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In situ measurements of photosynthetic irradiance responses of two Red Sea sponges growing under dim light conditions

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Abstract Photosynthetic responses to irradiance by the photosymbionts of the two Red Sea sponges *Theonella swinhoei* (Gray) and *Cliona vastifica* (Hancock) growing under dim light conditions were measured in situ (in September 1997) using a newly developed underwater pulse amplitude modulated (PAM) fluorometer. Relative rates of photosynthetic electron transport (ETR) were calculated as the effective quantum yield of photosystem II (Y) multiplied with the photosynthetic photon flux (PPF). Photosynthesis versus irradiance ($P-I$) curves, obtained within minutes, showed that individual specimens of both sponges, growing under very low light conditions, feature lower light saturation points as well as lower maximal ETRs than individuals growing under higher light. Evaluations of such curves using low irradiances of the actinic light source (20 to 130 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) showed a general decrease in Y , with a shoulder from the lowest irradiance applied till 20 to 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Point measurements yielded ETRs close to what could be estimated from the $P-I$ curves. These point measurements also revealed good correlations between the diurnally changing ambient irradiances (1 to 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and average ETR values for both species. Further analysis showed that although Y values varied considerably between the different point measurements, they did not decrease significantly with light under these very low irradiances. Therefore, PPF rather than Y seems to determine the in situ diel photosynthetic performance at the low ambient irradiances experienced by these sponges.

Introduction

Although sponges are animals, many species feature symbiotic relationships with cyanobacteria or unicellular algae. These photosymbionts provide the sponge with organic photosynthate; this has been considered especially important for species inhabiting oligotrophic waters where planktonic foods are scarce (Wilkinson 1983). For example, it was argued that some species from the Great Barrier Reef were restricted to depths where irradiance levels were expected to be high enough to yield net diel O_2 evolution (Cheshire and Wilkinson 1991). Similarly, it was found that substantial portions of the energy requirement of a freshwater sponge could be provided by the photosymbionts under sufficient light conditions (Sand-Jensen and Foldager Pedersen 1994). While such evaluations are based on assessments of gas exchange as measured under controlled conditions, supportive photosynthetic measurements in situ have been precluded largely by the lack of suitable portable underwater instrumentation.

Pulse amplitude modulated (PAM) fluorometry has recently been used in assessing photosynthetic properties of photosymbiont-containing invertebrates such as corals (Warner et al. 1996), and the development of a portable underwater PAM fluorometer (the Diving-PAM, Walz GmbH, Germany) has made in situ measurements possible as reported both for ascidians (Schreiber et al. 1997) and other organisms including several corals (Beer et al. 1998; Ralph et al. in preparation). While the advantage of using the Diving-PAM includes non-intrusive, fast and reliable in situ measurements (see preceding article of this issue), the method can only estimate rates of photosynthetic electron transport (ETR), ignoring any respiratory components; thus gross rather than net rates of photosynthesis are measured. Therefore, it can not per se replace O_2 or CO_2 (including longer-incubation $^{14}\text{CO}_2$) techniques in research where, e.g., gas exchange balances are sought. It could, however, be applied for

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investigating the gross photosynthetic component in, for example, adaptations of photosymbiont-containing organisms to ambient light. In this work, we used PAM fluorometry in situ for measurements of photosynthetic parameters in two marine sponges growing under dim light conditions.

Materials and methods

The same Diving-PAM (Walz GmbH, Germany) as in the preceding paper of this issue (Beer et al. 1998) was used for measuring chlorophyll fluorescence parameters of the two marine sponges *Theonella swinhoei* (Gray) and *Cliona vastifica* (Hancock). These species were chosen because of their photosynthetically active symbionts; *T. swinhoei* is known to contain cyanobacteria (Wilkinson 1979) while *Cliona* spp. have been reported to harbour zooxanthellae (Rosell and Uriz 1992). The investigated sponges grew at depths of 2 to 4 m in crevices of the coral reef near Eilat, Israel (northern Red Sea, 34°54'E; 31°35'N), and were accessed by snorkelling. Three specimens of *T. swinhoei* (designated The 1 to 3) and two of *C. vastifica* (Cli 1 and 2), measuring 10 to 30 cm² each, were identified in habitats featuring variable, but low (1 to 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ photosynthetic photon flux, PPF), irradiances throughout the day, and all measurements were done on those individuals. The measurements were carried out during September 1997, at an ambient water temperature of 25 °C.

