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Spatial heterogeneity in active chlorophyll fluorescence and PSII activity of coral tissues

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Abstract Chlorophyll-*a* fluorescence was measured in six species of coral, using pulse-amplitude-modulated fluorometers employing fibre-optic probes with diameters of 8 mm, 1 mm and 140 μm. The 8-mm probe integrated responses over a large area, giving more weight to coenosarc than polyp tissue for *Acropora nobilis*. With 1-mm and 140-μm fibre-optic probes, the photosynthetic responses of zooxanthellae in the coenosarc and the polyp tissue of *Acropora nobilis* were distinguished. The polyp tissue exhibited a lower maximum in relative electron transport rate than did the coenosarc tissue, and was subject to down-regulation at higher irradiances. Coenosarc and polyp tissue (both containing zooxanthellae) showed a wide range of responses in the other corals. Down-regulation of photosynthesis in a single polyp of *Pocillopora damicornis* was followed after exposure to moderate irradiance, with recovery occurring over a further 4 h of shade conditions. All the corals (*Acropora millepora, A. nobilis*, *Cyphastrea serailia*, *Montipora tuberculosa, Pocillopora damicornis* and *Porites cylindrica*) showed evidence of strong down-regulation of photosynthesis under high irradiance, and little evidence of photoinhibitory damage to photosystem II.

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Introduction

Heterogeneous distribution of photosynthetic activity in many reef-dwelling symbioses is a common observation (Kühl et al. 1995; Rowan and Knowlton 1995; Helmuth et al. 1997). Generally, corals are rigid and symmetrical. Polyps are fixed in their orientation to the solar irradiance and therefore adapted to a regular diurnal change in light environment, but fixed in their position vis-à-vis light or shade (Jokiel and Morrissey 1986). This results in chlorophyll and peridinin stratification, especially in arborescent corals, where different parts become photoadapted to their particular microenvironment (Jokiel and Morrissey 1986), thus explaining the lighter colour of the growing tip and deeper brown colour of the lower parts of many branched corals.

Colony morphology can have substantial effects on the photobiology of the coral tissue, and the host morphology can influence the microhabitat of the symbionts on a very small spatial scale, from millimetres to centimetres (Helmuth et al. 1997). Polyps near the tip of a branching coral have been shown to receive greater insolation than polyps at the base of the branch, the latter generally being shaded (Helmuth et al. 1997); thus, the distribution of zooxanthellae and/or their adaptation to irradiance within these different polyps are spatially variable. The most exposed growing regions of the colony are sun-adapted, while most of the lower parts of the colony are shade-adapted (Jokiel and Morrissey 1986). Symbiotic tissues can become photosynthetically differentiated, based on their light history. Generally, the coenosarc, being directly light-exposed, non-gastrodermal tissue between polyps, is sun-adapted, whilst the polyp is shade-adapted (Jokiel and Morrissey 1986). Even the light field within a single polyp is heterogeneous (Kühl et al. 1995). Given the complex structure of the polyp and the fact that most of the zooxanthellae are located in the endodermis of the gastrodermal cavity, it is not surprising that there are marked differences in the photosynthetic characteristics of coenosarc tissue and

even within individual polyps. Kühl et al. (1995) found that gross photosynthetic activity varied significantly between polyp and coenosarc tissue of *Favia* sp. They found that the oxygen production was < 5 nmol O_2 cm^{-3} s^{-1} in the oral disk tissue and up to 32 nmol O_2 cm^{-3} s^{-1} in the thick tissue covering the inner ridges of the corallum. Similarly, gradients of respiration and primary production were found along the length of *Acropora palmata* branches (Gladfelter et al. 1989). Because of higher respiration, the terminal 5 cm had the lowest primary production, while the proximal 10 cm had the highest production, and the region in between had intermediate rates. Photosynthate was transported between polyps from the proximal region up to the terminal region, where growth occurs and production exceeds respiration. There were also differences between tissue on the upper versus the lower surface of a branch (Gladfelter et al. 1989).

Polyps, zooxanthellae and chloroplasts become adapted to the local light climate at a particular site. The light climate of a coral is influenced by its morphology, proximity to other objects (shading), depth, wave focusing, cloud shading, as well as tidal and solar oscillations (Falkowski et al. 1990). Polyp density varies with light climate, where tissue exposed to full sunlight has significantly more polyps than do shaded regions (Porter et al. 1984). Similarly, the shaded polyps have elevated chlorophyll-*a* content. Although the zooxanthellar density usually remains unchanged (Porter et al. 1984; Jones and Yellowlees 1997). This means that, in principle, short-term photo-acclimation and longer-term photo-adaptation and photo-protection can be accomplished, although little is known about such mechanisms. Here we use the term photo-adaptation to mean a genetic alteration in the population of zooxanthellae due to differences in growth and survival, as opposed to photo-acclimation which is a physiological short-term response to an altered light climate (Falkowski et al. 1990; Helmuth et al. 1997).

