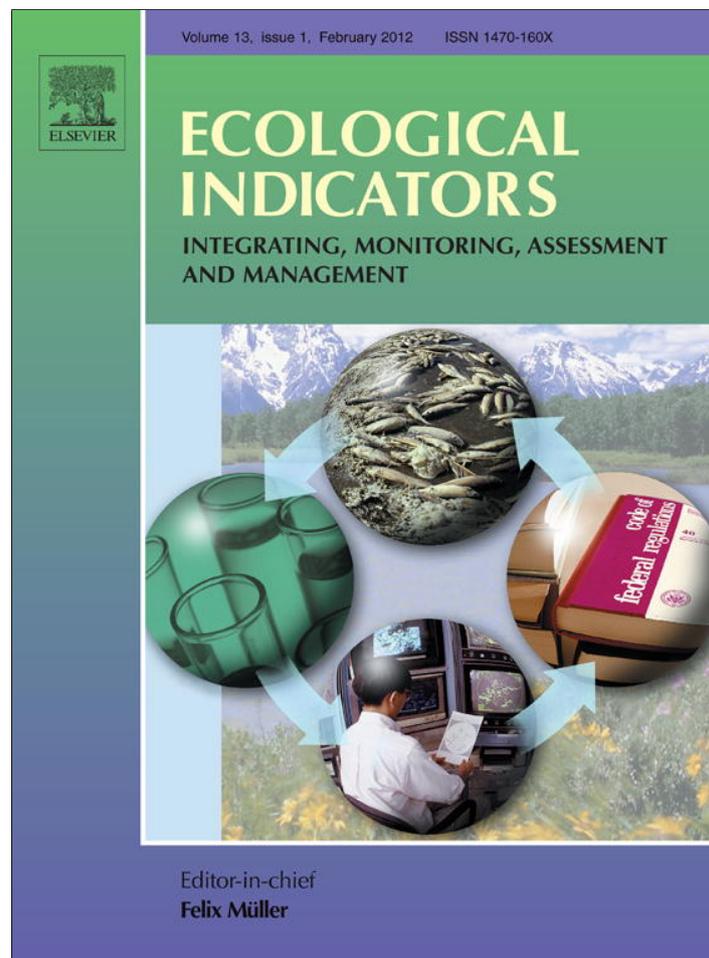


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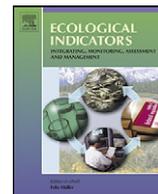
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Using PAM fluorometry for landscape-level assessment of *Thalassia testudinum*: Can diurnal variation in photochemical efficiency be used as an ecoindicator of seagrass health?

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ABSTRACT

Landscape-scale measurements of PSII photochemical efficiency ($\Delta F/F_m'$) by pulse-amplitude modulated (PAM) fluorometry were evaluated from 2006 to 2009 as a non-invasive method for monitoring the physiological condition of the seagrass, *Thalassia testudinum* Banks ex König, in Florida Bay, USA. Previous landscape-scale studies have shown that PAM-derived measurements of effective ($\Delta F/F_m'$) and maximum (F_v/F_m) photochemical efficiencies, as well as rapid light curves (RLC), exhibit significant diurnal variation. In this study, a regression approach was used to assess the magnitude of diurnal variation of $\Delta F/F_m'$ versus irradiance at the bay scale and among 12 sample basins. Variation in $\Delta F/F_m'$ versus irradiance reflects changes in the efficiency of dissipation of excess energy (photoprotection) and PSII inactivation (photodamage), which would be expected to vary with changes in physiological condition or photoacclimation state. $\Delta F/F_m'$ significantly decreased with increasing irradiance at both the bay and basin scales. Regression slopes of $\Delta F/F_m'$ versus irradiance were highly variable among years in 8 of the 12 sampled basins, ranging 5.6-fold from lowest to highest slopes. In contrast, regression derived y-intercepts varied only 1.7-fold over the 4-year period, but intercepts were significantly correlated with response slopes. Analyses of covariance indicated that diurnal response slopes significantly decreased from 2006 to 2009 at the bay scale, which corresponded to increasing water clarity and increased ambient irradiance at the leaf canopy as widespread phytoplankton blooms, which had initiated in the fall of 2005, dissipated. The results indicated a shift in the photo-acclimation state of *T. testudinum* with the cessation of phytoplankton blooms. They also suggest that regressions of $\Delta F/F_m'$ versus irradiance, rather than absolute $\Delta F/F_m'$ values, better reflect seagrass physiological condition when PAM-fluorescence measurements are obtained at landscape scales over diurnal periods.

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1. Introduction

Pulse-amplitude modulated (PAM) fluorometers provide quantitative information about photochemical efficiency by measuring chlorophyll fluorescence of photosystem II (PSII) (Schreiber and Schliwa, 1987). Because PSII is considered to be the most sensitive part of the photosynthetic pathway to stress (Becker et al., 1990), PAM fluorescence measurements may be used to identify changes in physiological status in response to varying environmental conditions before morphological or density-based changes are evident (Ralph et al., 2007a). In addition, PAM measurements are rapid, non-destructive and can be obtained without the need for

enclosures (Ralph et al., 1998; Schreiber, 2004; Ralph and Gademann, 2005). Thus, PAM fluorometry has become a powerful tool in the study of plant photosynthesis and it is increasingly being used for ecological monitoring (Durako and Kunzelman, 2002; Campbell et al., 2003; Adams and Demmig-Adams, 2004). The development of an underwater PAM fluorometer (Diving-PAM, Waltz) allows for the in situ assessment of photosynthetic condition in aquatic organisms, such as seagrasses and corals, under ambient conditions (Beer et al., 1998a,b, 2001; Ralph et al., 2007b; Chartrand and Durako, 2009).

