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Short communication

Among-shoot variability and leaf-specific absorptance characteristics affect diel estimates of in situ electron transport of *Posidonia australis*

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Abstract

Electron transport rate (ETR) calculations require values of irradiance, effective quantum yield of chlorophyll fluorescence ($\Delta F/F'_m$), the distribution of energy between PSII and PSI and leaf-specific photosynthetic absorptance. We conducted virtually simultaneous replicated measurements of diel changes in $\Delta F/F'_m$ of *Posidonia australis* in situ, and determined leaf-specific photosynthetic absorptance in vivo using integrating spheres. Leaf-specific non-photosynthetic and photosynthetic absorptance, corrected for spectral reflectance, comprised 16 and 68% of total spectral absorptance (84%); about 7% of photosynthetically active radiation incident on the leaves was reflected. Virtually simultaneous quantum-yield measurements of eight short shoots provided an estimate of among-sample variability in $\Delta F/F'_m$: generally the standard deviation was $\sim 10\%$ of the mean except for a few hours around midday when the variability rose to $\sim 25\%$ of the mean. Our results indicate that leaf-specific non-photosynthetic absorptance, spectral reflectance and shoot-to-shoot variability need to be taken into account when estimating ETR in the field.

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Keywords: Absorptance; Chlorophyll fluorescence; Electron transport rate; *Posidonia australis*

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1. Introduction

Chlorophyll fluorescence techniques have become a popular means for assessing photosynthetic activity of plants and algae in a wide variety of applications. While modulated fluorometers in particular are well suited for comparative photosynthetic measurements, the determination of absolute photosynthetic values is largely restricted to estimating the (effective and maximal) quantum yields of chlorophyll fluorescence (Genty et al., 1989; Schreiber, in press). Precise estimations of productivity have remained an attractive proposition, as determinations using fluorescence would be considerably faster and simpler than traditional techniques such as the use of polarographic oxygen electrodes (oxygen evolution, e.g. Cheshire et al., 1996) or labelled carbon (carbon fixation, Strickland and Parsons, 1972). As fluorescence can be directly measured in situ, artefacts caused by the placement of samples in chambers would be avoided. However, the technical difficulties involved in converting estimates of photosynthetic activity into productivity using fluorescence data remain unresolved (Beer et al., 2000b; Franklin and Badger, 2001). In this study, we describe an improved means of determining electron transport rate (ETR) – a parameter fundamental to any fluorescence-derived estimate of productivity – and demonstrate the magnitude of among-shoot variability using virtually simultaneous replicate measurements of chlorophyll fluorescence over a diel cycle.

Calculation of photosynthetic ETRs from effective quantum-yield ($\Delta F/F'_m$) measurements depends on knowing the flux of photosynthetically active radiation (PAR) at the leaf surface and the fraction of the PAR absorbed by the leaf (Beer et al., 1998, 2000a). The fraction of the incident irradiance absorbed by a tissue is defined as absorptance (Kirk, 1994; Mobley, 1994), which depends on both the transmittance and reflectance characteristics of the photosynthetic and non-photosynthetic tissue (in this study, we refer to the sum of leaf-specific photosynthetic [$A_L(\text{PAR})$] and non-photosynthetic absorptance [$A_L(720)$] as total leaf-specific absorptance, both of which have been corrected for reflectance [$R_L(\lambda)$]). However, what has been calculated in previous seagrass studies using modulated fluorescence techniques is a coefficient called the absorption factor (AF), which neither distinguishes photosynthetic from non-photosynthetic tissue absorptance nor accounts for leaf reflectance. Based on indirect estimates derived from transmittance measurements, Beer and Björk (2000) assumed leaf reflectance (measured underwater) to be negligible, but acknowledged that their calculated ETRs may have been under-estimated because the simple AF determinations they used may not have been valid for thick-leaved seagrasses or were possibly due to imprecise incident irradiance determinations. They suggested that more precise measurements of the irradiance absorbed by the photosynthetic pigments for thick-leaved seagrasses should be developed. We examine this limitation of estimating photosynthesis in the current study.

