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Photosynthetic characteristics of *Thalassia testudinum* measured in situ by pulse-amplitude modulated (PAM) fluorometry: methodological and scale-based considerations

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Abstract

Shoot-to-landscape scale sources of variation in photosynthetic characteristics of *Thalassia testudinum*, as measured in situ using a submersible pulse-amplitude modulated fluorometer (diving-PAM), were investigated. Shoot-scale variation was assessed to develop a standard-methods protocol for this species. Significant within-shoot and among-shoot scale variation was observed for several PAM-fluorescence parameters. The fraction of incident photosynthetically active radiation (PAR) absorbed by rank 1 (youngest) leaves, 0.67 ± 0.03, was significantly lower than the PAR absorbed by rank 2 and 3 leaves (0.78 ± 0.04 and 0.77 ± 0.04, respectively). Quantum yields (Y) and photosynthetic efficiency (F_v/F_m) exhibited greater variability and generally decreased with increasing leaf age ($Y = 0.78 \pm 0.02$, 0.78 ± 0.03, and 0.76 ± 0.06 and $F_v/F_m = 0.79 \pm 0.02$, 0.77 ± 0.5, and 0.75 ± 0.06 for rank 1, 2, and 3 leaves, respectively). Maximum fluorescence of light-acclimated leaves (F'_m) significantly decreased from the base to the tip of leaves (1032 ± 203, 1059 ± 139, and 793 ± 107 for the base, middle and tip of the leaf) and Y was significantly reduced from 0.68–0.76 to 0.27–0.62 when measured where leaf lesions were present. However, paired comparisons (with versus without lesions at the tip of the leaf) for the base, middle, and tip areas of rank 2 leaves indicated that lesions effects on photosynthesis were localized only to the area of the lesion (tip). The Y exhibited relatively high variability for short-shoots within seagrass die-off patches and were significantly reduced for short-shoots exhibiting die-off symptoms (rotten leaf base grayish-green leaf color, but no lesions). Negative response slopes for Y and F_v/F_m, measured as part of a landscape-scale sampling program, revealed the presence of significant diurnal (time-of-day) and photosynthetic photon flux density (PPFD) related variation, with a more negative slope for Y. These results indicate that the selection of leaf tissue, short-shoot location, and time of

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measurement need to be considered when determining photosynthetic rates for seagrasses in situ.
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1. Introduction

The use of chlorophyll-fluorescence techniques, such as pulse-amplitude modulated (PAM) fluorescence, are attractive for assessing photosynthetic characteristics of seagrasses because they are non-invasive, quantitative, and provide information about the photosynthetic efficiency ($F_{\mathrm{v}}/F_{\mathrm{m}}$) of photosystem II (PSII) (Kooten van and Snel, 1990; Beer et al., 1998; Ralph et al., 1998). In addition, PAM fluorescence correlates with both O_2 evolution (Ralph and Burchett, 1995; Beer and Björk, 2000) and ^{14}C uptake (Hartig et al., 1998). The recent development of an underwater PAM fluorometer allows for the assessment of seagrass photosynthesis in situ, without the need for enclosures (Masini et al., 1995; Ralph et al., 1998; Beer and Björk, 2000). However, Beer and Ilan (1998) warned that PAM fluorescence cannot totally replace O_2 or CO_2 methods, especially where net production or gas exchange balances are sought.

In PAM fluorometry, the leaf is subjected to a pulse of saturating light and two fluorescence measurements are made: one before (F'—the fluorescence at a given photosynthetic photon flux density (PPFD) or F—the fluorescence for dark-acclimated tissue) and one during (F'_{m}—the maximum fluorescence at a given PPFD or F_{m}—the maximum fluorescence for dark-acclimated tissue) the pulse. Effective Y ($F'_{\mathrm{m}} - F'/F'_{\mathrm{m}}$) is a measure of electron transport through PSII at a given irradiance, when part of the reaction centers are closed (Beer and Björk, 2000). Photosynthetic efficiency, or potential Y, $F_{\mathrm{v}}/F_{\mathrm{m}}$ (where $F_{\mathrm{v}} = F_{\mathrm{m}} - F$), is measured using dark-acclimated leaves; dark-acclimation allows for all of the reaction centers to open and all primary electron acceptors to be oxidized. The $F_{\mathrm{v}}/F_{\mathrm{m}}$ has been used as a sensitive indicator of photosynthetic stress in seagrasses (Ralph, 1999). Ralph and Burchett (1995) reported that fluorescence was more sensitive than oxygen electrode techniques for monitoring irradiance stress. PAM fluorescence has also been used to measure salinity stress (Kamermans et al., 1999), and carbon limitation (Hellblom and Björk, 1999; Schwarz et al., 2000) in seagrasses.

