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Photosynthetic impact of hypoxia on *in hospite* zooxanthellae in the scleractinian coral *Pocillopora damicornis*

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ABSTRACT: Shallow water coral reefs may experience hypoxia under conditions of calm weather doldrums. Anaerobic responses of endosymbionts (i.e. zooxanthellae) within *Pocillopora damicornis* coral colonies were tested using both slow and fast chlorophyll *a* fluorescence induction kinetics. Zooxanthellae were examined *in hospite* when exposed to control conditions (26°C, 200 µmol photons m^{-2} s^{-1}, 100% air-saturation, 4 cm s^{-1} flow) and to 2 treatments of reduced air content (40 and 0%), achieved by controlling the N_2:O_2 ratio in water circulating at 2 cm s^{-1}. Furthermore, the impact of water flow on photosynthesis was examined at 0% air saturation by turning off the flow entirely (0 cm s^{-1}), thereby mimicking the environmental conditions of calm weather doldrums. Corals exposed to depleted air content (0% with and without flow) showed a significant decrease ($p < 0.001$) in effective quantum yield (ϕ_{PSII}) in comparison with controls. Maximum quantum yield was significantly reduced when gas exchange was inhibited (0% without flow), whereas non-photochemical quenching (NPQ) was not affected. Fast polyphasic fluorescence transients of chlorophyll *a* fluorescence showed a significant increase in minimum dark-adapted fluorescence, F_0, when corals were exposed to anaerobic conditions. Furthermore, an increase in the J peak (2 ms) corresponding to the reduction of the primary electron acceptor, Q_A, was observed in 0% air-saturation with flow. We found that the most sensitive parameters for detecting physiological change associated with hypoxia were ϕ_{PSII} using slow (pulse-amplitude modulation) fluorescence kinetics, as well as an increase in the O peak, φ_{Po} (electron transport efficiency before Q_A), and an elevation of the J peak on a double-normalised transient using fast (Plant Efficiency Analyser) induction kinetics.

KEY WORDS: Fluorescence · Fast-induction kinetics · Pulse-amplitude modulation · Photosystem II · PSII

INTRODUCTION

The effect of hypoxia (defined as the absence of air or free oxygen) on Photosystem II (PSII) in photosynthesising aquatic organisms is largely unexplored (Schreiber & Vidaver 1974, Kühl et al. 1995, Schreiber et al. 2002). This is paradoxical, especially in corals, considering the frequent and sometimes prolonged periods of calm weather often encountered in shallow water environments such as reef flats and lagoons (e.g. Lesser et al. 1994, Nakamura & van Woesik 2001, Nakamura et al. 2003). *In situ* flow conditions measured in the reef environment range between 1 and 39 cm s^{-1} (Dennison & Barnes 1988, Patterson et al. 1991). An increase in the thickness of the diffusive boundary layer (DBL) is linked to a reduction in water flow across an organism's surface (e.g. Patterson et al. 1991, Kühl et al. 1995) leading to reduced gas-exchange, which can induce anaerobiosis (Schreiber & Vidaver 1974, Kühl et al. 1995). Anaerobiosis is the physiological condition induced by hypoxic water. Coral–algae symbioses are complex systems where both components are inter-related and are mutually affected by abiotic factors, such as dissolved gas con-

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tent which fluctuates with flow (Dennison & Barnes 1988, Kühl et al. 1995, Nakamura & van Woesik 2001, Brown et al. 2002, Nakamura et al. 2003). Thus, water motion has been found to increase both the respiration of the host and photosynthesis of the algae known as zooxanthellae (*Symbiodinium* spp.) (Patterson et al. 1991). Coral colonies are more prone to bleaching (whitening of corals due to loss of either symbiotic algae or their pigment, or both) when enduring low-flow conditions (Nakamura & van Woesik 2001, Nakamura et al. 2003). Under such conditions, zooxanthellae may become photo-inhibited due to a build-up of harmful oxygen free radicals (Nakamura & van Woesik 2001), which target the D1 protein in PSII reaction centres.

