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In situ measurement of gross photosynthesis using a microsensor-based light-shade shift method

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Abstract

We present a measuring procedure that allows the quantification of gross photosynthesis at the ambient light intensity, P(I), from light transition measurements similar to those employed in the light-dark shift method but without the necessity of achieving complete darkness. The method is thus more readily applicable in situ, where, during daylight, complete sample darkening is very difficult or even impossible to achieve. The procedure involves rapid microsensor-based monitoring of oxygen in the sample during a series of light transitions from ambient light intensity to a few intermediate levels, and determination of the initial rates of oxygen decrease during each partial darkening (shading) period. P(I) is then recovered by fitting the measured rates with a function derived from the model describing the change in photosynthesis rate with light intensity (P-I curve) and extrapolating it to the full light-to-dark transition. We validated this approach in the lab on coral and microbial mat samples and found that a satisfactory estimate of P(I) can be obtained with as few as 4-5 shade levels. We also applied the procedure in situ and showed that the gross photosynthesis rate at the ambient sunlight in the microbial mat and in the coral reached, respectively, approximately 50% to 65% and 94% to 97% of the saturated rate.

Introduction

The microsensor-based light-dark shift method, introduced more than two decades ago by Revsbech and Jørgensen (1983, 1986), has been widely used to quantify with submillimeter spatial resolution the primary production in densely stratified biological systems, such as microbial mats (e.g., Camacho and De Wit 2003; Jonkers et al. 2003; Wieland et al. 2003; Benthien et al. 2004; Fourçans et al. 2004; Ludwig et al. 2005, 2006; Pringault et al. 2005; Wieland and Kühl 2006; Abed et al. 2006), biofilms (Kühl et al. 1996; Hancke and Glud 2004), coral zooxanthellae (Kühl et al. 1995; De Beer et al. 2000; Al-Horani et al. 2003), and sponges (Schönberg et al. 2005). It involves rapid monitoring of oxygen concentrations in the sample during its prolonged exposure to constant photosynthetically active radiation (PAR) followed by a short (2-3 s) period of complete darkness. Oxygen concentration in the measured sample point, denoted as c, which reaches steady state during the prolonged PAR exposure, will start to decrease immediately after PAR is switched off, as a result of the disturbed balance between photosynthesis, respiration, and diffusive transport in and around the measuring point. Assuming that respiration rates in the sample in the light and during the few seconds of darkness succeeding the light period do not change, the volume-specific rate of gross photosynthesis, P, in the sample point during the illumination period equals the initial rate of O_2 decrease measured during the dark period (Revsbech and Jørgensen 1983, 1986):

$$P(I) = -[dc/dt]_0, \quad (1)$$

where subscript 0 refers to the light transition I→0.

This approach has been widely used under laboratory conditions, where the light-dark transitions can easily be achieved either by blocking the light source (e.g., a lamp) illuminating the sample with an opaque object (e.g., Revsbech and Jørgensen 1983), or by rapidly switching the light on/off electronically (e.g., Polerecky et al. 2007). Such "luxury" of a complete control

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over the illuminating light is, however, very difficult or even impossible to achieve in situ, where the sample is illuminated by the sunlight. Since rapid and complete darkening of the sample, i.e., the transition I→0, is a fundamental prerequisite for the light-dark shift method to give accurate values of P at the given illumination intensity I, implementation of this method is not straightforward outside a lab.

Using a standard laboratory microsensor system, which typically comprises a fast Clark-type oxygen microelectrode attached to a (motorized) micro-manipulator mounted on a heavy stand, a pA-meter, and a data recording unit (see, e.g., Polerecky et al. 2007), one can readily measure physico-chemical gradients not only in the lab but also in shallow-water systems, such as microbial mats and biofilms found in shallow ponds or rivers (Jonkers et al. 2003; Fourçans et al. 2004; Bissett et al. 2008). A recent technical development of a diver-operated submersible microsensor system (Weber et al. 2007) made it possible to conduct delicate microsensor measurements that can be fully controlled by the operator (e.g., to accurately position the microsensor tip to a selected point in the sample) also under water. Thus, one logical application of such systems would be to quantify the rates of gross photosynthesis in situ. However, considering the difficulties associated with the need of a complete sample darkening mentioned above, a new approach is required to allow such measurements.

As an immediate solution to this practical problem, one may propose using partial darkening (shading) of the sample, i.e., decreasing the ambient PAR intensity I to an intermediate level $0 < I_j < I$, and calculating the gross photosynthesis rate as a linear extrapolation of the measured initial rate of the O_2 concentration decrease $[dc/dt]_j$ to the full I→0 transition, i.e.,

$$P(I) \approx -[dc/dt]_j \times I/(I-I_j). \quad (2)$$

This procedure is, obviously, correct only under the assumption that the relation between the gross photosynthesis rate, P, and the PAR intensity, I, is linear. Such an assumption is valid, and thus this measurement principle is applicable, only over a limited range of intensities. It is well documented that as a result of finite rates of electron transfer in the photosynthetic apparatus of a phototrophic cell, the increase of P with I slows down above a certain intensity level, and photosynthesis eventually reaches some saturated rate P_{max}. This saturation of photosynthesis with increasing light intensity can be modeled by several suitable functions (Jassby and Platt 1976), one of which is the mono-exponential function proposed by Webb et al. (1974):

$$P(I) = P_{max}\,[1 - \exp(-I/E_a)], \quad (3)$$

where E_a is the onset of photosynthesis saturation. Thus, a correct approach for the estimation of P(I) should emerge by combining the idea of partial sample darkening (shading) with the knowledge of the nonlinear behavior of P versus I.

Here we present a measuring procedure that allows the quantification of gross photosynthesis at any ambient PAR intensity but does not require achieving complete darkening of the sample. The procedure involves rapid microsensor-based monitoring of O_2 in the sample during a series of light transitions from the ambient light intensity to a few intermediate levels (shades), and determination of the initial rates of O_2 decrease during each partial darkening (shading) period. Since rapid shading of the measured sample, e.g., by moving an opaque obstacle above or next to the sample so as to partially block the illuminating PAR (e.g., the ambient sunlight), is much easier than complete darkening, the method is more readily applicable for in situ measurements. We validate this new approach under laboratory conditions using a lamp as the source of PAR, and apply it to quantify gross photosynthesis rates in microbial mats and coral zooxanthellae under in situ and natural light conditions.

Materials and procedures

Theoretical basics—The new method is based on the assumption that the dependence of the gross photosynthesis rate (P) on the ambient PAR intensity (I) is described by some generally nonlinear function P(I), referred to as the P-I curve. Although the method is, in general, independent of the actual form of the function, we chose the exponential function described by Eq. 3. Based on this assumption, it is straightforward to realize that by measuring gross photosynthesis at several light levels, one can reconstruct the P-I curve, determine the parameters P_{max} and E_a in Eq. 3, and thus allow prediction of P at any given light intensity I.