Photosynthesis can rapidly respond to changes in the light environment (MacIntyre et al., 2000). The sensitivity of PAM fluorometry in detecting these rapid changes may result in highly variable signals when assessing the photophysiology of plants at the landscape scale. In a landscape-scale assessment of seagrass condition in Florida Bay, Durako and Kunzelman (2002) detected

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significant diurnal variation in both effective and maximum (dark acclimated) photochemical efficiencies of the dominant seagrass in the bay, *Thalassia testudinum* (turtle grass). This species was studied because its abundance and condition are perceived by the public as an indicator of the health of the bay (Goerte, 1994; Boesch et al., 1993; Durako et al., 2002). Significant negative slopes in regressions of effective and maximum (dark acclimated) photochemical yields against ambient irradiance or time of day illustrated the importance of diurnal variations in irradiance on the photosynthetic responses of *T. testudinum*.

Florida Bay is a shallow subtropical estuary, thus, seagrasses are exposed to large ranges of irradiances throughout the day. Belshe et al. (2007) reported that rapid light curve (RLC) derived parameters: photosynthetic efficiency (α), relative maximum electron transport rate ($rETR_{max}$), and saturation irradiance (I_k) also exhibited diurnal variability in *T. testudinum* in Florida Bay, but the magnitude, direction and significance of the variations were inconsistent among the ten sampled basins and between two sampling years. They were unable to discern which time of day was best suited for assessing the photophysiological status of *T. testudinum* using a statistical approach, but indicated that irradiance during measurements and light history effects must be factored into the interpretation of PAM fluorescence data. In a subsequent study, Belshe et al. (2008) investigated the use of calculated diurnal photochemical yields and diurnal rapid light curves (Longstaff et al., 2002), in an attempt to incorporate daily irradiance variability into their physiological assessment of *T. testudinum*. The diurnal yield method estimated unrealistically high predictions of $rETR_{max}$ and I_k . In contrast, the diurnal RLC method produced realistic predictions of $rETR_{max}$ and I_k , but was unable to predict α if ambient irradiances were $>I_k$ during sampling, which was relatively common at shallow and clear basins. They suggested that sampling earlier or later in the day when irradiances were reduced could overcome this problem, but acknowledged that this may not be possible for some field-sampling programs because of safety and navigational reasons (Belshe et al., 2008).

Although photosynthesis is dependent on irradiance, photosynthetic rates are not simply proportional to the rate of photon capture (Kirk, 1994). The number of electrons that can be funneled through PSII is affected by the number of reaction centers, whether the primary electron acceptor (Qa) is open to accept electrons and the time required to reopen Qa, among other factors. As irradiance increases, the number of open reaction centers in PSII decreases, resulting in a greater amount of fluorescence being emitted, an increase in alternative forms of energy dissipation (i.e., photoprotection and non-photochemical quenching; Demmig-Adams, 1998; White and Critchley, 1999; Marshall et al., 2000; Ort, 2001), and decreasing photochemical efficiencies (Ralph and Gademann, 2005). The relationship between irradiance and fluorescence is direct, but not always proportional. If non-photochemical energy dissipation mechanisms are not efficient, then PSII photoinactivation due to net photodamage may occur. A plant's photoacclimation state or whether it is stressed or not both may affect photosynthesis and thereby change the efficiency of these energy dissipation processes (Ralph and Gademann, 2005).

The seagrass *T. testudinum* exhibits photoacclimatory responses to changes in irradiance (Major and Dunton, 2002; Belshe et al., 2007). Changes in optical water quality, solar zenith angle, tides, wave focusing, and variable weather patterns may expose this species to a wide range of irradiances during each diurnal cycle. Thus, both efficient photon capture and photoprotective responses are required for its growth and survival (Ralph and Gademann, 2005). In this study, diurnal variation in ambient irradiance and PSII photochemical efficiencies of *T. testudinum* in Florida Bay were measured over a four-year period, as part of a landscape-scale ecological monitoring program. The relationships between

Table 1

List of basins sampled in Florida Bay and their abbreviations.

Abbreviation	Basin
Barnes	Barnes Sound
Blackwater	Blackwater Sound
Calusa	Calusa Key Basin
Crane	Crane Key Basin
Duck	Duck Key Basin
Eagle	Eagle Key Basin
Johnson	Johnson Key Basin
Manatee	Manatee Bay
Rabbit	Rabbit Key Basin
Rankin	Rankin Lake
Twin	Twin Key Basin
Whipray	Whipray Bay

irradiance and PSII photochemical efficiencies, at both the bay and basin scales, were examined using regression analyses and analysis of covariance to determine the utility of this approach in assessing the photophysiology of *T. testudinum* in response to changes in water clarity within Florida Bay. The objective of this examination was to determine if the slopes and intercepts from the PSII photochemical efficiency versus irradiance regressions, rather than individual $\Delta F/F_m'$ values, which depend on light history, would serve as better ecoindicators of seagrass physiological condition when measurements have to be obtained over diurnal periods.

2. Study area

This study was conducted within Florida Bay (ca. 25°05'N, 81°45'W), a shallow lagoonal estuary at the southern tip of Florida, USA. Florida Bay is characterized by over 50 shallow basins (water depth ca. <1–2 m) divided by carbonate mud banks and mangrove islands (Fourqurean and Robblee, 1999). Sampling was conducted as part of the South Florida Fish Habitat Assessment Program (FHAP-SF, see Durako et al., 2002 for a description of FHAP). FHAP-SF is a status and trends monitoring program for benthic macrophytes, which annually samples 17 basins extending from Lostman's River and Whitewater Bay in the western Everglades to Florida Bay (Fig. 1). Sampling was conducted at the same time each year, during the end of the dry season (May 12–June 3), when salinities tend to be highest (Boyer et al., 1997) and *T. testudinum* is approaching its maximum leaf area (Durako, 1995). The twelve basins (Table 1) chosen for this study are representative of the range of conditions across Florida Bay (Hackney and Durako, 2004) and all contain extensive beds of *T. testudinum*. Each basin was divided into 30 tessellated hexagonal subunits, and one station was randomly chosen within each subunit for each annual sampling (Fig. 1). Sampling subunits and station locations were generated using algorithms developed by the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) and were provided by Dr. Kevin Summers (EPA, Gulf Breeze, FL). This type of sampling design results in systematic random sampling and it scales the sampling effort to the size of the basin.