Reported values of AF for seagrasses range from 0.44 ± 0.02 for *Zostera marina* L. (Beer et al., 1998) to 0.78 ± 0.04 S.D. for *Thalassia testudinum* Banks ex König (Durako and Kunzelman, 2002); all are lower than the instrument (PAM fluorometer, Walz GmbH, Germany) default value of 0.84, which is a representative value of total leaf-specific absorptance (i.e., accounts for spectral reflectance but not non-photosynthetic tissue absorptance) for terrestrial plant leaves (Björkmann and Demmig, 1987; Knapp and Carter,

1998). Absorbance values corrected for non-photosynthetic leaf-specific absorbance (Enriquez et al., 1994; Olesen et al., 2002) and spectral reflectance (Cummings and Zimmerman, 2002; Drake et al., 2003) have been reported for seagrass leaves, yet we are not aware of any study that has considered both factors in conjunction with ETR estimates. Olesen et al. (2002) reported >0.90 leaf-specific spectral absorbance [$A_L(\lambda)$] for both *Cymodocea nodosa* (Ucria) Aschers and *Posidonia oceanica* (L.) Delile at 680 nm, the chlorophyll *a* peak. Leaf-specific photosynthetic absorbance was found to vary by less than 10% between *T. testudinum* (0.47) and *Z. marina* (0.56) despite five-fold differences in pigment content of leaves (Cummings and Zimmerman, 2002). Here we present data showing the contribution of both leaf-specific spectral reflectance and non-photosynthetic tissue absorbance to reducing $A_L(\text{PAR})$ in the thick-leaved seagrass *Posidonia australis* Hooker and compare $A_L(\text{PAR})$ to AF.

We measured $\Delta F/F'_m$ of eight seagrass samples virtually simultaneously in situ over a two-day period using a multi-channel fluorometer, and using (spectrally derived) $A_L(\text{PAR})$ values we were able to calculate ETR at different times of the day. The ability to simultaneously measure multiple samples enabled us to assess among-shoot variability in $\Delta F/F'_m$ (and ETR) under the same environmental conditions, and also to determine how mean values changed over the course of a day. An important facility of the multi-channel device was its ability to collect data independently of divers. After the field assessment, the leaves were removed from the field and assessed for in vivo absorbance in the laboratory.

2. Materials and methods

2.1. Sampling location and conditions

P. australis was examined at 4 m depth in calm waters in Jervis Bay, NSW, Australia (35° 07'S, 150° 42'E). Eight separate short shoots were selected at the same depth within an area of 1.5 m radius, and rank 2 leaves were positioned within the leaf holders of a custom-built multi-channel fluorometer (described in Runcie and Riddle, 2004). Rank 2 leaves were chosen as they represent the youngest fully developed leaves and have much lower surface fouling than higher rank leaves (Durako and Kunzelman, 2002; Drake et al., 2003). The multi-channel fluorometer provides virtually simultaneous measurements of $\Delta F/F'_m$ (ca. 6 s apart) thereby providing estimates of among-shoot variation that are largely uninfluenced by short-term changes in irradiance and environmental conditions (e.g. cloud movements, water temperature). Leaves were gently cleared of debris and epiphytes and were positioned to face upwards. The fluorometer was programmed to measure $\Delta F/F'_m$ of each sample every 90 min, and it was left to record data for just over 48 h (from 08:00, 11 February 2004 to 08:15, 13 February 2004). Water temperature was 23 °C.

On ending the experiment, we measured $\Delta F/F'_m$ of each sampled leaf with a Diving-PAM (Walz GmbH, Germany) both at the same region of the leaf that was measured with the multi-channel device and at locations on either side of this region. Leaf clips (Walz) were used to minimise the area examined and ensured measurements were obtained from the same tissue as that measured by the multi-channel device. All measurements were

made on material with minimal visible epiphyte coverage. Leaves were then removed and transported back to the laboratory for further treatment.