Estimations of light absorption by the sponges were attempted by measuring the reflectance of light by the tissue in the same way as for corals (see Beer et al. 1998). However, it was found that only about 1% of the incident light was reflected by *Theonella swinhoei*, while much of the light reaching *Cliona vastifica* was visibly absorbed by a non-photosynthetic orange pigment. Because of these complications (as pointed out by a critical reviewer), it was decided not to include light absorption fractions in the calculations of ETR, and therefore only relative ETRs are given.

Photosynthesis versus irradiance (*P-I*) curves were generated by attaching the main light guide of the Diving-PAM to the surface of a sponge via the "coral clip" (Walz's terminology) with hooked rubber bands, and then initiating automatic light curve measurements using the internal light source of the instrument. In order to obtain lower than default actinic (photosynthesis-inducing) light levels, the Diving-PAM settings were changed to minimal values for the "actinic light intensity" and "actinic light factor". However, it was found that even lower irradiances were needed in order to obtain *P-I* curves with enough points below light saturation, and therefore the light curves were run with a circular, neutral density filter between the tip of the light guide and the sponge tissues. This filter, which reduced light by ca. 70%, was simply cut out as a circular disk from the negative of an exposed and developed film, and was inserted into the "coral clip". Under such conditions, the "measuring light intensity" and "gain" had to be increased to their maximal settings in order to obtain an adequate fluorescence signal. The effective quantum yield of photosystem II (PSII) (*Y*) was measured by the Diving-PAM as $Y = (F'_m - F)/F'_m$, where F'_m is the maximal fluorescence in light-adapted photosymbionts following the application of a 0.8 s for photosynthesis saturating light pulse (which reduces, or "closes", all reaction centres of PSII), and *F* is the fluorescence yield of the photosymbiont at a given irradiance. Relative ETRs were calculated as $Y \times \text{PPF}$.

Point measurements were carried out for different areas of the three *Theonella swinhoei* and two *Cliona vastifica* sponges throughout the day in the same way as described earlier for corals (Beer et al. 1998). As in that work, the light guide cable of the PPF sensor (calibrated against the quantum sensor of a Li-Cor LI-18189 light meter) was fixed to the "leaf distance clip" so as to measure ambient light perpendicular to the sponge tissue. Both *Y* and ETR were also plotted against PPF in order to evaluate which factor determined the photosynthetic rate at the low ambient irradiances at which these sponges grew.

Results

In experiments where different light exposure times were compared (20 to 60 s), it was found that exposure of the sponges to the actinic light beam for 30 s at each irradiance level was enough to generate optimal *P-I* curves. Therefore, the "actinic light width" was set to 30 s for all subsequent light curve measurements.

Responses of ETR to increasing PPF (*P-I* curves) of the three *Theonella swinhoei* and two *Cliona vastifica* sponges are depicted in Fig. 1. While *T. swinhoei* growing at the highest light level showed saturation of photosynthetic electron transport at 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the two individuals growing in dimmer light showed saturation at only about half that irradiance. Also the photosynthetic rate at light saturation decreased gradually with decreasing ambient irradiances for the three sponges. Similarly, *C. vastifica* growing at very low light showed a considerably lower light saturation level, as well as a lower maximal photosynthetic rate, than did the specimen growing under the higher light level.