Several methodological considerations have already been identified for diving-PAM measurements of the photosynthetic characteristics of seagrasses. Calculation of absolute photosynthetic electron transport rates (ETR) from Y measurements depends on knowing the PPFD at the leaf surface and the fraction of the PPFD absorbed by the leaf (Beer et al., 1998). Beer et al. (1998) reported absorption factors (AF) for seagrasses range from 0.44 for *Zostera marina* L. to 0.72 for *Cymodocea nodosa* (Ucria) Aschers (Beer et al., 1998); all are lower than the instrument default value of 0.84 which is a representative value for terrestrial plant leaves. Ralph et al. (1998) suggested that recent light-history plays a significant role in seagrass photosynthetic responses measured by PAM fluorometer. They observed significant diurnal variation in maximum ETR in three Australian seagrasses. Maximum ETR increased in the morning, declined to a plateau in the mid-day, and then declined in dusk. In contrast, Y remained steady over the day or were high early in the morning, declined to a trough in the mid-day and increased toward in dusk. Beer and Björk (2000) observed that both F_v/F_m and ETR tended to decrease from early morning toward noontime in *Halophila ovalis* (R. Br.) J.D. Hook and *Halodule wrightii* Ashers.

We have begun assessing the utility of the diving-PAM for investigating the physiological condition of *Thalassia testudinum* Banks ex König in Florida Bay. PAM-derived fluorescence characteristics are being evaluated as non-destructive ecoindicators (i.e. indicators of health or early indicators of stress; *sense* (Durako, 1995; Ralph and Burchett, 1995), in the context of identifying beds which may be susceptible to the widespread die-off affecting this dominant species within the Bay (Robblee et al., 1991) versus beds which may have high recovery potential. In this study, several sources of variation in photosynthetic characteristics of *T. testudinum*, as measured in situ using a diving-PAM, were investigated. We initially investigated within-shoot leaf-scale variation to develop a standardized measurement protocol. We then examined larger-scale spatial variation for short-shoots within, and adjacent to, die-off patches and from a large Florida Bay scale data set obtained during sampling conducted as part of the Fish Habitat Assessment Program (FHAP). The goal of FHAP, which has been in existence since 1995, is to assess the status and trends of seagrass and macroalgae distribution, abundance, and condition within 10 basins in Florida Bay (as shown in Fourqurean et al., 2001; Durako et al., 2001 for detailed descriptions of FHAP).

2. Materials and methods

2.1. Chlorophyll fluorescence

Photosynthetic characteristics of *T. testudinum* were measured in situ in Florida Bay, FL, USA. All measurements were obtained at shallow depths (0–2 m) between dawn and dusk. PAM chlorophyll fluorescence was measured with a diving-PAM (Walz, Germany), using SCUBA. The tip of the instrument's optical fiber was placed 10 mm from, and perpendicular to the adaxial surface of leaves, using dark leaf clips. Effective quantum yield (*Y*) was determined by the saturating-light method as $F_m' - F'/F_m'$. The F_v/F_m was determined on dark-acclimated leaves. ETR may be estimated by multiplying *Y* by the PPFD received by the leaf, by 0.5 (which assumes half the PPFD is absorbed by PSII), and by the fraction of PPFD absorbed by the leaf AF. The light sensor of the diving-PAM is a cosine collector and, thus, sensitive to any variations in angle relative to the radiant flux (Kirk, 1994). Because of the constantly changing angle of *T. testudinum* leaves within the light field in situ, coupled with the difficulty we experienced in placing the sensor with a fixed angle at the position of the fluorescence measurements to obtain representative PPFD, we did not attempt to estimate and compare ETR values here.