Irradiance conditions at the study site were estimated (not measured) over the two-day sampling period because the logging quantum meter failed. At the termination of the experiment, irradiance was measured at 4.1 and 3.1 m depth and in the air using the light sensor supplied with the Diving-PAM; depth was measured to the decimetre with a Suunto dive computer. K_d was calculated from this data (Kirk, 1994) and we assumed this to be constant over the sampling period. Surface irradiance over the two-day period was then estimated assuming zero irradiance after sunset and before sunrise, maximum noon PAR of $1500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for both days, and that irradiance varied over the course of a day according to a sine function. Incident irradiance on the leaves was calculated using the estimated surface irradiance values with corrections for light attenuation through the water column (as described by K_d) and changing depth due to tidal fluctuation. Although these calculations assumed an undisturbed sea surface layer with minimal diffusion and cloudless conditions, on the second day there were periods of rain and wind; thus, values of incident irradiance of the second day are likely to be overestimates relative to irradiance estimates of day one.

2.2. Leaf optical properties

The rank 2 leaves of *P. australis* measured with the multi-channel device were collected on 13 February 2004, immediately after $\Delta F/F'_m$ measurements were obtained using the Diving-PAM. Individual leaves were placed in numbered plastic containers and kept moist and in the dark until optical properties were measured (within 2–3 h). Epiphytes were removed by gently scraping the leaf surface with the edge of a glass slide. Leaves were gently patted with a paper towel to remove excess water before being placed on the sample ports of integrating spheres. Leaf-specific spectral transmittance [$T_L(\lambda)$] and reflectance [$R_L(\lambda)$] were measured from 350 to 750 nm at 2 nm resolution using a fiber optic spectrometer (Ocean Optics USB2000) interfaced (50 μm diameter fiber) with FOIS-1 [$T_L(\lambda)$] and ISP-REF [$R_L(\lambda)$] integrating spheres (Ocean Optics, USA).

For transmittance measurements, a collimated beam from a tungsten halogen light source was adjusted to completely irradiate the 9.5 mm diameter sample port (100% T). Leaf samples were then placed over the sample port and positioned to determine $T_L(\lambda)$ at approximately the same area as sampled during the fluorescence measurements. Spectral leaf reflectance [$R_L(\lambda)$] was calculated by dividing the portion of diffuse light reflected from a leaf sample by the diffuse light reflected from a >98% reflective polytetrafluoroethylene (PTFE) Lambertian reference surface (Labsphere OSRS-99-010, USA), both placed over the 10.3 mm sample port. All spectra were corrected for electrical noise using dark spectra. Irradiance levels, optic-fiber diameters and spectral-integration times were optimized to attain a maximum signal of 3500 counts in scope mode. Leaf-specific transmittance [$T_L(\text{PAR})$] and reflectance [$R_L(\text{PAR})$] of photosynthetically active radiation (PAR) were calculated as the spectral average of $T_L(\lambda)$ and $R_L(\lambda)$ from 400 to 700 nm.

A spectral photosynthetic absorption factor (AF_{PAR}), which accounts neither for reflectance nor for non-photosynthetic absorbance, was derived by converting $T_L(\text{PAR})$ to

absorptance:

$$AF_{\text{PAR}} = 1 - \left[\frac{T_{\text{L}}(\text{PAR})}{100} \right]$$

The absorption factor ($AF_{\text{B\&B}}$) and estimate of reflection were also calculated using a modification of the method of Beer and Björk (2000). $AF_{\text{B\&B}}$ was derived by measuring in air (to be directly comparable to the measurements from the integrating spheres) the incident irradiance from the collimated beam of a tungsten halogen lamp before and after the Diving-PAM's irradiance sensor was covered with one layer of *P. australis* leaves. Reflection was estimated by placing one, two, and three layers of *P. australis* leaves over the Diving-PAM sensor and measuring the transmitted light (T). $\ln(T)$ was regressed against the number of leaf layers. The value of T extrapolated to zero leaves (y -intercept) was compared to the measured incident irradiance. Comparisons of leaf-specific diffuse reflectance conducted in air and under water, using a R200 reflectance probe (Ocean Optics, USA), indicated no significant differences in $[R_{\text{L}}(\text{PAR})]$ (Durako, unpublished data).