At even lower actinic irradiances, obtained with the improvised neutral density filter (see "Materials and methods"), Fig. 2 shows that *Y* decreased with PPF except for the first three light levels following darkness, and that normal *P-I* curves could be obtained which

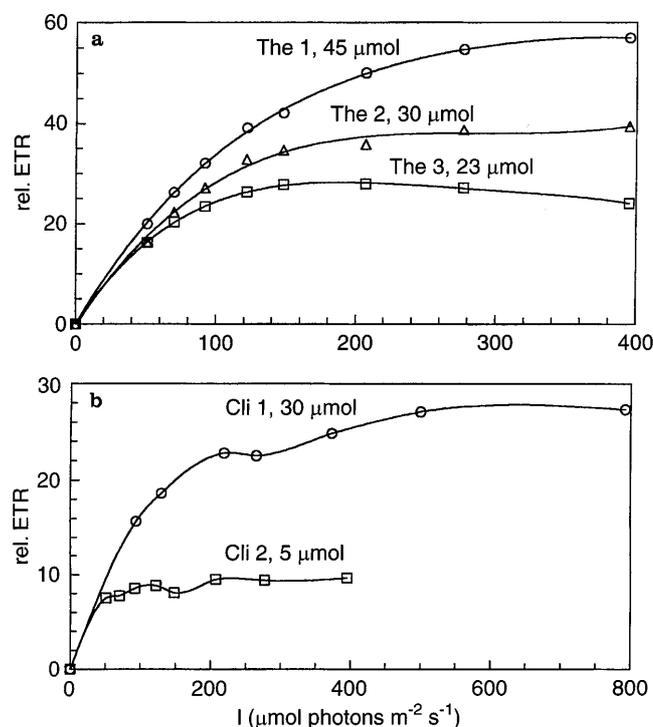


Fig. 1 *Theonella swinhoei*, *Cliona vastifica*. Relative photosynthetic electron transport rates (*rel. ETR*) as functions of incident irradiance (*I*, measured as PPF) for the various **a** *T. swinhoei* (*The 1–3*) and **b** *C. vastifica* (*Cli 1–2*) individuals. Ambient incident irradiance (in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PPF) at the time of the light curve measurements of each sponge is indicated on the graphs

