissue kit (Qiagen) following the manufacturer's protocol for animal tissues, using 200 µl elution buffer and two elution steps. To qualitatively assess the zooxanthella diversity and relative dominance of *A. valida*, 1 µl of the second DNA elution was used in a 25 µl PCR reaction with zooxanthella-specific primers to amplify the rDNA internal transcribed spacer 1 (ITS1) region, followed by single stranded conformation polymorphism (SSCP) analysis as described in Ulstrup and van Oppen (2003). The relative dominance of genotypes detected was determined by the strongest band for each sample. No assignment of dominance was given where multiple bands were discriminated by less than the equivalent of a ten times dilution (sensu Fabricius et al. 2004). SSCP is unable to detect relative abundances of less than 5–10% (Fabricius et al. 2004). In samples where only one zooxanthella genotype was detected, an ITS1 quantitative PCR (qPCR) assay, modified from Ulstrup and van Oppen (2003), was used to validate the results of the SSCP assay. The assay employed the primers described in Ulstrup and van Oppen (2003) for clade C, as well as a new clade A-specific primer (5′-CAG GTTCACGACAAGTTTTGGATA-3′). Quantitative PCR reactions were performed on the Rotor-Gene RG-3000A (Corbett Research), using Invitrogen's Platinum SYBR Green 2× PCR Master Mix. Twenty microlitre reactions were used consisting of 2 µl universal forward primer (1.8 µM), 2 µl A- or C-specific reverse primer (1.8 µM), 10 µl Platinum SYBR Green 2× PCR Master Mix (Invitrogen), 4 µl MilliQ water and 2 µl DNA template. Cycle conditions were as follows: 2 min at 50°C, 2 min at 95°C followed by 15 s at 95°C and 30 s at 60°C for 40 cycles.

Experimental setup

A custom-made combined O_2 microelectrode and tapered fibre-optic microsensor (combined sensor diameter 50–100 µm) (Kühl 2005) was used to conduct simultaneous measurements of O_2 concentration, gross photosynthesis rate and quantum yield of PSII at the surface of individual coral polyps (Ulstrup et al. 2006b) as a function of time and irradiance.

The O_2 microelectrode was connected to a picoamperemeter (PA2000, Unisense A/S, Denmark) and measuring signals were recorded on a strip chart recorder (Kipp and Zonen, Holland). A linear calibration of the microelectrode was performed at ambient temperature (20 ± 0.5°C) by recording signals in air-saturated and O_2-free seawater, respectively. The latter was obtained by adding sodium dithionite to a small volume of seawater. The O_2 concentration of air-saturated seawater at experimental temperature and salinity (230.9 µmol l^{-1}) was obtained from tabulated values (http://www.unisense.com).

The fibre-optic microprobe was connected to a PAM fluorometer (Microfibre-PAM, Walz, Germany; Schreiber et al. 1996). The fluorometer also controlled a red light emitting diode (LED) ring, which was used as an actinic (650 nm) light source. The fluorometer was interfaced with a windows-based PC and the system software (WinControl v2.08, Walz, Germany) for the measurement of chlorophyll *a* fluorescence in real time at defined irradiance and time intervals. The instrument settings of the Microfibre-PAM were adjusted so that a fluorescence signal above 100 units was obtained on dark-acclimated sample (photomultiplier gain 30, output gain 8 and measuring light 8).

The combined microsensor was positioned through the centre of the LED ring, which illuminated the coral sample with actinic light from oblique angles, thus minimising any self shadowing effects on the measurements (Ulstrup et al. 2006b). The microsensor was placed in direct contact with the coral tissue perpendicular to the direction of branch growth using a manual micromanipulator (MM33, Märtzhäuser, Germany) while observing the sample with a dissecting microscope (Leica, Germany).