Leaf-specific photosynthetic absorptance $[A_{\text{L}}(\text{PAR})]$ was determined by converting transmittance to absorptance (see above) followed by corrections for leaf-specific reflectance and non-photosynthetic absorptance at 720 nm $[A_{\text{L}}(720)]$ (Cummins and Zimmerman, 2002). Non-photosynthetic absorptance at 720 nm was used rather than $A_{\text{L}}(750)$ because the low irradiance output of the halogen light source at $\lambda > 720$ nm resulted in a high noise-to-signal ratio.

$$A_{\text{L}}(720) = [1 - T_{\text{L}}(720)] - R_{\text{L}}(720)$$

$$A_{\text{L}}(\lambda) = [1 - T_{\text{L}}(\lambda)] - [R_{\text{L}}(\lambda)] - A_{\text{L}}(720)$$

Leaf-specific photosynthetic absorptance $[A_{\text{L}}(\text{PAR})]$ was calculated as the spectral average of the corrected leaf-specific spectral absorptance values $[A_{\text{L}}(\lambda)]$ from 400 to 700 nm.

2.3. ETR calculations

$$\text{ETR} = \frac{\Delta F}{F_{\text{m}'}} \times \text{PAR} \times A_{\text{L}}(\text{PAR}) \times 0.5$$

ETR was calculated for each sample at each time as the product of $\Delta F/F_{\text{m}'}$, estimated incident irradiance for leaf-specific photosynthetic absorptance (generally measured as close to the leaf as possible), $A_{\text{L}}(\text{PAR})$ for each individual sample and 0.5, assuming equal sharing of electrons between PSII and PSI (Schreiber, in press).

2.4. Data analysis

Differences in $\Delta F/F_{\text{m}'}$ estimates were determined using analysis of variance preceded by tests for homogeneity. Post hoc comparisons on $\Delta F/F_{\text{m}'}$ values were determined using Dunnett's test, where $\Delta F/F_{\text{m}'}$ values were compared to the highest value of $\Delta F/F_{\text{m}'}$

measured at midnight during the first night. Optical properties calculated by the various methods were compared using *t*-tests. Tests were conducted using Sigma Stat (SPSS, Inc.) and Minitab (Minitab Inc.). Significance for all tests was assessed at $P < 0.05$ and variance is expressed in terms of standard deviation (S.D.) unless stated otherwise.

3. Results

The vertical attenuation coefficient for downward quantum irradiance of PAR (K_d) was 0.35 m^{-1} . At the end of the two-day period, some leaves were lightly covered with sand, which presumably prevented a portion of the incident irradiance from reaching these leaves. Over the two days, $\Delta F/F'_m$ varied from a maximum of 0.757 ± 0.070 at midnight to a minimum of 0.422 ± 0.105 at noon (Fig. 1A): $\Delta F/F'_m$ was significantly lower during the daytime. Among-shoot variability in $\Delta F/F'_m$, as expressed by S.D., was generally about 10% of the mean except during midday when variability increased and S.D. was approximately 25% of the mean. These results clearly demonstrate a diel decline in photosynthetic efficiency of *P. australis* coincident with the midday irradiance maximum, and are in agreement with both a decline in $\Delta F/F'_m$ and increased variance at midday for marine macroalgae (Runcie and Riddle, 2004).