2.2. Within-shoot scale measurements

Several series of measurements were conducted to examine sources of variability in the photosynthetic characteristics of *T. testudinum*, at differing scales. The first measurement series examined within-shoot leaf-scale variability and was conducted in Sunset Cove (25°5.4′N, 80°27.0′W), during July 2000. Chlorophyll *a* fluorescence, leaf AF, and derived photosynthetic responses (i.e. Y and F_v/F_m) were examined with respect to: (1) leaf age, from the youngest, second youngest and third youngest fully-developed leaves (first–third leaf ranks); (2) position of measurement along individual leaves (base, middle, tip); and (3) the presence or absence of necrotic lesions within the field of measurement. For F_v/F_m measurements, 5 and 10 min dark acclimation times were compared. Variability in leaf AF was also investigated. Here, AF values for the mid-leaf region of *T. testudinum* leaves of differing ages (ranks) were estimated by the method of Beer and Björk (2000).

2.3. Among-shoot scale measurements

A second series of measurements examined shoot-scale variability within, and adjacent to (along the ecotone and 1 m inside an apparently healthy bed), a recent die-off patch. It was assumed that the patch was recent, but probably not active, based on the condition of the rhizome material in the patch, the lack of standing-dead short-shoots with still-attached leaf sheaths, the absence of fresh leaf litter, and the sharp density ecotone (Durako, 1995). Measurements were carried out on Cross Bank (25°0.6′N, 80°34.8′W), also during July 2000. Shortly, thereafter, a series of measurements were made in an area with active die-off patches north of Barnes Key (24°56.4′N, 80°47.4′W), during September 2000. Active die-off was verified by the presence of fresh dead, but still green, leaves in the litter, standing-dead shoots with attached leaf sheaths, and by the presence of short-shoots with easily dislodged leaves with decaying leaf bases. The objectives of the die-off patch measurements were to determine the level of chlorophyll fluorescence variability of short-shoots within, and immediately adjacent to, recent and active, die-off patches and to determine if PAM fluorometry can be used as a tool to detect die-off related physiological stress in short-shoots. In the seagrass beds surrounding the die-off patches at the Barnes Key site, a fourth series of measurements were conducted to examine variability in Y as a function of position of measurement on the leaf (base, middle, tip) for short-shoots with, and without leaf lesions. Because the earlier (July 2000) measurements demonstrated a significant reduction in PAM fluorescence for leaves with lesions, the objective of this fourth series of measurements was to determine the extent of the lesion effect on Y.

2.4. Landscape-scale measurements

This series of measurements was conducted during the spring 2001 FHAP sampling conducted during May 2001. In this series, temporal and spatial variability due to sampling logistics associated with a large-scale sampling program were examined. The basins sampled in FHAP are representative of the range of environmental conditions within the Bay. The basins also exhibit a range of severity of die-off. Sampling in FHAP is conducted during the spring and fall each year in a manner that allows for unbiased interpolation of the actual sample points. Each basin is divided into 27–33 tessellated hexagonal sub-units; one random location is sampled from within each hexagonal sub-unit (Fourqurean et al., 2001). Thus, 275–330 stations are sampled each season (as shown in Durako et al., 2001 for a map of sampling locations). During the spring 2001 FHAP sampling, Y and F_v/F_m (5 min dark acclimation) were measured for four short-shoots at each of 285 stations. Scalar irradiance was measured at the level of the *T. testudinum* canopy at each station using quantum PAR scalar sensors (LiCor LI-193S). Generally, one basin per day (28–33 stations) was sampled (approximately 20 min per station), thus, measurements were obtained throughout the day (8:00 a.m.–7:00 p.m.).

2.5. Statistical analyses

Shoot-scale variability in leaf AF and chlorophyll fluorescence as a function of leaf rank and position were assessed using one-way ANOVA. All data were tested for normality and homogeneity of variances by the Kolmogorov–Smirnov test with Lilliefors' correction and the Levene Median test, respectively. If normality failed and homogeneity passed, transformations were attempted. If no transformations were successful in achieving normal distributions, then parametric analyses were performed on the raw data. If homogeneity of variances failed, data transformations were again attempted. If no transformations were successful, then non-parametric analyses (Mann–Whitney rank sum or Kruskal–Wallis one-way ANOVA on ranks) were used to assess treatment effects. If significant differences were found among treatments, then Tukey's tests were used to determine specific treatment differences. Variability due to differences in dark-acclimation times (5 min versus 10 min) and the presence or absence of lesions was evaluated using *t*-tests. Linear regression analyses were used to assess time-of-day and PPFD-related variability in Y and F_v/F_m in the FHAP data. All statistics were calculated using SigmaStat 2.0 (Jandel Scientific, San Rafael, CA) with significance determined at the 95% probability level ($P < 0.05$). Values are all reported as means ± S.D.