Measuring sequence

A batch program (Wincontrol, Walz GmbH, Germany) was written to automate the incremental change in actinic irradiance (0, 25, 50, 100, 170, 240, 470, 1100 µmol photons m^{-2} s^{-1}) from the LED ring as well as the initiation of a rapid light curve (RLC, see below). The actinic light levels were calibrated against a quantum irradiance meter with a quantum sensor (Li-190SA, LiCor, USA). Ten minutes of irradiation at each actinic light level was found to be sufficient time to reach steady-state oxygen conditions at the tissue surface, as monitored using the strip chart recorder and the WinControl chart function (data not shown). At the end of each light period the O_2 concentration at the polyp tissue surface was recorded first, followed by a RLC. Rapid light

curves were obtained from a series of saturating flashes >2,000 µmol photons m^{-2} s^{-1} in rapid succession (10 s) over the following irradiances: 0, 50, 80, 100, 170, 240, 340, 470, 780 µmol photons m^{-2} s^{-1}. Such RLC's provide insight into the momentary acclimation capacity for light utilisation of PSII (Schreiber 2004; Ralph and Gademann 2005). The initial saturating flash of each RLC also provided measures of steady-state quantum yield of PSII enabling comparative measures of relative electron transport rate (rETR) derived from RLCs and steady state light curves (SSLCs) where rETR = PAR × quantum yield of PSII.

After oxygen levels again reached steady-state, a sudden and short (2–3 s) light–dark shift was applied to measure the gross photosynthesis rate (P_g). Briefly, the light–dark shift technique is based on the assumption that the immediate O_2 depletion following the light–dark shift is equal to the gross photosynthetic O_2 production during the previous light period [see more details in Revsbech and Jørgensen (1983)]. Using this technique and taking advantage of the small sensor size along with the low stirring sensitivity and fast response time of the O_2 microelectrode, enabled us to measure of gross photosynthesis rate in nmol cm^{-3} s^{-1} at ~100 µm spatial resolution, i.e. at a scale smaller than a single polyp.

Curve fitting

The numerical functions described by Platt et al. (1980) were fitted to rETR versus irradiance (both RLC- and SSLC-derived) and P_g versus irradiance data derived from steady-state measurements. From fitted curves the descriptive parameters of chlorophyll *a* fluorescence: maximum rETR ($rETR_{max}$), initial slope (α_f), and minimum saturating irradiance (E_{kf}) and O_2: maximum P_g (P_{gmax}), initial slope (α_{Pg}) and minimum saturating irradiance (E_{kPg}) were collected. Fitted curves were derived from the empirical data (Sigmaplot v6.1 [Systat, USA]). For further details see Kühl et al. (2001) and Ralph et al. (2002).

Statistical analysis

Polyp samples were grouped together according to (1) their orientation towards light, and (2) the presence or absence of clade A. There were ten sun-adapted polyps, seven shade-adapted polyps, 12 polyps that harboured clades A + C and, finally, five polyps that only harboured clade C. The unequal sample size meant that the physiological data were analysed using the non-parametric Mann–Whitney *U* test to detect differences between sun- and shade-adapted polyps and polyps with clades A + C versus clade C, respectively. Significant differences were accepted at $P < 0.05$. Differences in quantitative parameters ($rETR_{max}$, α_f and E_{kf}) of chlorophyll *a* fluorescence-derived curves (both RLCs and SSLCs) for each group were analysed using repeated measures ANOVA. Box's test of equality of covariance matrices was used to test the homogeneity of variances across groups, and data were log transformed where necessary. All data complied with the assumption of sphericity. Post-hoc comparisons of means for significant factors were carried out using Tukey's HSD tests. All analyses were performed with STATISTICA v 7.1 (SysSoft Inc., USA).

Results

Genetic composition and distribution of *Symbiodinium*

The presence and relative dominance of zooxanthella types found in single polyps across an individual colony of *A. valida* is shown in Table 1. Seven out of ten sun-adapted and five out of seven shade-adapted polyps harboured clades A [GenBank Accession # AF380513, as determined in Ulstrup et al. (2006a)] and C simultaneously. However, a larger fraction of the sun-adapted (50%) than of the shade-adapted polyps (29%) were dominated by clade A. The relative dominance of clade C (either C1 or C2 sensu van Oppen et al. 2001) was higher in shade-adapted (71%) than in sun-adapted polyps (50%) with C1 being dominant in one polyp and C2 in 4 polyps in both sun- and shade-adapted polyps, respectively (Table 1). The qPCR assay showed no amplification of clade A in the five samples that had been observed only to have clade C using SSCP.

