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S. Beer · M. Ilan · A. Eshel · A. Weil · I. Brickner

Use of pulse amplitude modulated (PAM) fluorometry for in situ measurements of photosynthesis in two Red Sea faviid corals

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Abstract Measurements of the photosynthetic activity of symbiotic zooxanthellae in corals under natural growth conditions has been limited until recently, and this is one of the first reports on utilising a newly developed underwater pulse amplitude modulated (PAM) fluorometer (the Diving-PAM, Walz Gmbh, Germany) for such studies in situ. Photosynthetic responses to irradiance (photosynthetic photon flux, PPF) of the two faviid corals *Favia favus* (Forskål) and *Platygyra lamellina* (Ehrenberg) were measured while snorkelling or SCUBA diving (in August 1997), and we report here the results in terms of effective quantum yields of photosystem II (Y) and estimated rates of photosynthetic electron transport (ETR, calculated as $Y \times 0.5 \times \mathrm{PPF} \times \mathrm{FA}$, where FA is the estimated fraction of light absorbed by the photosymbiont-containing tissue). Both species showed a reduction in Y with increasing actinic irradiances produced by the instrument above 500 μmol photons m^{-2} s^{-1}, and the corresponding ETR values yielded apparently typical photosynthesis versus irradiance (*P-I*) curves, which saturated between 1500 and 2000 μmol photons m^{-2} s^{-1}. It was found that 30 s irradiation at each PPF level was sufficient to give optimal ETR values and, therefore, each *P-I* curve could be obtained within a few minutes. In situ point measurements from various areas of colonies under ambient light showed average ETR values within the range expected from the *P-I* curves. In order to test the Diving-PAM in an eco-physiologically relevant experiment, photosynthetic ETR versus PPF was measured for three sections of a large *P. lamellina*, each section of which received different natural irradiance levels. The results clearly demonstrated adaptations to the ambient light field in that vertical and downward-facing portions of the colony showed gradually lower maximal ETRs, steeper initial slopes of the *P-I* curves and, accordingly, lower light saturation points than upward-facing areas receiving higher light levels. Based on these trials, some evaluations are given as to the applicability of the Diving-PAM for photosynthetic measurements when monitoring similar corals.

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S. Beer (✉) · A. Eshel · A. Weil
Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel

M. Ilan · I. Brickner
Department of Zoology, Tel Aviv University, Tel Aviv 69978, Israel

Introduction

Many corals contain photosymbiotic unicellular algae, generally called zooxanthellae, within their tissues. These algae both enhance calcification and provide photosynthate for the nutrition of the colony and are, thus, an essential component of reef-building corals growing in oligotrophic waters (Barnes and Chalker 1990). The photosynthetic performance of this constituent of corals is usually evaluated in studies where either O_2 evolution or $^{14}CO_2$ uptake are measured (see Muscatine 1990 for a review). However, such measurements are hampered by the need for detaching and/or enclosing the corals or parts thereof. Therefore, non-intrusive in situ photosynthetic measurements under ambient conditions have been limited so far.

Measurements of in vivo chlorophyll fluorescence induction has been used for estimating the potential quantum yield of photosystem II (PSII) in dark-adapted macrophytes, and the development of pulse amplitude modulated (PAM) fluorometry has also made it possible to measure the effective quantum yield of PSII (Y) under ambient light (Schreiber et al. 1986; reviewed by Schreiber and Bilger 1993). In this method, Y is defined as $(F'_m - F)/F'_m$, where F'_m is the maximal fluorescence yield in a light-adapted plant following a saturating-light pulse and F is the normal fluorescence in the light; this parameter (Y) has been shown experimentally to be directly proportional to the quantum yield in some

terrestrial plants as measured by CO_2 uptake (Genty et al. 1989). Good correlations between photosynthetic rates based on Y and those measured by O_2 evolution were also recently reported for the cyanobacterial photosymbionts of some lichens under limited light conditions (Sundberg et al. 1997).