Most coral-photobiology research has assumed that the response of a gross portion of a coral is representative. This approach fails to recognise that spatial variation of the zooxanthellae and of their environment exists at a micro-scale (millimetres to micrometres). The microhabitat is affected by light climate, flow dynamics, gas exchange and nutrient availability, which vary widely within a single colony (Kühl et al. 1995; Helmuth et al. 1997; de Beer et al. 2000). With the advent of chlorophyll fluorometers for use with single optical fibres (Schreiber et al. 1996; Microfibre-PAM, Walz, Effeltrich, Germany), it is now possible to assess photosynthetic activity of substantially smaller regions of tissue and even of different regions of polyps. For instance, a 1-mm acrylic fibre-optic probe can be placed on top of an individual polyp or between two adjacent polyps. Alternatively, a 140-µm fibre-optic probe can be inserted into a single polyp to measure the local photosynthetic activity of the contracted tentacle tissue and the gastrodermal tissue.

We present the first assessment of the fine-scale variation of active chlorophyll fluorescence in corals, as measured with pulse-amplitude-modulated (PAM) fluorometers equipped with optical fibres of three different diameters. The aim of this study was to demonstrate the utility of microscale chlorophyll-fluorescence measurements in coral studies, and to address the following questions: are the polyps and coenosarc tissues different in their photosynthetic activity, do these patterns vary between species, and how does a single polyp respond to exposure to high irradiance?

Materials and methods

All coral specimens were collected from Heron Island Lagoon (152°6′ E, 20°29′S) in water of depths < 2 m and were maintained (in the same orientation as collected) in flow-through aquaria under natural and shaded lighting and at 25–27°C.

Effect of fibre-optic probe diameter

Acropora nobilis samples ($n = 4$) were maintained in aquaria under an irradiance of 50 µmol photons m^{-2} s^{-1}. The fibre-optic probes used in these experiments were (1) the standard 8-mm multi-stranded glass-fibre-optic probes supplied with the Diving-PAM (Walz, Effeltrich, Germany), and (2) the 1-mm acrylic-fibre-optic probe (F1, Walz) and 140-µm single-strand glass-fibre-optic cables (Radiall, Germany) used in conjunction with the PAM-Control fluorometer (Walz). Fibres were positioned using a manually operated micro-manipulator (Märtzhäuser, MM33) in conjunction with a dissecting microscope (Wild MS, Switzerland). Actinic irradiance was provided by a separate fibre-optic with a quartz halogen light source (Intralux 5000, Volpi). All measurements with the 1-mm and the 140-µm fibre-optic probes were performed in a small Perspex flow-through chamber (100×40×40 mm). The water in the chamber was circulated using a submersible pump (Eheim 1048, Germany) with a flow rate of ~5 l min^{-1}.

Photosynthetic activity was assessed using rapid-light curves (RLC), where samples were exposed to eight incremental steps of irradiance ranging from either 0 to 1,070 µmol photons m^{-2} s^{-1} (1-mm and 140-µm fibre-optic probe; Microfibre-PAM) or 0 to 2,700 µmol photons m^{-2} s^{-1} (8-mm fibre; Diving-PAM). During the RLC measurement, samples were exposed to 10 s of irradiance at each incremental step. For terrestrial plants, electron transport rate (ETR) has been determined according to the following formula: $ETR = \phi_p \times 0.84 \times 0.5 \times PAR$, where $\phi_p = (F_{m'} - F_t)/F_{m'}$ is the effective PSII quantum yield, 0.84 is the assumed absorption coefficient, 0.5 is the correction for two photosystems absorbing photons, and PAR is the photosynthetic active radiation (Genty et al. 1989). Beer et al. (1998) found the absorption coefficient of corals to be 0.023–0.036, compared to the standard value of 0.84, as determined for terrestrial leaves by Björkman and Demmig (1987). Since the absorption coefficient of various coral species will be significantly different from that of terrestrial leaves, we think that Beer et al. (1998) have underestimated this coefficient. Until a widely accepted method is developed for determining absorption coefficients, we recommend using "relative ETR", as determined by $\phi_p \times PAR$.