3. Methods

3.1. Chlorophyll fluorescence

Photosynthetic characteristics of *T. testudinum* were measured in situ with an underwater pulse-amplitude modulated fluorometer, Diving PAM (Walz, Germany). Fluorescence measurements were obtained during annual spring FHAP-SF sampling from 2006 through 2009. At each station where *T. testudinum* was present, effective PSII photochemical efficiencies ($\Delta F/F_m' = [F_m' - F]/F_m'$, where F is the steady-state chlorophyll fluorescence in ambient

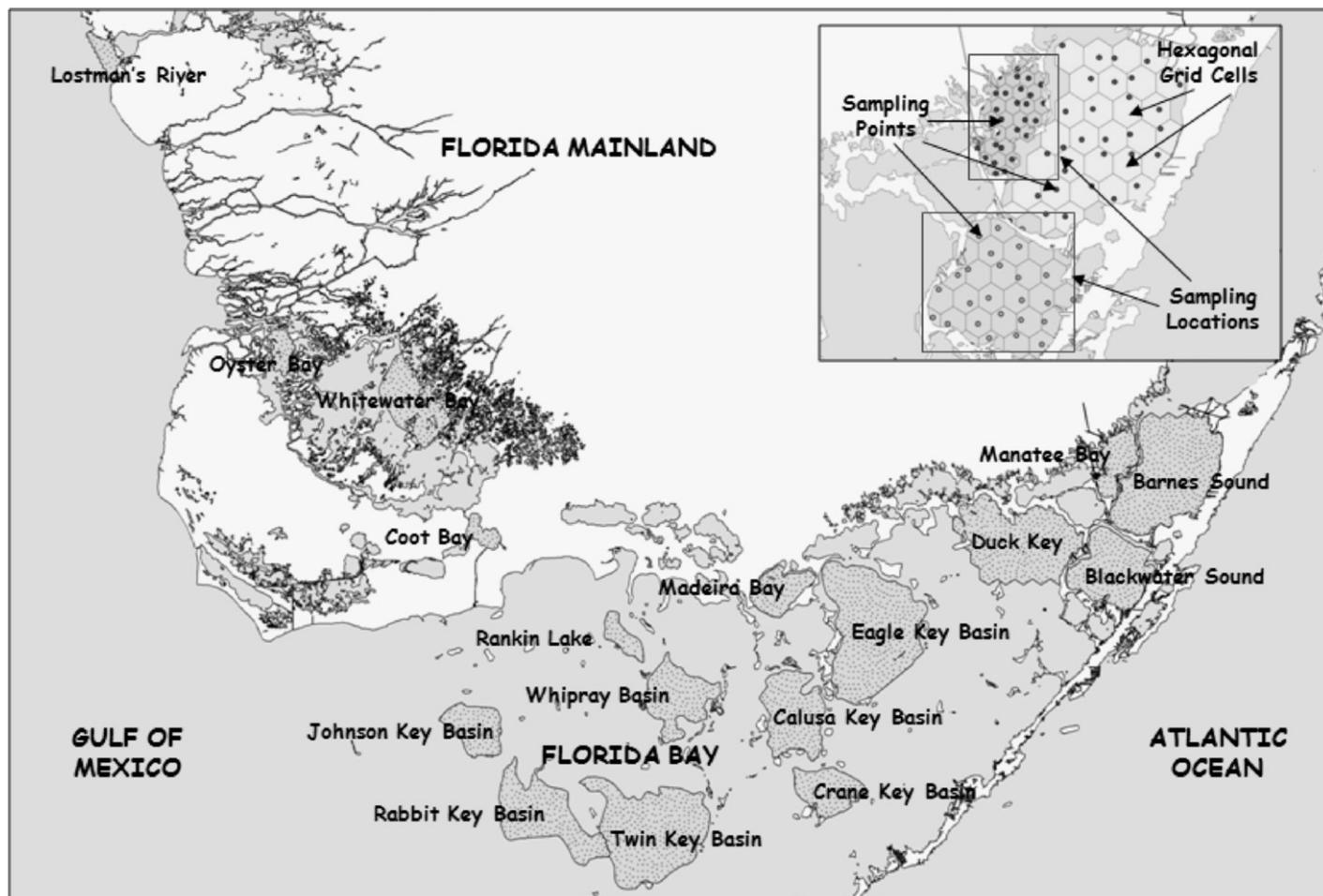


Fig. 1. Basin locations for the South Florida Fish Habitat Assessment Program (FHAP-SF). Inset shows detail of how each basin is subdivided into 30 tessellated hexagonal subunits from which a random sampling point is selected.

light and F_m' is the maximum fluorescence in ambient light following a saturating light pulse), were determined on the middle of the rank 2 leaf of six haphazardly chosen short shoots (Durako and Kunzleman, 2002). Sampled short shoots were separated by at least 2 m. A clear acrylic leaf clip held the Diving PAM fiber optic approximately 5 mm from the leaf surface and at an angle of 60° relative to the surface to minimize shading during yield measurements. Because Florida Bay is approximately 2000 km² and the sampled basins range in size from 5.8 to 62.4 km² (Durako et al., 2002), stations within each basin were sampled systematically over the course of a single day (~0800–1600 h) in order to minimize station-to-station travel time.

3.2. Irradiance

Photosynthetically active radiation (PAR, 400 to 700 nm) during PAM measurements was determined at each station at the top of the *T. testudinum* leaf canopy using an underwater scalar quantum PAR sensor (LiCor LI-193S) attached to a LiCor LI-1000 datalogger (Lincoln, NE, USA). Light profiles were also conducted at each station to calculate vertical attenuation coefficients of scalar irradiance [$K_0(\text{PAR})$] for the water column. Secchi and water depths were measured using a calibrated PVC pole with a Secchi disk attached to its end. Florida Bay is generally shallow (<2 m) so the secchi disk was frequently visible on the bottom. Because of among-basin variability in water depth, functional water clarity was estimated as the

water depth divided by the Secchi depth. This provided a depth-normalized estimate of available irradiance and water clarity.