While PAM fluorometers have been used for estimating photosynthetic efficiencies of coral photosymbionts in vivo (Warner et al. 1996), a newly developed underwater PAM fluorometer has now made it possible to perform such measurements on benthic organisms in situ. Because this so-called Diving-PAM was introduced only in April 1997, data resulting from its use in research of photosymbiotic photosynthesis are still restricted; one report on an ascidian (Schreiber et al. 1997) has been published and Ralph et al. (in preparation) have contributed some data on reef dwellers, including several corals, but other studies are probably in progress. Here, we report results of initial trials using the Diving-PAM for in situ measurements of photosynthetic responses to light in two Red Sea faviid corals.

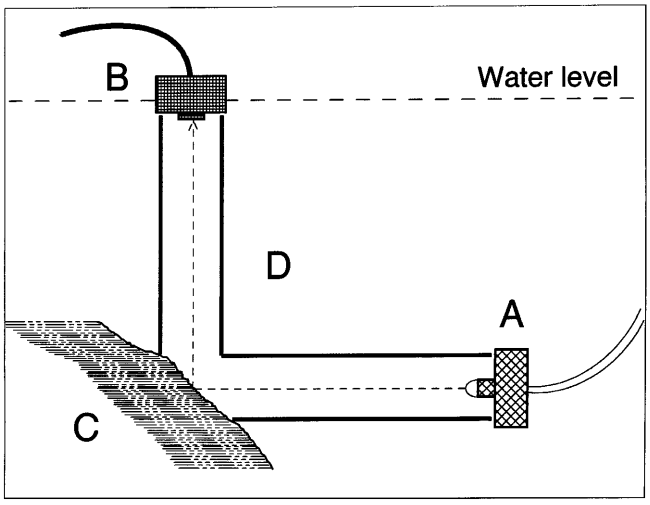

Fig. 1 Schematic drawing of the installation used for measuring intensity of reflected light (*A* light source; *B* light sensor; *C* live coral colony or skeleton; *D* black plastic tubing, 10 mm i.d., the two arms are at 90° to one another and 45° to the surface)

Materials and methods

Two faviid corals, *Favia favus* (Forskål) and *Platygyra lamellina* (Ehrenberg) from the Red Sea, were studied in situ at the coral reef off Eilat, Israel (37°54′E; 31°35′N), either by snorkelling or SCUBA diving. These species were chosen because of their relative abundance, and because their flat surfaces (compared with branching corals) facilitate measurements with the Diving-PAM. Usually, 0.1 to 0.3 m diameter coral heads were measured at depths of 3 to 4 m; in one case, photosynthetic responses to light were measured on distinct areas of a large *P. lamellina* (about 1 m diameter) which received differing levels of ambient irradiances. This hemispherical colony was part of a large knoll, and had surface areas which faced upward (at 0.5 m depth), to the side and downward (at a depth of 1.5 m). All measurements were done during August 1997, at an ambient water temperature of 25 °C.

The portable underwater fluorometer Diving-PAM (Walz Gmbh, Germany) was used for chlorophyll fluorescence measurements of the photosymbiotic components of the studied corals. For point measurements under ambient light, the main optical fibre was connected to a "leaf distance clip" (Walz's terminology), which allows for measurements of fluorescence at a 60° angle to the coral surface. The photosynthetic photon flux (PPF) light meter fibre was attached to the clip in such a way that it measured incident light at a 90° angle to the coral surface; this meter was always pre-calibrated against a quantum sensor of a Li-Cor (USA) LI-189 light meter. The Diving-PAM settings of the "measuring light intensity" and "gain" had to be increased to 10 and 6, respectively, in order to give optimal fluorescence signals for these two corals.

For the generation of photosynthesis versus irradiance (*P-I*) curves, the "coral clip" was attached to the coral surface by rubber-banded hooks. After 10 to 20 s of darkness, an automatic series of measurements was initiated using the internal actinic (photosynthesis-inducing) light source. The irradiance levels (determined by the "actinic light intensity" and "actinic light factor") were varied between different runs so as to obtain curves which reached into the saturation range, and the irradiance duration at each light level was also varied. For these *P-I* curves, the actual actinic light levels that had reached the measured coral surface were determined separately. Here, the tip of the Diving-PAM's PPF meter light guide was attached to the bottom of the coral clip so that it received the same irradiances as had the coral.

