Journal of Experimental Marine Biology and Ecology 342 (2007) 253–268

Journal of
EXPERIMENTAL
MARINE BIOLOGY
AND ECOLOGY

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Photosynthetic rapid light curves (RLC) of *Thalassia testudinum* exhibit diurnal variation

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Received 13 March 2006; received in revised form 1 September 2006; accepted 30 October 2006

Abstract

The use of pulse-amplitude-modulated fluorometry (PAM) and rapid light curves (RLC) were evaluated for monitoring the physiological condition of the seagrass, *Thalassia testudinum*, at the landscape scale in Florida Bay, USA. PAM fluorometry provides rapid, non-invasive, and quantitative physiological information on the state of photosynthesis. Yet, previous studies of effective and maximum quantum yields have shown that problems arise when expanding measurements from the organismal scale to the landscape scale, mainly due to temporal and irradiance-induced changes in photophysiology. Here, the magnitude of diurnal and spatial variation of photosynthetic characteristics among 10 sample basins and between two sample years was investigated using RLCs. Because RLCs measure effective quantum yields over a range of changing actinic irradiances, we hypothesized that the response parameters might be less sensitive to diurnal light history effects. Our results indicate that the RLC parameters, alpha and ETR_{max}, significantly changed diurnally, as was previously found for both maximum and effective quantum yields, but the diurnal patterns were variable among the 10 basins. Both among-basin and between-year comparisons were confounded by diurnal variation and statistical analyses comparing morning, mid-day, and afternoon time periods were unable to definitively discern which time of day was best suited for assessing the relative photophysiological status of *T. testudinum*. However, pooling RLC data at the basin scale revealed among-basin differences and landscape scale trends that were consistent with basin-level morphometric variation in this seagrass. Thus, PAM fluorometry may be useful as a landscape scale monitoring tool within certain constraints. When using this approach over large spatial and temporal scales, diurnal variability must be considered.

Keywords: Diurnal variation; PAM fluorometry; Photosynthesis; Seagrass

1. Introduction

Pulse-amplitude-modulated (PAM) fluorometers provide quantitative information about photosystem II (PSII) by measuring chlorophyll fluorescence. If the sample is dark acclimated, which allows all PSII reaction centers to relax, the maximum quantum yield

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doi:10.1016/j.jembe.2006.10.056

can be calculated ($F_v/F_m=[F_m-F_o]/F_m$), where F_o is the minimum fluorescence under the PAM measuring light and F_m is the maximum fluorescence following a saturating light pulse. This provides information about the maximum photochemical efficiency of PSII (Maxwell and Johnson, 2000). Alternatively, if the sample is acclimated to the light of its environment, then the effective quantum yield (Φ_{PSII}) can be calculated ($\Delta F/F_m'=[F'_m-F]/F'_m$), where F and F'_m are the corresponding light-acclimated minimum and maximum fluorescence. This gives an approximation of the proportion of absorbed energy being used for photochemistry, at a given point in time (Genty et al., 1989; Maxwell and Johnson, 2000). PAM fluorometry is attractive as a monitoring tool because it is rapid, non-destructive, and can provide in-depth, quantitative physiological information about photosynthetic organisms (Ralph and Gademann, 2005). This information may be used to identify changes in photosynthetic status before morphological or density-based changes are evident.

Durako and Kunzelman (2002) initially used PAM fluorometry in the Fish Habitat Assessment Program (FHAP) during the spring 2000 sampling to assess the physiological state of *Thalassia testudinum* Banks ex König (Hydrocharitaceae). *T. testudinum* is the dominant macrophyte in the shallow waters of Florida Bay (Zieman, 1982) and its abundance is perceived by the public as an indicator of the health of the bay (Durako et al., 2002). FHAP samples ten basins, which represent the range of conditions within Florida Bay and due to the large area covered each basin takes an entire day to sample. Although stations are located using a systematic random sample design, to efficiently sample such a large area, stations must be visited systematically in order to minimize station-to-station travel time. However, Durako and Kunzelman (2002) found that this introduced a significant source of diurnal variation that confounded the assessment of the seagrasses' physiological state. When effective and maximum quantum yields were regressed against actinic irradiance or time of day, both resulted in significant negative slopes, illustrating the importance of recent light history on seagrass photosynthetic responses (Ralph et al., 1998; Ralph and Gademann, 2005).

Photosynthesis is dependent on the quantity and quality of light received, but the rate of carbon fixation is not simply proportional to the rate of photon capture (Kirk, 1994). The amount of energy that can be funneled through PSII is dependent on whether the primary electron acceptor (Qa) is open to accept electrons. As the amount of captured light increases, the possibility of being closed (not able to accept electrons) increases, which results in a greater amount of fluorescence being emitted or an increase in alternative forms of energy dissipation (i.e., non-photochemical quenching; White and Critchley, 1999; Marshall et al., 2000; Ralph and Gademann, 2005). This makes fluorescence yield dependent on the previous light history of the sample. Though this is a direct relationship, it is not always proportional. Therefore, interpreting the measurements taken by PAM fluorometers under varying environmental conditions can be difficult, especially when taking numerous measurements throughout a range of actinic intensities. The sensitivity of PAM fluorometry to plants' previous light histories and diurnal fluctuations pose a special problem when sample regimes are expanded to the landscape scale, such as the FHAP sampling of Florida Bay (Durako and Kunzelman, 2002).

T. testudinum is a high-light adapted plant that has the ability to photoacclimate in response to changes in irradiance (Major and Dunton, 2002). Because of *T. testudinum*'s blade architecture and growth habit, each leaf blade is exposed to a large irradiance gradient throughout its lifetime (Durako and Kunzelman, 2002; Enríquez et al., 2002). Shallow depth, wave focusing, variable weather patterns, and tidal and solar oscillations expose the plants to a wide range of irradiances during each diurnal cycle. Given the extreme fluctuations in light reaching the seagrass leaf blade, both efficient photon capture and use along with photoprotective responses are required for growth and survival (Ralph and Gademann, 2005).

Photosynthesis responds to environmental fluctuations using an array of processes that affect light-harvesting efficiency and photosynthetic capacity. Regulation can be accomplished by variations of the relative abundance of the constituents of the photosystems or on shorter time scales by varying the efficiency of their activities (MacIntyre et al., 2000). Plants have a range of adaptations, which allow them to change photosynthetic activity and capacity in response to prevailing light conditions. Structural and functional adaptations to long-term light conditions, including changes in pigment and protein (Rubisco) concentrations, determine the photoacclimation of the plant (sun-shade). There is also a complex group of rapid responses, which either decrease the absorption of light energy or provide alternative energy sinks when photosynthetic capacity is exceeded (MacIntyre et al., 2000). These protective processes (down-regulations) appear to limit damage to the photosystems but result in lower quantum yields (Gorbunov et al., 2001). Down-regulations of photosynthesis allow plants to tolerate and utilize their rapidly changing light environment, while sustaining their photosynthetic systems (White and Critchley, 1999; Gorbunov et al., 2001). When protective responses are exceeded, proteins in the reaction centers can be damaged by excess excitation energy and re-synthesis of these proteins is required (MacIntyre et al., 2000). The levels of regulation of photosynthetic processes can be distinguished by the time scales needed for them to take place and recover. Yet the occurrence and magnitude of environmental changes dictate the relative importance of the regulatory mechanisms. Because environmental factors such as light and temperature vary independently from photosynthetic changes, but on time scales that coincide with these changes, the results can be complex and unpredictable (MacIntyre et al., 2000).