Photo-physiology of individual polyps with contrasting branch orientation and symbiont composition: steady-state light curves

The O_2 concentration reached at 1,100 µmol photons m^{-2} s^{-1} showed no significant difference (Mann–Whitney *U*) between sun- and shade-adapted polyps (Fig. 1a). In contrast, polyps that harboured a combination of *Symbiodinium* clades A and C showed significantly higher O_2 concentration ($P = 0.002$, Mann–Whitney *U*) at 1,100 µmol

Table 1 Observed SSCP genotype frequencies (*n*) of clade A, C1, and C2 (f_A, f_{C1} and f_{C2}, respectively) are shown as well as their relative proportion (%) to total samples of either sun- or shade-adapted polyps

	Sun-adapted ($n = 10$)			Shade-adapted ($n = 7$)		
	f_A	f_{C1}	f_{C2}	f_A	f_{C1}	f_{C2}
N	7(**5**)	5(**1**)	9(**4**)	5(**2**)	5(**1**)	7(**4**)
%	70(**50**)	50(**10**)	90(**40**)	71(**29**)	71(**14**)	100(**57**)

Bolded values denote the number (or proportion) of polyps in which a given type was dominant

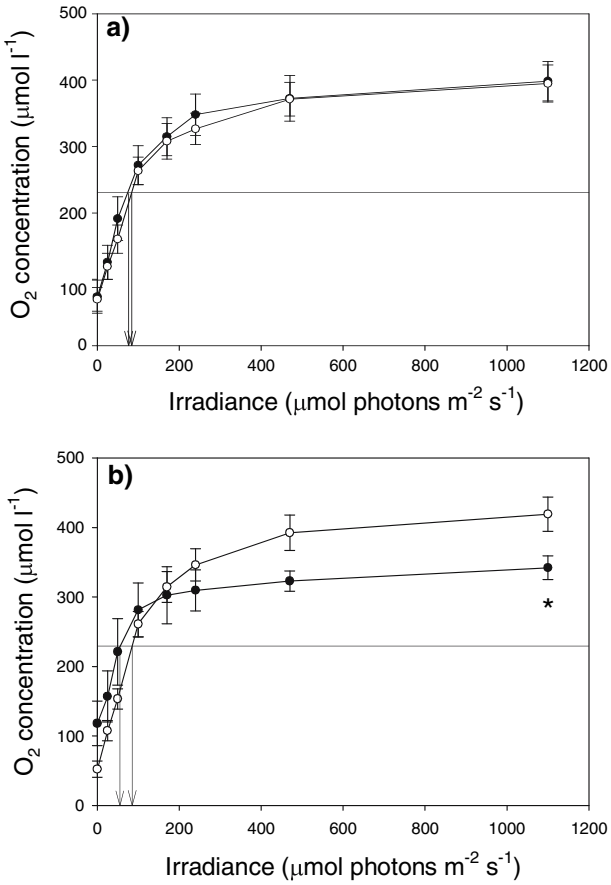

Fig. 1 a–b Steady-state light curves of O_2 concentration (µmol l^{-1}) ± SE at the surface of **a** individual sun- (*open circle*) and shade-adapted (*filled circle*) polyps, **b** clade A + C (*open circle*) and clade C polyps (*filled circle*) of *A. valida.* The *horizontal line* indicates the level of the air-saturation at the surface of the coral (230.9 µmol l^{-1}) and the *vertical arrows* indicate the compensation irradiance, E_c, estimate. *Asterisks* indicates significant difference ($P = 0.002$)

Table 2 Quantitative parameters derived from fitted steady-state relative electron transport rates (rETR) and O_2 curves of individual polyps of *A. valida*