Typically, the P-I curve in dense microbial systems is determined by measuring gross photosynthesis using the traditional microsensor-based light-dark shift approach at gradually increasing intensities of the ambient light (e.g., Wieland and Kühl 2006). Here, we propose a different approach, which does not require manipulation of the ambient light. Namely, we propose to maintain the ambient light intensity constant and, instead, use shading to decrease the light intensity illuminating the sample.

When the sample is illuminated by the full ambient light intensity, I, photosynthesis operates at a full rate, P(I). When the illumination is decreased, e.g., by shading the sample with a filter of transmission T_j, photosynthesis will operate at a decreased rate, $P(T_jI)$. Using Eq. 3, it is easy to see that for a given ambient light intensity, I, the decrease in the gross photosynthesis rate, denoted as $\Delta P_j = P(I) - P(T_jI)$, decreases exponentially with T_j:

$$\Delta P_j(T_j) = P_{max}\,[\exp(-T_jI/E_a) - \exp(-I/E_a)]. \quad (4)$$

In the same way the initial rate of the O_2 decrease upon sample darkening corresponds to the local gross photosynthesis rate in the traditional light-dark shift method (Eq. 1; Revsbech and Jørgensen 1983), the initial rate of the O_2 decrease upon sample *shading* represents the *decrease* in the local gross photosynthesis rate, i.e.,

$$\Delta P_j(T_j) = -[dc/dt]_j, \quad (5)$$

where subscript j refers to the light transition $I \rightarrow T_jI$. Note that

the quantification of $\Delta P_j(T_j)$ does not require that the steady state O_2 concentration at the measured point has been fully reached. When the O_2 concentration is slowly increasing or decreasing before shading, e.g., due to slowly increasing or decreasing ambient light intensity, the decrease in photosynthesis rate, $\Delta P_j(T_j)$, can be evaluated as the difference between the rates of O_2 decrease immediately before and after shading. This follows from the same mass balance considerations as those thoroughly described by Revsbech and Jørgensen (1983).

At this point, it is useful to note that shading, i.e., $0 < T_j < 1$, is not the only possible way of altering the sample illumination. In the same way as shading leads to a decrease in photosynthesis rate, $\Delta P_j > 0$, addition of light to the sample, e.g., by illuminating it with an extra lamp, will increase the photosynthesis rate, i.e., $\Delta P_j < 0$. Assuming that the increase in photosynthesis from the rate at the ambient light level to that at the increased light intensity is immediate (within ~1 s), this approach will provide additional experimental points on the P-I curve, and may thus further increase confidence of the P-I curve prediction, especially in the high intensity range.

Samples—Two types of microbial mat samples and one coral species were used in this work. The first microbial mat sample originated from the hypersaline lake La Salada de Chiprana (NE Spain). It was collected in September 2005 and stored in an aquarium filled with in situ water (salinity 80 g L^{-1}, temperature 20°C) under a 16 h light/8 h dark illumination regime (250-300 μmol photons m^{-2} s^{-1}) for many months prior to the measurements. The composition and a more detailed functional description of the mat are given elsewhere (Jonkers et al. 2003). This mat was used only in the laboratory measurements.

The second mat sample originated from the Island of Elba (Italy) and was found in a rockpool, approx. 50 cm above the mid-water line. The mat has not yet been characterized in greater detail, but was dominated by *Rivularia atra*, a filamentous, sheath-forming cyanobacterium that can form dense irregular crusts up to 8 mm thick. The mat also contains carbonate precipitates at depth of ~2 mm, presumably as a result of calcification coupled to photosynthesis (Bissett et al. 2008). The salinity and temperature of the pool water varies greatly during the day (38-50 g L^{-1}, 23-35°C), as a result of exposure to intense sunlight (2000-2700 μmol photons m^{-2} s^{-1} on a sunny day) and, depending on the wind, frequent or sporadic flushing with the water from the sea. The investigated coral *Cladocora caespitosa* was found several meters off-shore of Elba Island at depth of ~5 m (salinity 39 g L^{-1}, temperature 23°C, in situ scalar irradiance on a sunny day of 1300-1500 μmol photons m^{-2} s^{-1}), which was also the location of the in situ measurements conducted on the coral. For laboratory measurements, small samples of the rockpool mat and the coral were collected in a Petri dish together with the in situ water, and brought to a nearby field station, where they were stored under in situ light conditions until the measurements.

Measurement setup—A fast Clark-type oxygen microelectrode (tip size ~2 μm, response time <0.5 s, stirring sensitivity <1.5%) with a guard cathode (Revsbech 1989) was used to measure oxygen concentrations inside the mat/coral tissue. For the laboratory and ex situ measurements, the microelectrode was assembled in a laboratory microsensor system, comprising a heavy stand, motorized stage, motorized micromanipulator, sensitive pA-meter, data acquisition device, and a portable computer (see, e.g., Polerecky et al. 2007). In situ measurements were conducted using a recently developed diver-operated microsensor system (Weber et al. 2007), comprising an adjustable stand, a motorized micromanipulator, a microsensor connected to an amplifier, and a data logger, all powered by a battery. Two-point linear sensor calibration was based on the readings in the water used in the measurements, which was bubbled with air and N_2 gas.

Light during the laboratory measurements used for method validation was provided by a fiber-optic halogen light source (KL 2500, Schott AG) equipped with a short-pass NIR filter, while all the remaining measurements were conducted in direct sunlight. Shading of the light produced by the lamp was done manually using gray filters (10 × 10 cm), which were made by laser-printing various shades of gray on standard transparency foils (Lexmark). Shading of the sunlight, both in air and under water, was done by placing gray neutral density foils (LEE filters #209, 210, 211, 298, and 299) attached to frames (~50 × 50 cm) between the sun and the sample, ~40 cm above the sample. Enhanced illumination was provided by underwater video lights (HID, Multitec). The scalar irradiance at the sample surface, at full light (I) or at the various shade/enhanced levels (I_j), was quantified using a spherical micro quantum sensor (US-SQS/L, Walz), which was connected to a light meter (LI-250A, LI-COR Biosciences) and positioned a few centimeters from the measuring point. Transmission coefficients were then calculated as $T_j = I_j/I$.