Polyp versus coenosarc tissue

Six colonies of six coral species [*Acropora millepora* (Ehrenberg), *Acropora nobilis* (Dana), *Cyphastrea serailia* (Forskal), *Montipora tuberculosa* (Lamarck), *Pocillopora damicornis* (Linnaeus) and *Porites cylindrica* (Dana)] were maintained in aquaria, at 200–300 µmol photons m^{-2} s^{-1}. *Acropora millepora*, *A. nobilis* and *Pocillopora damicornis* were branching corals, *M. tuberculosa* was a plate-growth form, and *C. serailia* and *Porites cylindrica* were both

massive-growth forms. RLCs were measured with a 140-µm fibre-optic probe of a Microfibre PAM. The region sampled on each colony was generally 3–10 cm distal from the tip for branching growth forms and randomly on the upper surface of massive and plate forms. Tips were not used, since they are low in zooxanthellar density (Gladfelter et al. 1989).

Curve fitting of relative ETR versus irradiance curves

RLCs were fitted by the function of Platt et al. (1980).

$$P = P_S[1 - \exp(-\alpha E_d/P_S)]\exp(-\beta E_d/P_S) \quad (1)$$

E_d is the downwelling irradiance (wavelength 400–700 nm). P_s is a scaling parameter defined as the maximum potential ETR in the absence of photoinhibition. α is the initial slope of the light curve before the onset of saturation and provides a measure of the efficiency of light utilisation. β is the slope of the light curve beyond the onset of photoinhibition. In the absence of photoinhibition ($\beta = 0$) equation 1 simplifies to:

$$P = P_m[1 - \exp(-\alpha E_d/P_m)] \quad (2)$$

The maximal rate of relative ETR at light saturation is termed the photosynthetic capacity, P_m, and can be derived as

$$P_m = P_S[\alpha/(\alpha+\beta)][\beta/(\alpha+\beta)]^{\beta/\alpha} \quad (3)$$

Curves were fitted with a non-linear Levenberg–Marquardt regression algorithm (Origin 6.1, OriginLab Corp., Northhampton, USA). In case of fitting average values, the fitting was constrained by weighing each mean value with its standard deviation. This gives points with a small standard deviation more influence on the curve fit than points with larger associated standard deviation.

Down-regulation of a single *Pocillopora damicornis* polyp

Pocillopora damicornis colonies were shade-adapted (1–2 days at ~50 µmol photons m^{-2} s^{-1}), then exposed to 500 µmol photons m^{-2} s^{-1} (Intralux 5000, Volpi) and RLCs were constructed at 5-min intervals for 30 min on the same polyp. Recovery was monitored by RLCs at 10, 30 min, 1, 2, 4 and 6 h after high light exposure. Dark–light induction curves were constructed before high light exposure, after 30 min of high light exposure, and again after 2 and 4 h of recovery at < 50 µmol photons m^{-2} s^{-1}. Induction curves provided details about the rate of Calvin cycle activation and about non-photochemical quenching (q_N), which was determined according to the following equation (Schreiber et al. 1994): $q_N = (F_m - F_m')/(F_m - F_o)$, where F_m is the dark-adapted maximum fluorescence yield, F is the instantaneous fluorescence yield, F_o is the minimal fluorescence yield and F_m' is the light-adapted maximum fluorescence yield. Samples were dark-adapted for 20 min before constructing the induction curve and were initially pulsed with a saturating flash, and after 40 s darkness the actinic irradiance (350 µmol photons m^{-2} s^{-1}) was applied for 4 min with a saturating flash every 20 s. Fluorescence measurements were obtained with the 140-µm fibre-optic probe of the Microfibre-PAM fluorometer.

Results

The PAM-detected photosynthetic response of shade-adapted *Acropora nobilis* varied according to the relative diameter of the fibre-optic probes used (Fig. 1). The rapid-light curves from the 8-mm and the 1-mm fibre optic (positioned over the coenosarc) were similar, whilst the polyp tissue of *A. nobilis* exhibited a substantially reduced relative ETR_{max}. This indicated that the majority of the fluorescence signal measured with the 8-mm fibre-optic probe originated from the coenosarc tissue, with relatively little signal coming from the polyp tissue. However, when polyps were singled out using a 1-mm or 140-µm fibre-optic probe, a substantial down-regulation response was found with a lowered relative ETR at elevated irradiance. Coenosarc tissue did not show this response. The photosynthetic efficiency (α, slope of the light-limiting region of the RLC) of the 8-mm, 1-mm coenosarc and 140-µm coenosarc samples was similar, whilst the 1-mm polyp and the 140-µm polyp sample appear to have a slightly less acute α. The six species of coral had a range of different RLC responses (Fig. 2). For *Cyphastrea serailia* and *Montipora tuberculosa*, the polyp samples had much higher relative ETR_{max} than the coenosarc tissue (Fig. 2d and e). For *A. millipora* (Fig. 1a) this difference was less