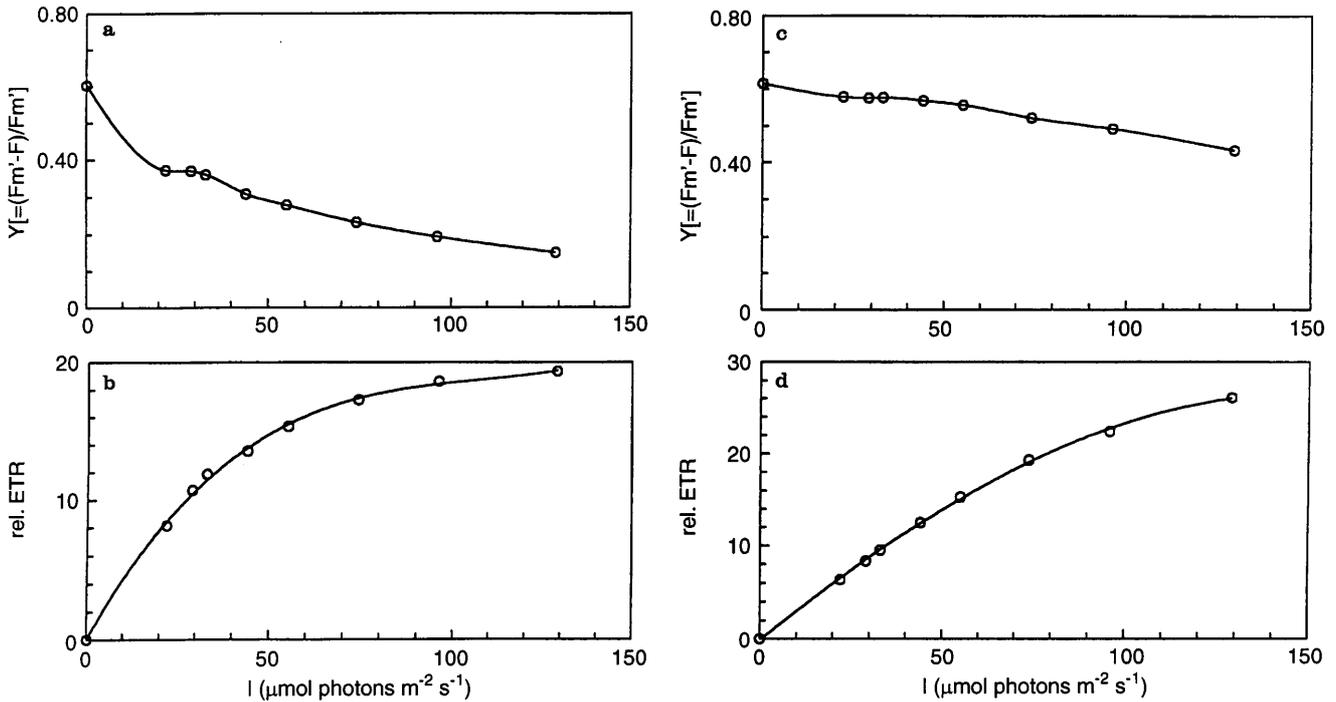


Fig. 2 *Theonella swinhoei*, *Cliona vastifica*. Effective quantum yields of PSII (Y) and relative photosynthetic electron transport rates (*rel. ETR*) as functions of incident irradiance (I , measured as PPF) for **a**, **b** *T. swinhoei* and **c**, **d** *C. vastifica* as measured at low actinic irradiances. Ambient irradiances at the time of the measurements were 23 and 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *T. swinhoei* and *C. vastifica*, respectively

merged well with the curves generated at higher light for those sponges growing under similar light conditions (cf. Fig. 1). The shoulders in the Y curves are similar to those obtained for corals at higher irradiances (Beer et al. 1998); as shown below, they represent a range of PPF in which Y is not restricted significantly by the irradiance (see Figs. 4, 5).

Point measurements on the different *Theonella swinhoei* and *Cliona vastifica* specimens showed that average photosynthetic ETRs related positively to changes in irradiance throughout the day (Fig. 3). These ETR values also correspond well with those expected from the P - I curves of Figs. 1 and 2, showing that both point measurements in situ (expressed as average values of several measurements) and photosynthetic rates derived from P - I curves as measured here may be valid ways to estimate the gross photosynthetic performance of these sponges.

In order to evaluate the relative contributions of Y and PPF in determining the ETRs within the ambient irradiances experienced by these sponges during the day, the Y and ETR values of all point measurements contributing to Fig. 3 were plotted against PPF (Figs. 4, 5). Figure 4 shows that although the Y values of *Theonella swinhoei* tended to decrease with PPF, they were very scattered within the PPF range. A very similar picture was obtained for *Cliona vastifica* (Fig. 5). Here, the possible downward trend of Y versus PPF was even less evident. Following multiplication of Y with PPF, the

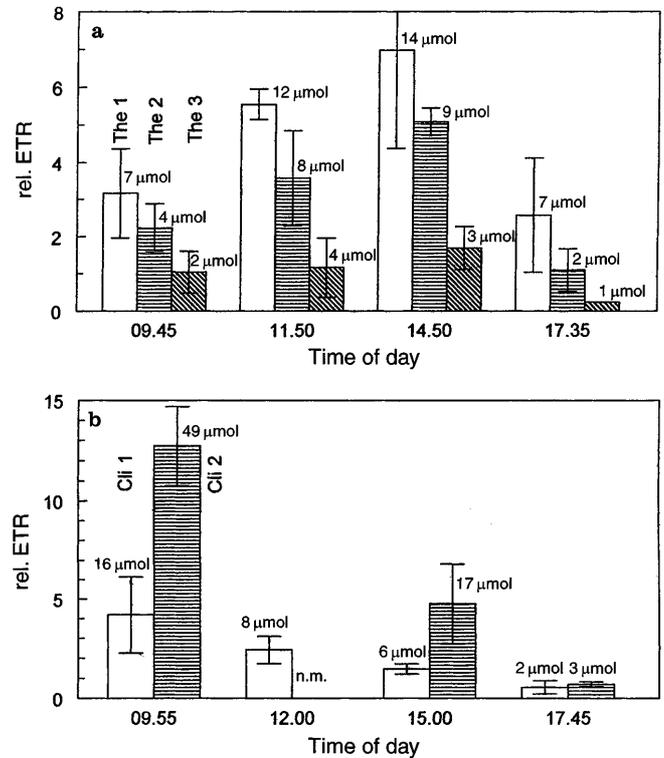


Fig. 3 *Theonella swinhoei*, *Cliona vastifica*. Relative electron transport rates (*rel. ETR*) of **a** *T. swinhoei* and **b** *C. vastifica* measured during various times of the day. Average ambient irradiances (in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PPF) experienced by each individual of *T. swinhoei* (The 1–3) and *C. vastifica* (Cli 1–2) during the point measurements, as recorded by the Diving-PAM's PPF sensor, are indicated for each bar. Data are averages of 10 to 15 measurements \pm SD; n.m., not measured