The effect of hypoxic conditions on photo-physiological performance has been addressed on numerous occasions with terrestrial plants using PAM (pulse-amplitude modulation) fluorescence techniques (e.g. Schreiber & Vidaver 1974) and, to a lesser extent, fast-induction kinetics (e.g. Kirilovsky & Etienne 1991, Haldimann & Strasser 1999). Reported physiological impacts on PSII under hypoxic conditions include the formation of reductants due to the onset of chlororespiration (Garab et al. 1989, Miyake et al. 1994). Under anaerobic conditions, the plastoquinone (PQ) pool becomes reduced in the dark, which leads to the reduction of the primary electron acceptor of PSII, Q_A (Schreiber & Vidaver 1974, 1975). This ultimately results in a decline in the photochemical efficiency of PSII ($F_v/F_m = [F_m - F_0]/F_m$, where F_v is variable fluorescence, F_v/F_m is maximum quantum yield, and F_m and F_0 are maximum and minimum dark-adapted fluorescence, respectively), as a large proportion of the electron transport chain is pre-reduced, thus increasing the number of closed PSII reaction centres; this reduces F_m. Effects such as increased F_0 and a rise in the amplitude of the J peak (see 'Materials and methods') along a fast-induction curve have also been reported (Kirilovsky & Etienne 1991, Haldimann & Strasser 1999).

In corals, PAM fluorometry has been mainly used to address stress responses in connection with bleaching, by examining the PSII maximum and effective quantum yields of zooxanthellae as proxies of health (e.g. Jones et al. 2000, Jones & Hoegh-Guldberg 2001, Ralph et al. 2001) and using non-photochemical quenching (NPQ) as an indicator of dissipation of excess energy (Warner et al. 1996, Jones et al. 1998). Fast-induction kinetics using the Plant Efficiency Analyser (PEA fluorometer; Hansatech Instruments) are less commonly employed, but have been used to address photo-physiological issues of both isolated zooxanthella (Iglesias-Prieto 1995) and zooxanthellae *in hospite* (Hill et al. 2004a). Both technologies utilise chlorophyll *a* fluorescence measurements and rely on the dark-adapted 'Kautsky' effect to induce fluorescence emission (e.g. Haldimann & Strasser 1999, Hill et al. 2004a,b). Therefore, we expect that induction-curve analysis, along with fast-induction kinetics analysis, will identify photosynthetic responses of endosymbionts to anaerobic conditions.

This paper investigates the photosynthetic effect of air content and flow in the water surrounding corals, as well as identifying the most sensitive biomarker of anaerobic stress in corals. Using slow (PAM) and fast (PEA) induction kinetic techniques, we examine coral endosymbionts at a range of air saturation and flow conditions to investigate impacts of hypoxia on PSII of zooxanthellae *in hospite*.

MATERIALS AND METHODS

Pocillopora damicornis (Linnaeus) colonies were collected from Heron Island lagoon (<2 m deep) (152° 06′ E, 20° 29′ S) over the period 26 January to 4 February 2003, and transported to the University of Technology, Sydney where they were allowed to acclimate for 2 mo in a 500 l aquarium supplied with recirculating artificial seawater (carbonate [140 ppm] and synthetic sea salt [Aquasonic 'Ocean Nature'] to 33 ppm in reverse-osmosis water, 26 ± 1°C and 200 µmol photons m^{-2} s^{-1}). A nubbin was broken from 4 *P. damicornis* colonies and held in the 500 l aquarium to acclimatise for 14 d before experimentation. Induction-curve analysis (PAM) and fast-induction kinetics (PEA) were performed for each coral replicate (n = 4).

Experimental protocol. One nubbin from each of the 4 colonies was transferred into a purpose-built dark-acclimation chamber (2.8 l; see Hill et al. 2004b) with continuously circulating 100% air-saturated seawater at 26 ± 1°C, at a flow rate of 4 cm s^{-1} (23 l min^{-1} into an 11 cm diameter chamber). This was deemed to be an intermediate flow rate around a *Pocillopora damicornis* colony by Lesser et al. (1994). Each sample was acclimated for 10 min under 200 µmol photons m^{-2} s^{-1} (Li-Cor) with actinic light provided by a halogen lamp (12 V, 150 W with UV filter). A pilot study showed that 10 min of dark incubation was sufficient to fully dark-adapt the samples. Water temperature was maintained using a temperature-regulated water bath (TH3 Thermoregulatory, Ratek Instruments) connected to the dark-acclimation chamber. Samples were dark-adapted for 10 min after which fast-induction-kinetic transients (PEA) were obtained. Actinic light of 200 µmol photons m^{-2} s^{-1} was re-applied for a further 10 min, after which the nubbin was dark-acclimated for 10 min. Finally, induction-curve analysis (PAM) was performed. This procedure was repeated for the con-

trol (100% air-saturation with flow) and each of the 3 treatments: 40 ± 5% air content with a flow regime of ca. 2 cm s^{-1}, 0 to 2% air content with a flow regime of approximately 2 cm s^{-1}, and 0% air content with no flow. Air saturation was regulated by adding N_2 to the flow stream (Schreiber & Vidaver 1974) and measured using oxygen-sensing 100 µm optodes (Precision Sensing). It was assumed that CO_2 was not limiting during N_2 flushing, due to the volume of the chamber, the recirculation of water and the buffering capacity of bicarbonate in seawater. The optodes were calibrated linearly to 100% air saturation in 200 ml of seawater, aerated with an air stone for at least 10 min, and 0% air saturation in seawater, which had been flushed with N_2 for at least 10 min.