Rate measurements—The measuring protocol for the light-shade shift method is essentially the same as for the light-dark shift method (Revsbech and Jørgensen 1983). Namely, the microsensor was positioned in the point of interest inside the sample, and O_2 concentrations were recorded every 0.2-0.3 s. The sample was illuminated by ambient light intensity until the O_2 concentration in the measuring point reached steady state. Then the sample was shaded for a short period (2-3 s), during which the initial slope of O_2 decrease was determined, giving the value of ΔP_j (Eq. 5). This was repeated for as many different shade levels as possible (see below), so as to increase the confidence with which the P-I curve would eventually be reconstructed and thus the gross photosynthesis at the ambient PAR intensity predicted. The 2-3 s duration of the shading period was employed to limit the spatial resolution of the measured slopes $[dc/dt]_j$ to ~100 μm, as discussed by Revsbech and Jørgensen (1983). Each shading was carried out in triplicates to assess measurement's reproducibility.

To validate the concept of the proposed light-shade shift method, measurements were conducted in a dark laboratory, using a lamp as the source of PAR. Each sample was placed

separately in a small aquarium filled with in situ water bubbled with air. A pump was used to induce defined water flow above the sample surface. The measurements were realized using two protocols, each simulating a different quality of ambient light illumination that can be encountered in situ. In the first protocol, applied in the rockpool mat and coral measurements, the illumination intensity was adjusted to the peak value measured in situ during the day of the measurement, and a total of 5 transmission filters were used for shading (Table 1, lines 1-2). Two enhanced light levels were additionally used in the coral measurement (Table 1; $T_j > 1$). This protocol was employed to simulate the first and preferred measuring strategy, whereby the light-shade shift measurements are carried out during midday when the ambient illumination is maximum and approximately constant for a few hours, and with as many filters and enhanced light levels as possible. In contrast, the measurements in the Chiprana mat were conducted at 3 different ambient light intensities and using only 3 filters (Table 1, line 3). This was done to simulate the second possible strategy, whereby the measurements are done earlier or later in the day, when the ambient light intensity changes relatively fast, and the number of shadings performed when the illumination is approximately constant is limited.

Immediately after these measurements, traditional light-dark shift measurements were conducted at several defined intensities (corresponding to the intermediate light intensities during the light-shade shift measurements) to determine the true P-I curve. To facilitate direct comparison, the light-shade and light-dark shift measurements were conducted in the same point of the sample and with the same data acquisition timing protocol (see above).

Ex situ and in situ measurements were conducted during a clear day in July 2007 on Elba Island. On the measuring day, the disturbance of the rockpool by waves from the sea was frequent, which posed a high risk of damage to the microsensor system. Therefore, a small mat sample was collected in a glass beaker together with the in situ water (salinity 39 g L^{-1}, temperature 23°C), and the measurements were performed ex situ, ~5 m away from the site, immediately after the collection. Ambient sunlight intensity during the measurement and the applied transmissions are summarized in Table 1, lines 4-6. The light-shade shift measurements were conducted in 100 μm steps from the mat surface down to the carbonate precipitate layer (depth interval of 0-2 mm), always starting with the shading of the lowest transmission at each depth. Only when the lowest transmission resulted in a detectable change in oxygen concentrations were the shades with the higher transmission applied. Afterward, steady state O_2 profiles were measured at the ambient light intensity and in the dark (after sunset). All measurements were conducted in the same spot to allow comparative estimation of areal rates of gross and net photosyntheses and respiration, which were calculated as described previously (Kühl et al. 1996; Polerecky et al. 2007).

The in situ measurements in the coral tissue were performed while diving. One diver controlled the microsensor setup and gave instructions, while the other diver, when instructed, carefully shaded the sample with a filter and/or illuminated the sample with additional light, taking care to not disturb the water flow above the sample. The in situ scalar irradiance was monitored and recorded by a third person from a boat above (Table 1, lines 7-8). The measurements were conducted in several points in the coral tissue but only at a single depth to avoid sensor breakage by the coral skeleton.

Data analysis—For the data obtained at constant ambient light intensity, the measured pairs $[T_j, \Delta P_j]$ were fitted by Eq. 4, giving the best estimates of P_{max} and E_a together with their 95% confidence intervals δP_{max} and δE_a. These values were subsequently used to extrapolate Eq. 4 to $T_j = 0$, giving the best estimate of the predicted gross photosynthesis at the ambient light intensity, P(I), (note that $\Delta P[0] = P[I]$) and its 95% confidence interval δP(I). The measured values of ΔP_j were subsequently subtracted from the predicted value of P(I), giving the best estimates of the gross photosynthesis rate P_j at intensity T_jI. Similarly, the best estimate of the reconstructed P-I curve was calculated by subtracting the fitted curve $\Delta P_j(T_j)$ from the predicted value P(I), as follows from Eq. 4. The 95% prediction

Table 1. Summary of the experimental conditions during the laboratory (used for method validation), ex situ and in situ light-shade shift measurements, together with the parameters characterizing the P-I curve (Eq. 3) recovered for each measurement.*†

	Location	Sample	Light source	I	Transmission coefficients, T_j							P_{max}	E_a	Position
1	lab	rockpool mat	lamp	2630	0.04	0.06	0.09	0.21	0.38	–	–	5.67±0.10	950±108	–
2	lab	coral	lamp	1350	0.24	0.50	0.64	0.78	0.87	1.13	1.28	32.0±6.6	1771±692	–
3	lab	Chiprana mat	lamp	40, 130, 310	0.26	0.55	0.74	–	–	–	–	11.1±1.1	250±35	–
4	ex situ	rockpool mat	sunlight	1900	0.10	0.22	0.31	0.56	0.68	1.25	1.5	6.91±0.42	1425±211	1.8 mm
5				=	=	=	=	=	=	=	=	13.3±1.6	2726±536	1.9 mm
6				=	=	=	=	=	=	=	=	6.55±0.27	1529±154	2.0 mm
7	in situ	coral	sunlight	1000	0.10	0.20	0.4	0.50	0.75	1.8–2.6	–	11.2±0.6	488±77	spot 1
8				600	=	=	=	=	=	2–2.8	3.3–3.8	8.5±0.3	393±33	spot 2

*The parameters are shown as the best estimate ± the 95% confidence interval. Ambient scalar irradiance above the sample surface, I, at which the measurements were conducted, and E_a are given in μmol photons $m^{-2}s^{-1}$, P_{max} is in mmol $m^{-3}s^{-1}$.
†Symbols = and – refer to the same value as on the preceding line of the table and to no value, respectively.