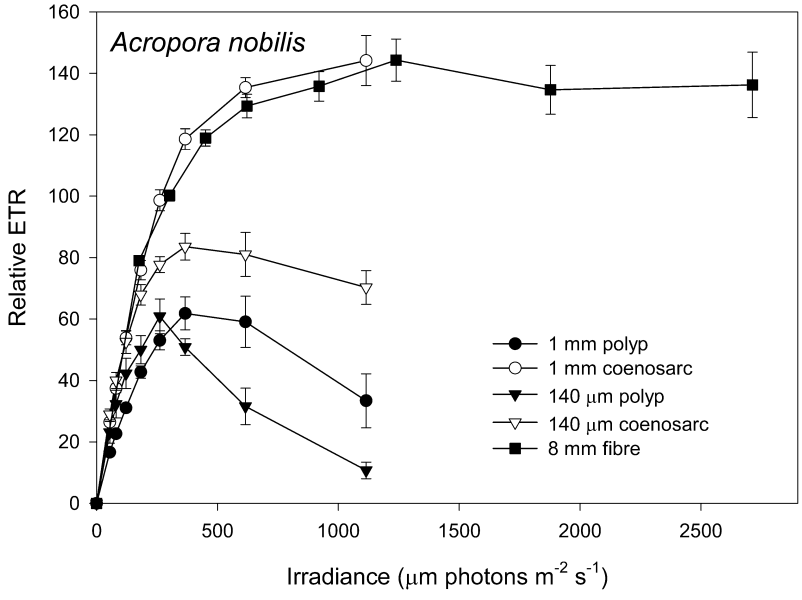

Fig. 1. Average rapid-light curve (RLC) of *Acropora nobilis* ($n = 4 \pm$ SEM) measured with three different fibre-optic probes. The 8-mm fibre-optic probe measured a combined response from polyp and coenosarc, whereas the 1-mm and 140-µm fibre-optic probes both measured the polyp and coenosarc separately. Units of relative electron transport rate (ETR) are µmol electrons m^{-2} s^{-1} and of irradiance are µmol photons m^{-2} s^{-1}. *Inverted triangles* represent the 140-µm fibre (*open* coenosarc, *solid* polyp); *circles* represent the 1-mm fibre (*open* coenosarc, *solid* polyp) and *solid squares*, the 8-mm fibre-optic probe