Slow (PAM) fluorescence measurements. Fluorescence measurements were performed using a Diving-PAM — Walz settings: saturating intensity (SI) = 8, saturating width (SW) = 0.8 s, actinic intensity (AI) = 3 (= 200 µmol photons m^{-2} s^{-1}), actinic width (AW) = 300 s, absorption factor (AF) = 1, gain (G) = 4, damping (D) = 2, induction delay (ID) = 40 s, induction width (IW) = 20 s. The Diving-PAM employs a 3 µs pulse from a red light-emitting diode (LED) with a peak emission at 650 nm as the measuring light (0.15 µmol photons m^{-2} s^{-1}). Saturation pulses were of >4500 µmol photons m^{-2} s^{-1} and actinic light was of 100 µmol photons m^{-2} s^{-1}. Chlorophyll fluorescence was detected at wavelengths above 710 nm.

Dark–light transition curves (induction curves) demonstrate the capacity of a tissue to regulate photosynthesis at a known level of irradiance. Tissue was dark-adapted initially for 10 min, then actinic light of 200 µmol photons m^{-2} s^{-1} was applied for 5 min. In order to monitor the effective quantum yield (ϕ_{PSII}) and quenching parameters, a saturating pulse was applied every 20 s. ϕ_{PSII} and non-photochemical quenching (NPQ) were determined according to the following equations (Schreiber 2004):

$$NPQ = (F_m - F_m')/F_m' \quad (1)$$

$$\phi_{PSII} = (F_m' - F_t)/F_m' \quad (2)$$

where F_m is maximum dark-adapted fluorescence, F_m' is maximum light-adapted fluorescence and F_t is minimum light-adapted fluorescence. Maximum non-photochemical quenching (NPQ_{max}) was recorded 4 min into the induction curves of all treatments.

Fast (PEA) fluorescence measurements. The OJIP transient (nomenclature by Strasser et al. 1995) can be divided into 2 phases, the fast rise from the minimum fluorescence (O ≈ F_0) to J and onwards to the intermediate step (I), and a slower rise to F_m (= P). The fluorescence rise from O to J corresponds to the reduction of the primary electron acceptor of PSII, Q_A, to Q_A^- (Strasser et al. 1995, Hill et al. 2004a). Fast-induction kinetics provide detailed information on the photochemical state of PSII, as well as the filling of the PQ pool (Govindjee 1995).

Fast-induction kinetics were measured using the Plant Efficiency Analyser (PEA) with coral samples after 10 min dark adaptation. The array of 6 red LEDs (peak wavelength 650 nm) provided the saturating illumination (3200 µmol photons m^{-2} s^{-1}) and focused on an area 4 mm in diameter. A PIN-photodiode (shielded by a long-pass filter >720 nm) detected the fluorescence signal from the coral. The fluorescence signal was obtained over 2 s and recorded every 10 µs 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), and P (F_m) was recorded as the maximum fluorescence reached after a 1000 ms sampling period. Curves for each treatment were normalised to F_0(F_t/F_0) to illustrate the change in variable fluorescence (F_v). Furthermore, the relative variable fluorescence was calculated by double normalising to O and P $(F_t - F_0)/(F_m - F_0)$ to illustrate the accumulation of the reduced form of Q_A (Q_A^-) (Haldimann & Strasser 1999). The efficiency of electron transport before Q_A was calculated using the following formula (Lazár 1999):

$$\varphi_{Po} = 1 - (F_0/F_m) \quad (3)$$

where F_0 is minimum dark-adapted fluorescence and F_m is maximum dark-adapted fluorescence.

Statistical analyses. One-way analysis of variance (ANOVA, Glantz 2002) tests were used to determine if significant differences were present among the different air-content treatments in F_v/F_m, ϕ_{PSII}, and NPQ_{max} (see Fig. 1), as well as 6 individual PEA parameters (O, J, I, P, F_v and φ_{Po}, see Table 1) and OJIP points (see Fig. 3A) along the curve. Where the assumptions of normality and equal variance failed ($p < 0.05$), data was transformed using natural log. Transformed data successfully met the assumptions of normality and equal variance. Post-hoc comparisons were performed using the Holm test, which is less conservative than Tukey's HSD or Bonferroni and also controls the overall risk of a false-positive conclusion at the nominal level (Glantz 2002).