(a)	Sun-adapted ($n = 10$)	Shade-adapted ($n = 7$)	*P* value
$rETR_{max}$	85 ± 13^{a}	$75 \pm 12^{a,b,c}$	0.380
α_f	0.99 ± 0.09^{a}	1.00 ± 0.12^{a}	0.961
E_{kf}	$92 \pm 18^{a,b}$	81 ± 13	0.922
P_{gmax}	30 ± 7	31 ± 4	0.495
α_{Pg}	0.22 ± 0.06	0.33 ± 0.08	0.172
E_{kPg}	160 ± 32	122 ± 26	0.205
(b)	A + C ($n = 12$)	C ($n = 5$)	*P* value
$rETR_{max}$	86 ± 12^{a}	$69 \pm 9^{a,b}$	0.527
α_f	0.98 ± 0.07^{b}	1.03 ± 0.18^{a}	0.598
E_{kf}	$91 \pm 15^{a,b}$	78 ± 18	0.527
Pg_{max}	32 ± 5	25 ± 7	0.598
α_{Pg}	0.21 ± 0.04	0.41 ± 0.13	0.246
E_{kPg}	172 ± 25	81 ± 21	0.073

Chlorophyll *a* fluorescence, $rETR_{max}$ (a.u), α_f, E_{kf} (µmol photons m^{-2} s^{-1}); O_2, Pg_{max} (nmol cm^{-3} s^{-1}), α_{Pg}, E_{kPg} (µmol photons m^{-2} s^{-1}). (a) sun- and shade-adapted comparison, (b) clade A + C and clade C comparison. Sample size (*n*) averages and standard errors are given. Tukey's HSD comparison of chlorophyll *a* fluorescence parameters with those of RLC are given as superscript letters

photons m^{-2} s^{-1} than polyps that only contained clade C (Fig. 1b). Differences in compensation irradiance, E_c, were not statistically tested. However, the difference in mean E_c was greater between polyps with clade C symbionts and those that harboured clade A and C symbionts simultaneously than between sun- and shade-adapted polyps (Fig. 1b).

The fluorescence-based steady-state rETR versus irradiance curve showed no difference in maximum rETR, α_f, or E_{kf} (Mann–Whitney *U*) between sun- and shade-adapted polyps (Table 2a). Comparisons of quantitative parameters derived from O_2-based gross photosynthesis rate (P_g) versus irradiance curves (P_{gmax}, α_{pg}, E_{kPg}) also did not yield any differences between these groups (Mann–Whitney *U*) (Table 2a). The quantitative parameters of the rETR curve (ETR_{max}, α_f, E_{kf}) were not significantly different between polyps harbouring clades A + C versus those that only harboured clade C (Mann–Whitney *U*) (Table 2b). However, there was a trend ($P = 0.079$, Mann–Whitney *U*) towards a higher E_{kPg} in gross photosynthesis rate versus irradiance curves between polyps harbouring clades A + C versus clade C (Table 2b).

In all groups, P_g continued unchanged or rose even further at intermediate irradiance >200 µmol photons m^{-2} s^{-1}, whereas rETR became inhibited at irradiance >200 µmol photons m^{-2} s^{-1} for all groups except for polyps harbouring only clade C where rETR started to become inhibited at irradiance <200 µmol photons m^{-2} s^{-1} (Fig. 2a–d). Irrespective of group, there was no clear correlation between P_g and rETR at moderate to high irradiances (Fig. 2).

Rapid light curves

Relative ETR_{max}, α_f and E_{kf} calculated for each RLC fitted to the hyperbolic tangent function of Platt et al. (1980) yielded no significant difference between sun- and shade-adapted polyps (Table 3a) or between polyps that harboured clades A + C versus those that only harboured clade C (Mann–Whitney *U*) (Table 3b).