In this study, we investigated the sensitivity of rapid light curves (RLC) to landscape scale variations previously detected when measuring effective and maximum quantum yields (Durako and Kunzelman, 2002). By measuring effective quantum yields over a range of increasing actinic light intensities, RLCs provide additional information about the efficiency (alpha) and capacity (ETR_{max}) of photosynthesis (Marshall et al., 2000). RLCs can be used to illustrate the acclimation of the photosynthetic apparatus to a range of light intensities, and also provide a reliable assessment of photosynthetic activity (Ralph and Gademann, 2005). We broadened the scope of our analyses from the station level examined by Durako and Kunzelman (2002) to the basin level and incorporated the diurnal cycle. Through comparisons of mean measured and derived photosynthetic parameters among basins and between years, we were able to establish some applications and functional limitations of using this physiological approach for landscape scale assessment. The goal of this study was to assess diurnal changes in photosynthetic parameters measured with PAM fluorometry and to determine the effect of these changes on the interpretation of measurements taken at the landscape scale.

2. Materials and methods

2.1. Study site

This study was conducted in Florida Bay (ca. 25°05′ N, 81°45′W), a shallow lagoonal estuary at the southern tip of Florida, USA. The bay is characterized by shallow basins (ca. <1 m) divided by carbonate mud banks and mangrove islands (Fourqurean and Robblee, 1999). Seagrass community development increases in a gradient from the enclosed northern sections to the more open western sections of the bay. This gradient coincides with changing environmental characteristics within the bay. Sediment type and depth change from northeast (shallow fine-grained) to southwest (deeper muddy-sand) (Zieman et al., 1989). Light attenuation is generally greatest in the eastern and south-central regions and lowest in the north-central and western regions (Phlips et al., 1995). Water temperature is on average more constant throughout the bay but can show substantial variation at a small scale due to the shallow depth (Zieman, 1982).

Table 1
List of basins sampled in Florida Bay and their abbreviations

Abbreviation	Basin
BLK	Blackwater Sound
CAL	Calusa Key
CRN	Crane Key
EAG	Eagle Key
JON	Johnson Key
MAD	Madeira Bay
RAB	Rabbit Key
RKN	Rankin Lake
TWN	Twin Key
WHP	Whipray Bay

The Fish Habitat Assessment Program (FHAP) samples 10 basins (Table 1) that lie within the borders of the Everglades National Park (ENP). The basins were chosen to represent the range of conditions within the bay. Each basin is divided into 27–33 tessellated hexagonal subunits, and one station is randomly chosen within each subunit. This results in 275–330 stations that are randomly sampled throughout the bay (see Durako et al., 2002, for a map of sampling stations and more information on FHAP).

2.2. Photosynthetic parameters

Photosynthetic characteristics of *T. testudinum* were measured with an underwater fluorometer, Diving PAM (Walz, Germany), in spring of 2002 and 2004, during FHAP sampling. Rapid light curves (RLC) were performed on four haphazardly chosen short shoots of *T. testudinum* at each station. The short shoots that were chosen were representative of the shoots observed at the station. The middle of the rank 2 blade of each *T. testudinum* short shoot was gently scraped to remove epiphytes before attaching the dark leaf clip (DIVING-LC). The leaf clip held the Diving PAM fiber optic 5 mm from the surface of the blade in 2002. This distance was reduced to 2 mm in 2004 in order to allow a reduction in instrument gain and to obtain a higher signal to noise ratio. Each rapid light curve exposed the leaf to eight incremental steps of irradiance ranging from 0 to 2060 μmol photons m^{-2} s^{-1} in 2002, and 0 to 1735 μmol photons m^{-2} s^{-1} in 2004. The reduction in irradiance levels in 2004 was due to slight damage to the fiber optic causing a decrease in light transmission at the same instrument settings. An effective quantum yield measurement (Φ_{PSII}) was taken at the beginning of each curve, before actinic light from the Diving PAM was applied, and at the end of each 5s irradiance step, resulting in nine Φ_{PSII} measurements for each rapid light curve. Each Φ_{PSII} measurement was used to calculate the electron transport rate (ETR) through photosystem II using the following equation:

$$\mathrm{ETR} = \Phi_{PSII} \times \mathrm{PAR} \times \mathrm{AF} \times 0.5,$$

where PAR is the actinic photosynthetically active radiation generated by the internal halogen lamp of the

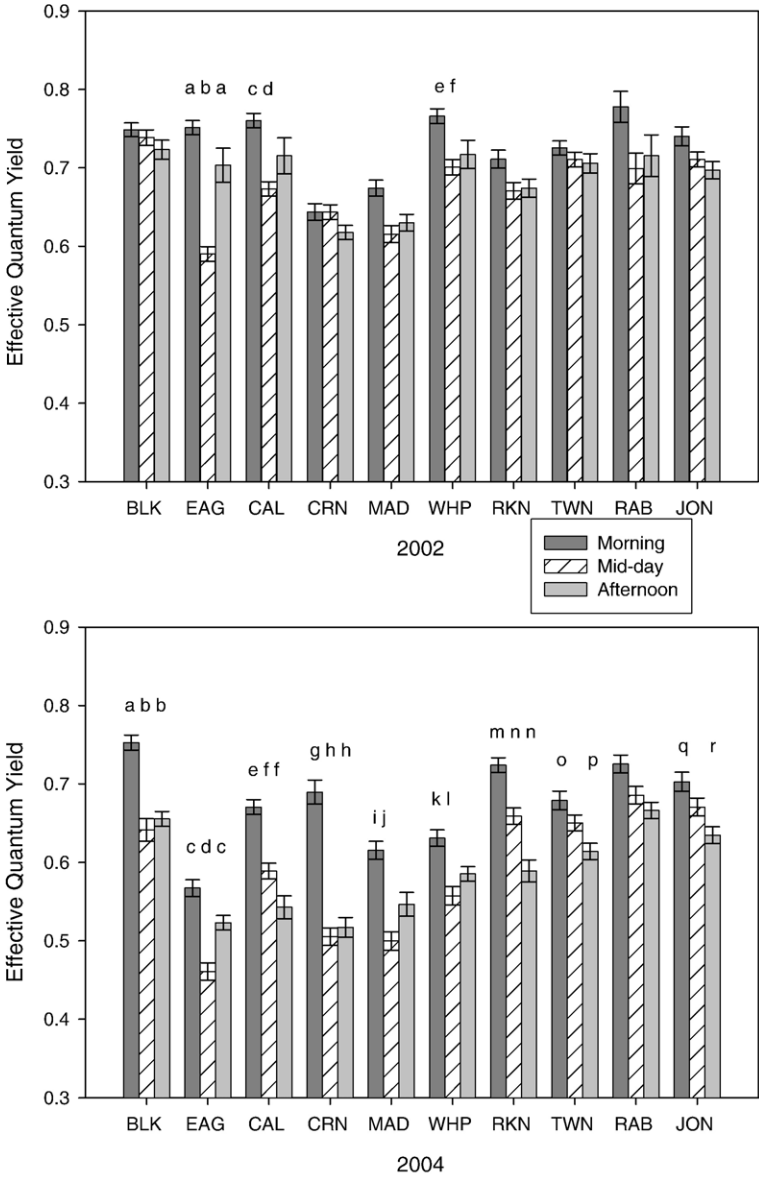

Fig. 1. Comparison of mean morning (0800–1100 h), mid-day (1100–1400 h), and afternoon (1400–1700 h) effective quantum yield (Φ_{PSII}) of *T. testudinum* blades measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically relative to their spatial position within the bay (east to west). Letters denote significant differences among time periods ($p<0.05$, Tukey's post hoc test).