confidence interval of the reconstructed P-I curve, shown in Figs. 1-3 below by dash-dotted lines, was calculated as a sum of the 95% confidence interval of the fitting curve $\Delta P_j(T_j)$ and $\delta P(I)$. Alternatively, the lower and upper 95% confidence band of the predicted P-I curve, shown in Figs. 1-3 below by dotted lines, was estimated as the minimum and maximum photosynthesis rate calculated from Eq. 3, using all combinations of fitted parameters $P_{max} \pm \delta P_{max}$ and $E_a \pm \delta E_a$, respectively. Nonlinear fitting and predictions, including the 95% confidence intervals, were done in Matlab (version 7.0; Mathworks) using functions 'fit' and 'predint'. Fitting of the data obtained at variable ambient light intensity I, i.e., triplets [I, T_j, ΔP_j], was done using Matlab's function 'fminsearch', which, unlike function 'fit', supports fitting by a function with more than one independent variable (I and T_j in this case). The Matlab source codes of the fitting programs are available as supplementary material (LSShift.zip; http://www.mpi-bremen.de/Lubos_Polerecky.html). Statistical significance of the fitting parameters was evaluated in SigmaPlot (version 10.0; Systat Software Inc.). Prediction of the photosynthesis rate at the ambient light intensity, P(I), was considered statistically significant if both estimates of the fitting parameters P_{max} and E_a were statistically significant ($p < 0.05$).

Assessment

Method validation—The oxygen concentration at the measured point started to decrease immediately after the sample was shaded. It returned to the steady state level (i.e., that which had been reached before the shading) within 1-2 min after the shading was removed and the original ambient light level was restored (data not shown). In general, the rate of the O_2 decrease was higher when a darker shade (i.e., lower T_j) was used, as expected from Eq. 4. The situation was opposite when additional light was shined onto the sample, resulting in an immediate increase in local O_2 concentration. This indicated that the microsensor tip was located at a point where the gross photosynthesis was non-zero and exhibited dependence on light intensity. In contrast, if the measured change in the rate of O_2 evolution after shading or light-addition was not immediate but delayed for a few seconds, it was concluded that the local gross photosynthesis was zero and the delayed change occurred as a result of diffusive transport between the measured point and a photosynthetically active volume located in close proximity (Revsbech and Jørgensen 1983). This happened, for example, when the sensor tip was in the diffusive boundary layer or in the photosynthetically inactive zones in the sample.

The experiments conducted at constant illumination show that the measured decrease in photosynthesis, ΔP_j, decreased exponentially with the filter transmission, T_j, in both the rockpool mat and coral experiments (circles in Figs. 1A and 1B, top-right axes). The data were fitted with Eq. 4, from which the best estimates of P_{max} and E_a were obtained (Table 1, lines 1-2). Both estimates were statistically significant ($p < 0.05$). The measured data and the fit were subtracted from the estimate of P(I), which was calculated by extrapolating the fit to $T_j = 0$. This resulted in predicted rates of gross photosynthesis (squares) and in the best estimate of the reconstructed P-I curve (solid curve, both in Figs. 1A and 1B, bottom-left axes). When the gross photosynthesis rates were measured directly by the light-dark shift method, the values for all measured intensities closely followed this reconstructed P-I curve and fell within the 95% confidence intervals (compare crosses with solid, dash-dotted, and dotted lines in Figs. 1A–B). In particular, the rates predicted for the rockpool mat and coral tissue at the ambient illumination of 2630 and 1350 μmol photons m^{-2} s^{-1} were 5.3 ± 0.3 and 16.4 ± 2.4 mmol m^{-3} s^{-1}, respectively, while the rates measured by the light-dark shift method at the same point and light intensity were 5.5 ± 0.4 and 17.1 ± 0.7 mmol m^{-3} s^{-1}, respectively.

In the measurements conducted at three defined light intensities in the Chiprana mat (Fig. 1C), the ΔP_j values decreased approximately linearly with T_j for the two lowest light intensities (40 and 130 μmol photons m^{-2} s^{-1}), whereas a trend of exponential decrease was apparent at the highest PAR intensity (310 μmol photons m^{-2} s^{-1}). The data did not lie on a single exponential curve, but rather followed three distinct trends (shown by dashed lines in Fig. 1C). This was expected from Eq. 4, which predicts that both the rate of decrease as well as the offset of the ΔP_j versus T_j curve are parameterized by the ambient light intensity I. However, after the complete dataset was processed as described in *Data analysis* section, a single P-I curve was recovered (squares and solid line in Fig. 1C), characterized by statistically significant ($p < 0.05$) values of P_{max} and E_a (Table 1, line 3). The gross photosynthesis rates measured by the light-dark shift method at the three ambient light intensities closely followed this predicted P-I curve (compare crosses with solid, dash-dotted, and dotted lines in Fig. 1C).

Thus, it can be concluded that the new light-shade shift approach and the traditional light-dark shift method give equivalent results regarding the light dependence of the gross photosynthesis rate. This was confirmed for all photosynthetic systems studied in this work and over the range of illumination intensity typically encountered naturally in these systems. This finding is important, and it needs to be verified before quantifying gross photosynthesis rates in full sunlight to validate the applicability of the light-shade shift procedure for such measurements in the studied system.

Method application—After successful validation of the method, light-shade shift measurements were conducted in the full sunlight. The ex situ measurements in the rockpool mat showed that the decrease in photosynthesis with the transmission coefficient (Fig. 2A), and thus the reconstructed P-I curve (Fig. 2B), varied with depth in the mat. This was due to a significant variation of P_{max} with depth, which changed between 6.55 ± 0.27 and 13.3 ± 1.6 mmol m^{-3} s^{-1} (Table 1) and was most likely related to a variation in biomass of the phototrophic cells. The best-fit values of E_a were similar for the