3. Results

3.1. Within-shoot scale variability

Significant within-shoot scale variation was observed for several parameters. Values of AF for leaves of differing ages (ranks) are shown in Table 1. All AF values for the *T. testudinum* leaves were lower by 7–21% than the diving-PAM default value 0.84. Leaf AF values for the youngest leaves were significantly lower than those of the second and third youngest leaves. The youngest leaves were visibly lighter green in color and had lower mean minimum fluorescence (F) values (Table 1), indicating that lower chlorophyll contents may be partially responsible for the lower AF values. A comparison between 5

Table 1
Fractions of incident light absorbed (AF) and F of the mid-blade region of leaves of differing ages (ranks) in *Thalassia testudinum*

Leaf age	Leaf Rank	AF*	F
Youngest	1	0.67 ± 0.03 b	169 ± 62
Second youngest	2	0.78 ± 0.04 a	172 ± 76
Third youngest	3	0.77 ± 0.04 a	202 ± 94

[*] Values are means ± S.D., ($n = 10$); values with the same letter are not significantly different as determined by Tukey's multiple comparison test ($P < 0.05$).

(0.71 ± 0.12) and 10 min (0.71 ± 0.10) dark-acclimation times for F_v/F_m measurements of the mid-blade portion of rank 2 leaves, indicated no significant time effect (paired *t*-test, d.f. $= 32$, $P = 0.93$).

The *Y* and F_v/F_m with 5 min dark acclimation for *T. testudinum* in Sunset Cove tended to decrease, but exhibited greater variability, with increasing leaf age ($Y = 0.78 \pm 0.02$, 0.78 ± 0.03, and 0.76 ± 0.06 and $F_v/F_m = 0.79 \pm 0.02$, 0.77 ± 0.5, and 0.75 ± 0.06 for ranks 1–3 leaves, respectively), although the decreases were not statistically significant. Variability in *Y* as a function of position along the leaf (0.76 ± 0.02, 0.75 ± 0.04 and 0.70 ± 0.04 for the base, middle and tip, respectively) was also not significant ($F_{2,17} = 3.55$, $P = 0.055$), however, F'_m did significantly vary as a function of leaf position ($F_{2,17} = 4.45$, $P = 0.03$, Fig. 1), being lowest for the tip region of the leaves. Effective *Y* for short-shoots at both Sunset Cove and Cross Bank were significantly reduced when measured where