Diving PAM, AF is the light absorption capacity of the leaf, and 0.5 assumes that the photons absorbed are equally partitioned between PSII and PSI (Genty et al., 1989). Because of the constraints of sampling at such a large scale, it was not feasible to measure absorption of all seagrass blades. Instead the instrument default AF value of 0.84 was used to standardize absorption across the bay. Without knowledge of the actual amount of light being absorbed, fluorescence measurements can only be used as an approximation for electron transport (Beer et al., 1998a,b; Runcie and Durako, 2004). This is further complicated by the heterogeneity among samples taken throughout the landscape in this experiment. Therefore, information provided by the ETRs is an integrated approximation of landscape-level photosynthetic characteristics.

Rapid light curves (RLC) were generated from the calculated ETRs and the irradiances applied during the rapid light curve steps. Each RLC was fitted to a double exponential decay function in order to quantify the

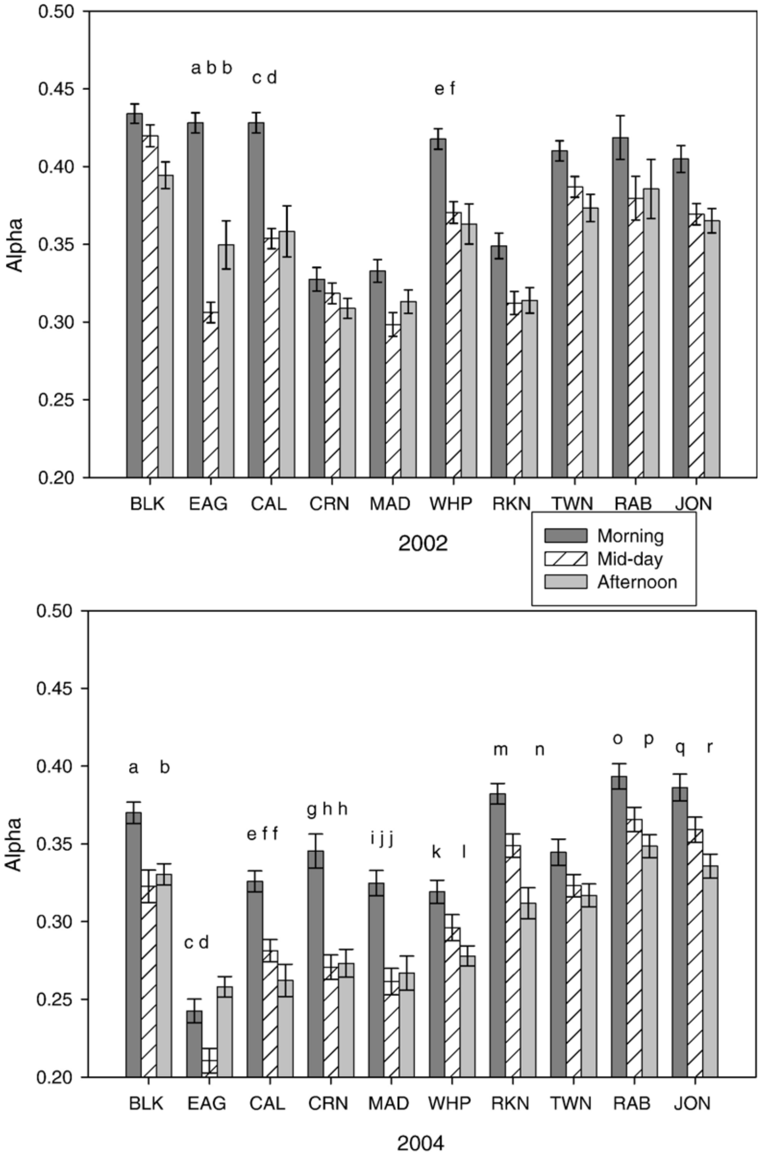

Fig. 2. Comparison of mean morning (0800–1100 h), mid-day (1100–1400 h), and afternoon (1400–1700 h) rapid light curve generated efficiency (alpha, μmol e^{-} m^{-2} s^{-1}/μmol photon m^{-2} s^{-1}) of *T. testudinum* blades measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically relative to their spatial position within the bay (east to west). Letters denote significant differences among time periods ($p<0.05$, Tukey's post hoc test).

characteristic parameters, alpha and ETR_{max} (Platt et al., 1980). The initial slope of the RLC (alpha) is a measure of the light harvesting efficiency of photosynthesis and the asymptote of the curve, the maximum rate of photosynthesis (ETR_{max}), is a measure of the capacity of the photosystems to utilize the absorbed light energy (Marshall et al., 2000). All curves were fit using the NLIN procedure, SAS version 8.2 (SAS Institute, Cary, NC). The use of RLCs enabled us to compare the spatial and temporal variability of effective quantum yield (the first yield measurement taken before light is applied) and the derived parameters (alpha and ETR_{max}).

2.3. Physical parameters

Temperature, salinity, water depth, and irradiance at the seagrass canopy (PAR_{canopy}) were measured at each station. Ambient irradiance at the seagrass canopy was determined using a scalar quantum PAR sensor (LiCor LI-193S) attached to a LiCor LI-1000 data logger

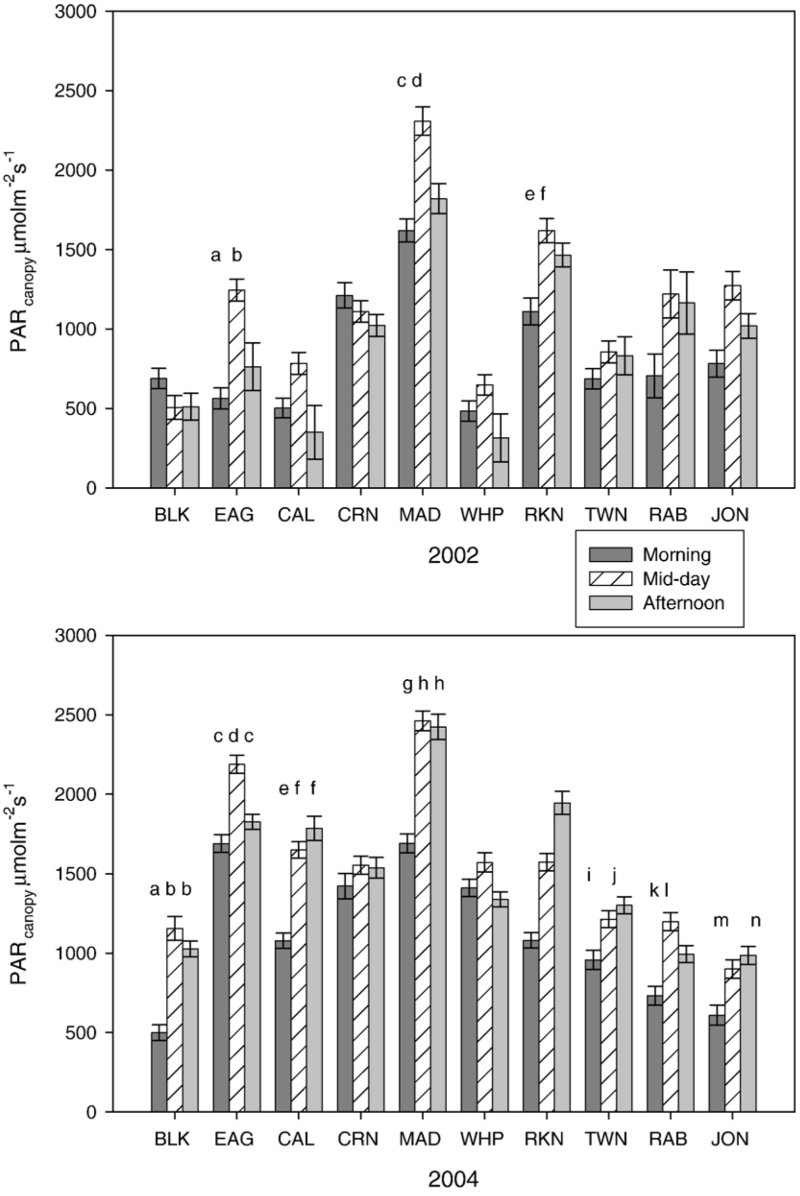

Fig. 3. Comparison of mean morning (0800–1100 h), mid-day (1100–1400 h), and afternoon (1400–1700 h) irradiance (µmol photon m^{-2} s^{-1}) measured at the seagrass canopy (PAR_{canopy}) in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically relative to their spatial position within the bay (east to west). Letters denote significant differences among time periods ($p<0.05$, Tukey's post hoc test).