The fraction of light absorbed by the photosymbiont-containing coral tissue was estimated in the laboratory using the installation shown in Fig. 1. The tip of a fibre-optically guided halogen light source (KL1500, Schott, Germany) and the quantum sensor of a Li-Cor LI-189 light meter were inserted into the two ends of a V-shaped black plastic tube (10 mm i.d.). The cut base of the V-tube was pressed against the surface of the coral colony, or a skeleton of the same coral from which the tissue had been removed by a water-pick and Na-hypochlorite, or a mirror, allowing only light reflected at 45° to reach the sensor. The fraction of the incident light absorbed by the living tissue (FA) was calculated as $(R_s - R_c)/R_t$, where R_t is the incident irradiance reflected by the mirror, R_c is the irradiance reflected by the live coral and R_s that reflected by the coral skeleton. These measurements were carried out under water, and it was assumed that R_c resulted mainly from light reflected by the skeleton underlying the tissue, and not from the live tissue surface. This value, FA, was then used instead of the standard "ETR-factor" for estimating absolute values of ETR (in μmol electrons m^{-2} s^{-1}) as $Y \times$ PPF (in μmol photons m^{-2} s^{-1}) $\times$ FA $\times$ 0.5 (the ratio of electrons transported per photons PPF absorbed by the two photosystems, assuming that they each utilised 50% of the absorbed photons).

Results

The FA values of corals growing at 3 m depth were found to be 0.036 ± 0.005 for *Favia favus* and 0.023 ± 0.003 for *Platygyra lamellina*, and they were used here as the "ETR-factors" for estimating photosynthetic ETRs. Although these values are far lower than the 0.84 default value of the Diving-PAM given for a "normal" terrestrial leaf, they may still be overestimated due to the assumption that light absorbed by the living tissue was absorbed by the pigment antennae of the zooxanthellae only and that there was no reflection from the live tissue surface (but from the skeleton only). On the other hand, these values may be underestimated due to a shallow penetration of the "measuring light" into the tissue (see below). Also, light absorption was measured only from a 45° angle to the average coral surface, and FA was determined only for colonies growing at 3 m depth; possible differences between

colonies depending on varying topography or adaptations to varying light fields for their growth were ignored.

Response curves of *Y* and ETR versus PPF are given for *Favia favus* in Fig. 2 and for *Platygyra lamellina* in Fig. 3. The shapes of the *Y* versus irradiance curves are similar in all cases, with a general decrease in *Y* at higher irradiances preceded by a shoulder from the lowest light applied, between 500 and 1000 µmol photons m^{-2} s^{-1}. This range, in which there is no clear reduction in *Y* with increasing light, represents the range of PPF for which the low frequency of closure of the PSII reaction centres does not reduce the photosynthetic electron flow significantly and where, therefore, the photosynthetic rate is in direct relationship to the PPF level. There was no substantial difference in the response of *Y* and ETR to irradiance times of 30 and 60 s for *F. favus*; 30 and 50 s irradiance times gave similar values for *P. lamellina*. From these and similar experiments, it was determined that 30 s of actinic irradiation at each light level was enough to yield reliable *P-I* curves for these two faviid corals, and the "actinic light width" was therefore set to 30 s for all subsequent experiments.

The capability of the Diving-PAM to furnish rapid *P-I* curves is further illustrated in Figs. 4 and 5. Each of the colonies of *Favia favus* and *Platygyra lamellina* yielded a different curve, but the general shape of all curves was similar. Again, a characteristic shoulder in *Y* was usually observed at low irradiances, and light