RESULTS

In a pilot study, the air content at the coral surface under 4 cm s^{-1} flow rates was monitored using optodes. Results showed that this was reduced from 100 to 35 ± 5% air-saturation after 10 min in darkness. This was the lowest air content achievable (under flow) without N_2 manipulation, thereby defining the 40% intermedi-

ate air treatment. In a subset (n = 2) where the flow was re-established (100% air), the coral was able to completely reverse the impact of turning off the flow.

Slow (PAM) fluorescence

Maximum (F_v/F_m) and effective (ϕ_{PSII}) quantum yield, as well as NPQ_{max} of the 4 different treatments, are shown in Fig. 1. F_v/F_m was significantly reduced ($p = 0.017$) in the 0% air-saturation without flow treatment compared to the 100, 40 and 0% air-saturation treatments with flow. ϕ_{PSII} values for the severely air-depleted treatments (0% air-saturation with and without flow) were significantly lower ($p < 0.001$) than those for the 100 and 40% air-saturation treatments. NPQ_{max} was not significantly different ($p = 0.536$) among all 4 treatments.

The induction curves for ϕ_{PSII} and NPQ showed similar shapes for the 100 and 40% air-saturation treatments, but differed for the air-depleted samples (0% air saturation with and without flow) (Fig. 2). The most conspicuous difference between these 2 groups was the fast down-regulation of ϕ_{PSII} at the dark–light transition for the air-depleted samples (Fig. 2C,D), most likely due to a large rise in F_0 and a smaller decline in F_m. NPQ declined after 100 s actinic light in the 100 and 40% air-saturation treatments, whereas it increased or remained steady in the 0% air-saturation treatments (with and without

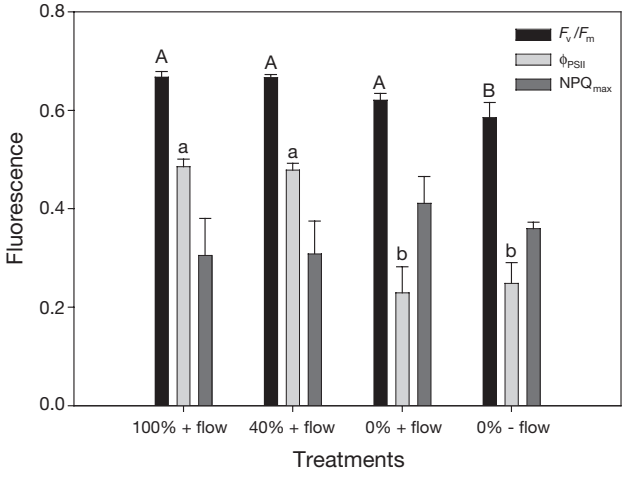

Fig. 1. *Pocillopora damicornis*. Effects of air-saturation and flow on maximum quantum yield, F_v/F_m, (black bars), effective quantum yield, ϕ_{PSII}, (light grey bars) and maximum non-photochemical quenching, NPQ_{max}, (dark grey bars) obtaining during induction curve analysis (n = 4). The 4 treatments, 100% air-saturation, 40% air-saturation, 0% air-saturation with flow and 0% air-saturation without flow are shown including standard error bars. Values with different letters were found to be significantly different ($p < 0.001$) by the Holm test