(Lincoln, Nebraska, USA). Measurements used to calculate ETRs were taken at the top of the canopy as part of light profiles, which were conducted at each station to calculate attenuation coefficients for the water column. Therefore, we did not take into account self-shading due to canopy development and seagrass density. It should be noted that *T. testudinum* density increases from east to west in the bay and self-shading may have more of an affect on the light field in the western basins. Salinity and temperature were measured with a handheld digital water-quality meter (YSI 30; Yellow Springs, Ohio, USA).

2.4. Statistical analysis

To assess the effect of diurnal fluctuations during each basin sampling period, within-basin variation of Φ_{PSII}, RLC parameters (alpha, $\mathrm{ETR_{max}}$), and physical

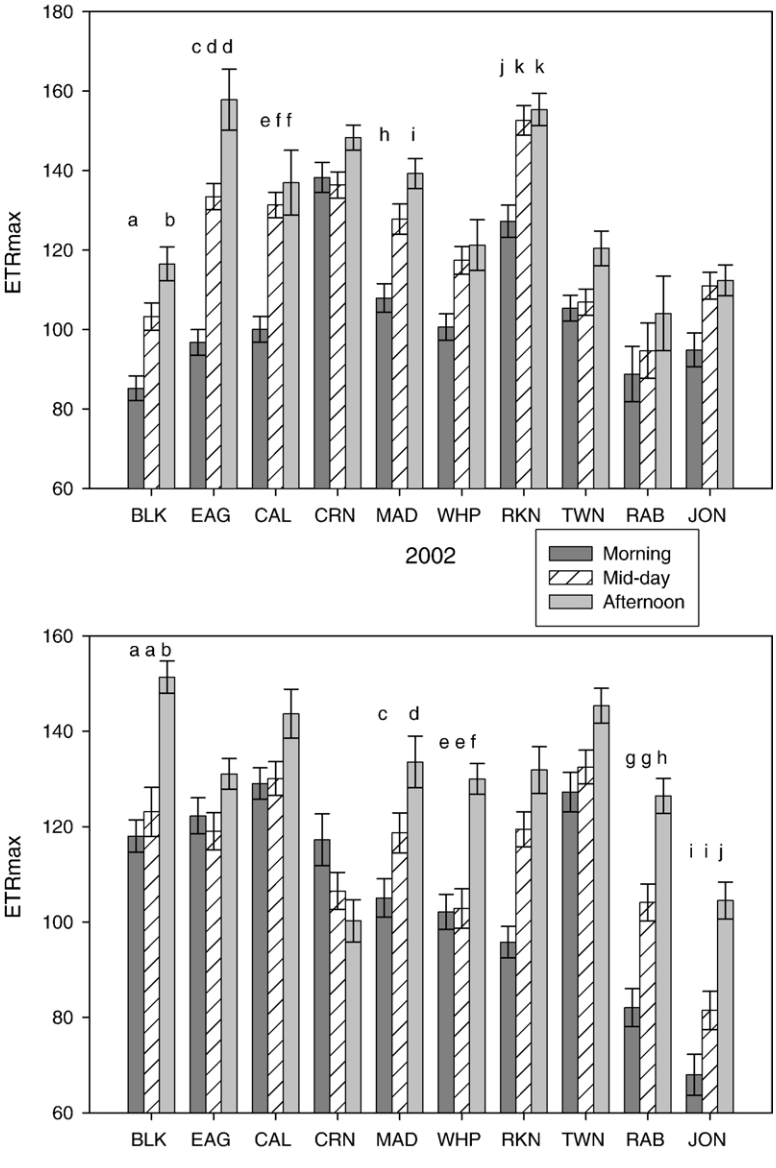

Fig. 4. Comparison of mean morning (0800–1100 h), mid-day (1100–1400 h), and afternoon (1400–1700 h) photosynthetic capacity ($\mathrm{ETR_{max}}$) (μmol e^{-} m^{-2} s^{-1}) of *T. testudinum* blades measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically relative to their spatial position within the bay (east to west). Letters denote significant differences among time periods ($p < 0.05$, Tukey's post hoc test).

parameters (temperature, $\text{PAR}_{\text{canopy}}$) were compared. Measurements of photosynthetic and physical parameters were grouped by morning (0800–1100 h), mid-day (1100–1400 h), and afternoon (1400–1700 h), and means for these three time periods were compared within each basin. To assess variability in photosynthetic parameters among basins and years, mean Φ_{PSII} and RLC parameters (alpha and ETR_{max}) for the three time periods were also compared among basins and between years (2002, 2004). Only means of similar time periods were compared. Daily mean parameters were also compared among basins and between years in order to incorporate the entire basin-level spatial scale into the analysis, and to contrast with separate time period comparisons. All comparisons were made via repeated measures ANOVA with a Tukey–Kramer adjustment for pairwise comparison. Also, Pearson correlations were calculated to examine possible relationships between photosynthetic and physical parameters.