3.3. Statistical analysis

Regressions of $\Delta F/F_m'$ versus PAR were calculated for each basin, the pooled bay-scale data for each year, and the total 4-year dataset using Sigma Plot for Windows 11.0. All data were tested for normality and homogeneity of variances by the Kolmogorov–Smirnov test with Lilliefors' correction and the Levene test, respectively. Analysis of covariance (ANCOVA) was used to compare regression slopes and intercepts between and among years ($p < 0.05$). ANCOVAs were calculated using PROC GLM in SAS 9.1. Interannual variation of bay mean $\Delta F/F_m'$ and PAR was compared by one-way analysis of variance in Sigma Plot for Windows 11.0. If significant among-year differences were detected, Tukey's pair-wise comparisons were used to determine the significance for year-to-year comparisons. All results are reported as means \pm standard error.

4. Results

4.1. Interannual variation in $\Delta F/F_m'$ versus PAR at the bay scale

Regression analyses indicated that $\Delta F/F_m'$ for *T. testudinum* significantly ($p < 0.001$) decreased with increasing PAR across Florida Bay for all 4 years of sampling (Fig. 2). ANCOVA indicated that

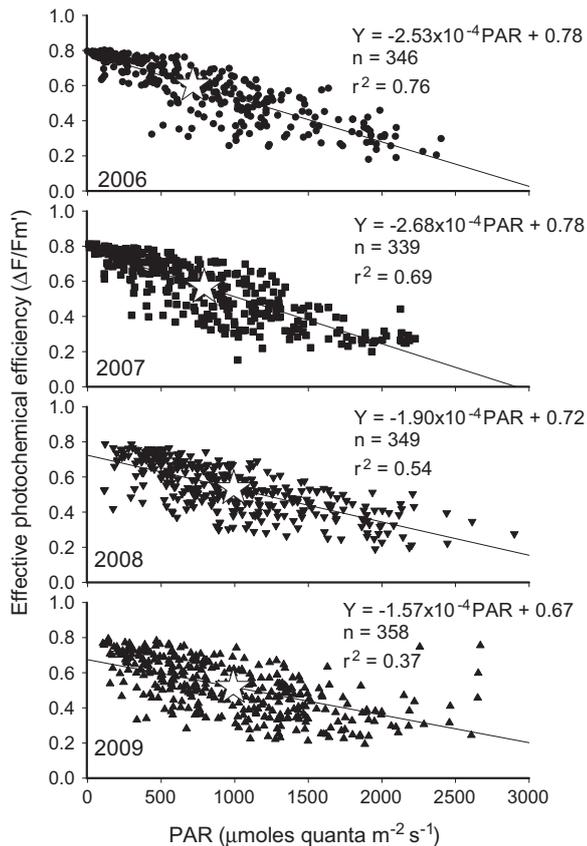


Fig. 2. Mean effective yields ($\Delta F/F_m'$) versus irradiance at the leaf canopy of *Thalassia testudinum* measured during 2006, 2007, 2008, and 2009 FHAP-SF sampling in Florida Bay. The solid lines represent the fitted regression equations ($p < 0.001$ for all four years). The stars represent the bay-scale mean effective yields ($\Delta F/F_m'$) and irradiances for each year.

the negative slopes and intercepts of the $\Delta F/F_m'$ versus PAR bay-scale regressions were not significantly different between 2006 and 2007. Regression slopes and intercepts decreased from 2007 to 2008 and from 2008 to 2009 (i.e., the slope became less negative); both decreases in regression slopes were significant (ANCOVA, $p < 0.001$ for 2007 vs 2008 and $p < 0.05$ for 2008 vs 2009). The significant reduction in negative slopes from 2006–2007 compared to 2008–2009 corresponded with a significant ($p < 0.001$) 25% increase in mean daily bottom irradiances measured during sampling (716 ± 31 and $792 \pm 291 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 2006

and 2007, $p = 0.254$, compared to 993 ± 29 and $990 \pm 31 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 2008 and 2009, $p = 1.0$). The reduced bottom irradiances in 2006 and 2007 reflected the presence of widespread phytoplankton blooms during this period (Fig. 3, Boyer et al., 2009). Mean $\Delta F/F_m'$ for the bay decreased significantly each year ($p < 0.05$) from 2006 to 2008 (0.604 ± 0.009 , 0.568 ± 0.009 , 0.535 ± 0.007 , for 2006, 2007, 2008, respectively) then leveled off in 2009 (0.518 ± 0.008 for 2009, 2008 vs 2009 $p = 0.49$; see Fig. 2).

4.2. Interannual variation in $\Delta F/F_m'$ versus PAR at the basin scale

The relationships between $\Delta F/F_m'$ and PAR were much more variable among years, at the basin scale. Regressions of $\Delta F/F_m'$ versus PAR were significant for all basins and years, with the exception of Johnson Key basin in 2007 and Manatee Bay in 2008 (Table 2). The lack of a significant regression for Johnson Key basin in 2007 was due to an instrument malfunction, such that measurements were only obtained during a short portion of the day (1000–1400 h) when irradiances were high. Vessel logistical issues during the Manatee Bay sampling in 2008 resulted in measurements only being taken during a short period in the afternoon (1400–1700 h) when irradiances were high with relatively little variation. Slopes of $\Delta F/F_m'$ versus PAR for significant regressions varied over five-fold (-3.27×10^{-4} to -0.58×10^{-4}) among the twelve basins, over the 4-year sampling period (Table 2 and Fig. 4). Intercepts were less variable, ranging less than two-fold, from 0.531 to 0.919 (Table 2 and Fig. 5). Interannual variation in regression slopes was not significant between 2006 and 2007 or between 2008 and 2009 in eight of the twelve basins (Table 3), five of which were the same for both time periods. The regression slopes for Crane, Duck and Rabbit Key basins did not vary significantly over the 4-year period (Table 2). The regression intercepts also did not significantly vary for these three basins, between 2008 and 2009, indicating very stable photoacclimation states for *T. testudinum* in these relatively clear basins, which were largely unaffected by the phytoplankton blooms (Fig. 3).