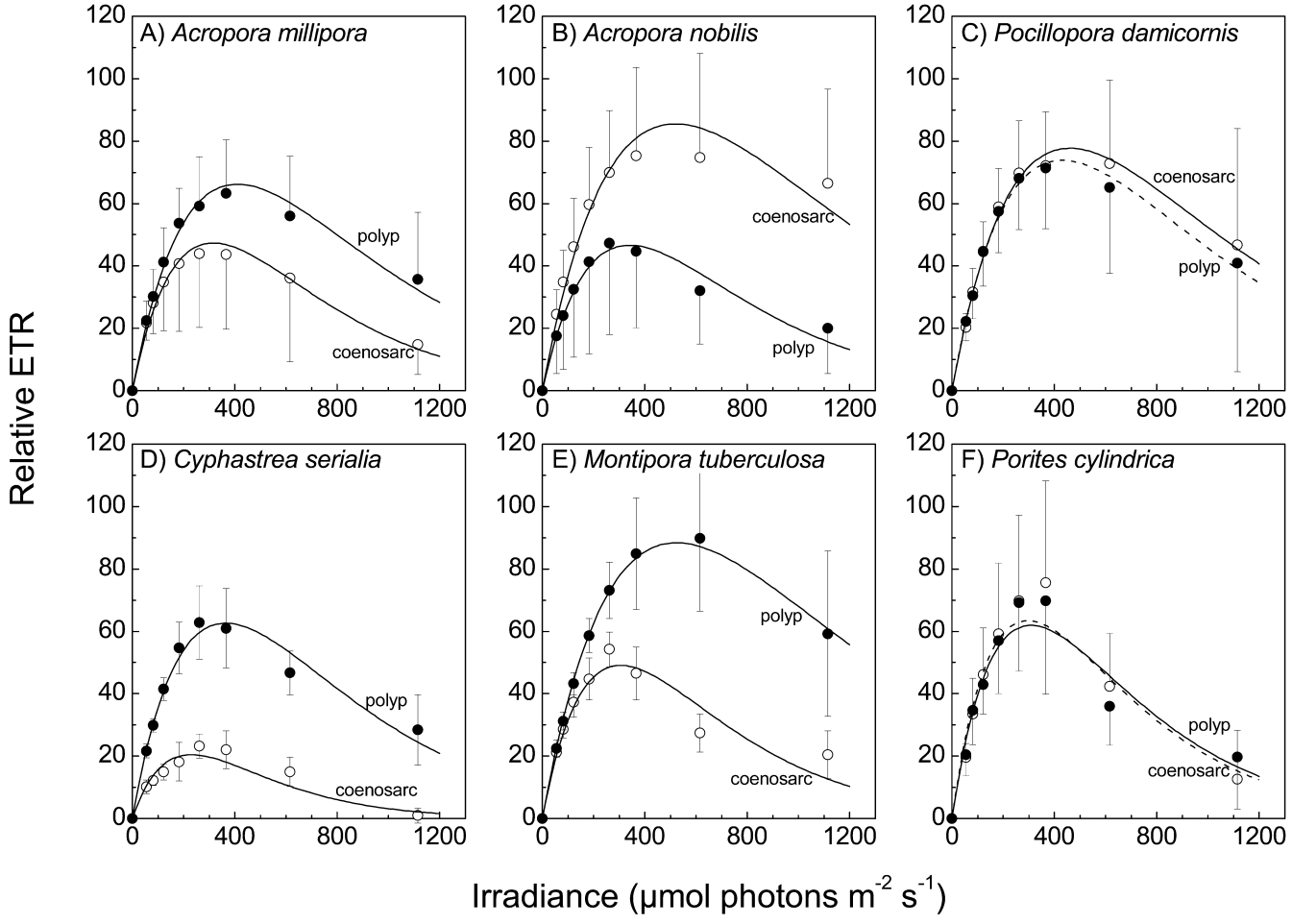

Fig. 2. Rapid-light curve for six species of coral, **A** *Acropora millepora*, **B** *Acropora nobilis*, **C** *Pocillopora damicornis*, **D** *Cyphastrea serailia*, **E** *Montipora tuberculosa*, **F** *Porites cylindrica*, separated into polyp and coenosarc tissues ($n = 6$), using the 140-µm fibre-optic probes. *Open symbols* represent coenosarc and *solid symbols* represent polyp tissue samples. Units of relative ETR are µmol electrons m^{-2} s^{-1} and units of irradiance are µmol photons m^{-2} s^{-1}. Curve fitted to average RLC for coenosarc and polyp, with *error bars* representing standard deviation of the mean

pronounced. *Acropora nobilis* showed the opposite pattern: the coenosarc tissue had a higher relative ETR_{max} than the polyp (Fig. 2b). The remaining two coral species (*P. damicornis* and *P. cylindrica*) showed no apparent difference between the photosynthetic activity of the polyp and that of the coenosarc tissue (Fig. 2c and f). For all species there was significant down-regulation of photosynthesis at high light intensities, with *P. cylindrica* (Fig. 1f) being the most extreme.

Down-regulation of a single polyp of *Pocillopora damicornis*

Figure 3 illustrates the down-regulation for a single coral polyp. After 30 min exposure, the relative ETR_{max} was less than 25% of the original shade-adapted response. After the colony was returned to low light conditions (~50 µmol photons m^{-2} s^{-1}), recovery was relatively fast, and after 4 h the polyp was in a similar condition to that before exposure.

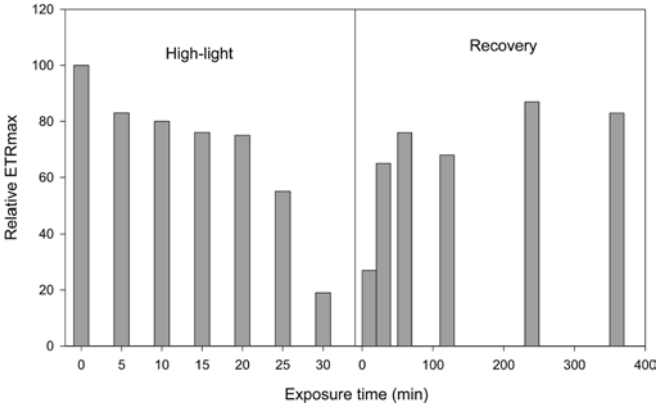

Fig. 3. Relative ETR_{max} derived from a series of RLCs during 30-min exposure of a single *Pocillopora damicornis* polyp to 500 µmol photons m^{-2} s^{-1}. RLCs were performed at 5, 10, 15, 20, 25 and 30 min exposure. After exposure, recovery (under an irradiance of 50 µmol photons m^{-2} s^{-1}) was monitored, with RLCs performed at 10, 30 min, 1, 2, 4 and 6 hr . Relative ETR_{max} was plotted against recovery time. Units of relative ETR are µmol electrons m^{-2} s^{-1} and units of irradiance are µmol photons m^{-2} s^{-1}