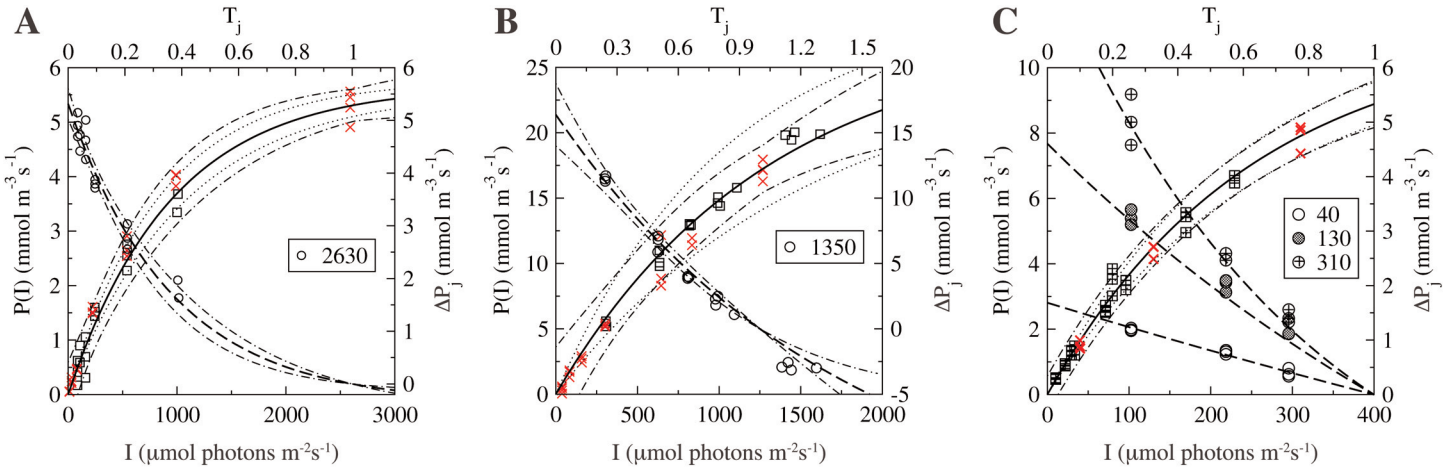

Fig. 1. Raw data obtained from the light-shade shift measurements (ΔP_j vs. T_j; circles), and the light dependence of the gross photosynthesis rate (P vs. I, squares) recovered from them using Eqs. 3–4, measured (A) in a rockpool microbial mat (dominated by *Rivularia atra*), (B) in the tissue of coral *Cladocora caespitosa*, and (C) in a Chiprana microbial mat, using a laboratory lamp as the illuminating light source (PAR intensity in μmol photons $m^{-2}s^{-1}$ is specified in legend). Note that the measuring points with $T_j > 1$ were obtained by temporarily increasing the sample illumination using an additional lamp, as opposed to shading it with a neutral density filter ($T_j < 1$). Top-right axes: dashed lines represent the best fits of the experimental data by Eq. 4, with the 95 % confidence interval indicated by the dash-dotted line. Bottom-left axes: solid line represents the predicted P-I curve (Eq. 3), with the 95% confidence interval, calculated in two alternative ways, shown by the dash-dotted and dotted lines (see text for more details). Gross photosynthesis values measured in the same point of the sample by the conventional light-dark shift method at varying PAR intensities are depicted by crosses for comparison.

two depths with lower P_{max} (~1500 μmol photons m^{-2} s^{-1}), which suggests similar light adaptation, but they were substantially lower than that corresponding to the highest P_{max} (~2700 μmol photons m^{-2} s^{-1}; Table 1). Although the estimates of both parameters were statistically significant ($p < 0.05$) for all depths, this difference was probably due to the reconstructed P-I curve being insufficiently constrained at the high-intensity end. This is also suggested by the relatively large uncertainty of the E_a estimate, which was related to the fact that even the highest intensity applied during the measurement (~3000 μmol photons m^{-2} s^{-1}) did not result in close-to-saturating photosynthesis rate. At all measured depths, the value of E_a was very high and comparable to the maximum intensities experienced by the mat during a clear summer day. This suggests that the photosynthetic activity in the mat is limited by light throughout the day. For example, the photosynthesis rate at the ambient PAR intensity of 1900 μmol photons m^{-2} s^{-1} reached only 50% to 65% of the maximum (saturated) value of P_{max} (Fig. 2B). Similar light limitation was observed in other photosynthetic systems such as microphytobenthic

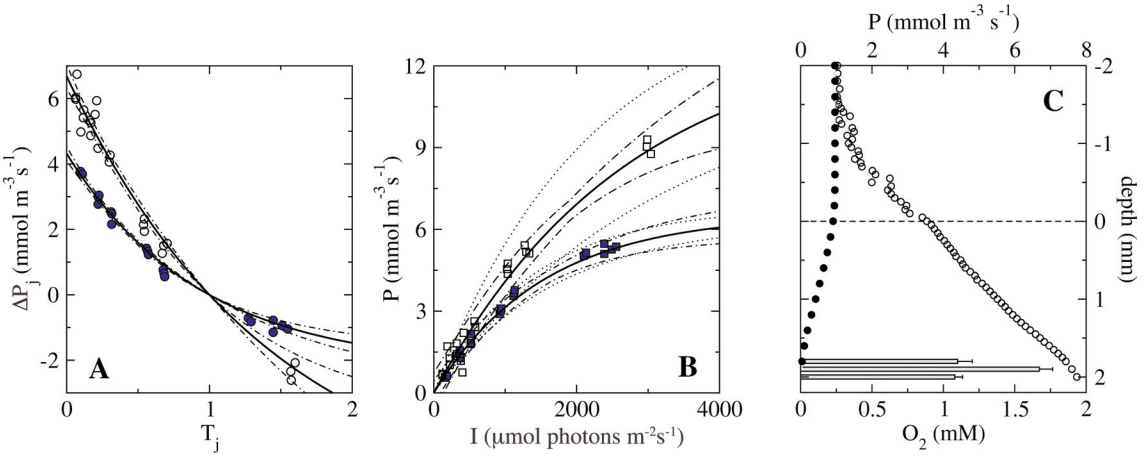

Fig. 2. Measurements in a rockpool microbial mat (dominated by *Rivularia atra*) conducted in a glass beaker at full sunlight (scalar irradiance above the mat surface 1900 μmol photons $m^{-2}s^{-1}$) a few meters away from the site from which the mat was collected. Examples of (A) the raw data fitted with Eq. 4 and (B) the recovered P-I curves, obtained at selected depths (1.8 mm = filled symbols; 1.9 mm = open symbols) are shown. The 95% confidence intervals are depicted in the same way as explained in Fig. 1. Panel C shows a vertical profile of the gross photosynthesis rate in the mat, as predicted from the light-shade shift measurements exemplarily shown in panels A-B for the light intensity 1900 μmol photons $m^{-2}s^{-1}$. Error bars represent the 95% confidence intervals. Light (open circles) and dark (filled circles) profiles of O_2 concentrations in the same spot are shown for completeness.

assemblages (Lassen et al. 1997; Dodds et al. 1999) or microbial mats (Wieland and Kühl 2006).

The gross photosynthesis rates predicted for the ambient light intensity ranged from 4.3 to 6.7 mmol m^{-3} s^{-1} and were localized over a depth interval 1.8-2.0 mm (Fig. 2C). Photosynthetic activity outside this interval was either not detectable (<1.8 mm) or was not measured (>2.0 mm) because of the risk of breaking the sensor. Thus, the photic zone that could be investigated in our measurements was 0.3 mm thick. The oxygen profile in the mat measured at the same light intensity showed enormous concentration values in the photic zone, reaching approximately 10 times the air-saturated values, or ~2 times the ambient atmospheric pressure. This result was not an artifact, as the sensor was carefully calibrated after the measurements using in situ water saturated with nitrogen, air, and pure oxygen, as well as checked for possible cross-sensitivity to temperature. Although this observation is interesting, its explanation and possible implications are beyond the aims of this work.