High interannual variation in $\Delta F/F_m'$ versus PAR regression slopes in Barnes, Blackwater, Twin and Whipray corresponded with high interannual variation in chlorophyll, $K_0(\text{PAR})$ and available irradiance (compare Figs. 3, 4 and 6). Available irradiance was lower for the bay in 2006 and 2007 ($85.7 \pm 5.0\%$ and $84.3 \pm 4.3\%$, respectively) compared to 2008 and 2009 ($93.9 \pm 3.3\%$ and $98.9 \pm 0.8\%$, respectively). 2006 through 2007 was a period unusually severe phytoplankton blooms, which began to abate in 2008 (Fig. 3, Boyer et al., 2009). As a result of the turbid conditions, the secchi disk was visible on the bottom at all stations in only three western bay

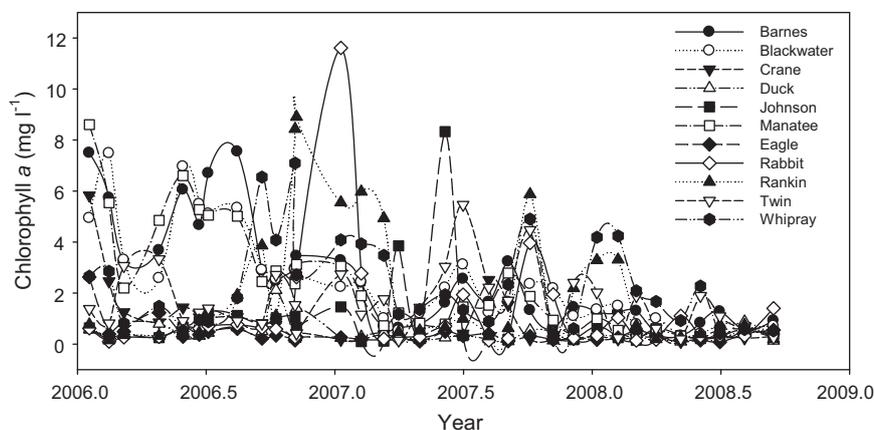


Fig. 3. Monthly chlorophyll a concentrations from January 2006 to September 2008 in eleven of the twelve basins sampled for chlorophyll fluorescence of *Thalassia testudinum*. There is no water quality monitoring station in Calusa Key Basin. See Table 1 for full basin names.

Table 2

Regression results comparing $\Delta F/F_m'$ versus PAR relationships within twelve Florida Bay basins during 2006–2009 FHAP sampling ($n=27-31$). Slope and intercept (int) estimates presented as mean \pm (s.e.). n.s. = nonsignificant regression.

Basin	2006			2007			2008			2009		
	Slope ($\times 10^{-4}$)	Int	r^2	Slope ($\times 10^{-4}$)	int	r^2	Slope ($\times 10^{-4}$)	int	r^2	Slope ($\times 10^{-4}$)	int	r^2
Barnes	-2.94 (.33)	0.80 (0.01)	0.48	-2.86 (0.28)	0.85 (0.15)	0.61	-1.55 (0.32)	0.75 (0.02)	0.45	-3.22 (0.41)	0.92 (0.04)	0.69
Blackwater	-0.58 (0.12)	0.79 (0.01)	0.87	-2.88 (0.49)	0.82 (0.31)	0.88	-1.71 (0.44)	0.73 (0.04)	0.43	-0.75 (0.21)	0.73 (0.03)	0.34
Calusa	-2.47 (0.18)	0.87 (0.01)	0.61	-3.01 (0.21)	0.84 (0.02)	0.56	-1.36 (0.55)	0.72 (0.04)	0.18	-1.56 (0.42)	0.68 (0.05)	0.33
Crane	-1.67 (0.27)	0.78 (0.01)	0.69	-1.92 (0.33)	0.72 (0.03)	0.59	-1.73 (0.33)	0.72 (0.05)	0.51	-1.34 (0.22)	0.69 (0.03)	0.56
Duck	-2.32 (0.19)	0.73 (0.03)	0.84	-2.15 (0.28)	0.69 (0.04)	0.68	-2.22 (0.20)	0.78 (0.03)	0.81	-1.73 (0.20)	0.70 (0.02)	0.72
Eagle	-2.37 (0.24)	0.79 (0.04)	0.77	-2.61 (0.21)	0.76 (0.03)	0.84	-1.48 (0.28)	0.68 (0.05)	0.48	-1.48 (0.21)	0.76 (0.02)	0.65
Johnson	-2.21 (0.58)	0.63 (0.06)	0.34	n.s.	–	–	-1.36 (0.56)	0.55 (0.05)	0.18	-1.57 (0.43)	0.62 (0.04)	0.33
Manatee	-1.74 (0.40)	0.77 (0.02)	0.40	-2.39 (0.35)	0.78 (0.02)	0.63	n.s.	–	–	-2.02 (0.29)	0.72 (0.04)	0.63
Rabbit	-2.00 (0.61)	0.67 (0.05)	0.27	-2.79 (0.61)	0.65 (0.06)	0.42	-3.19 (0.34)	0.77 (0.03)	0.75	-2.51 (0.33)	0.71 (0.03)	0.66
Rankin	-1.09 (0.44)	0.69 (0.05)	0.18	-2.32 (0.24)	0.79 (0.02)	0.77	-1.13 (0.05)	0.6 (0.05)	0.16	-2.14 (0.32)	0.60 (0.04)	0.62
Twin	-2.10 (0.24)	0.76 (0.02)	0.73	-3.27 (0.47)	0.81 (0.03)	0.63	-2.32 (0.58)	0.8 (0.03)	0.37	-1.27 (0.54)	0.56 (0.06)	0.17
Whipray	-2.52 (0.10)	0.78 (0.01)	0.18	-2.41 (0.52)	0.8 (0.02)	0.46	-2.79 (0.26)	0.84 (0.03)	0.81	-0.95 (0.30)	0.53 (0.04)	0.26

basins in 2006 (Johnson, Rabbit and Rankin); this increased to nine basins located across the bay by 2009 (Fig. 6).