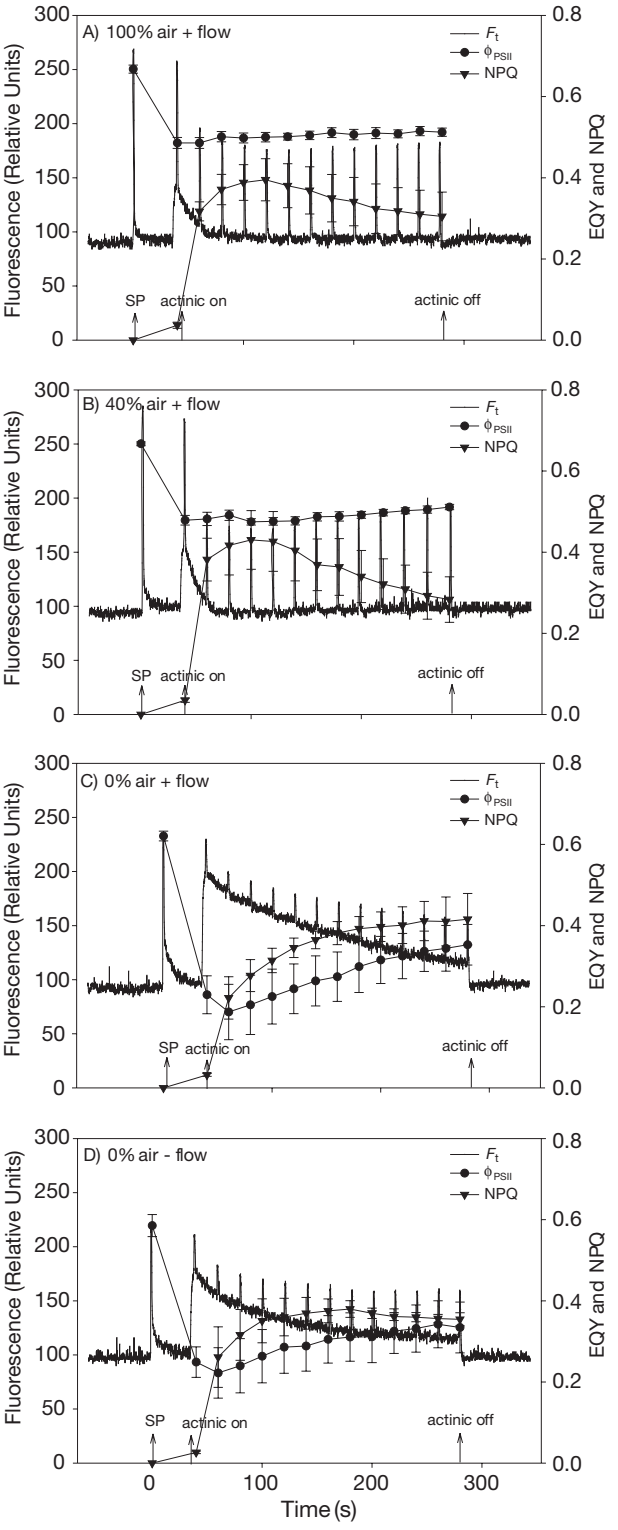

Fig. 2. *Pocillopora damicornis*. Induction-curve analysis of the 4 treatments, (A) 100% air-saturation, (B) 40% air-saturation, (C) 0% air-saturation with flow and (D) 0% air-saturation without flow. The curves represent mean values, including standard error bars (n = 4) of ϕ_{PSII} (EQY) and non-photochemical quenching (NPQ), and a representative curve showing F_t is superimposed on the data. SP: saturating pulse

flow). Fig. 2 also shows a trace of F_t during the induction. Differences here were also dependent on air content, rather than flow. That is, there was no difference in curve shape between the 100 and 40% air-saturation treatments, in contrast to the 0% with and without flow treatments. The difference between the 2 groups was evident after the dark–light transition.

Fast (PEA) fluorescence

The fast-induction kinetics of the 4 treatments are shown in Fig. 3. When exposed to 100 and 40% air saturation, corals showed a classical OJIP curve (Fig. 3A) with no significant differences in the amplitudes of the peaks (Table 1). An analysis of the amplitudes of the O, J, I and P peaks allowed the detection of significant differences with the other 2 treatments (Table 1). The O peak was found to increase under anaerobic conditions ($p < 0.001$) and the Holm post-hoc test revealed that there were also significant differences between the 0% air-saturation treatments, where the treatment with flow had a higher O (Table 1). A significantly higher J peak amplitude was also observed in the 0% air-saturation with flow treatment, compared to the other 3 treatments ($p < 0.005$). Following the J peak, the corals exposed to 0% air saturation with and without flow showed a pronounced dip, which was not apparent in the 100 and 40% treatments (Fig. 3A). The I and P peaks were greatly reduced ($p < 0.05$) in the 0% air-saturation without flow treatment compared to the other treatments. Fig. 3B shows the fast-induction curves of the 4 treatments normalised to F_0. This presentation of the data (also see Table 2) allows a clearer understanding of the change in variable fluorescence ($F_v = F_m - F_0 \approx P - O$) among the treatments, where the values for the 0% air-saturation with and without flow treatments were significantly lower than those for the 100 and 40% treatments. When double-normalised (Fig. 3C), the J peak was considerably higher in the 0% air-saturation with and without flow treatments than in the 100 and 40% treatments.

Variation in the time at which each peak occurred was also investigated. Table 1 shows that the timing of the O, J and P peaks remained constant among treatments (0.05 ms, 2 ms and 1000 ms, respectively), but the timing of the I peak varied. In the 100 and 40% air-saturation treatments, the I peak occurred at 50 ms, I_A, whilst in the 0% with and without flow treatments, the I peak took longer to reach (150 ms), I_B.

An analysis of the electron transport efficiency before Q_A (φ_{Po}) also separated the treatments into the 2 groups of with air (100 and 40%) and without air (0% with and without flow) (Table 2, $p < 0.05$). This shows that under conditions of 0% air, the electron transport efficiency before Q_A had declined.