Mean values of $\Delta F/F'_m$ of leaves measured with the multi-channel device at the end of the two-day period were compared with in situ measurements of the same leaves performed up to 30 min later with the Diving-PAM. Diving-PAM-derived $\Delta F/F'_m$ values of leaf areas previously sampled by the multi-channel device (0.726 ± 0.023) were significantly greater than adjacent tissues (0.656 ± 0.065 ; $t = 2.85$, $P < 0.05$). Values taken from the part of the leaf that was already sampled by the multi-channel device were excluded from the analysis. Mean values derived from both devices were not significantly different (multi-channel device: 0.656 ± 0.065 , $n = 7$; Diving-PAM (distal and proximal samples): 0.698 ± 0.060 , $t = 1.53$, $P = 0.150$, $n = 16$). Estimates of $\Delta F/F'_m$ of individual leaves obtained by either device were therefore similar.

3.1. Leaf optical properties

Transmittance, reflectance, and absorptance spectra for the rank 2 leaves of *P. australis* were typical of vascular plants (Björkmann and Demmig, 1987; Knapp and Carter, 1998, Fig. 2). Highest total leaf-specific absorptance (low transmittance) was observed in the Soret band (400–500 nm) and around the chlorophyll *a* peak of 660–670 nm. Slightly less than 80% of the incident irradiance was absorbed in this spectral region (Fig. 2B). Lowest total leaf-specific absorptance occurred around the green wavelengths (550 nm), the spectral region with highest transmittance and reflectance. In this region of the spectra, less than 50% of the incident irradiance was absorbed.

Estimates of the spectral absorption factor (AF_{PAR}) derived by averaging leaf-specific spectral transmittance from 400 to 700 nm (i.e., no correction for leaf-specific reflectance or non-photosynthetic absorptance) indicated that up to 92% (0.90 ± 0.02 , $n = 8$) of PAR was absorbed by *P. australis* leaves (Table 1). While higher than any AF previously reported for seagrass leaves, the spectral AF derived from spectral transmittance was not

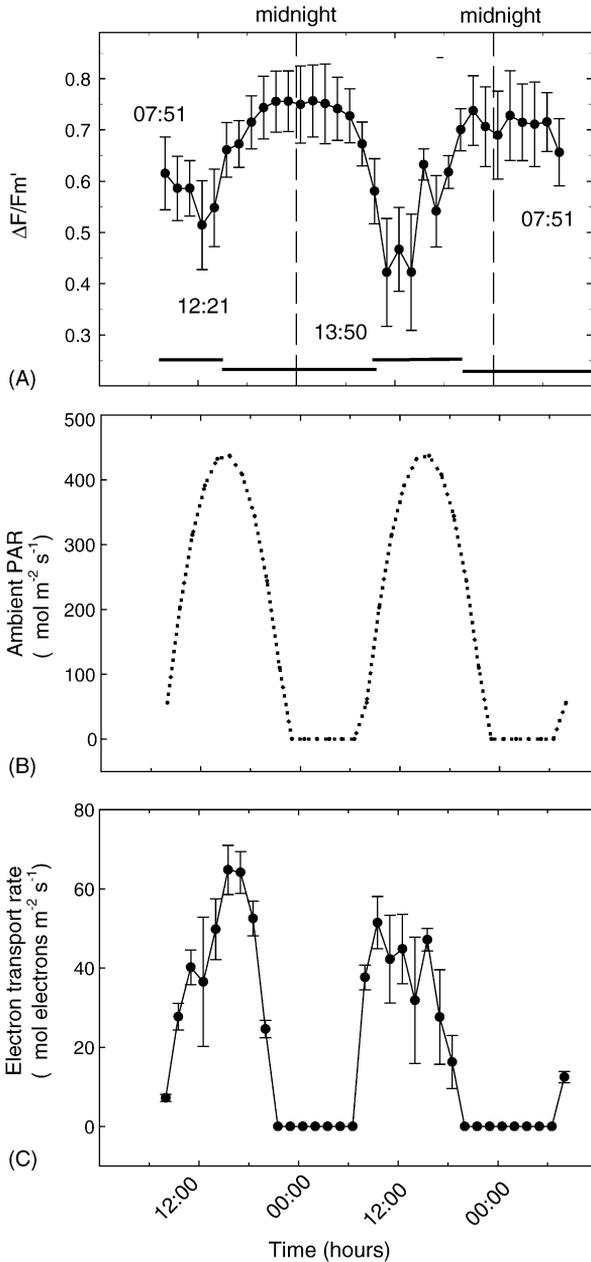


Fig. 1. *Posidonia australis* in Jervis Bay, NSW, over a two-day period. (A) Shows diel variation in $\Delta F/F_m'$ (horizontal bars represent values that are not significantly different), (B) shows the estimated ambient irradiance at the site of measurement and (C) shows calculated ETR. Values of $\Delta F/F_m'$ and ETR are means \pm S.D., $n = 8$.