The $rETR_{max}$ of the SSLCs was lower than of the RLCs conducted at 50, 100, 170 and 240 µmol photons m^{-2} s^{-1} ($F = 4.344$, $P < 0.001$, rmANOVA) irrespective of previous light exposure of sun-adapted polyps (Tables 2a, 3a). The initial slope, α_f, declined in RLCs obtained at 1,100 µmol

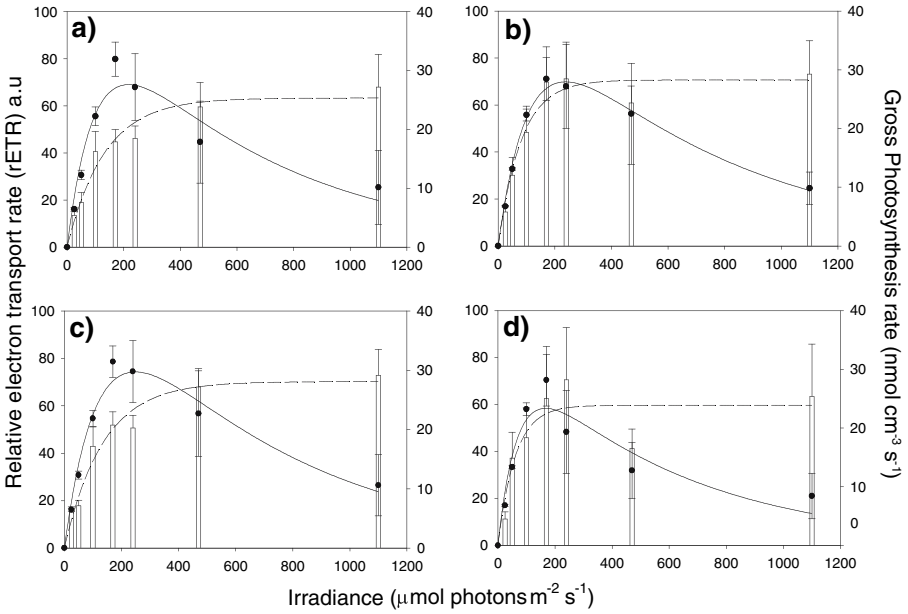

Fig. 2 **a**–**d** Steady-state relative electron transport rate (rETR) (*filled circle*) and gross photosynthesis rate (*white bars*) as a function of increasing irradiance (µmol photons m^{-2} s^{-1}) of individual polyps of *A. valida* for **a** sun-adapted polyps, **b** shade-adapted polyps, **c** clade A + C polyps, and **d** clade C polyps. The fitted rETR curves are superimposed with a *solid line*. The fitted gross photosynthesis rate curves are superimposed with a *broken line*

photons m^{-2} s^{-1} ($F = 11.058$, $P < 0.001$, rmANOVA). Minimum saturating irradiance (E_{kf}) was significantly lower for RLCs obtained at 25 µmol photons m^{-2} s^{-1} than at 470 and 1,100 µmol photons m^{-2} s^{-1} ($F = 3.294$, $P = 0.029$, rmANOVA) (Table 3a). Relative ETR_{max} for shade-adapted polyps was lower at 1,100 µmol photons m^{-2} s^{-1} than at any other irradiance ($F = 11.998$, $P < 0.001$, rmANOVA) (Table 3a). The initial slope, α_f, obtained 1,100 µmol photons m^{-2} s^{-1} was also significantly reduced compared to α_f obtained at all other irradiances ($F = 7.515$, $P < 0.001$, rmANOVA) including the SSLC (Tables 2a, 3a).

Relative ETR_{max} for polyps that harboured a mix of clade A + C was reduced in rapid light curves conducted at 1,100 µmol photons m^{-2} s^{-1} ($F = 7.941$, $P < 0.001$, rmANOVA, Table 3b). The initial slope, α_f, became reduced in RLCs carried out 470 µmol photons m^{-2} s^{-1} ($F = 13.103$, $P < 0.001$, rmANOVA) (Table 3b), whereas E_{kf} increased only in the RLC obtained at 470 µmol photons m^{-2} s^{-1} ($F = 2.797$, $P < 0.009$, rmANOVA) (Table 3b). In polyps that harboured only clade C, RLCs obtained at 470 and 1,100 µmol photons m^{-2} s^{-1} were significantly reduced ($F = 5.600$, $P < 0.001$, rmANOVA), whereas α_f became reduced ($F = 4.350$, $P < 0.001$, rmANOVA) in RLCs obtained at irradiances >240 µmol photons m^{-2} s^{-1} (Tables 2b, 3b).