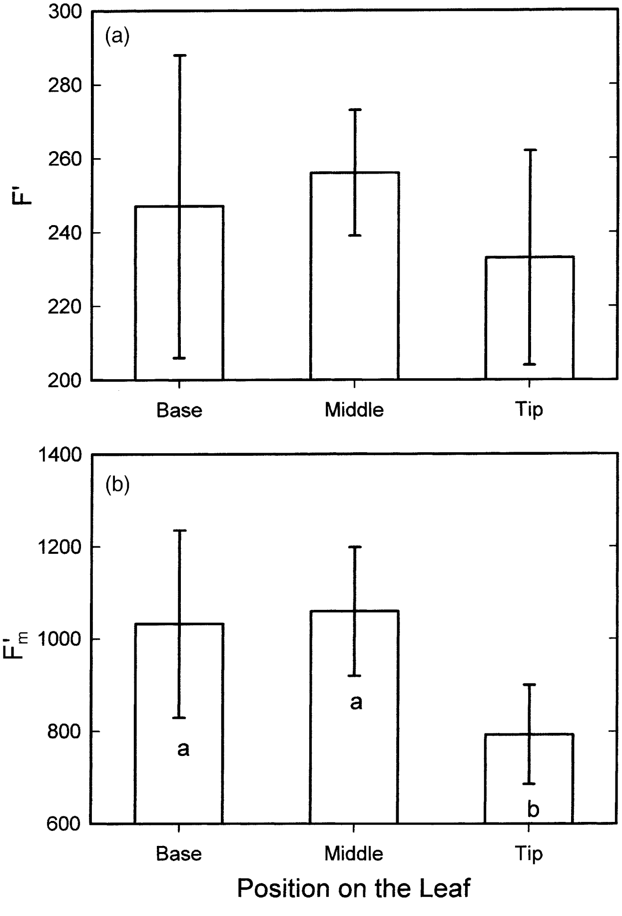

Fig. 1. (a) Minimum and (b) maximum light-acclimated fluorescence of *T. testudinum* as a function of position of PAM fluorescence measurement along the rank 2 leaf (mean ± S.D., $n = 4$, bars with the differing letters are significantly different as determined by Tukey's multiple comparison test).

Table 2
Shoot-scale variability in effective Y for short-shoots with leaf lesions and for short-shoots located adjacent to or within die-off patches in *Thalassia testudinum*[a]

Location	Treatment group	Y	d.f.	T/F	P
Sunset Cove	Clean	0.76 ± 0.02 a	6	54.00	0.015
	Lesion	0.62 ± 0.11 b			
Cross Bank	Clean	0.68 ± 0.03 a	6	57.00	0.002
	Lesion	0.27 ± 0.12 b			
	1 m Inside bed	0.74 ± 0.02	2,17	2.84	0.09
	Ecotone	0.72 ± 0.03			
	Die-off patch	0.69 ± 0.06			
Barnes Key	Green shoot	0.77 ± 0.03 a	2,19	13.54	<0.001
	Ecotone	0.71 ± 0.09 a			
	Die-off shoot	0.58 ± 0.107 b			
	Shoot with no leaf lesions				
	Leaf base	0.74 ± 0.01	2,11	1.06	0.39
	Leaf middle	0.73 ± 0.03			
	Leaf tip	0.71 ± 0.04			
	Shoot with leaf lesions				
	Leaf base	0.74 ± 0.01 a	2,11	22.68	<0.001
	Leaf middle	0.72 ± 0.01 a			
	Leaf tip with lesion	0.68 ± 0.01 b			

[a] Values are means ± S.D.; values with the same letter are not significantly different as determined by Mann–Whitney rank sum t-test (T) or Tukey's multiple comparison test ($P < 0.05$).

leaf lesions were present (Table 2); Y was reduced by >50% by lesions in the Cross Bank population. Based on these results the sampling protocol that was adopted for most of our subsequent measurements utilized the mid-blade region of lesion-free, rank 2 leaves; 5-min dark acclimation periods were used for F_v/F_m determinations.

3.2. Among-shoot scale variability

The Y exhibited relatively high variability for the isolated short-shoots within the older die-off patch at Cross Bank (Table 2). Mean Y decreased from 1 m inside an apparently healthy bed to the shoots at the ecotone and to the isolated shoots within the die-off patch, but because of the high variability the decrease was not statistically significant (Table 2). In contrast, Y was significantly reduced (Table 2) for short-shoots exhibiting die-off symptoms (rotten leaf base grayish-green leaf color) within an active die-off patch at Barnes Key, relative to healthy-appearing shoots within the patch (green shoot) and shoots along the ecotone between the die-off patch and a healthy-appearing bed (Table 2). These measurements were taken within a small (approximately 2 m diameter) patch having many shoots with easily dislodged leaves. No lesions were observed within the active die-off patch.

Short-shoots with leaf lesions were abundant in the seagrass beds surrounding the die-off patches at Barnes Key. The general trend of decreasing Y from the base to the tip of the leaf was again observed at Barnes Key for both short-shoots with and without lesions (Table 2). The decrease was significant for shoots with lesions at the tip region of their leaves and only for the tip area. Thus, the lesion effects were localized only to the area of the lesion and photosynthesis for the rest of the leaf was not significantly affected.