Owing to the limited dataset available for the oxygen concentrations and gross photosynthesis rates, only an incomplete oxygen budget in the mat could be estimated. Areal rate of gross photosynthesis, obtained by depth-integrating the volumetric rates over the photic zone thickness, amounted to $P_{a,ph}$ ~ 1.5 μmol m^{-2} s^{-1}, whereas the total oxygen flux into the overlying water in the light was $NP_{a,l}$ ~ 1.0 μmol m^{-2} s^{-1}. Considering the relatively constant oxygen gradient and complete absence of photosynthesis at depths 0-1.8 mm, respiration activity was comparatively very low or absent in this zone. Assuming that the decrease from the gross oxygen production rate $P_{a,ph}$ to the net production rate $NP_{a,l}$ occurred in the photic zone, which is equivalent to the assumption that the oxygen flux at depth 2.0 mm was zero, the areal respiration rate in the photic zone in the light would be $R_{a,l} = P_{a,ph} - NP_{a,l}$ ~ 0.5 μmol m^{-2} s^{-1}. In the dark, oxygen penetrated down to the top boundary of the photic zone, again suggesting that it was consumed only at depths below 1.8 mm. The dark oxygen flux at the mat-water interface amounted to $R_{a,d}$ ~ 0.3 μmol m^{-2} s^{-1}, suggesting that the respiration in the photic zone between 1.8 and 2.0 mm was enhanced in the light by a factor of ~1.67 compared to the respiration in the dark. This enhancement was possibly underestimated because the flux at 2.0 mm depth was assumed to be zero. Although it could not be determined more accurately due to the insufficient amount of data points at depths around 2 mm, the oxygen gradient seemed to change only minimally between depths 1.8 and 2.0 mm (Fig. 2C), suggesting lower net photosynthesis $N_{a,l}$, i.e., higher respiration $R_{a,l}$, and thus even more intensive oxygen recycling in the photic zone in the mat under illumination. As suggested in other studies (e.g., Kühl et al. 1996), this enhancement of respiration in the light was most likely due to the release of photosynthesis products which stimulate respiration by heterotrophic microorganisms, or may have been partly due to an increased depth of O_2 penetration in the light.

The in situ measurements in the coral showed that the decrease in photosynthesis with the transmission coefficient (Fig. 3A), and thus the reconstructed P-I curve (Fig. 3B), varied across the coral tissue. This was due to a significant variation of P_{max}, which decreased from 11.2 ± 0.6 mmol m^{-3} s^{-1} inside the polyp to 8.5 ± 0.3 mmol m^{-3} s^{-1} between the polyps (Table 1) and was probably related to different densities of algal symbionts in the coral tissue. The value of E_a was relatively low and similar for both locations, suggesting similar light adaptation (Table 1). The gross photosynthesis rates in the coral tissue determined for the maximum light intensity encountered by the coral during a sunny day (1350 μmol photons m^{-2} s^{-1}) amounted to 8.2-10.5 mmol m^{-3} s^{-1} (Fig. 3C). In contrast to the microbial mat (see above), these values were 94% to 97% of the maximum rate P_{max}, indicating that the symbiotic zooxanthellae operated at close to saturated rates at typical daylight intensities.

The areal rate of net photosynthesis in the light, determined ex situ from the profiles measured at a similar PAR intensity (Fig. 3C), amounted to 0.7 μmol m^{-2} s^{-1}, whereas the respiration in the dark was ~0.5 μmol m^{-2} s^{-1}. Since the gross photosynthesis was measured only at a single depth in the coral tissue, no information about its depth variation and extent is available. Thus, no comparison between the areal respiration rates in the light and dark is possible.

Minimum and optimum requirements—To assess the sensitivity of the light-shade shift method to the amount and combination of the shade levels, the fitting procedure described in the *Data analysis* section was conducted for all possible combinations of at least two shades employed in the coral laboratory experiment. The results of this assessment are summarized in Table 2 and Fig. 4.

Overall, the proportion of statistically significant predictions of P(I) (i.e., those based on the estimates of P_{max} and E_a derived from the fit of the measured data pairs $[T_j, \Delta P_j]$ with $p < 0.05$) increased with increasing number of shades (Fig. 4). Both of the fitted values of P_{max} and E_a were usually statistically significant when the combination included shades with low or both low and high transmission coefficients (values marked with an asterisk in Table 2, data points with small error bars in Fig. 4). This was possible even with 2-3 shades combined. On the other hand, when the transmissions of the combined shades were close to each other or did not include the darkest shade, the p value of at least one of the parameters P_{max} and E_a was > 0.05, leading to statistically insignificant predictions of P(I). These were typically marked with an unacceptably large 95% confidence interval (values not marked with an asterisk in Table 2, data points with large error bars in Fig. 4), and often underestimated the real P(I) value. This happened relatively often for the combination of 2-4 shades, but was possible also for 5-6 shades (e.g., lines 16 and 18 in Table 2). All statistically significant predictions of P(I) were accurate, i.e., fell within the 95% confidence interval of the photosynthesis rate measured by the light-dark shift method. The 95% confidence