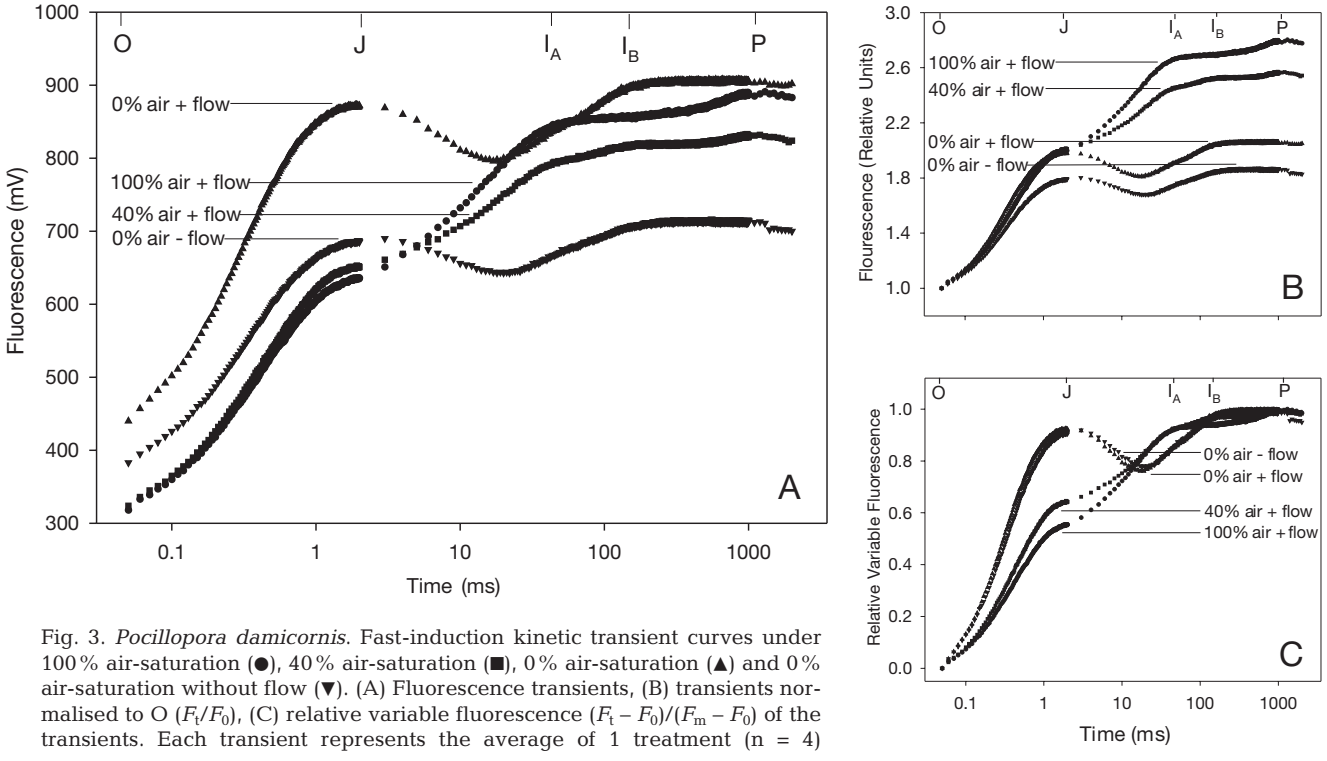

Fig. 3. *Pocillopora damicornis*. Fast-induction kinetic transient curves under 100% air-saturation (●), 40% air-saturation (■), 0% air-saturation (▲) and 0% air-saturation without flow (▼). (A) Fluorescence transients, (B) transients normalised to O (F_t/F_0), (C) relative variable fluorescence $(F_t - F_0)/(F_m - F_0)$ of the transients. Each transient represents the average of 1 treatment (n = 4)

Table 1. *Pocillopora damicornis*. Amplitude and time of occurrence of the O, J, I and P peaks along the fast-induction curves for the 4 treatments, 100, 40 and 0% air-saturation with flow and 0% air-saturation without flow. Averages ± SE of mean are shown (n = 4). Values with different superscript letters were found to be significantly ($p < 0.05$) different by the Holm test