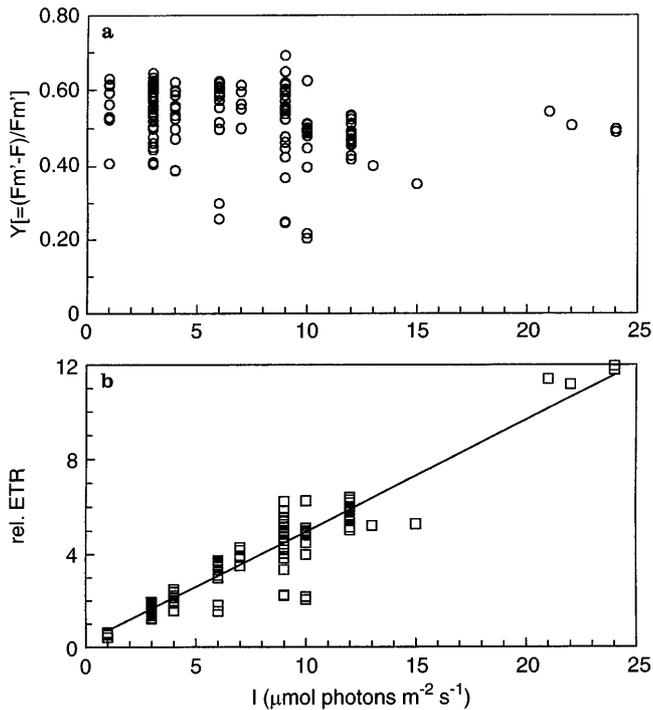


Fig. 4 *Theonella swinhoei*. **a** Effective quantum yields of PSII (Y) and **b** relative photosynthetic electron transport rates (*rel. ETR*) as functions of the ambient irradiance (I , measured as PPF) as recorded for each measurement by the Diving-PAMs PPF sensor. Data are from the same measurements as averaged in Fig. 3a

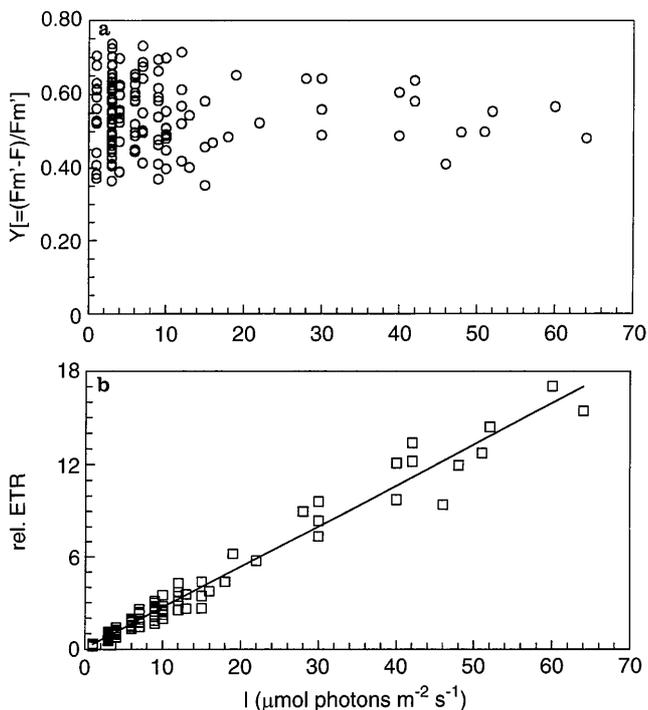


Fig. 5 *Cliona vastifica*. **a** Effective quantum yields of PSII (Y) and **b** relative photosynthetic electron transport rates (*rel. ETR*) as functions of the ambient irradiance (I , measured as PPF) as recorded for each measurement by the Diving-PAMs PPF sensor. Data are from the same measurements as averaged in Fig. 3b

resulting relative ETR values of both species showed a positive linear correlation with PPF within this low-irradiance range. In all, this means that principally PPF alone (and not Y) determines the photosynthetic ETR response to light at the low ambient irradiances at which these sponges grow.