3.3. Landscape-sampling scale variability

Regression analyses of Y and F_v/F_m measured during the May 2001 FHAP sampling indicated significant diurnal variation (Fig. 2). The response slopes for both characteristics as a function of time of day were negative for the entire morning-to-dusk data set, with a slightly more negative slope for Y. When only the afternoon measurements were examined, the fluorescence response slopes were not significantly different from zero. Mann–Whitney rank sum t-test comparisons of pooled data for the morning (8–13 h) and afternoon (13–20 h) indicated that $Y(T_{498,695} = 256760, P < 0.001)$ and F_v/F_m $(T_{502,685} = 23763, P < 0.001)$

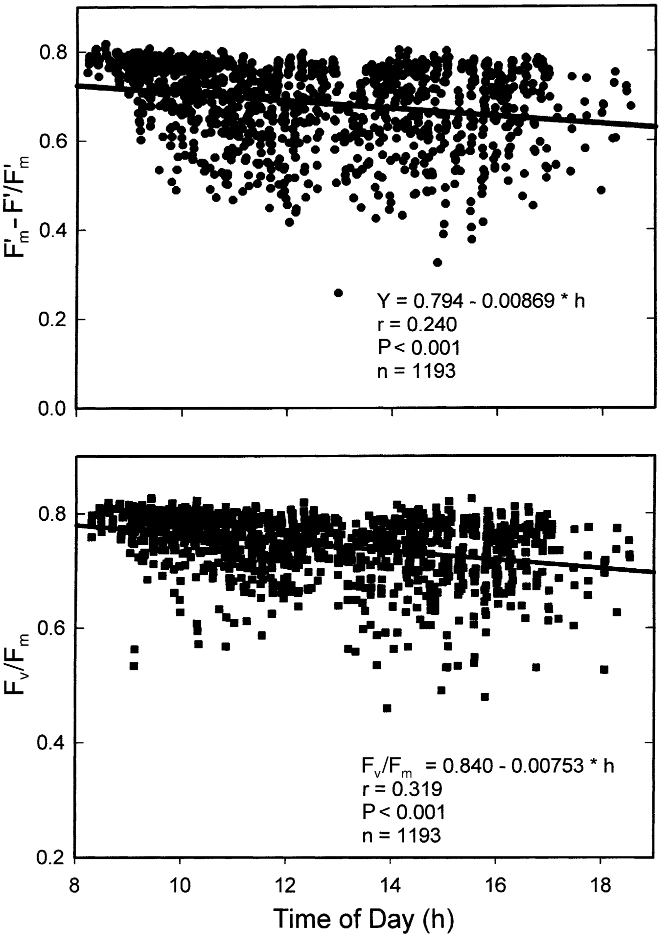

Fig. 2. Photosynthetic quantum yields $(F'_m - F'/F'_m)$ and F_v/F_m of *T. testudinum* sampled from 08:00 to 19:00 h in 10 basins in Florida Bay: the solid line represents the fitted regression equation.

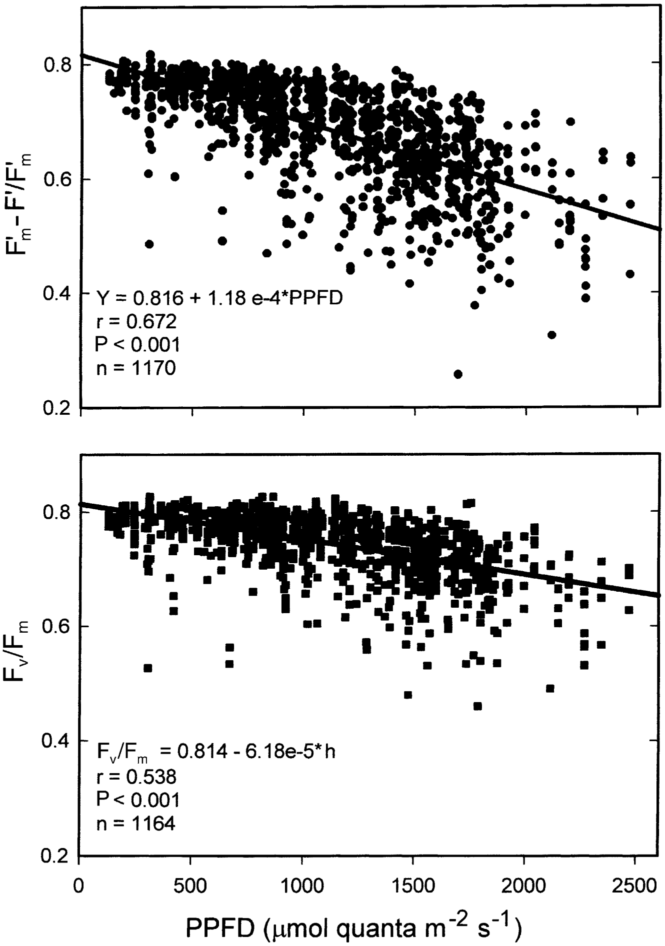

Fig. 3. Photosynthetic quantum yields ($(F'_m - F'/F'_m)$) and F_v/F_m of *T. testudinum* with respect to PPFD at the level of the leaf canopy in 10 basins in Florida Bay: the solid line represents the fitted regression equation.