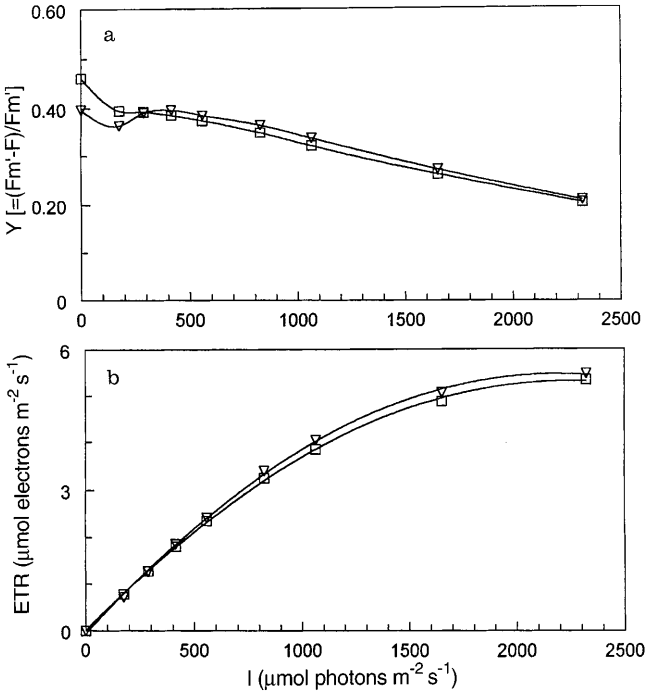

Fig. 3 *Platygyra lamellina.* **a** Effective quantum yields of PSII (*Y*) and **b** photosynthetic electron transport rates (*ETR*) as functions of irradiance (*I*, measured as PPF) for *P. lamellina* growing at 3 m depth. The two curves were obtained for different areas of the same colony by supplying either 30 s (▽) or 50 s (□) of actinic light at each of the different irradiance levels

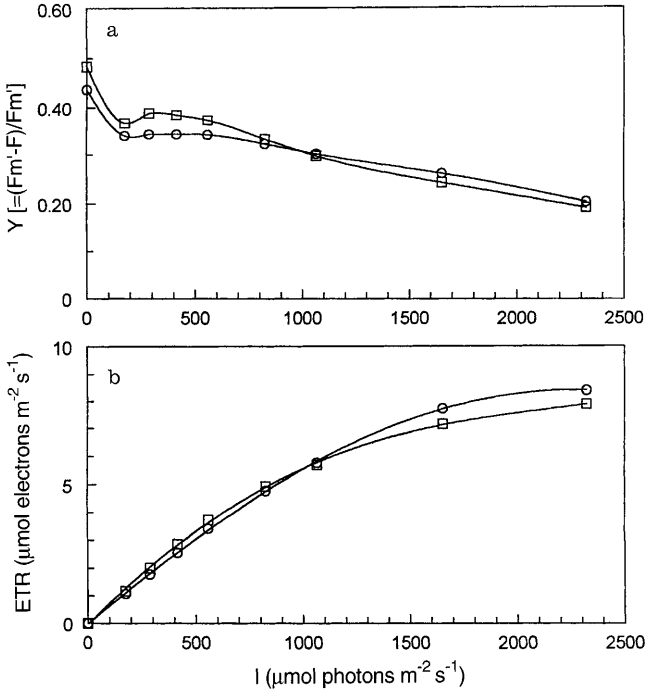

Fig. 2 *Favia favus.* **a** Effective quantum yields of PSII (*Y*) and **b** photosynthetic electron transport rates (*ETR*) as functions of irradiance (*I*, measured as PPF) for *F. favus* growing at 3 m depth. The two curves were obtained for different areas of the same colony by supplying either 30 s (○) or 60 s (□) of actinic light at each of the different irradiance levels

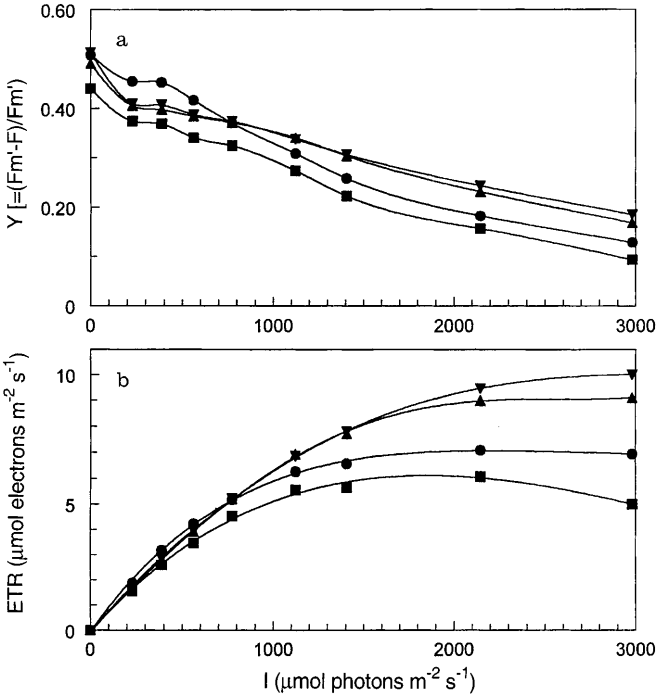

Fig. 4 *Favia favus.* **a** Effective quantum yields of PSII (*Y*) and **b** photosynthetic electron transport rates (*ETR*) as functions of irradiance (*I*, measured as PPF) for four different colonies of *F. favus* growing at 4 m depth. The duration of irradiance at each level of actinic light was set to 30 s