Treatment	O		J		I		P	
	Time (ms)	*F* (mV)	Time (ms)	*F* (mV)	Time (ms)	*F* (mV)	Time (ms)	*F* (mV)
100% + flow	0.05	337.8 ± 8.5^a	2	678.5 ± 21.7^a	50	903.0 ± 27.6^a	1000	946.3 ± 25.5^a
40% + flow	0.05	355.5 ± 15.9^a	2	722.0 ± 36.0^a	50	878.5 ± 28.9^a	1000	914.3 ± 28.0^a
0% + flow	0.05	458.7 ± 5.5^b	2	909.0 ± 13.4^b	150	930.7 ± 9.6^a	1000	937.0 ± 7.7^a
0% – flow	0.05	410.8 ± 1.9^c	2	734.5 ± 35.4^a	150	755.0 ± 25.1^b	1000	762.3 ± 25.3^b

Table 2. *Pocillopora damicornis*. Fast-fluorescence transient parameters for the treatments 100, 40 and 0% air-saturation with flow and 0% air-saturation without flow. Averages ± SE bars are shown (n = 4). All parameters showed p-values <0.05. Values with different superscript letters were found to be significantly ($p < 0.05$) different by the Holm test

Parameter	100% + flow	40% + flow	0% + flow	0% – flow
F_v	608.5 ± 19.7^a	558.8 ± 15.5^a	478.3 ± 10.9^b	351.5 ± 20.0^b
φ_{Po}	0.64 ± 0.01^a	0.61 ± 0.01^a	0.51 ± 0.01^b	0.46 ± 0.02^b

DISCUSSION

We set out to determine whether anaerobic conditions ultimately induce a decrease in the photosynthetic activity of zooxanthellae *in hospite*, whilst also identifying the most sensitive fluorescence biomarker of hypoxic conditions. Induction-curve analysis showed that ϕ_{PSII} decreased significantly ($p < 0.01$) after short periods of anoxic conditions (Fig. 1). This caused a reduction in the PQ pool, leading to increased PSII reaction centre closure. The rate at which dissipation of excess energy (NPQ_{max}) occurred did not increase concurrently with a decline in ϕ_{PSII}, which is often the case during bleaching events (Warner et al. 1996, Jones et al. 1998). However, the shape of the induction curves clearly indicates an increased rate of NPQ saturation in air-depleted treatments. This can also be inferred from the shape of the F_t curves, in which the impact of low air-saturation (0%) can be seen in the rate at which F_t is quenched in the dark–light transition (Fig. 2C,D). This was also observed by Schreiber & Vidaver (1975), who proposed that the recovery of photosynthesis, as manifested in F_t, is dependent upon the dark-limiting steps of the re-oxidation of the PSII primary acceptor pool. The increase in F_t is thought to be linked to the water-splitting enzyme, hydrogenase, catalysing the endogenous electron reactions on the donor side of PSII, which is slowly induced during anaerobiosis (Schreiber & Vidaver 1974).

Anaerobiosis resulted in an increase in F_t after a saturating pulse in the dark (seen in Fig. 2C,D), and this fluorescence signal was slowly quenched when exposed to moderate actinic light (200 µmol photons m^{-2} s^{-1}). This is explained by the reduction of PSII reaction centres, which is linked to dark reduction of the PQ pool, a phenomenon known as chlororespiration (Harris & Heber 1993). In light, fluorescence is slowly quenched due to Photosystem I (PSI) gradually re-oxidising PSII (opening reaction centres) via the electron transport chain (Kaftan et al. 1999).

The fast-induction curves revealed several physiological changes, which occur in corals when exposed to anaerobic conditions (Fig. 3). For the 100 and 40% air-saturation treatments (Table 1), no significant differences were observed in the amplitudes of the O, J, I or P peaks, nor in the timing of each peak. This indicates that the various components of the photosynthetic apparatus are functioning similarly under these conditions. However, under conditions of 0% air-saturation with and without flow, changes in the fast-induction curves became apparent (Fig. 3). When the air-saturation was reduced to 0%, a significant rise in the O peak was seen (Fig. 3A). This rise was maximal under 0% air-saturation with flow (Table 1). An increase in F_0 (O) has been suggested to occur as a result of the dissociation of the light-harvesting complex of PSII (LHC II) from the PSII reaction centres (Armond et al. 1978, Yamane et al. 2000). This response is common with high-temperature stress (Schreiber & Berry 1977), although an alternative explanation for an increase in F_0 is the inhibition of electron flow from Q_A to Q_B (Bukhov et al. 1990, Yamane et al. 2000). This occurs in PSII reaction centres in darkness through the reduction of Q_A by PQ. The reduction of PQ may result from cyclic electron transport around PSI (Ravenel et al. 1994) or from chlororespiration (Garab et al. 1989). These 2 mechanisms result in the reduction of the elec-

tron acceptors of PSII in the dark. Another possible contributor to a rise in F_0 is the decline in efficient energy trapping by PSII (Havaux 1993, Yamane et al. 2000).