Discussion

Relative occurrence of clade A

Zooxanthellae belonging to clade C are the most commonly observed types world-wide (LaJeunesse 2001; LaJeunesse et al. 2003), whereas members of clade A are generally rare in the Indo-Pacific and an association between *Symbiodinium* clade A and Indo-Pacific *A. valida* has not been reported previously. However, Visram and Douglas (2006) observed two *A. valida* colonies from the West Indian Ocean that harboured a mixed symbiont community of clades A and C and two colonies in which only clade A was detected.

Colonies of *A. valida* harbouring a mixed zooxanthella community have been found in other parts of the Great Barrier Reef (clades C and D, Ulstrup and van Oppen 2003) suggesting that the symbiotic relationship of *A. valida* is flexible and may therefore be shuffled (sensu Baker 2003). *Symbiodinium* clade D was found to co-occur with clade C in *A. valida* on inshore reefs generally known for higher light attenuation and temperature than offshore reefs (Berkelmans 2002) such as Heron Island, which is located 80 km from the coast at the cooler southern end of the Great Barrier Reef. The difference in *Symbiodinium* clades harboured by *A. valida* at different locations may therefore be related to different light and temperature optima.

There was no clear pattern in the presence of clade A versus C within individual polyps across the colony in relation to their orientation towards light. Thus, both clades were detected in sun- as well as in shade-adapted polyps. However, clade A was relatively more dominant in sun- (50%) than in shade-adapted polyps (29%) indicating opportunistic proliferation in sun-exposed tips, possibly due to competition with less adapted clade C symbionts. To support the notion of high light-preference of *Symbiodinium* clade A, several coral species in the Caribbean have been shown to contain clade A in shallow but not in deep conspecifics (Rowan et al. 1997; Baker 2001; Toller et al.

Table 3 Quantitative parameters [$rETR_{max}$ (a.u), α, E_k (μmol photons m^{-2} s^{-1})] of RLCs at different irradiances derived from fitted relative electron transport rates (rETR)

(a)	$rETR_{max}$			α			E_k		
Light intensity	Sun-adapted	Shade-adapted	*P* value (MW)	Sun-adapted	Shade-adapted	*P* value (MW)	Sun-adapted	Shade-adapted	*P* value (MW)
0	$74 \pm 12^{a,b}$	51 ± 7^{c}	0.203	$0.77 \pm 0.07^{a,b}$	0.70 ± 0.08^{a}	0.418	$101 \pm 20^{a,b}$	75 ± 7	0.355
25	$63 \pm 10^{a,b}$	$64 \pm 10^{a,b,c}$	0.770	0.89 ± 0.08^{a}	0.85 ± 0.09^{a}	0.495	76 ± 10^{b}	81 ± 12	0.845
50	75 ± 9^{a}	$76 \pm 9^{a,b,c}$	0.922	0.93 ± 0.09^{a}	0.88 ± 0.07^{a}	0.626	$83 \pm 10^{a,b}$	86 ± 7	1
100	83 ± 10^{a}	84 ± 12^{b}	0.845	0.86 ± 0.08^{a}	0.85 ± 0.03^{a}	0.435	$97 \pm 9^{a,b}$	98 ± 12	0.770
170	81 ± 11^{a}	$81 \pm 12^{a,b}$	0.626	0.82 ± 0.07^{a}	0.85 ± 0.06^{a}	0.922	$95 \pm 10^{a,b}$	95 ± 11	0.922
240	82 ± 15^{a}	$79 \pm 15^{a,b}$	0.922	$0.67 \pm 0.07^{a,b}$	0.79 ± 0.11^{a}	0.380	$124 \pm 20^{a,b}$	111 ± 24	0.696
470	$70 \pm 15^{a,b}$	$56 \pm 10^{a,c}$	0.874	$0.47 \pm 0.07^{b,c}$	0.67 ± 0.11^{a}	0.125	182 ± 48^{a}	88 ± 13	0.223
1100	34 ± 7^{b}	24 ± 8^{d}	0.367	0.32 ± 0.07^{c}	0.23 ± 0.06^{b}	0.491	146 ± 30^{a}	88 ± 25	0.222
P value (rmANOVA)	***P* < 0.001**	***P* < 0.001**		***P* < 0.001**	***P* < 0.001**		***P* = 0.029**	*P* = 0.453	