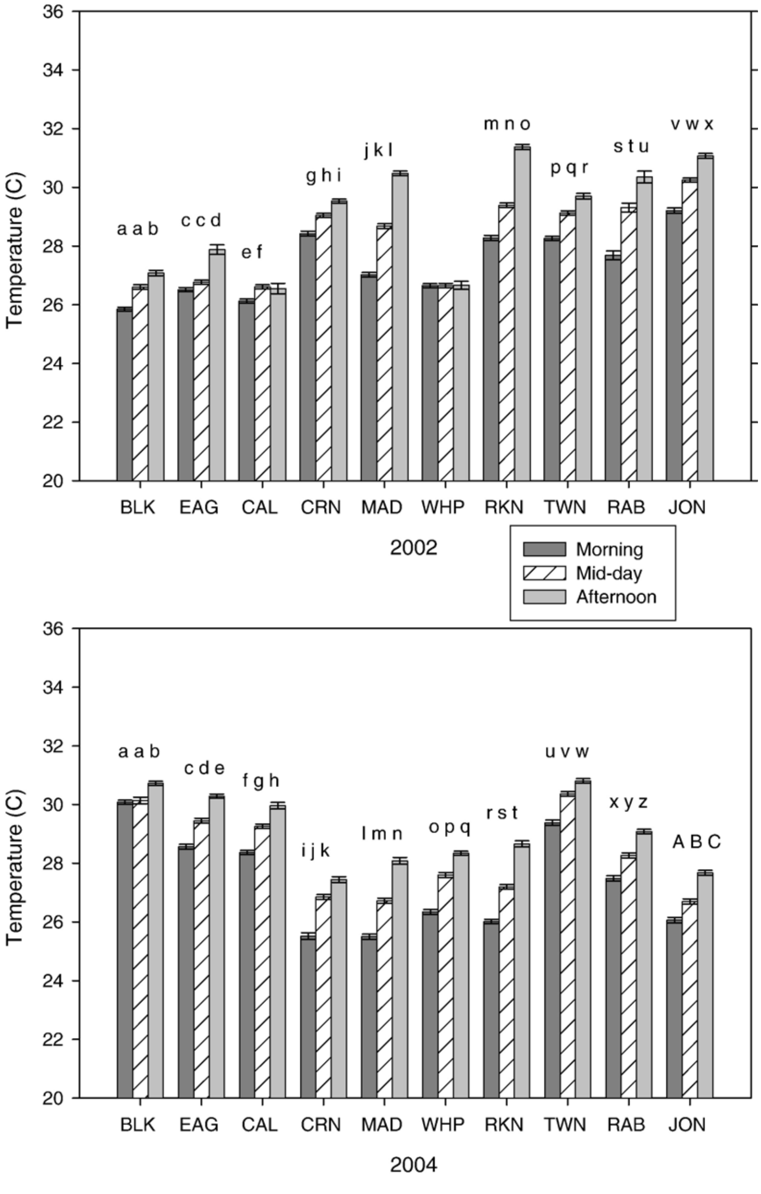

Fig. 5. Comparison of mean morning (0800–1100 h), mid-day (1100–1400 h), and afternoon (1400–1700 h) water temperature (°C) measured in the ten basins sampled during spring FHAP 2002 and 2004. Basins arranged graphically relative to their spatial position within the bay (east to west). Letters denote significant differences among time periods ($p<0.05$, Tukey's post hoc test).

3. Results

3.1. Diurnal variation of photosynthetic parameters

Effective quantum yield (Φ_{PSII}) exhibited relatively consistent diurnal patterns in all ten basins and between years (Fig. 1). Φ_{PSII} was always highest in the morning (0800–1100 h) and decreased during the mid-day (1100–1400 h). Φ_{PSII} then either exhibited a slight recovery in the afternoon (1400–1700 h) or continued to decrease in the afternoon. Though there were diurnal patterns in all of the basins, the differences among time periods were not always significant and differences in the number of basins that showed significant diurnal

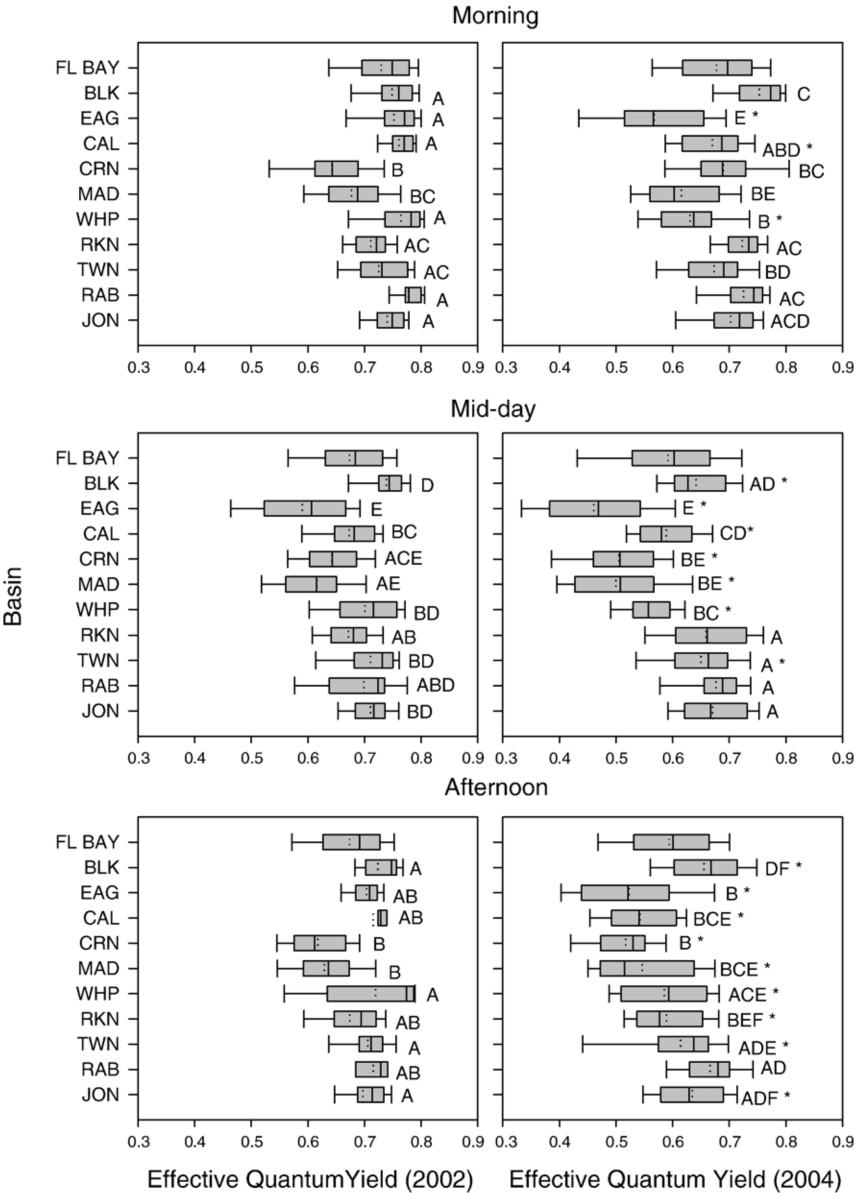

Fig. 6. Effective quantum yield (Φ_{PSII}) comparisons among the 10 basins sampled in Florida Bay measured within similar time periods in 2002 and 2004. Basins with different letters are significantly different within a time period and basins marked with an asterisk (∗) exhibited significant differences between years during the specific time period ($p < 0.05$, Tukey's post hoc test). Basins arranged graphically relative to their spatial position within the bay (east to west). Box and whisker diagrams: boxes enclose the 25th and 75th percentile, whiskers enclose the 10th and 90th percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire bay during each time period are shown for comparison.

variation also varied between years. Only three basins in 2002 had significant differences among time periods, while in 2004 all but one basin showed significant diurnal variation. Photosynthetic efficiency (alpha) also displayed a diurnal trend (Fig. 2). Alpha exhibited a pattern similar to Φ_{PSII}, with highest values in the morning, a decrease in efficiency during mid-day, and either a slight recovery in the afternoon or a continued decrease. Even though all basins illustrated patterns of fluctuation throughout the day, not all basins showed significant differences among time periods. As was found in Φ_{PSII}, the number of basins that had significant variation among time periods increased from three basins in 2002 to nine basins in 2004.