A greater J peak amplitude occurred under the 0% air-saturation with flow treatment compared to the other 3 treatments, which all had similar J peak amplitudes (Fig. 3A). The J peak is indicative of the photochemical phase, where Q_A is reduced to Q_A^- under illumination (Govindjee 1995, Hill et al. 2004a). The increase in J indicates that there was a large increase in the rate of Q_A reduction under 0% air-saturation with flow. This response is probably due to the accumulation of redox components in the electron transport chain, which become reduced in the dark (Haldimann & Strasser 1999). The probable mechanism responsible for this dark pre-reduction of the electron transport chain is chlororespiration or PSI cyclic electron transport (Yamane et al. 2000). An elevated J peak was only observed in the 0% air-saturation with flow treatment. The lack of a similar response in the 0% treatment without flow suggests that elevation of the J peak may not be a stable biomarker of anaerobiosis in zooxanthellae *in hospite* (Fig. 3A).

The J peaks in the 0% air-saturation with and without flow treatments were 97 and 96%, respectively, of the maximum fluorescence (P peak). In contrast, the same rise in the 100 and 40% air-saturation with flow treatments only contributed 72 and 79%, respectively, of the maximum fluorescence. The transients of the air-depleted (0% air-saturation with and without flow) treatments were thus dominated by the O–J rise, where J = I = P (Haldimann & Strasser 1999). This indicates that the maximal fluorescence yield occurred at the J peak, due to the blockage of electrons beyond Q_A. The reduction in the efficiency of electron transport before Q_A (φ_{Po}) (Table 2) indicates that the dark reduction of the electron acceptors limits the capacity for Q_A reduction. Also, the P peak was reduced in amplitude to the level of the J peak, due to the limited capacity for Q_A re-oxidation. This provides further evidence that anaerobic conditions cause the reduction of the electron transport chain in the dark.

The anaerobic (0% air-saturation with and without flow) treatments caused a significant dip to occur after the J peak (Fig. 3A). Schreiber & Vidaver (1974) proposed that this dip occurs in response to enhanced PSI activity, which functions optimally under conditions of anaerobiosis. The dip after the J peak in the fast-induction curves signifies that PSI cyclic electron transport is operating, possibly along with chlororespiration, causing the dark reduction of the electron acceptors and PQ pool (Schreiber & Vidaver 1974). We speculate that this possible increase in PSI activity could also explain the delayed appearance of the I peak (I_B) in the air-depleted treatments. In comparison, PSII becomes inhibited, which is demonstrated by the decline in F_v/F_m (Fig. 1) and F_v (Table 2, Fig. 3B). Furthermore, I and P peak signals were lower in 0% air-saturation without flow compared to the other treatments, as a result of reduction of the PQ pool (Fig. 3A). The relative variable fluorescence (Fig. 3C) demonstrates that there is an elevation of the J peak with a decline in air saturation, due to the accumulation of Q_A^- under hypoxic conditions in the dark. From these fast-induction curves, the most sensitive biomarkers of the onset of anaerobiosis are a rise in F_0, a reduction in φ_{Po} and an elevated J peak in double-normalised transients.

Future research is needed to demonstrate whether far-red illumination is able to re-oxidise PQ and open the PSII reaction centres. Under far-red illumination, PSI activity would be stimulated, thus removing effects of dark-reduced electron acceptors in PSII under anaerobic conditions. Introducing O_2 would also remove the anaerobiosis-dependent increase in fluorescence signal by oxidising the PQ pool (see Schreiber & Vidaver 1974).

In conclusion, we have demonstrated that hypoxia indeed induces deviations from normal functioning PSII systems, through the measurements of both fast (PEA) and slow (PAM) chlorophyll *a* fluorescence. The 2 instruments were shown to complement each other in giving detailed information on the response of *Pocillopora damicornis* when exposed to decreased air and flow levels. Anaerobiosis can develop rapidly under low-flow conditions (Patterson et al. 1991, Kühl et al. 1995, Nakamura & van Woesik 2001, Nakamura et al. 2003). This has important implications for coral bleaching events, which are intensified under such conditions. We observed similar photochemical impacts as those which occur during bleaching events (e.g. Jones et al. 1998, Hill et al. 2004b). Hypoxia and bleaching conditions in conjunction (i.e. doldrums, elevated temperature and high light) may intensify this response, which would suggest increased susceptibility of coral to bleaching. Furthermore, an important technical outcome of this research is that the use of dark-adapted coral samples held in low-volume facilities could lead to overestimation of the F_0 level (O peak, Table 1) and, thereby, distortion of other fluorescence parameters such as φ_{Po}, F_v and F_v/F_m.

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