(b)	$rETR_{max}$			α			E_k		
Light intensity	**A + C**	C	*P* value (MW)	A + C	C	*P* value (MW)	A + C	C	*P* value (MW)
0	64 ± 9^{a}	$61 \pm 13^{a,b,c}$	0.624	$0.69 \pm 0.05^{a,c}$	0.83 ± 0.13^{a}	0.066	$94 \pm 16^{a,b}$	76 ± 12	0.713
25	60 ± 8^{a}	$73 \pm 12^{a,b}$	0.399	$0.88 \pm 0.08^{a,b}$	0.85 ± 0.12^{a}	0.833	75 ± 10^{a}	87 ± 10	0.292
50	74 ± 8^{a}	80 ± 14^{a}	0.673	$0.89 \pm 0.07^{a,b}$	0.96 ± 0.09^{a}	0.343	$84 \pm 9^{a,b}$	84 ± 8	0.752
100	84 ± 9^{a}	82 ± 0.18^{a}	1	$0.81 \pm 0.06^{a,b}$	0.96 ± 0.06^{a}	0.092	$103 \pm 8^{a,b}$	84 ± 12	0.114
170	81 ± 10^{a}	80 ± 12^{a}	1	$0.80 \pm 0.06^{a,b}$	0.92 ± 0.05^{a}	0.140	$98 \pm 9^{a,b}$	87 ± 12	0.461
240	85 ± 14^{a}	$69 \pm 12^{a,b}$	0.598	$0.70 \pm 0.07^{a,b,c}$	$0.76 \pm 0.13^{a,b}$	0.916	$122 \pm 16^{a,b}$	111 ± 34	0.598
470	73 ± 13^{a}	$43 \pm 4^{b,c}$	0.126	$0.51 \pm 0.07^{c,d}$	$0.65 \pm 0.15^{a,b}$	0.462	171 ± 39^{b}	74 ± 10	0.079
1100	27 ± 7^{b}	37 ± 3^{c}	0.609	0.29 ± 0.07^{d}	0.25 ± 0.03^{b}	0.955	$101 \pm 24^{a,b}$	164 ± 36	0.233
P value (rmANOVA)	***P* < 0.001**	***P* < 0.001**		***P* < 0.001**	***P* < 0.001**		***P* < 0.009**	*P* = 0.168	

Averages and standard errors are given as well as *P* value for Mann–Whitney *U* test (MW). (a) sun- and shade-adapted comparison, (b) clade A + C and clade C comparison. *P* value (repeated measures ANOVA) and Tukey's HSD comparisons of RLCs at different irradiances are given as superscript letters and are comparable to those given for SSLCs in Table 2. Significant *P* values are bolded

2001). Likewise, possible microscale changes in irradiance across a colony during growth may drive a continuous shuffling of mixed *Symbiodinium* communities to partition clades into specific light microhabitats. In this case, as polyps bud at the apex of a young colony, older polyps are likely to become more shaded which may coincide with a shift in the *Symbiodinium* type predominance in the symbiont community.

Photo-physiology of individual polyps with contrasting orientation and symbiont composition

The significant differentiation observed in O_2 concentration at 1,100 μmol photons m^{-2} s^{-1} and dark O_2 concentration between polyps which harboured clade C only and clades A + C simultaneously suggest higher metabolic activity of respiration and photosynthesis in polyps harbouring both clades A + C. The latter is also confirmed by the steady-state rETR curves which saturated at lower irradiances <200 μmol photons m^{-2} s^{-1} for polyps containing only clade C suggesting adaptation to relatively lower irradiances (Table 2b).