Among-year and among-basin variations in regression intercepts and slopes were significantly correlated ($p < 0.001$), although the relationship was weak ($r^2 = 0.35$, Fig. 7). Similar to the bay-scale relationships, individual basin negative slopes and intercepts were generally highest in 2006 or 2007 and lowest in 2008 or 2009 (Figs. 4 and 5), corresponding with changes in available irradiance (Fig. 6).

5. Discussion

Durako and Kunzelman (2002) showed that diurnal variation of $\Delta F/F_m'$ and F_v/F_m (maximum photochemical efficiency, which is measured under a dark-acclimated state such that all reaction

centers are open and non-photochemical quenching is minimal) with PAR introduced significant sources of variation in landscape-scale sampling of *T. testudinum* photosynthesis in Florida Bay. The results presented here show that regression slopes and y-intercepts of the diurnal $\Delta F/F_m'$ versus PAR relationship seem to vary with changes in water clarity and light availability (and possibly nutrient levels, Boyer et al., 2009). Thus, diurnal regressions rather than individual measurements of $\Delta F/F_m'$, may provide more representative measures of the photophysiological state of *T. testudinum* when PAM-fluorescence measurements are obtained over the day. The negative slope of $\Delta F/F_m'$ versus PAR regressions reflects a reduction in the fraction of light energy that is used photochemically with increasing irradiance. This is due to the inherent effect of an increase in energy dissipation through non-photochemical quenching, which may serve as a photoprotection response (Adams and Demmig-Adams, 2004), or photodamage to PSII resulting from

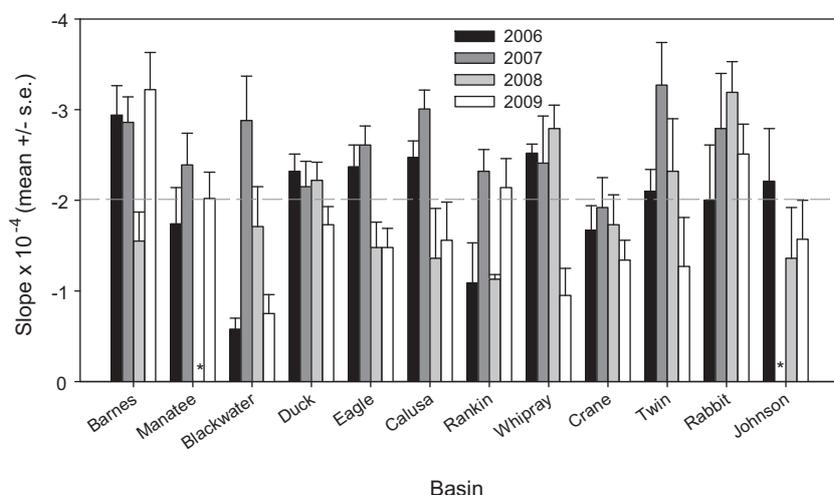


Fig. 4. Basin-specific slopes from mean effective yields ($\Delta F/F_m'$) versus irradiance regressions calculated from measurements of *Thalassia testudinum* during 2006, 2007, 2008, and 2009 FHAP-SF sampling in Florida Bay. Horizontal dashed line represents the slope for a regression of the entire dataset. Basins are arranged from northeast to southwest. See Table 1 for full basin names.

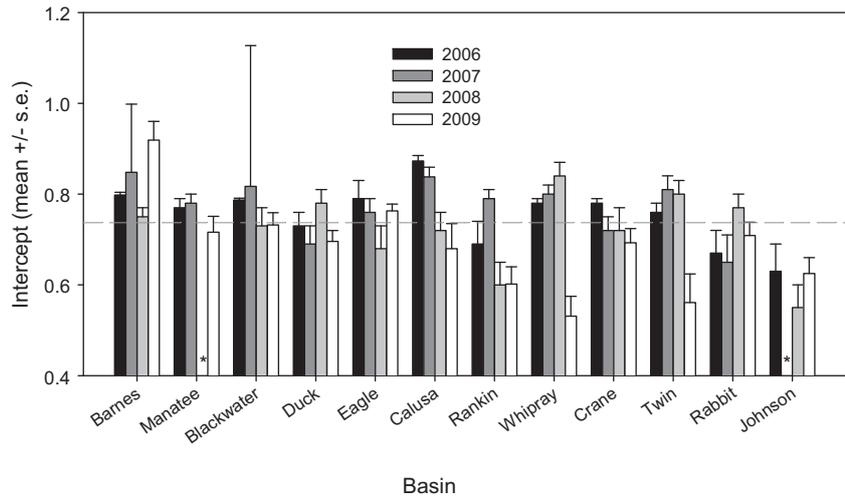


Fig. 5. Basin-specific intercepts from mean effective yields ($\Delta F/F_m'$) versus irradiance regressions calculated from measurements of *Thalassia testudinum* during 2006, 2007, 2008, and 2009 FHAP-SF sampling in Florida Bay. Horizontal dashed line represents the intercept for a regression of the entire dataset. Basins are arranged from northeast to southwest. See Table 1 for full basin names.

the production of reactive oxygen species (Demmig-Adams et al., 1999). The magnitude of this slope is a measure of the efficiency of energy dissipation by PSII (Ralph and Gademann, 2005). In contrast, the y-intercept of the $\Delta F/F_m'$ versus PAR regressions may serve as an estimate of F_v/F_m . Measurement of F_v/F_m requires a dark acclimation period of greater than 30 min for each sample and thus is not amenable to landscape scale sampling.