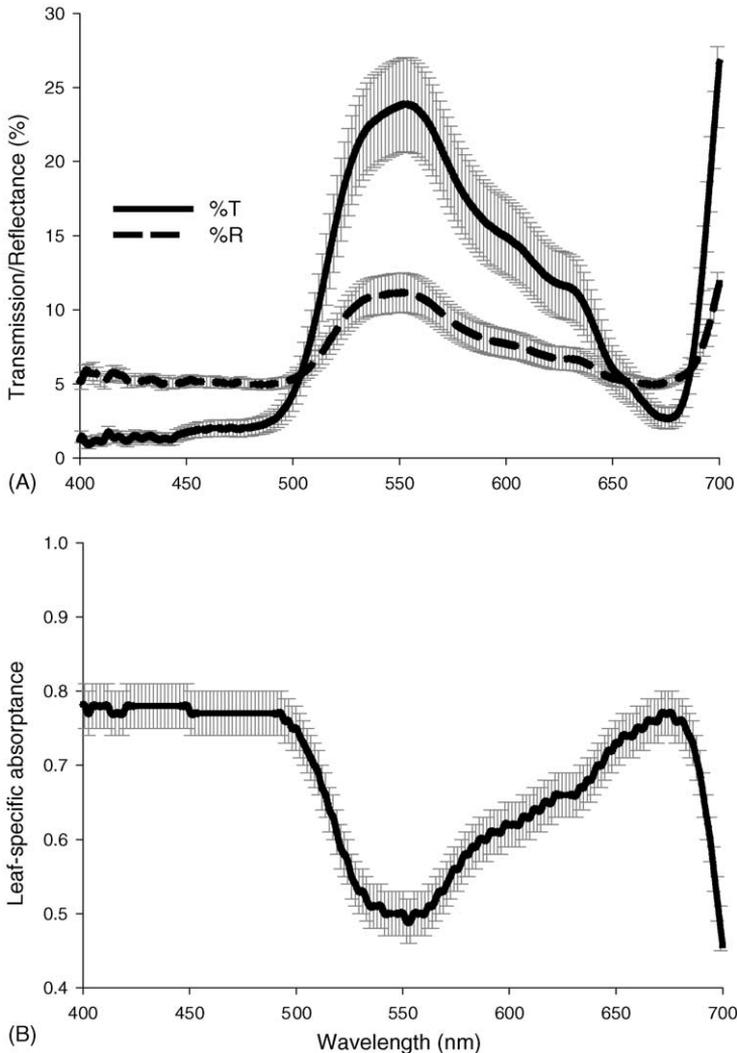


Fig. 2. *Posidonia australis*. Average (\pm standard deviation) leaf-specific spectral transmittance, reflectance (A) and absorbance (B) for rank 2 leaves sampled by the multi-channel fluorometer ($n = 8$).

significantly different from $AF_{B\&B}$ (0.84 ± 0.04 , $P = 0.08$, Table 2) calculated using the method of Beer and Björk (2000). However, when reflected incident PAR ($7 \pm 1\%$) and non-photosynthetic absorbance ($16 \pm 3\%$) are considered, the resulting leaf-specific photosynthetic absorbance [$A_L(\text{PAR})$] values (0.68 ± 0.03 , Table 1) are significantly lower than AF_{PAR} ($P < 0.001$).