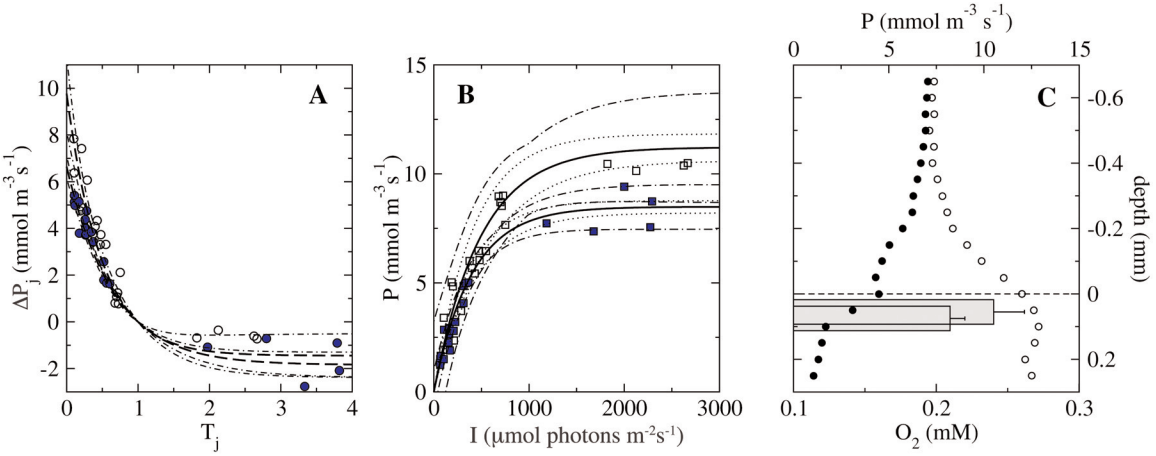

Fig. 3. Measurements in the tissue of the coral *Cladocora caespitosa* conducted in situ at full sunlight using a diver-operated microsensor system. Examples of (A) the raw data fitted with Eq. 4, and (B) the recovered P-I curves, obtained in a point inside the coral polyp (open symbols) and between two polyps (filled symbols) at ambient light intensities 1000 and 600 μmol photons $m^{-2}s^{-1}$, respectively, are shown. The 95% confidence intervals are depicted in the same way as explained in Fig. 1. Panel C shows the gross photosynthesis rates in the two points predicted for the maximum ambient light measured in situ during a sunny day (1350 μmol photons $m^{-2}s^{-1}$). Error bars represent the 95 % confidence intervals. Examples of light (open circles) and dark (filled circles) profiles of O_2 concentrations in another spot in the coral polyp (measured ex-situ at the same ambient light intensity and in the dark) are also shown.

Table 2. Examples of gross photosynthesis rates (P[I] in mmol $m^{-3}s^{-1}$) at the ambient light intensity and the parameters characterizing the recovered P-I curve (P_{max} in mmol $m^{-3}s^{-1}$, E_a in μmol photons $m^{-2}s^{-1}$), predicted from the light-shade shift measurements using selected combinations of shades.†

	N	T_1	T_2	T_3	T_4	T_5	T_5	T_6	Predicted				
Line		(0.24)	(0.50)	(0.64)	(0.78)	(0.87)	(1.13)	(1.28)	P(I)	P_{max} (*p*)		E_a (*p*)	
1	2	*	*						17.3 ± 3.2 (*)	28.0 ± 3.8	(0.002)	1318 ± 483	(0.035)
2	2	*						*	16.5 ± 0.5 (*)	35.0 ± 2.2	(<0.0001)	1993 ± 207	(<0.0001)
3	2		*			*			24.5 ± 25.2	28.4 ± 7.4	(0.01)	637 ± 388	(0.41)
4	2			*				*	11.6 ± 0.7	115 ± 53	(0.0756)	11916 ± 6150	(< 0.0001)
5	3	*	*		*				17.7 ± 2.1 (*)	26.6 ± 2.0	(<0.0001)	1159 ± 259	(0.0036)
6	3	*		*		*			18.5 ± 0.8 (*)	24.8 ± 0.4	(<0.0001)	923 ± 60	(< 0.0001)
7	3	*		*				*	16.8 ± 1.3 (*)	29.8 ± 2.5	(<0.0001)	1523 ± 269	(0.0002)
8	3		*	*	*				22.6 ± 11.0	27 ± 3.0	(<0.0001)	715 ± 234	(0.1139)
9	3			*	*			*	11.3 ± 2.2	156 ± 379	(0.6002)	16962 ± 44257	(0.0011)
10	4	*	*	*			*		16.8 ± 1.4 (*)	29.5 ± 2.4	(<0.0001)	1500 ± 268	(< 0.0001)
11	4	*		*	*		*		16.4 ± 3.1 (*)	33.2 ± 11.4	(0.0135)	1871 ± 1120	(0.0302)
12	4	*			*	*		*	16.9 ± 1.1 (*)	30.1 ± 2.4	(< 0.0001)	1542 ± 252	(< 0.0001)
13	4			*	*	*	*		11.7 ± 11.6	>100	(0.9820)	>5000	(0.4367)
14	4			*		*	*	*	11.7 ± 6.6	>100	(0.9030)	>5000	(0.1078)
15	5	*	*		*	*		*	16.9 ± 1.2 (*)	29.6 ± 2.3	(< 0.0001)	1504 ± 249	(< 0.0001)
16	5		*	*	*		*	*	13.2 ± 4.0	81 ± 148	(0.5902)	7123 ± 15358	(0.0205)
17	6	*	*	*	*	*		*	17.1 ± 1.3 (*)	28.1 ± 1.6	(< 0.0001)	1353 ± 190	(< 0.0001)
18	6		*	*	*	*	*	*	13.3 ± 3.8	66 ± 86	(0.4510)	> 5000	(0.0125)
19	7	*	*	*	*	*	*	*	16.3±1.9 (*)	32.4±5.1	(<0.0001)	1811±540	(0.0001)

†The different shades are characterized by transmission coefficients T_j, and those selected for the prediction of P(I) for a given line are marked by asterisks. Calculations were based on the raw data shown by circles in Fig. 1B. Numbers after ± represent the 95% confidence interval of the predictions. N = number of selected shades. The gross photosynthesis rate at the same light intensity, determined by the traditional light-dark shift method, was 17.1 ± 0.7 mmol $m^{-3}s^{-1}$. Statistical significance of the fitting parameters P_{max} and E_a are shown by *p* values.
*Indicates statistically significance (see text for further explanation).

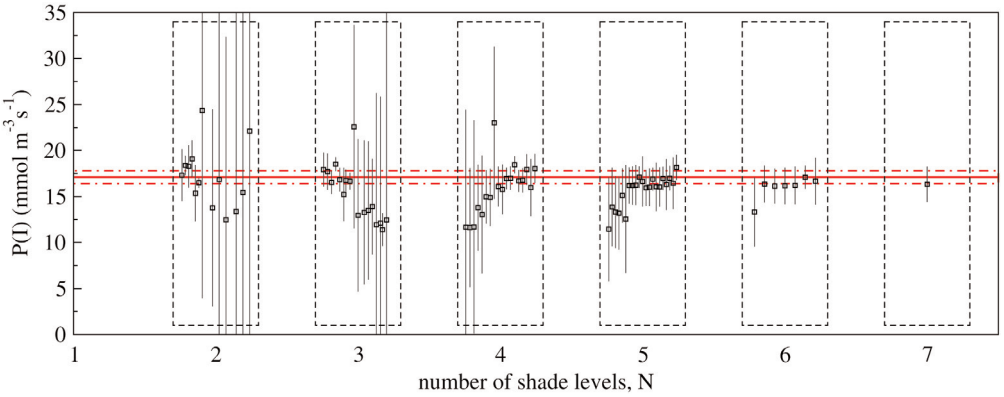

Fig. 4. Gross photosynthesis rates in a coral tissue at ambient light intensity of 1350 μmol photons $m^{-2}s^{-1}$, predicted from a combination of N different shade levels using data points in Fig. 1B. Values selected randomly from predictions determined from *all* possible shade combinations for a given N ($7!/N!(7-N)!$, $N=2,\ldots,7$) are shown. Error bars indicate the 95% confidence interval. Predictions from selected shade combinations are listed in Table 2. Gross photosynthesis rate measured by the traditional light-dark shift method (replicate measurements displayed by crosses at light intensity 1350 μmol photons $m^{-2}s^{-1}$ in Fig. 1B) is depicted here by horizontal lines (solid = mean, dash-dotted = 95% confidence interval).

interval of these predictions was lower for fits characterized by lower p values of the fitted parameters P_{max} and E_a, and was typically 2-3 times larger than the 95% confidence interval of the P(I) value measured directly by the light-dark shift method (Fig. 4). The precision of the P(I) prediction did not improve beyond this level even when 6-7 shades were applied.