were significantly lower in the afternoon. There were also significant negative response slopes for Y and F_v/F_m versus PPFD at the canopy (Fig. 3). Again, yield was more negatively correlated with PPFD than F_v/F_m.

4. Discussion

4.1. Within-shoot scale variability

In order for a parameter, such as chlorophyll fluorescence, to be utilized as an ecoindicator, parameter responses must be representative of the physiological condition or "health" of the organism and sources of variation must be partitioned between natural variability and variability associated with stress or deteriorating condition (Durako, 1995). Leaf-age related variability contributed to significant differences in within-shoot PAM-fluorescence characteristics of *T. testudinum*. Thus, choice of leaf tissue is an important methodological consideration. To calculate an absolute photosynthetic ETR using PAM fluorometry, the AF of the leaves and the incident irradiance at the point of the fluorescence measurement need to be determined (Beer et al., 1998). Interspecific variability in seagrass leaf AF is known to be significant (Beer et al., 1998; Beer and Björk, 2000). However, intraspecific (among-leaf) variability in leaf AF for seagrasses has not been previously reported. The significantly lower AF for youngest leaves measured for *T. testudinum* would reduce calculated ETRs by 15%. Except for the youngest leaf, the leaf AF measured for *T. testudinum* leaves were higher than previously reported for seagrasses (0.44–0.72, Beer et al., 1998; Beer and Björk, 2000; Schwarz et al., 2000), but lower than the diving-PAM default AF of 0.84, a value that represents the average absorption of photosynthetically active radiation (PAR) in terrestrial vascular plant leaves (Björkman and Demmig, 1987). Although rank 2 and 3 blades had similar leaf AF, rank 3 blades of *T. testudinum* in Florida Bay exhibit lower growth and higher surface fouling (sediments, detritus, and epiphytes) than rank 2 leaves, and they frequently exhibit signs of senescence, such as chlorotic or necrotic leaf tips. Rank 3 leaves exhibited greater variability in both Y and F_v/F_m. Maximum photosynthetic rates (P_{max}) also decline with increasing leaf age in *T. testudinum* (Durako and Kuss, 1994). Thus, rank 2 blades (second youngest) seem to be generally preferable for PAM fluorescence measurements to characterize short-shoot photosynthesis.

Because the short-shoot leaf meristem in *T. testudinum* is intercalary (Tomlinson, 1972), leaf age also varies from the base (youngest) to the tip (oldest) of individual leaves. Leaf tips of older rank leaves are frequently chlorotic, broken off, and more heavily fouled than lower blade regions. Our measurements showed a general trend of declining Y from the base to the tip of even rank 2 leaves, with a significant reduction in maximum fluorescence at the leaf tip. This reduction in maximum fluorescence may indicate an increase in non-photochemical quenching, rather than a destruction of PSII reaction centers (Dawson and Dennison, 1996). In addition to an age effect, the reduction in maximum fluorescence at leaf tip may also be a response to higher irradiance at the top of the canopy (Ralph and Burchett, 1995). The logistics of placing the leaf clip and optical fiber of the diving-PAM at the leaf base were more difficult due to physical interference from leaf sheaths enclosing the shoot base and from neighboring shoots. In addition, because of the presence of steep vertical gradients in physical parameters (e.g. light, current velocity, diffusive boundary layer, etc.) within seagrass canopies (Koch, 1996, 2001), photosynthetic rates at the base of the short-shoot may be light, or carbon, limited. Thus, we adopted the middle portion of rank 2 blades as the most representative tissue for assessing shoot-specific photosynthesis.