The occurrence of clade A in polyps may therefore influence the light-tolerance of corals at a small spatial scale. This corresponds with the fact that clade A was found to be dominant in half of the sun-adapted polyps whereas only 29% of shade-adapted polyps were dominated by clade A (Table 1). The effective PAR levels for downward-facing, shaded polyps in situ are likely to be greater, e.g. due to the high reflectance of coral sands in shallow waters, than estimated E_c, E_{kf} and E_{kPg} values which did not exceed 200 μmol photons m^{-2} s^{-1}. Shaded polyps may thus have been adapted to relatively high irradiances which may have resulted in the presence of clade A.

The statistical analyses did not detect any differences between polyps that harboured clades A + C versus those

that only harboured clade C for dark-acclimated O_2 concentration, rETR and P_g curves versus irradiance. This was most likely due to a lack of statistical power and it is probable that a greater sampling intensity could have resolved the trends observed.

Chlorophyll *a* fluorescence versus gross photosynthesis rate: technical considerations

While every method has its limitations, the use of microsensors clearly permit minimally invasive mapping of oxygen and photosynthesis activity at the intra-polyp scale with a minimal bias between measurements. Oxygen microsensors exhibit negligible O_2 consumption and a fast response time enabling the accurate measurements of microenvironments and spatio-temporal oxygen dynamics. Fiber-optic microscale measurements of PSII activity enable intra-polyp measurements and allow separate mapping of activity, e.g. in coenosarc and polyp tissue (Ralph et al. 2002), which is impossible with larger sensors.

In contrast to the high gross photosynthesis rates observed at high irradiances >200 µmol photons m^{-2} s^{-1}, rETR showed a substantial decline over the course of the steady-state light curve, suggesting dynamic photo-inhibition (Gorbunov et al. 2001) (Fig. 2a–d). Disparate curve shapes for rETR and P_g have been shown previously for corals (Ulstrup et al. 2006b) who suggested that cyclic PSII electron flow was partially responsible for the observed non-linearity (Franklin and Badger 2001; Lavaud et al. 2002; Longstaff et al. 2002).

Measuring properties of the two sensors used here may also contribute to the different responses observed. The microfibre sensor measures chlorophyll *a* fluorescence immediately adjacent to the fibre tip (Schreiber et al. 1996), whereas the gross photosynthesis measurement using the O_2 microsensor has a spatial resolution of ~100 µm (Revsbech and Jørgensen 1983). This may result in underestimating the gross photosynthesis rate of zooxanthellae in deeper tissue, which are likely to be more shaded than the zooxanthellae in the top layers. In addition, measurement performed with a fibre-optic probe may be affected by the optical properties of the tissue, i.e. the balance between absorption and multiple scattering, which is likely to be different between sun and shade-adapted polyps (Kühl et al. 1995; Enriquez et al. 2005) resulting in a potential difference in spatial resolution.

Influence of light history on capacity for photo-acclimation

At high irradiances, the quantitative parameters of SSLCs were different from RLCs. At low to moderate irradiances, RLCs performed similar to SSLCs. This is in contrast to Ulstrup et al. (2007) who found that dark-acclimated RLCs were significantly reduced compared to SSLCs suggesting that if zooxanthellae are allowed to acclimatise for an extended period of time to each irradiance level, their photosynthetic performance yields higher rETR values. The contrasting results indicate that zooxanthellae may have widely different abilities and mechanisms of photo-acclimation at various time scales, which may have implications for the decline of health of corals during bleaching conditions when the light levels experienced by zooxanthellae are exacerbated due to paling of coral tissue (Enriquez et al. 2005).

In summary, this study elucidates some of the causes of heterogeneous photo-acclimatisation at the scale of single polyps within an *A. valida* colony. We conclude that the light climate (branch orientation) experienced by the symbiont communities in individual polyps may not be the only factor responsible for small scale variations in photosynthetic capacities, but that light preference, and therefore photosynthetic capacity, of *Symbiodinium* differs between the members of clades A and C studied here. It remains to be experimentally determined whether specific *Symbiodinium* types in mixed communities perform differently in response to synergistic effects of temperature and light. Such studies would provide new insight into the acclimatisation and adaptation to climate change potentially facilitated through shuffling of mixed symbiont communities.

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