This result is a direct consequence of the P-I curve shape (Fig. 1B), and the relative position of the ambient and shaded light intensities on the curve. Specifically, shades which are insufficiently dark ($T_j > 0.5$) probe only the leveling-off part of the P-I curve, missing its (possibly) steeper gradient at lower intensities and thus generally leading to underestimated P(I) predictions based on the model given by Eq. 4. On the other hand, the combination of similar shade levels probes only a narrow region on the P-I curve, which generally results in a poorly constrained prediction of the P-I curve, leading to P(I) estimates with unacceptably large 95% confidence intervals.

Thus, taking into account the accuracy of P(I) prediction, we conclude that 2-4 shade levels can be sufficient to accurately estimate the gross photosynthesis rate at ambient light intensity, provided that these shades are selected so as to cover as wide an intensity range on the P-I curve as possible, especially toward the low-intensity end. Considering also the precision of the prediction and the practical aspects (e.g., measurement time and experimental effort), 4-5 properly selected shade levels seem to be optimal for satisfactory P(I) estimation. The optimum choice of the shade levels should include as dark a shade as possible, and the transmission coefficients should be spread relatively evenly between the lowest and highest value, covering as wide a range of the illumination intensity as possible. Whether or not the choice of shades leads to satisfactory P(I) estimates based on the model given by Eq. 4 can be checked by evaluating the p values of the fitting parameters P_{max} and E_a. For example, in our case, a combination of shades and light additions with corresponding transmission coefficients around 0.25, 0.5, 0.6-0.8, and 1.2 allowed satisfactory reconstruction of the P-I curve, i.e., with both P_{max} and E_a statistically significant ($p < 0.05$), and thus accurate estimation of the gross photosynthesis rate over the entire range of light intensities experienced by the studied system in situ (see Fig. 1B).

Comments and recommendations

The presented method allows high spatial resolution quantification of volumetric gross photosynthesis rates in a sample exposed to ambient light. In contrast to the microsensor-based light-dark shift method that has been traditionally used for such measurements under laboratory conditions, our new method does not require full darkening of the sample. Therefore, it is suitable for in situ applications conducted at full sunlight. The execution of the light-shade shift measurement is straightforward: using an oxygen microelectrode and a calibrated light sensor, one needs to accurately and rapidly (at least every 0.5 s) monitor the oxygen concentration and illuminating light intensity in the studied system, while placing objects with different transmissions (e.g., neutral density transmission foils) between the sample and the sun for a few seconds in a few minutes intervals (usually every 1-2 min). From the rate of oxygen decrease measured during the short shading period, and by applying several different shade levels, one can essentially reconstruct the light-dependence of the gross photosynthesis rate (the so-called P-I curve) and thus predict the rate at the measured point in the sample at the ambient light level. Additional information about the P-I curve, in particular at the high-intensity end, can be obtained by illuminating the sample with additional light instead of shading.

From the practical point of view, an accurate and reasonably precise estimate of gross photosynthesis at one measuring point can be accomplished within 15-30 min, considering that 4-5 different shade levels are applied in triplicates and that a typical "recovery" phase after the shade removal lasts 1-2 min. Thus, the measurements should preferably be carried out during

midday of a clear or very cloudy day, when the ambient light intensity practically does not change. In such case, the data analysis is simple, as one only needs to fit the measured pairs $[T_j, \Delta P_j]$ with Eq. 4, without considering its explicit dependence on the ambient light intensity I (see Figs. 1A–B). When the ambient light intensity is not stable over such long time periods, e.g., in the morning or later in the afternoon, it is recommended that the measurements are carried out with as many shade levels and as fast as possible, e.g., by decreasing the number of replicate measurements at each shade level to 2 or 1. In such case, data analysis must be slightly modified, as the explicit dependence of the fitting Eq. 4 on I must be considered when fitting the complete dataset $[I, T_j, \Delta P_j]$.

The measurements are easy to handle when done in the air where the sensor and filter positioning as well as the communication is simple. Underwater measurements are more difficult, but possible when done by experienced divers and with a well thought-through measuring protocol. Because of the higher friction, filter positioning under water is not as simple as in the air but can be done without disturbing the water flow conditions above/in the sample.

Because the method employs microsensor-based monitoring of O_2 concentrations in the sample and its temporal variation induced by the change in illumination, it is very important that the changes in O_2 concentration are not disturbed by other factors, such as sudden changes in water flow in or above the sample, which may be difficult to control. Thus, the method is best suited for systems found in habitats with well-defined and relatively constant flow conditions, such as microbial mats or biofilms in lakes or slow rivers, corals in areas sheltered against too high water currents, intertidal sediments at low or high tide, etc. Using the newly developed diver-operated microsensor system (Weber et al. 2007), the measurements are not restricted to shallow waters but can be relatively easily conducted completely under water, as practically demonstrated in this work.

Although the primary aim of the light-shade shift measurement is to quantify gross photosynthesis, P(I), at the ambient light intensity, I, we showed that the measurement also leads to a useful byproduct, namely to the P-I curve characterizing the response of the studied photosynthetic system to light intensity in the range from 0 to at least I. The 95% confidence interval of the P-I curve recovered from the light-shade shift measurement is generally larger than that recovered from the light-dark shift measurements, typically by a factor 2-3. This is because the photosynthesis rates at variable light intensities are not measured directly, as with the light-dark shift method, but determined indirectly from a model that fits the decrease in photosynthesis measured at variable shade levels. Nevertheless, considering that such estimation is possible from data collected in situ, the light-shade shift method may be useful also for in situ studies of light adaptation. Here, the use of light addition (as opposed to shading) may be particularly useful, especially for the assessment of the P-I curve at the high-intensity end, if the studied system is not light adapted or if the ambient light intensity happens to be too low at the time of the measurement. However, the validity of this conceptual approach over the intensity range intended for the P-I curve assessment should be checked ex situ under controlled laboratory conditions before it is applied in situ.

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