Comparing measured incident irradiances to those estimated using the reflectance-estimate method of Beer and Björk (2000) resulted in similar means (Table 2) but paired samples were not correlated ($P = 0.81$ for slope of linear regression), suggesting random variation.

Table 1

Posidonia australis: among-shoot variability in optical properties of leaves sampled by the multi-channel fluorometer in Jervis Bay, NSW, on 13 February 2004

Short-shoot no.	AF _{PAR}	T _L (PAR)	R _L (PAR)	A _L (720)	A _L (PAR)
1	0.92	0.08	0.06	0.17	0.68
2	0.88	0.12	0.05	0.13	0.69
3	0.90	0.10	0.07	0.16	0.67
4	0.88	0.11	0.06	0.21	0.61
5	0.89	0.11	0.08	0.11	0.71
6	0.92	0.07	0.07	0.15	0.70
7	0.92	0.07	0.08	0.16	0.68
8	0.90	0.10	0.07	0.15	0.68
Mean	0.90	0.10	0.07	0.16	0.68
S.D.	0.02	0.01	0.01	0.03	0.03

AF_{PAR} is the spectral photosynthetic absorption factor; T_L(PAR) and R_L(PAR) are the leaf-specific transmittance and reflectance of photosynthetically active radiation (PAR), respectively; A_L(720) is non-photosynthetic absorbance at 720 nm, and A_L(PAR) is the leaf-specific photosynthetic absorbance.

Table 2

Posidonia australis: comparisons between the Diving-PAM derived absorption factor (AF_{B&B}, see text), spectral photosynthetic absorption factor (AF_{PAR}) and extrapolated incident irradiance ($I_{0\text{ Lvs}}$) vs. measured incident irradiances (I_0)

Short-shoot no.	AF _{B&B}	AF _{PAR}	$I_{0\text{ Lvs}}$ ($\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$)	I_0 ($\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$)
1	0.82	0.92	1619	923
2	0.79	0.88	2321	1417
3	0.88	0.90	906	1418
4	0.89	0.88	1380	2557
Mean	0.84	0.90	1556	1579
S.D.	0.04	0.02	589	692

Maximum ETR values on the first and second days were $64.7 \pm 6.2 \mu\text{mol electrons m}^{-2}\text{ s}^{-1}$ and $47.1 \pm 2.8 \mu\text{mol electrons m}^{-2}\text{ s}^{-1}$, respectively (Fig. 1C). Similar to estimates of $\Delta F/F'_m$, the residual standard deviation (S.D./mean) in ETR between samples was generally greatest during the middle of the day.

4. Discussion

In order to assess ETR, one requires estimates of irradiance, the ratio of PSII to PSI, $\Delta F/F'_m$ and leaf-specific photosynthetic absorbance [$A_L(\text{PAR})$]. Irradiance is (usually) measured proximal to the leaf sample(s) being measured. Although our irradiance values are estimates only, this does not influence the main thrust of this part of the study, which was not to obtain quantitative rates of ETR per second, but to compare differences in ETR calculations derived using leaf-specific photosynthetic absorbance and calculations derived using AF. The PSII:PSI absorption ratio is generally unity for seagrasses (Major and Dunton, 2002).

$\Delta F/F'_m$ measured in darkness is equivalent to the maximal quantum yield of chlorophyll fluorescence (F_v/F_m), and values measured in this study are similar to values for seagrasses reported in other studies (Beer et al., 1998; Beer and Björk, 2000; Durako and Kunzelman, 2002). As the multi-channel device has no facility for briefly darkening the samples immediately prior to measurement, all $\Delta F/F'_m$ measurements taken during the day necessarily incorporated energy-dependent non-photochemical quenching (Schreiber, in press). Therefore, $\Delta F/F'_m$ values obtained using the multi-channel device may be slightly lower than those obtained a few seconds after acclimation in the dark (when this quenching process is relaxed). This slight decline was evident when comparing measurements performed with the Diving-PAM and the multi-channel device. Nevertheless, replicate $\Delta F/F'_m$ values that are obtained inclusive of energy-dependent non-photochemical quenching provide a powerful description of the diel changes in $\Delta F/F'_m$, and in this study, the midday decline was significant over both daylight periods in spite of lower actual irradiances during the second day due to cloudy weather and surface waves. The midnight values of $\Delta F/F'_m$ during the second night were slightly lower than those of the first night. This may have been due to biofouling of the fluorescence probes with chlorophyllous material, or an effect of the clips (e.g. reduced water movement, structural stress).