Both Φ_{PSII} (-0.63, $p<0.001$, $df=1054$) and alpha (-0.57, $p<0.001$, $df=1054$) were negatively correlated

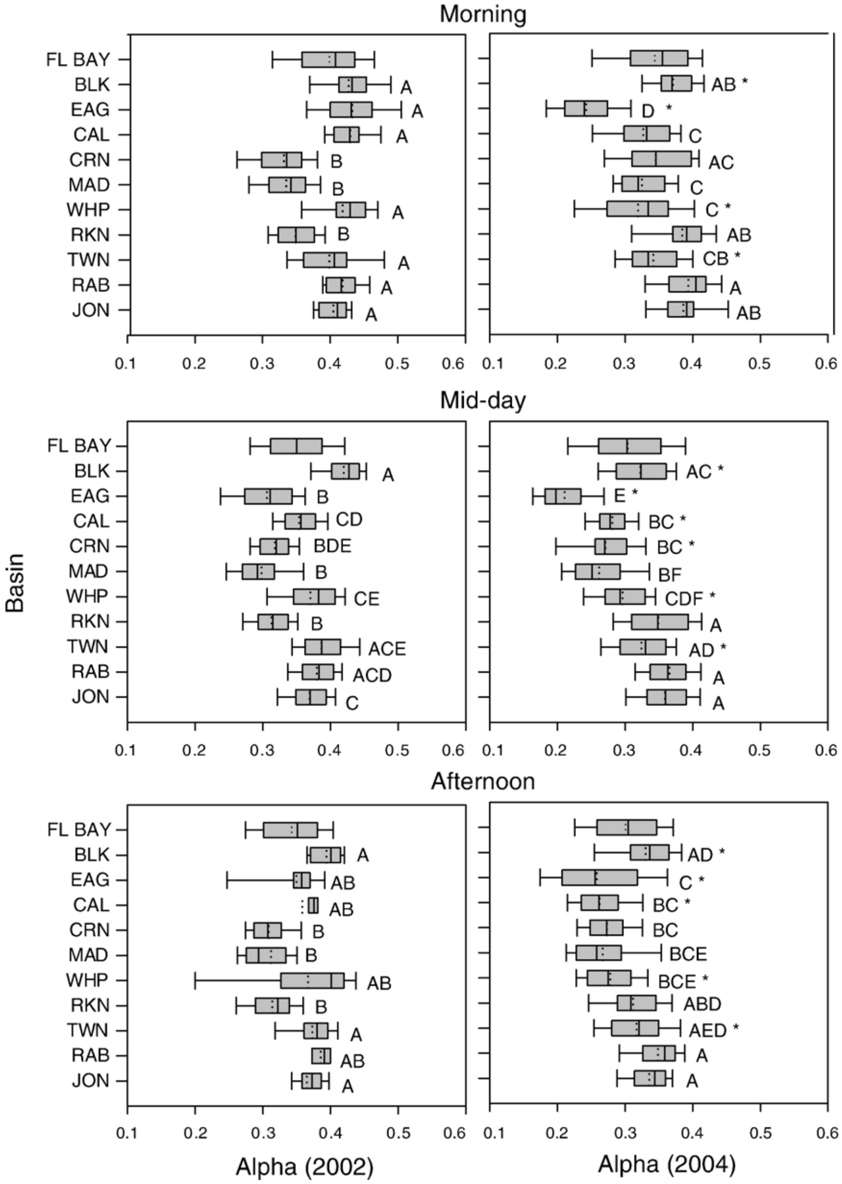

Fig. 7. Photosynthetic efficiency (alpha) (μmol e^{-} m^{-2} s^{-1}/μmol photon m^{-2} s^{-1}) comparisons among the 10 basins sampled in Florida Bay measured within similar time periods in 2002 and 2004. Basins with different letters are significantly different within a time period and basins marked with an asterisk (∗) exhibited significant differences between years during the specific time period ($p<0.05$, Tukey's post hoc test). Basins arranged graphically relative to their spatial position within the bay (east to west). Box and whisker diagrams: boxes enclose the 25th and 75th percentile, whiskers enclose the 10th and 90th percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire bay during each time period are shown for comparison.

with the irradiance measured at the seagrass canopy (PAR_{canopy} µmol m^{-2} s^{-1}; Fig. 3). While PAR_{canopy} in most basins exhibited a typical diurnal pattern with low irradiance in the morning, highest irradiance around mid-day and a decrease in irradiance as the day progressed, the differences among the time periods we sampled were not always significant. There was a marked increase in irradiance reaching the seagrass canopy in 2004 in most basins (though there was a 20% decrease in irradiance in JON, and RAB and RKN mean irradiance did not change between years, all other basins measured had a 14–161% increase in irradiance). The increase in the number of basins that exhibited a significant variation in PAR_{canopy} throughout the day

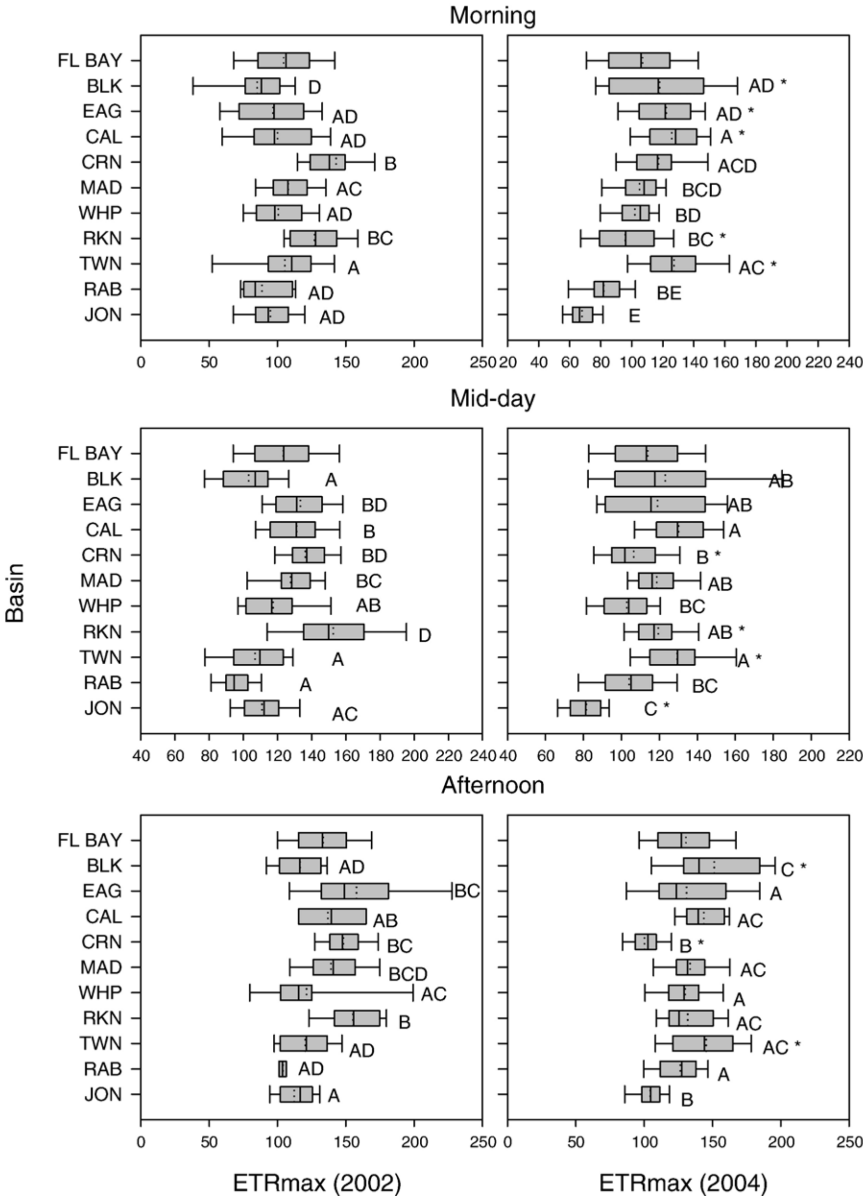

Fig. 8. Photosynthetic capacity (ETR_{max}) (µmol e^{-} m^{-2} s^{-1}) comparisons among the 10 basins sampled in Florida Bay measured within similar time periods in 2002 and 2004. Basins with different letters are significantly different within a time period and basins marked with an asterisk (∗) exhibited significant differences between years during the specific time period ($p < 0.05$, Tukey's post hoc test). Basins arranged graphically relative to their spatial position within the bay (east to west). Box and whisker diagrams: boxes enclose the 25th and 75th percentile, whiskers enclose the 10th and 90th percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire bay during each time period are shown for comparison.

also increased in 2004, which may be responsible for the increased diurnal variation observed in both Φ_{PSII} and alpha during the latter year.