A series of dark–light induction curves clearly illustrated the process of down-regulation in response to

high light intensity, and recovery under shaded conditions, of a single *P. damicornis* polyp (Fig. 4a–c). Figure 4a shows the polyp under shade-adapted conditions, with a moderately high initial effective PSII quantum yield ($\phi_p = 0.7$), high maximum fluorescence (F_m; 360 units) and high minimum fluorescence (F_o; 110 units), whilst q_N was induced and stablised rapidly. After 1 h of exposure to 500 μmol photons m^{-2} s^{-1}, the induction curve (Fig. 4b) showed significantly lower F_m and F_o, suggesting a slight down-regulation in the polyp (Fig. 4b). Figure 4c illustrates the induction curve after 4 h recovery in the shade; the curves were similar to the pre-exposure (Fig. 4a): q_N was functioning well, effective quantum yield was similar to the original, and F_m and F_o also recovered.

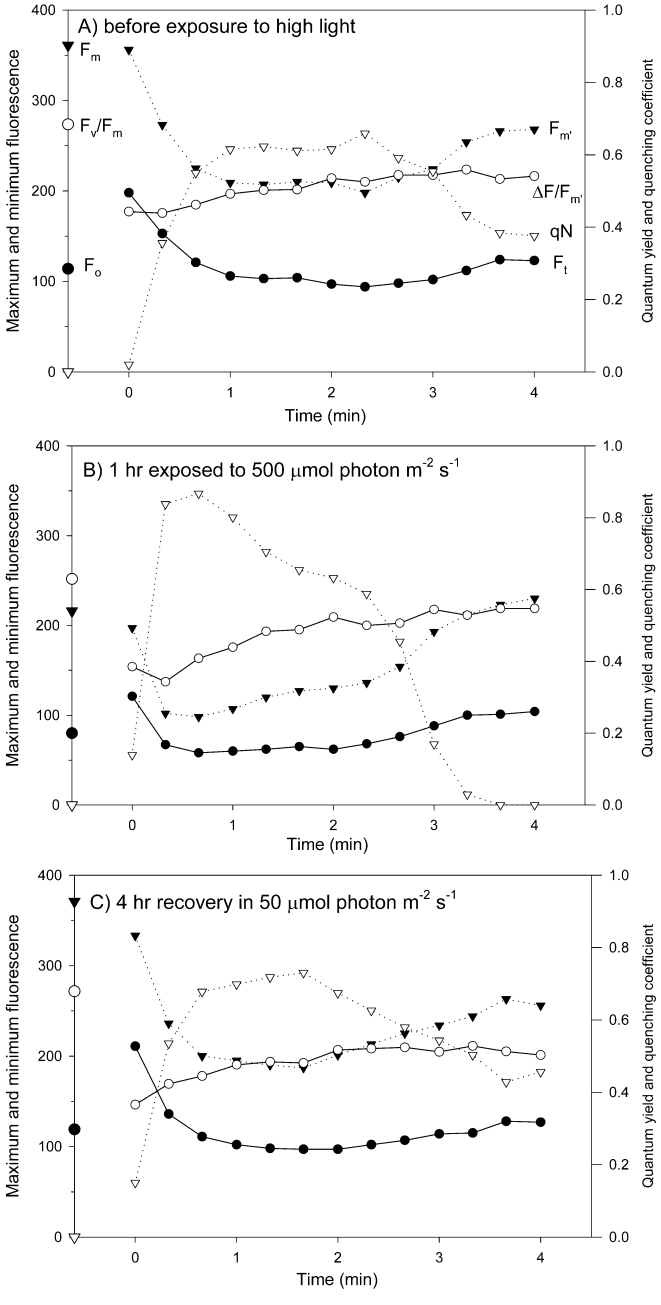

Fig. 4A–C. Dark–light induction curves of a single *Pocillopora damicornis* polyp exposed to high light (500 μmol photons m^{-2} s^{-1}) and its subsequent recovery (under an irradiance of ~50 μmol photons m^{-2} s^{-1}). Sample times were **A** before exposure to high light, **B** after 1 h exposure to 500 μmol photons m^{-2} s^{-1}, and **C** 4 h recovery at 50 μmol photons m^{-2} s^{-1}. *Open circle* = Effective quantum yield; *solid triangle* F_m or $F_{m'}$; *solid circle* F_o or F_t; *open triangle* q_N

Discussion

This study has used a new microfibre chlorophyll-fluorescence technique to investigate microscale heterogeneity in photosynthetic activity in corals. Scleractian corals have been investigated in a number of recent studies using PAM fluorometry (Warner et al. 1996; Beer et al. 1998; Jones et al. 1998; Hoegh-Guldberg and Jones 1999; Ralph et al. 1999), as well as fast-repetition-rate fluorometry (Gorbunov et al. 2000, 2001). In all the PAM-based studies, a large (8 mm) fibre-optic probe has been used. With the advent of microfibre technology it is now possible to use fibre-optic probes as small as 20 μm in diameter (Schreiber et al. 1996). With a 140-mm fibre, many new and interesting patterns of photosynthetic response have been revealed in polyp and coenosarc tissues of a variety of corals.