For leaf-specific absorptance estimation, the Diving-PAM uses a default value of 0.84, representative of 44 species of terrestrial leaves (Björkmann and Demmig, 1987). In a later study, leaf optical properties from 26 species of herbs to trees collected from shaded-to-open habitats also indicated average leaf-specific photosynthetic absorptances ranging from 0.83 to 0.87 (Knapp and Carter, 1998). This suggests there is striking overall similarity in terrestrial vascular leaf optical properties across a diversity of habitats. In contrast, reported AF values for seagrasses measured in situ vary by almost a factor of 2 (0.44 for *Z. marina*, Beer et al., 1998; to 0.78 for *T. testudinum*, Durako and Kunzelman, 2002). Seagrass AFs also exhibit significant within-shoot variability leading to the suggestion that in situ fluorescence measurements be restricted to rank 2 leaves (Durako and Kunzelman, 2002). By using integrating spheres to measure leaf optical properties in vivo, we can now account for light scattering in both transmittance and reflectance of incident irradiance. This leads to a more accurate estimate of the proportion of the incident irradiance that is absorbed in the leaf tissue. Resulting estimates of leaf-specific photosynthetic absorptance using this optical arrangement are lower and less variable than the AF values derived from simply placing leaves over a PAR sensor, as the latter incorporates both spectral reflectance (ca. 7% of PAR) and non-photosynthetic absorbance (ca. 16% of PAR). Our calculated value of $A_L(\text{PAR})$ for rank 2 leaves of the thick-leaved seagrass *P. australis* (0.68 ± 0.03) was significantly lower than AF estimates (0.84–0.90) derived using the latter method. As has previously been shown with *Z. marina* and *T. testudinum* (Cummings and Zimmerman, 2002), if non-photosynthetic absorbance is included, total leaf-specific absorptance of *P. australis* is similar to higher plants ($0.68 + 0.16 = 0.84$; Table 1).

The application of $A_L(\text{PAR})$ instead of AF in our ETR calculations suggests that, at least in the case of *P. australis*, previous estimates of ETR were about 24% too high (assuming that PSII:PSI absorption ratio is unity). Discrepancies of this magnitude are not trivial, especially when using fluorescence data to derive estimates of productivity. When determining ETR of a phototroph in situ, we suggest in vivo measurements of $A_L(\text{PAR})$ be

carried out afterwards to ensure that absorbance of photosynthetically active radiation within the leaf tissue is correctly estimated.

Although our assumption that a PSII:PSI absorption ratio of unity holds true for most higher plants including seagrasses (Falkowski and Raven, 1997; Major and Dunton, 2002 and references therein), this is not necessarily the case for some protists (rhodophytes) and prokaryotes (cyanobacteria) where the ratio can be between 0.5 and 0.25, while some chromophytes may have ratios of two or greater (Falkowski and Raven, 1997). Deviation from the assumed ratio of unity would provide ETR estimates that are five-fold greater (calculated from a ratio of 0.25) or two-thirds less (calculated from a ratio of 2) than that calculated using the default value provided in some modulated fluorometers. Evaluation of the PSII:PSI absorption ratio for a species, and understanding how this ratio may vary under different environmental conditions is the next step required in the search for accurate measurement of ETR.

Lastly, the simultaneous measurement of variable fluorescence of multiple samples is clearly important in assessing the variability in physiological state within a population of photosynthetic organisms. The combination of obtaining replicated virtually simultaneous sample measurements and determining the photosynthetically relevant absorbance values for individual samples takes us a few steps closer to a truly representative estimate of ETR.

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