Discussion

The photosynthetic responses of the two sponge species to light show very low saturating irradiances, being about 10% of those measured for some Red Sea corals (Falkowski and Dubinsky 1981; Beer et al. 1998). However, the sponges also received very low ambient light levels throughout even the bright summer days prevailing during our measurements. Within this irradiance range, photosynthesis was linearly correlated with PPF; the latter thus determines the measured ETR responses while Y is largely unaffected. Our point measurement data also indicate that there were no marked differences in the average photosynthetic responses to light between the different individuals of each species in response to the average daily light they received. On the other hand, maximal photosynthetic rates were higher in those sponges growing under the higher light levels. In all, these results show that within these species, the individuals were adapted to a generally low light exposure in terms of photosynthetic yields at low light, but that the photosynthetic capacity was higher in higher-light growing specimens. The latter may be due to elevated activities of enzymes in the photosynthetic CO_2 reduction cycle, but the ecological advantage of such a higher photosynthetic potential at high light levels remains obscure for these stationary low-light growing sponges.

The amount of organic energy provided by the photosymbionts to the entire sponge will depend on several factors. Since the investigated sponges are >100 mm thick, and since the photosymbionts are restricted to their surface cell layers (Ilan unpublished), it seems that the latter must be very efficient if they were to contribute as much to the growth of the sponge as indicated for other species (Wilkinson 1983; Cheshire and Wilkinson 1991; Sand-Jensen and Foldager Pedersen 1994). It is also possible that smaller amounts of essential organic compounds such as vitamins are derived mainly from the photosymbionts, while the bulk energy is acquired by ingesting particulate and/or dissolved organic material. While PAM fluorometry was found to be a suitable method for investigating photosynthetic properties of these marine sponges it will not replace, but rather complement, gas exchange methods in studies where diel energy balances are to be evaluated. Our results show, however, that the Diving-PAM can be used for in situ non-intrusive measurements of gross photosynthetic parameters related to adaptations of the photosymbiotic components of sponges to light.

In addition to the results presented here regarding sponges, the Diving-PAM has been shown suitable in

three previous studies for in situ photosynthetic measurements of photosymbiotic components of several other marine invertebrates (Schreiber et al. 1997; Beer et al. 1998; Ralph et al. in preparation). Besides the suggestions regarding its application for corals as reported earlier (Beer et al. 1998), we would like to point out a few special considerations when using this instrument for measurements at low irradiances. Even when the “actinic light intensity” and the “actinic light factor” were set to their minimal values, and when the main light guide was placed as far away from the sponge tissue as possible while using the “coral clip”, it was found to be too high to produce *P-I* curves with high resolution at the low-irradiance portions of the curves. This problem was circumvented in the present study by inserting a neutral density filter between the light guide and the sponge. This is, however, not an ideal solution since the modulated measuring light and the fluorescence transfer back through the light guide, then become very weak and, consequently, fluorescence values become too low. For the filter used (reducing light by ca. 70%), it was possible to increase the basic fluorescence signal to acceptable values by increasing the “measuring light intensity” and “yield” to their highest values, but this would not be possible if using darker filters. Thus, the Diving-PAM is presently limited to producing light curves starting at above ca. 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ or, if filters can be made, above ca. 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The PPF sensor is also relatively insensitive at low irradiances, and there is no resolution between single irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) readings. Because of this shortcoming of the sensor, the point-measured values of Figs. 4 and 5 are displayed in vertical rows, creating a high scatter of ETR along the PPF axis. Despite these limitations, the results presented here confirm the potential of underwater PAM fluorometry using the

Diving-PAM as a fast, reliable and non-intrusive means of monitoring photosynthetic parameters of photosymbionts in situ.

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