In this study, the most negative slopes and highest intercept values of $\Delta F/F_m'$ versus PAR regressions, indicative of high maximum photochemical efficiency but a decreased ability to dissipate excess light energy (i.e., a shade-acclimated or stressed state), were observed during periods with reduced irradiance associated with the presence of widespread phytoplankton blooms and elevated water column nutrient levels (Boyer et al., 2009). Both slopes and intercepts were lower, reflecting reduced maximum quantum efficiency but an increased ability to utilize higher irradiances (i.e., sun acclimated or healthy) when water clarity and bottom irradiances were higher. At the basin scale, these high-irradiance relationships were most clear in Duck, Crane, Rabbit and Johnson Key basins, which all had relatively uniform and high benthic light availabilities (Fig. 6), were largely unaffected by phytoplankton

blooms (Boyer et al., 2009) and had stable cover of *T. testudinum* (Hall and Durako, 2011). In contrast, the regression parameters for Blackwater Sound and Whipray Bay, which both exhibited large changes in water clarity (Fig. 6), indicated shifts from shade to sun-acclimation in *T. testudinum* (Figs. 4 and 5). The shifts corresponded with periods of loss and recovery of *T. testudinum* (Hall and Durako, 2011). The changes in the $\Delta F/F_m'$ versus PAR regressions are consistent with the view that this species is a high-light adapted plant that has the ability to photoacclimate in response to changes in irradiance (Major and Dunton, 2002) and they reflect the trade-offs between photosynthetic efficiency and photoprotection (Ralph et al., 2002).

Belshe et al. (2007) observed that coincident with an increase in irradiance between the two years that they sampled there was an increase in diurnal variation in $\Delta F/F_m'$ in *T. testudinum*. They also observed that depth-dependent irradiance history obscured some diurnal variations at the landscape scale. Silva and Santos (2003) observed greater diurnal variation in RLC parameters and quantum yields in intertidal *Cymodocea nodosa* (Ucria) Ascherson, compared to subtidal plants. Greatest variation in $\Delta F/F_m'$ occurred during the mid-day in subtidal *Posidonia australis* Hooker

Table 3
ANCOVA results comparing 2006–2009 $\Delta F/F_m'$ versus PAR regression coefficients for *Thalassia testudinum* in Florida Bay.

Basin	2006 vs 2007		2006–2008		2006–2009		2008 vs 2009	
	Slopes ^a	Intercepts ^a						
Barnes	n.s.	***	***	–	***	–	***	–
Blackwater	**	–	***	–	***	–	***	–
Calusa	n.s.	n.s.	**	–	**	–	n.s.	n.s.
Crane	n.s.	*	n.s.	*	n.s.	*	n.s.	n.s.
Duck	n.s.	n.s.	n.s.	***	n.s.	***	n.s.	n.s.
Eagle	n.s.	***	*	–	***	–	n.s.	**
Johnson	n.d. ^b	n.d. ^b	n.s. ^c	n.s. ^c	n.s. ^d	n.s. ^d	n.s.	n.s.
Manatee	n.s.	n.s.	n.d. ^e	n.d. ^e	n.s. ^f	*f	n.d. ^e	n.d. ^e
Rabbit	n.s.	***	n.s.	***	n.s.	***	n.s.	n.s.
Rankin	*	–	***	–	*	–	n.s.	***
Twin	***	–	***	–	***	–	n.s.	***
Whipray	n.s.	n.s.	n.s.	n.s.	***	–	***	–

^a n.s. = not significantly different at $p < 0.05$, significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$.

^b n.d. = no data 2007 regression n.s.

^c 2006 vs 2008.

^d 2006 vs 2008 vs 2009.

^e 2008 regression n.s.

^f 2006 vs 2007 vs 2009.

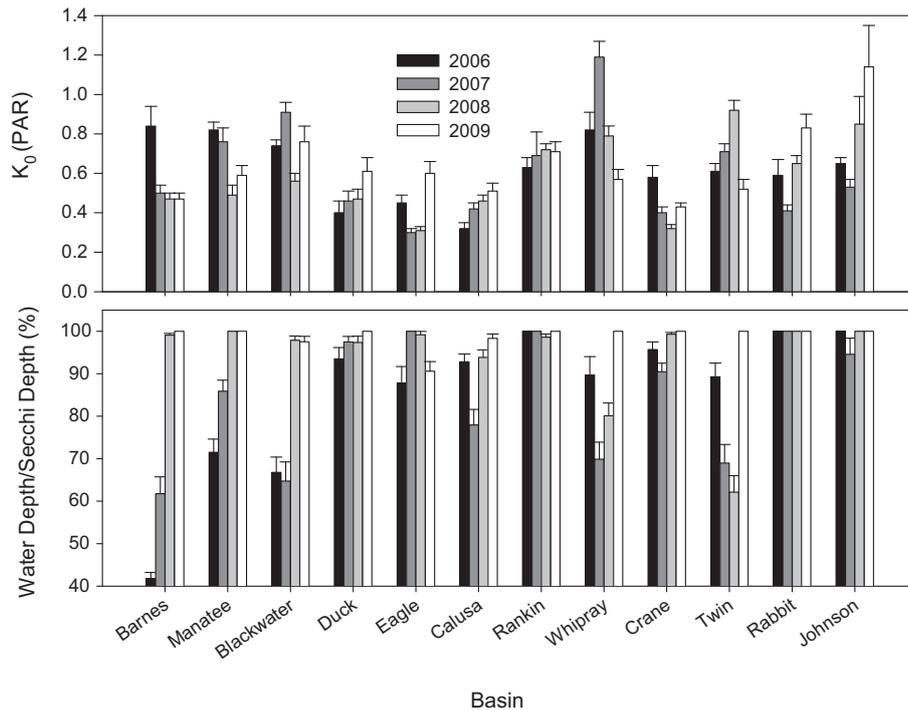


Fig. 6. Basin-specific water clarity characteristics measured during 2006, 2007, 2008, and 2009 FHAP-SF sampling. Basins are arranged from northeast to southwest. See Table 1 for full basin names.