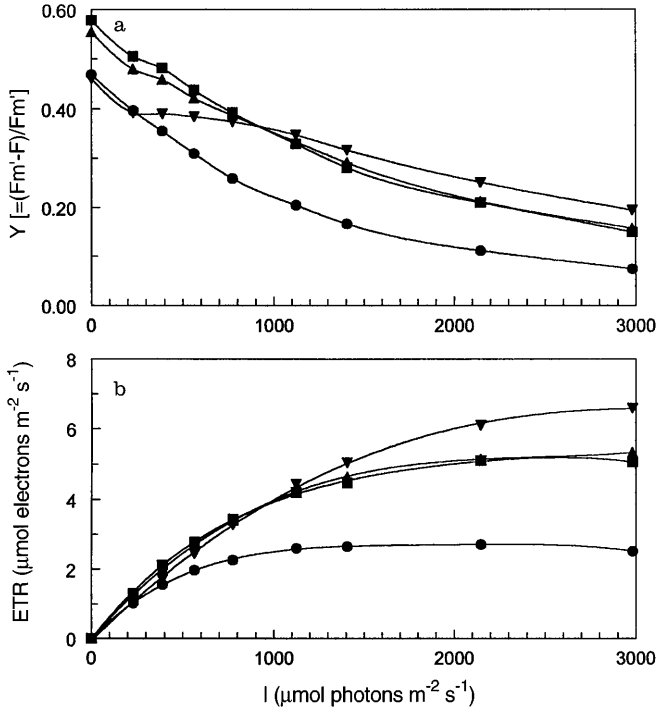

Fig. 5 *Platygyra lamellina*. **a** Effective quantum yields of PSII (*Y*) and **b** photosynthetic electron transport rates (*ETR*) as functions of irradiance (*I*, measured as PPF) for four different colonies of *P. lamellina* growing at 4 m depth. The duration of irradiance at each level of actinic light was set to 30 s