Comparing the results using the three different sizes of fibre-optic probes, the greatest difference was obtained for relative ETR_{max} and degree of down-regulation in *Acropora nobilis*. Explanations for this can be put forward in terms of (1) different mixtures of coenosarc/polyp zooxanthellae sampled, (2) the additional signal from endolithic algae in the corallum and (3) photoacclimation of the polyps and different parts of the polyp, to different light environments (Fig. 1). The smaller fibre-optic probes (1 mm and 140 μm) covered a small area of tissue (and tissue types) and therefore provided a more homogeneous signal. The optical properties of the large fibre-optic probe (8 mm), with a wider field of focus (numerical aperture), results in a signal being collected from several polyps and interconnected (coenosarc) regions, whereas the smaller numerical aperture of the smaller fibre-optic probes (1 mm and 140 μm) ensures that only very localised measurements are performed. Furthermore, in averaging over larger areas when measuring with the 8-mm probe, the coenosarc tissue is most probably contributing disproportionately to the signal, since this tissue is thinner and exhibits a higher degree of backscatter than the more fleshy polyp tissue (Kühl et al. 1995), resulting in a higher fluorescence signal. Fluorescence measurements with the 8-mm fibre and derived parameters of photosynthetic performance therefore represent an average of various tissues that contribute to the signal proportionally according to their optical properties. Changes in optical properties, when polyps expand or contract, alter the exposed area of coenosarc relative to polyp tissue, and this can be interpreted erroneously as changes in photosynthetic performance. Measurements obtained

with 8-mm probes should therefore be carefully interpreted in terms of intra-polyp heterogeneity.

We measured less acute initial slopes (α) in RLCs with the 1-mm and the 140-μm probe compared to the 8-mm probe, while α was constant at all levels when the 1-mm and 140-μm probes were used on coenosarc tissue. Given the corallite structure of *Acropora nobilis*, it can be suggested that the coenosarc tissue would be more sun-adapted than polyp tissue and therefore would have a less acute α than polyp tissue. Thus, for *A. nobilis* and probably other corals, it is clear that the 8-mm probe yields photosynthetic information heavily weighted towards coenosarc tissue in comparison with polyp tissue.

In addition to photoprotective responses of coral polyps, and the effects of contraction/expansion of polyps mentioned above, the presence of the fibre probe close to the coral will affect the local flow pattern and the complex diffusive boundary layer surrounding the coral. It has been found that boundary layers associated with coral polyps are heterogeneous, owing to the complex micro-topography of the surface (Patterson 1992; Shashar et al. 1993; de Beer et al. 2000). A change in the diffusive boundary layer due to the presence of the fibre-optic probe affects the local chemical gradients, which again affect the host and symbiont metabolism and therefore may lead to changes in fluorescence signals and derived parameters. Therefore, the assumption that the deployment of probes, especially the large fibre probes, is non-invasive should be regarded with caution, and the use of smaller fibres is recommended.

Polyp versus coenosarc tissue

In attempting to compare the six coral species (Fig. 2) some caution must be exercised: firstly, there is a range of different morphological forms, and secondly, the absorption coefficient of each species could be different and therefore we describe only the relative ETRs. The wide variation in polyp RLCs (Fig. 2) indicates the diversity of microhabitats within a single colony, and how the light history alters the photosynthetic activity of an individual polyp.

The occurrence of different types of symbionts in the same host suggests a possible explanation for changes in photosynthetic activity along a branch and between branches of a single colony (Rowan and Knowlton 1995). Spatial variation observed here can be explained both by genetic diversity (two distinct patterns in Fig. 2) and by photo-acclimation. Hosts with more than one type of symbiont could be linked to the variation in photo-adaptation of what we have previously called sun- and shade-adapted regions of the colony. The extreme down-regulation of *Porites cylindrica* at elevated irradiances could be explained as a protective mechanism to dissipate excess radiant energy in shallow high-light environments, or it could simply be due to the concentration of a particular taxon of symbionts in sun-lit regions. Since these specimens were from a massive coral and samples were taken from the upper surface, high-light levels would be a regular stress. The other massive coral, *Cyphastrea serailia*, had a similar series of RLC's.