Quantum yield ($F'_m - F'/F'_m$) is measured under light-acclimated conditions, whereas, F_v/F_m is measured following a dark-acclimation period. Both 5- and 10-min dark-acclimation periods have been used for seagrasses (Ralph et al., 1998; Ralph, 1999; Beer and Björk, 2000). Because of the lack of a significant difference between 5- and 10-min dark acclimation times for *T. testudinum*, we adopted the shorter times for our standard F_v/F_m measurement protocol.

4.2. Among-shoot scale variability

In examining among-shoot scale variability, we were most interested in using PAM fluorescence to compare levels of stress. Effective *Y* is the most consistent measure of photosynthetic stress from a combination of factors (Ralph, 1999). The *Y* of *T. testudinum* leaf material measured in situ at Sunset Cove and Cross Bank were significantly lower for regions of *T. testudinum* leaves that had visible lesions. These patterns agree with those previously reported using oxygen-flux measurements (Durako and Kuss, 1994). The necrotic leaf lesions indicate infection of the leaf by the marine slime mold *Labyrinthula*, an organism thought to play a role in the die-off of *T. testudinum* in Florida Bay (Porter and Muehlstein, 1989; Robblee et al., 1991; Durako and Kuss, 1994). Reduced photosynthesis caused by *Labyrinthula* lesions decreases oxygen available for transport to below-ground tissues, possibly making *T. testudinum* more susceptible to hypoxia and sulfide toxicity (Carlson et al., 1994; Durako and Kuss, 1994). Comparisons of close-interval PAM fluorescence measurements along individual rank 2 leaves with and without visible lesions, indicated that lesion-caused reductions in photosynthesis were restricted to the immediate area of the lesion and did not result in a whole leaf-blade level effect. The PAM data reveal that the severity of stress imposed by the leaf lesions will be a function of the proportion of total leaf surface that is necrotic.

The *Y* also declined for short-shoots along transects from dense, apparently-healthy beds, into recent and active die-off patches. The photosynthetic characteristics of solitary short-shoots within the recent die-off patch at Cross Bank were significantly lower than those of shoots along the ecotone and shoots 1 m inside the bed. This indicates that photosynthesis may be reduced in die-off patches, even in the absence of visible lesions. In the case of the recent die-off patch, the reduction in *Y* may partially be a density/light effect, rather than being indicative of die-off induced stress. Short-shoot density significantly declines from seagrass beds to die-off patches (Durako, 1995). This reduction in density reduces shading from neighboring shoots, increasing light availability to individual shoots. The FHAP data indicate that *Y* decreases with increasing PPFD, which agrees with previous PAM data (Ralph et al., 1998; Beer and Björk, 2000). In contrast, the significant reduction in *Y* of short-shoots within the active die-off patch at Barnes Key are probably not a density/light effect. Here, the die-off shoots were surrounded by living and recently-dead shoots that still had most of their leaves attached (i.e. there was not a visible density gradient). The effect of density on photosynthesis, from the standpoint of nearest-neighbor effects on the light field, needs to be more clearly defined in future PAM measurements.

4.3. Landscape-sampling scale variability

Although a systematic random-sampling approach is utilized in FHAP, the large area and number of stations (28–33) sampled within each basin necessitates that stations are sampled in a systematic manner, to minimize station-to-station travel time. Sampling along efficient travel routes from morning to dusk allows sampling for each basin to be completed in a day, but we were concerned that this approach might also introduce additional sources of variation that might confound a landscape-scale assessment of physiological condition. The decline in *Y* and F_v/F_m with time of day for the pooled FHAP data is similar to the significant diurnal variation in these parameters previously observed using sensitive repeated-measures approaches (Ralph, 1996; Ralph et al., 1998) and for measurements within a small area (Beer and Björk, 2000). This down-regulation of photosynthesis was largely related to PPFD at the level of the canopy, and it illustrates the importance of recent light history in seagrass photosynthetic responses (Ralph et al., 1998). Our measurements indicate that this diurnal down-regulation of photosynthesis may introduce a significant source of variation in landscape-scale sampling of photosynthesis, this variation being greater for Y than for F_v/F_m.

The rapid, non-invasive nature of PAM fluorometry for the measurement of photosynthesis in situ is attractive for determining the physiological health of seagrass ecosystems. The data presented here indicate that sources of variation must be understood and considered before the PAM-fluorometric parameters can be widely applied in ecosystem assessments.

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