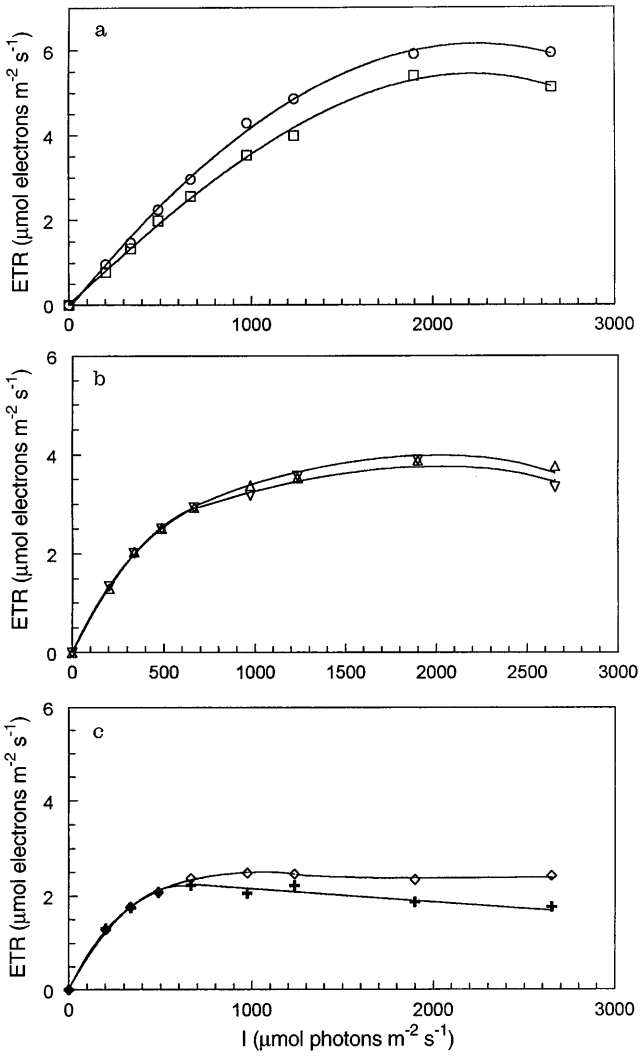

Fig. 6 *Platygyra lamellina*. Photosynthetic electron transport rates (*ETR*) as functions of irradiance (*I*, measured as PPF) measured at various areas of a large hemispherical-shaped *P. lamellina* colony. **a** The top, upward-facing, part of the colony (receiving ca. 500 µmol photons m^{-2} s^{-1} at midday); **b** the side (receiving ca. 100 µmol photons m^{-2} s^{-1}); **c** the downward-facing bottom (ca. 20 µmol photons m^{-2} s^{-1}). The two curves of each area of the coral represent two replicate *P-I* measurement series close to one another. The duration of irradiance at each level of actinic light was set to 30 s

saturation occurred between 1500 and 2000 µmol photons m^{-2} s^{-1}. Since the various corals grew at similar depths and, thus, under similar light climates, it is assumed that the discrepancies in saturation levels and maximal ETRs reflect intrinsic differences between the different colonies other than adaptations to irradiance.

Point measurements on two larger specimens (ca. 0.4 m diameter) under ambient light showed ca. 15 and 30% standard deviations for *Y* and ETR, respectively, at different areas of the *Favia favus* individual, while the *Platygyra lamellina* showed more uniform values (Table 1). The average *Y* and ETR values obtained were somewhat below those which could be derived from the *P-I* curves.

Photosynthetic responses to increasing actinic light were also measured for various areas of the same large *Platygyra lamellina* colony which received very different ambient irradiances (Fig. 6). The top part, which was exposed to ca. 500 µmol photons m^{-2} s^{-1} during midday, showed photosynthetic saturation at around 2000 µmol photons m^{-2} s^{-1}, and the ETR was 5.5 µmol

Table 1 *Favia favus*, *Platygyra lamellina*. Effective quantum yields of PSII (*Y*) and photosynthetic electron transport rates (*ETR*) for *F. favus* (growing at 4 m depth under 150 to 200 µmol photons m^{-2} s^{-1} photosynthetic photon flux, *PPF*, at 1430 hrs) and *P. lamellina* (growing at 3 m and 400 to 500 µmol photons m^{-2} s^{-1} at noontime). Point measurements were done in situ using the leaf distance clip to which both the main light guide and the tip of the PPF light guide were fixed. Data are averages of 12 measurements ± SD

	PPF (µmol photons m^{-2} s^{-1})	*Y* [$= (F'_m - F)/F'_m$]	ETR (µmol electrons m^{-2} s^{-1})
F. favus	173 ± 29	0.239 ± 0.032	0.76 ± 0.22
P. lamellina	431 ± 43	0.270 ± 0.017	1.33 ± 0.15

electrons m^{-2} s^{-1} at that light level. On the other hand, the lower part of the colony, facing downward and receiving only ca. 20 µmol photons m^{-2} s^{-1}, showed a steeper initial slope of the *P-I* curve, saturating at ca. 600 µmol photons m^{-2} s^{-1}, and reached an ETR of 2.3 µmol electrons m^{-2} s^{-1}. A middle, vertically facing section of the same coral which received ca. 100 µmol photons m^{-2} s^{-1} during mid-day showed values which were intermediate between the upward- and downward-facing sections.