The other growth forms (plate or branching) appear to offer the possibility either for genetic strains or photo-adaptation to protect and shade polyps, and in some, this even applies to coenosarc tissue. Thus, *Montipora tuberculosa* (plate-growth form) showed a response similar to that of the massives for the coenosarc (Fig. 2e), while the polyps showed less down-regulation. The branching species, such as *Acropora nobilis*, had polyps which showed little down-regulation and the coenosarc tissue samples had both high relative ETR_{max} and little down-regulation. While the mechanism for down-regulation has not been investigated here, Brown et al. (1999) have shown that q_N is associated with the xanthophyll cycle.

The routine use of a binocular microscope with the Microfibre PAM alerts the investigator to any retraction of the polyps; in the present investigation, retracted polyps were avoided. The expansion and contraction of *Aiptasia pulchella* tentacles altered the distribution and orientation of zooxanthellae, therefore affecting immediate light climate (Muller-Parker 1987). Movement of the tentacle during the elaboration of a RLC causes the data to vary unpredictably, and should be avoided. Polyps and tentacles contract when disturbed by the light or touch of the fibre-optic probe.

Down-regulation of a single *Pocillopora damicornis* polyp

The progressive down-regulation of a single polyp and its subsequent recovery have never been monitored in situ before (Fig. 3). The initial RLC of the control sample that was acclimated to ~50 μmol photons m^{-2} s^{-1} showed very efficient use of the lower intensity irradiance ($\phi_p = 0.63$), and became down-regulated at irradiances > 500 μmol photons m^{-2} s^{-1}. The down-regulation was rapid and therefore can be assumed to be associated with q_N and the xanthophyll cycle, which in other systems is rapidly entrained (Schreiber and Bilger 1987; Demmig-Adams and Adams 1996). The control induction curve (Fig. 4a) indicated that q_N was effectively entrained during exposure to 350 μmol photons m^{-2} s^{-1}, the actinic intensity during the induction curve. Non-photochemical quenching increased in the 1st min, and then decreased after 2 min (Fig. 4a and c), at which point it can be inferred that the Calvin cycle became fully operational (Schreiber et al. 1994). After 1 h of exposure to 500 μmol photons m^{-2} s^{-1} (not a particularly high irradiance) q_N was initially high; i.e. photosynthesis was strongly down-regulated, but during 3 min of moderate irradiance, this fell to zero, with concomitant increases in effective quantum yield (ϕ_p).

The recovery of the high-light-exposed coral polyp followed a similar pattern (Fig. 3), where there was rapid recovery over the first 20 min and a slower phase

of recovery lasting up to 3 h. However, the 4- and 6-h RLCs began to show increased down-regulation at high irradiances (Ralph et al., unpublished data). A much slower recovery period was found when the exposure period was longer. From the gradual increases in both F_o and F_m in the 4-h recovered sample, it can be shown that the tissue was returning to a photosynthetically active condition from the down-regulated state. Thus, our data support a dynamic down-regulatory mechanism common to all corals investigated, as has been previously suggested (Ralph et al. 1999).

The present results provide little evidence for photoinhibition in corals (i.e. damage to photosystem II). The down-regulatory response shown for the single polyp is supported by the recent findings of Hoegh-Guldberg and Jones (1999); however, these first results need further investigation. We show here that coral photobiology is not constant for a coral colony, and that different shade- and sun-adapted responses in coral polyp and coenosarc tissue confound the interpretations of many studies. This effect is also present for the 8-mm fibre-optic probe in our study, whilst the smaller fibre-optic probes (1 mm and 140 μm) reveal the more specific tissue responses to light.

The larger fibre-optic probe collected an integrated signal from a variety of tissues, whilst the smaller fibre-optic probes were able to target a particular tissue type. For *A. nobilis,* we conclude that the fluorescence signal measured by the 8-mm fibre-optic probe was dominated by coenosarc tissue, since the polyp signal was considerably smaller. This first photobiological investigation of the fine-scale variation of chlorophyll-*a* fluorescence in corals has indicated the usefulness and necessity of performing such analyses at the finer scale. The pattern of photosynthetic activity varied greatly between the polyp and coenosarc tissues for different species. We were able to follow the down-regulation of photosynthesis in a single polyp of *Pocillopora damicornis*, after exposure to moderate irradiance, and its recovery in shade over a further 4 h. Overall, our study indicates that coral photobiology at the scale of the polyp is strongly heterogeneous, and more studies of microenvironmental controls of coral photobiology are needed, now that various experimental tools for microscale analysis have become available (reviewed in Kühl and Revsbech 2001).

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