Photosynthetic capacity ($\mathrm{ETR}_{\mathrm{max}}$) exhibited an internally consistent diurnal pattern that was different than the patterns exhibited by Φ_{PSII} or alpha. $\mathrm{ETR}_{\mathrm{max}}$ was lowest in the morning and progressively increased through mid-day and afternoon (Fig. 4). There were a couple of exceptions to this general trend; both Crane Key (CRN) in 2002 and Eagle Key (EAG) in 2004 exhibited a slight decline between morning and mid-day. Also, CRN in 2004 showed a decline from morning through the afternoon. However, the differences among time periods were not significant. Though $\mathrm{ETR}_{\mathrm{max}}$ showed a consistent pattern in most basins, the difference among time periods was not always significant and the basins that showed significant differences changed between the years. $\mathrm{ETR}_{\mathrm{max}}$ was most correlated with temperature (0.49, $p<0.001$), which showed a very similar diurnal trend of increasing throughout the day (Fig. 5).

3.2. Among-basin and between-year comparisons

Variability in effective quantum yields (Φ_{PSII}) among basins differed among time periods and between years (Fig. 6). Among-basin differences in 2002, as determined by the number of significant Tukey post hoc differences, were greatest during mid-day, lowest in afternoon, and intermediate in morning. In 2004, among-basin differences were greatest in the afternoon, lowest in the morning, and showed intermediate variability during mid-day. Interannual variability was also distinct among time periods, with the greatest differences between years occurring during afternoon measurements and the least during the morning period.

Mean basin photosynthetic efficiency (alpha) also differed among time periods and years (Fig. 7). In 2002, the greatest number of among-basin differences occurred during mid-day, with less variation in morning and afternoon. Mid-day in 2004 also exhibited the greatest amount of among-basin variation, with morning displaying the least, and afternoon showing intermediate variability. Interannual variation in alpha exhibited differing patterns among time periods, with the highest number of significant differences detected during mid-day and the lowest in the morning.

Among-basin variability in photosynthetic capacity ($\mathrm{ETR}_{\mathrm{max}}$) exhibited similar heterogeneity among time periods as the other PAM-derived parameters (Fig. 8). Among-basin differences were the same during all three time periods in 2002, while morning exhibited the highest significant among-basin variability in 2004, with lower variation among basins occurring in both mid-day and afternoon. The greatest number of within-basin significant differences between years was detected during the morning, with a decrease in the number of significant changes as the day progressed.

Comparisons of the mean daily basin parameters allowed us to evaluate how basins differed if the time of day variation was pooled and the entire spatial scale for each basin was incorporated into the analyses. There was a larger range of mean daily Φ_{PSII} at the basin and bay scales in 2004 and Φ_{PSII} tended to be lower, but both years exhibited a similar pattern for among-basin differences (Fig. 9). Comparing daily mean Φ_{PSII} of both years to the three daily time periods indicated that daily mean Φ_{PSII} exhibited similar among-basin difference patterns as mid-day comparisons during 2002 and afternoon comparisons in 2004 (compare Figs. 9 and 6). When comparing daily mean alpha between years, 2002 exhibited a greater number of among-basin differences, but 2004 had a greater range (Fig. 10). Mean daily alpha

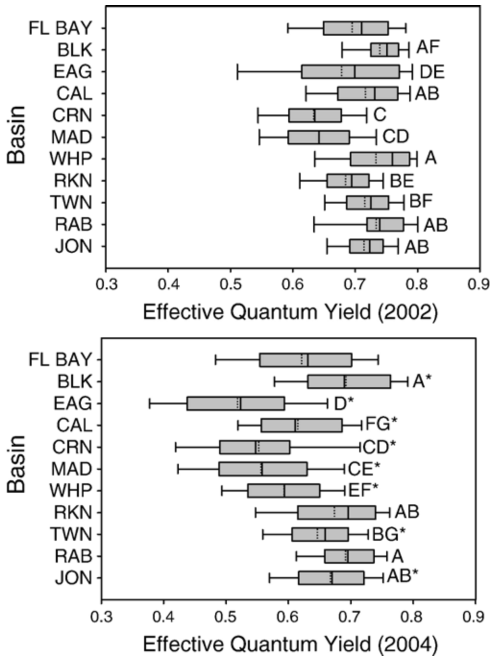

Fig. 9. Effective quantum yield (Φ_{PSII}) comparisons among the 10 basins sampled in Florida Bay measured throughout the entire day in 2002 and 2004. Basins with different letters are significantly different and basins marked with an asterisk (∗) exhibited significant differences between years ($p<0.05$, Tukey's post hoc test). Basins arranged graphically in conjunction with their spatial position with in the bay (east to west). Box and whisker diagrams: boxes enclose the 25th and 75th percentile, whiskers enclose the 10th and 90th percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire bay are shown for comparison.

in 2002 exhibited more significant variability among basins than any single time period, but in 2004 mean daily alpha showed similar among-basin variability as morning and less variability than mid-day or afternoon (compare Figs. 10 and 7). Mean daily $\mathrm{ETR}_{\max}$ in 2002 showed greater among-basin variability than in 2004, but there was a similar range in both years (Fig. 11). Mean daily $\mathrm{ETR}_{\max}$ displayed as much, or more, variation among basins than any separate time period alone in both 2002 and 2004 (compare Figs. 11 and 8).

The basins were arranged graphically (Figs. 7–11) relative to their spatial position within the bay (east to west) in order to determine if photosynthetic parameters reflected the gradient of environmental conditions within the bay. Excluding Blackwater Sound (BLK), there is a general trend of increasing Φ_{PSII} and alpha from east to west within the bay, which was most pronounced in 2004, during mid-day, afternoon, and pooled daily comparisons. Although BLK is located farthest east in the bay, the photosynthetic characteristics of *T. testudinum* in the sound more closely resembled the basins located in the north central bay. $\mathrm{ETR}_{\max}$ exhibited an opposite trend of decreasing capacity from east to west within the bay, though this trend was less apparent.

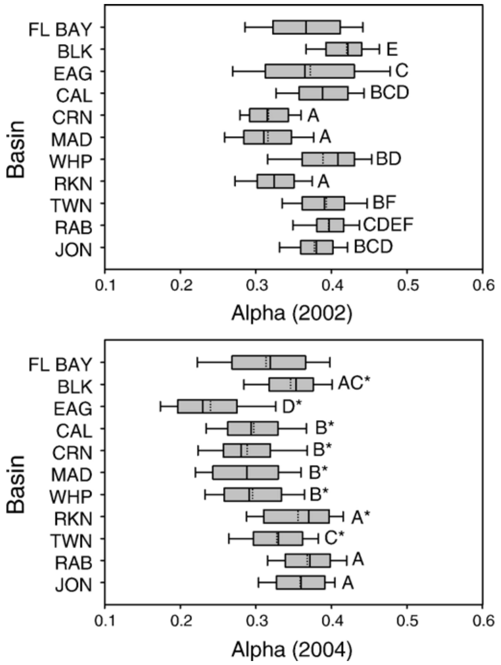

Fig. 10. Photosynthetic efficiency (alpha) (μmol e^{-} m^{-2} s^{-1}/μmol photon m^{-2} s^{-1}) comparisons among the 10 basins sampled in Florida Bay measured throughout the entire day in 2002 and 2004. Basins with different letters are significantly different and basins marked with an asterisk (∗) exhibited significant differences between years ($p<0.05$, Tukey's post hoc test). Basins arranged graphically relative to their spatial position within the bay (east to west). Box and whisker diagrams: boxes enclose the 25th and 75th percentile, whiskers enclose the 10th and 90th percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire bay are shown for comparison.