Discussion

The results presented here, together with those of another investigation (Ralph et al. in preparation), have demonstrated the feasibility of using PAM fluorometry for estimating photosynthetic responses in situ of corals bearing photosymbionts. The foremost advantage of the Diving-PAM is its non-intrusive mode of operation, and our results also support the notion of the previous workers that series of short-term irradiances result in reliable *P-I* curves for corals. However, a few points should be taken into account by potential users of the Diving-PAM for similar future work. For point measurements of ETR under ambient light, it is important to also measure the actual irradiance reaching the same spot on the colony for which *Y* is measured. The present version of the Diving-PAM does not provide for such an option, and we therefore had to adapt the tip of the fluorescence measuring fibre to accommodate the light-sensitive tip of the PPF meter light guide. Regarding the latter fibre-optical light guide, it was found to be sensitive to bending so that significantly (up to 30%) different values of PPF could be obtained depending on the degree of bending. This meter should also always be calibrated against a better PPF measuring instrument. Concerning the internal halogen light source which provides actinic light, it was found that irradiance levels decreased during the time of each irradiance step, and this was especially significant at high light levels. Here, it was decided to use the PPF value obtained at the end of each irradiance period as measured separately from the field measurements. It should also be noted that the light spectrum of the lamp changes with changing irradiance levels, and that this could somewhat affect the photosynthetic performance and, thus, the outcome of these measurements. On the brighter side, the instrument operated very reliably during the ca. 10 000 measurements we have done so far, and it was easy to use under water. Also, the option of creating successive levels of irradiances during user-defined time periods rendered it very convenient in obtaining repeatable uniform *P-I* curves within minutes.

While $Y \times$ PPF can be used as a relative measure of ETR, absolute ETR values calculated from PAM fluorescence measurements must be based on the amount of light absorbed by the photosynthetic antennae chlorophyll or, in the case of these corals, at least by the zooxanthella-containing tissue. While we agree with Falkowski et al. (1990) that "measuring the light absorbed [in corals] is almost impossible", we still tried to estimate the fraction of the incident light absorbed by the living tissue of "normal" corals growing 2 to 3 m deep. Although the presently studied species feature more smooth surfaces than most other corals, such estimations are hampered by the complex topography of the skeleton which reflects part of the incident light. Further complications in quantifying the ETR of any photosynthetic tissue by PAM fluorometry include that pigments other than chlorophyll may also absorb light and that the red "measuring light" of the Diving-PAM may penetrate the tissue more shallowly than the incident actinic PPF. Given these uncertainties, the absolute ETRs given here should be taken as approximations only pending better ways to determine FA. The values obtained (only 2.3 to 3.6% was absorbed) yielded, within these limitations, ETRs of 6 to 8 µmol electrons m^{-2} s^{-1}. In order to compare these rates with others, measured by the use of O_2 electrodes, we may use the range of 19 to 101 µg O_2 cm^{-2} h^{-1} as compiled by Muscatine (1990) for a number of corals (but not for faviid forms). This range translates to 3.3 to 18 µmol O_2 m^{-2} s^{-1} or, assuming 4 mol electrons mol^{-1} O_2 evolved in photosynthesis, 13 to 70 µmol electrons m^{-2} s^{-1}. While our rates thus are below those obtained for other corals by gas exchange measurements, especially if the latter were to be corrected for respiration, the high light saturation points are close to those measured for other species (e.g. Lesser 1997), including the intensively studied *Stylophora pistillata* from the Red Sea (e.g. Falkowski and Dubinsky 1981). The increasingly steeper initial slopes of the *P-I* curves for *Platygyra lamellina* when growing at decreasing irradiances also conforms with many such observations in other corals. This phenomenon has generally been attributed to enlarged chlorophyll antenna complexes (e.g. Falkowski and Dubinsky 1981) and, if so, then a higher fraction of incident light is also absorbed per surface area of a low-light growing coral. Again, the absolute ETRs resulting from these experiments should be used as a first approximation only, but relative ETR values ($Y \times$ PPF) would show exactly the same differences in initial slopes, light saturation points and rates.

The PAM fluorometer renders *Y* and, consequently, ETR values in the light by measuring chlorophyll fluorescence as quenched by photosynthetic electron flow through PSII. Therefore, only gross photosynthesis is measured. In studies where photosynthetic gas exchange or energy regimes are sought, PAM fluorometry should ideally be complemented by methods in which respiration rates can be measured. On the other hand, we have shown that PAM fluorometry alone can yield relevant results when investigating responses of the photosynthetic apparatus per se to light utilisation in experiments such as the one illustrating adaptations to varying ambient light conditions. This renders the Diving-PAM potentially useful also for in situ monitoring of the

general condition of corals as based on those photosynthetic parameters measured here.

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