(~25% of the mean), which in turn decreased the ability to detect changes in photosynthetic efficiency (Runcie and Durako, 2004). These trends suggest that as the magnitude of the driving environmental parameter increases there are corresponding increases in the magnitude and variability of photo-regulatory mechanisms. Although the magnitude of the slopes and intercepts of the $\Delta F/F_m'$ versus PAR regressions differed among the four years studied here, there was not a corresponding change in variability in these regression-derived parameters (Table 2). In addition, $\Delta F/F_m'$ versus PAR regressions were less sensitive to the specific range of irradiances that occurred during fluorescence measurements compared to absolute $\Delta F/F_m'$ values. As an example, ANCOVA indicated there was no significant among-year variation in $\Delta F/F_m'$ versus PAR regression slopes for *T. testudinum* in Crane Key Basin, a relatively stable population that was largely unaffected by the recent phytoplankton blooms (Boyer et al., 2009). In contrast, mean $\Delta F/F_m'$

($F_{3,103} = 26.7, p < 0.001$) and PAR ($F_{3,103} = 28.4, p < 0.001$) both exhibited significant among-year variations.

Plants have an array of processes and mechanisms, which allow them to vary photosynthetic efficiency and capacity in response to variations in irradiance (MacIntyre et al., 2000). Acclimation to long-term light conditions include structural and functional modifications, such as changes in leaf morphology, the number of light harvesting complexes, pigments (type and concentrations) and protein (Rubisco) concentrations. There are also rapid down-regulatory responses, which either decrease the absorption of light energy or provide alternative energy sinks when photosynthetic capacity is exceeded. This is normally associated with the xanthophyll cycle (Ralph et al., 2002). This photoprotective down-regulation limits damage to the photosystems under high irradiance conditions, but also results in lower $\Delta F/F_m'$ (Gorbunov et al., 2001). These modifications determine the photoacclimation

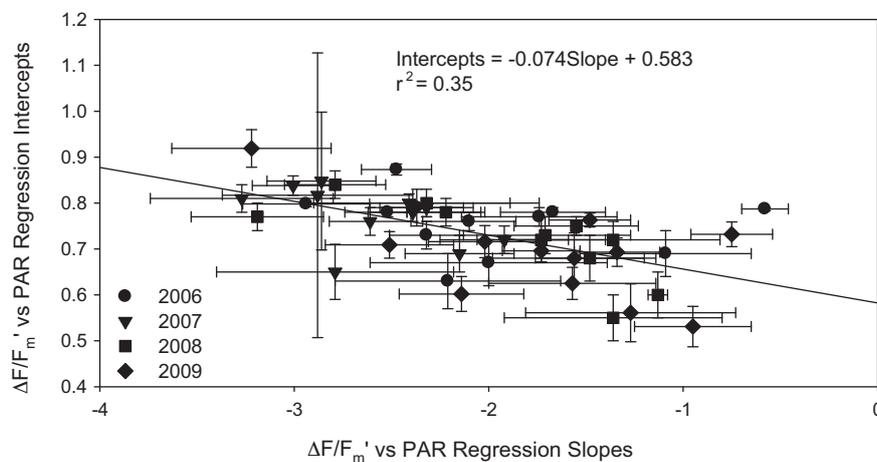


Fig. 7. Relationship between basin-specific regression slopes and intercepts calculated from mean effective yields ($\Delta F/F_m'$) versus irradiance measurements of *Thalassia testudinum* during 2006, 2007, 2008, and 2009 FHAP-SF sampling in Florida Bay.

state of the plant (sun- versus shade-acclimated), which affects the efficiency of dissipation of excess light energy and the rate at which $\Delta F/F_m'$ decreases with increasing irradiance (Ralph and Gademann, 2005). Here, the slopes and intercepts of $\Delta F/F_m'$ versus PAR were generally higher at both the bay and basin scales during 2006 and 2007 compared to 2008 and 2009 (Figs. 3 and 4) suggesting a change in the photophysiological state of *T. testudinum* in Florida Bay. This change coincided with higher light availability associated with the cessation of persistent phytoplankton blooms, which began in northeast Florida Bay in 2005 (Boyer et al., 2009). This bloom is believed to be the result of an interaction between road construction activities in the Florida Keys and hurricane-related disturbances. Three hurricanes passed through the region from August to October 2005. In anticipation of storm flooding, there was a large discharge of freshwater from the Everglades prior to the passage of Hurricane Katrina, which resulted in elevated nutrient levels that may have exacerbated and prolonged the phytoplankton blooms (Rudnick et al., 2006). Boyer et al. (2009) predicted that, in the absence of additional hurricane impacts, the phytoplankton blooms would decrease with a concomitant increase in water clarity. No hurricanes directly impacted Florida Bay in 2008 or 2009 (www.nhc.noaa.gov). Decreased water-column chlorophyll (Fig. 3) and increased water clarity in 2008 and 2009 (Fig. 6) reflect this lack of storms during this period and the $\Delta F/F_m'$ versus PAR responses of *T. testudinum* indicate a more high-light acclimated physiological state for this species.

6. Conclusions

Accurate physiological information from point measurements of photochemical efficiency can be masked by diurnal variations caused by the dynamic responses of PSII to changing environmental conditions. Parameters derived from rapid light curves are as sensitive as measurements of effective and maximum photochemical efficiency to diurnal fluctuations, even though RLC parameters are derived from measurements over a range of irradiances (Durako and Kunzleman, 2002; Belshe et al., 2007, 2008). This clearly indicates that time of day (or irradiance history) effects must be factored into the interpretation of fluorescence data when using PAM fluorimeters over large spatial and temporal scales. In ecosystems where the magnitudes of change are large and occur on much faster time scales than the ecosystem changes being measured, the resulting fluctuations may obscure the true physiological signal. Time-restricted approaches, such as measurements of F_v/F_m or sampling only during early morning, at mid day or late afternoon, place limits on the spatial scale which can be examined (Belshe et al., 2007). In order to evaluate the status of an ecosystem, it is preferable to comprehensively cover the entire spatial scale of the system. Evaluating mean daily $\Delta F/F_m'$ characteristics using data from the entire spatial sample may provide representative scale-appropriate information, but these values are sensitive to the specific range of irradiances encountered during sampling. The advantage of the $\Delta F/F_m'$ versus PAR regression approach described here is that it integrates diurnal irradiance variation into the physiological assessment and thus is less sensitive to specific irradiance conditions during sampling.

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