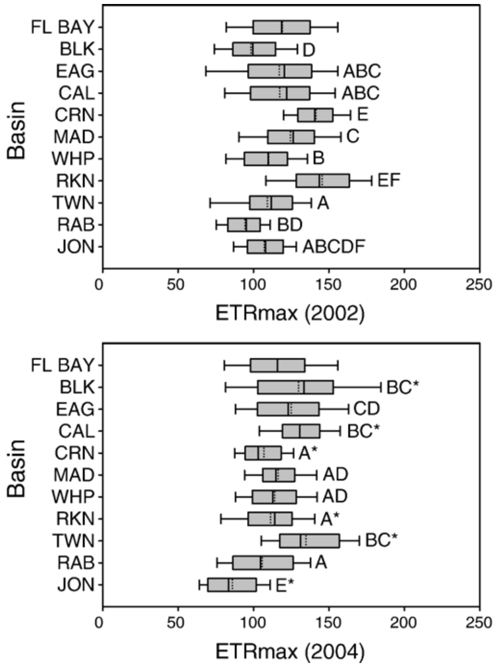

Fig. 11. Photosynthetic capacity ($\mathrm{ETR}_{\max}$) (μmol e^{-} m^{-2} s^{-1}) comparisons among the 10 basins sampled in Florida Bay measured throughout the entire day in 2002 and 2004. Basins with different letters are significantly different and basins marked with an asterisk (∗) exhibited significant differences between years ($p<0.05$, Tukey's post hoc test). Basins arranged graphically relative to their spatial position within the bay (east to west). Box and whisker diagrams: boxes enclose the 25th and 75th percentile, whiskers enclose the 10th and 90th percentile, vertical line within box represents median, and dashed vertical line represents the mean. Values for the entire bay are shown for comparison.

4. Discussion

Our results demonstrate that effective quantum yields (Φ_{PSII}) and rapid light curve derived photosynthetic parameters (alpha and $\mathrm{ETR}_{\max}$) vary throughout the day for *T. testudinum* in Florida Bay, yet the statistical significance of the variations differed among basins and between years. Φ_{PSII} and alpha provide measures of the efficiency of photosynthesis and both exhibited similar diurnal patterns in this study. The highest values of both parameters were observed in the morning with marked decreases during mid-day. In some basins, there was a partial recovery in the afternoon, while in others there was a continued decrease as the day progressed. In the basins that had a partial recovery in the afternoon, it is likely that this rapid recovery time indicates down-regulation and photoprotection had occurred, which prevented damage to the photosystems during high mid-day irradiances. This is normally associated with the xanthophyll cycle (Ralph and Gademann, 2005). However, the continued decrease in Φ_{PSII} and alpha observed in other basins suggests photodamage. Photoprotective mechanisms in the populations of *T. testudinum* in these basins seem to have been inadequate to dissipate the excess energy during mid-day, possibly reflecting stress (Ralph and Gademann, 2005). In addition, there may not have been enough time following the highest mid-day irradiances to re-establish the pool of functional reaction centers by the time our sample period ended. Beer et al. (1998a) measured Φ_{PSII} of shallow and deep growing coral colonies, over a diurnal cycle. They found that calculated ETR values for shallow high-light colonies were always lower in the afternoon than in the morning, at equal irradiances. Also, shallow colonies consistently had lower Φ_{PSII} than deeper colonies. These results, along with our observations, indicate there is a trade off between photosynthetic efficiency and photoprotection.

Both Φ_{PSII} and alpha were negatively correlated with the ambient irradiance at the seagrass canopy ($\mathrm{PAR}_{\mathrm{canopy}}$). There was a marked increase in irradiance reaching the seagrass canopy in 2004 in the majority of the basins. Coinciding with this increase in irradiance was an increase in the number of basins that exhibited a significant variation in $\mathrm{PAR}_{\mathrm{canopy}}$, Φ_{PSII}, and alpha throughout the day. These trends illustrate that as the magnitude and variability of the driving environmental parameter increased there were corresponding increases in the magnitude and variability of the photo-regulatory mechanisms.

Though trends were evident in the photosynthetic characteristics of *T. testudinum* their magnitude and significance differed among basins and between years, which made it difficult to ascertain any clear landscape scale physiological signals or to develop a correction for time of day effects. This was, in part, due to convolution of the fluorescence signals by measuring seagrasses growing at a wide range of depths throughout the sample period. Since water depth has a direct effect on irradiance reaching seagrasses and changes their irradiance pre-history, this obscured some diurnal variations at the landscape scale.

The capacity of photosynthesis ($\mathrm{ETR}_{\max}$) also exhibited a characteristic diurnal pattern but the significance of the changes varied among basins and between years. Photosynthetic capacity was lowest in the morning and steadily increased as the day progressed, resulting in the highest capacity in the afternoon. There were a few exceptions to this general trend though they were not significant. In CRN in 2002 and EAG in 2004 there was a decrease in $\mathrm{ETR}_{\max}$ from morning to mid-day, but both basins exhibited an increase in afternoon. The largest deviation from the general trend was in CRN in 2004, which showed the exact opposite trend as the majority of other basins. We believe this was an artifact due to battery problems with the Diving-PAM forcing us to split up the sample period for this basin into 2 days. $\mathrm{ETR}_{\max}$ was most closely correlated with temperature and as a general trend they both increased throughout the day. The rate of photosynthesis at light saturation is assumed to be dependent on the activity of Rubisco, which can be controlled by variations in the enzyme's concentration or in the short term by its activation state (MacIntyre et al., 2000). The observed pattern may reflect increased synthesis of Rubisco, which could result in an accumulation of enzyme as the day progresses and a subsequent increase in carbon fixation capacity. Most enzyme-catalyzed reactions show an exponential increase in rate as temperature increases (Taiz and Zieger, 1998), which could also explain the consistent morning to afternoon increases we observed. The general trend in photosynthesis of *T. testudinum* was increased capacity with decreasing efficiency as the day proceeded. This resulted in a negative correlation (-0.21, $p<0.0001$, $df=1054$) between alpha and $\mathrm{ETR}_{\max}$ and it suggests that excess excitation energy created by high irradiances may preferentially hinder efficiency of the light reaction (Ralph and Gademann, 2005).

Though RLC measurements exhibited significant diurnal variability, informative physiological patterns did emerge. Excluding Blackwater Sound, which is the most eastern basin within the bay, yet was found to be more similar to the north central basins, there was a general east to west trend of increasing photosynthetic efficiency. This is consistent with previous observations of the morphometric characteristics of *T. testudinum* across Florida Bay (Hackney and Durako, 2004, 2005). Standing crop, the ratio of aboveground-to-belowground biomass, leaf area index, leaf number, and size all increase from east to west within the bay. This spatial pattern of increase has been attributed to landscape scale differences in environmental parameters. In addition, like photosynthetic efficiency, morphometric characteristics of *T. testudinum* from Blackwater Sound are most similar to those of populations in north-central Florida Bay. In conjunction with increasing photosynthetic efficiency, a general trend of decreasing capacity was also observed from east to west, within the bay. This gradient could be an indication of a photoadaptive response to the general increase in depth along this gradient and may reflect a transition from sun- to shade-adapted plants. Also, the increased seagrass density in western basins may cause considerably more self-shading, which would contribute to the reduction of light reaching individual blades.

The ability to distinguish significant differences among basins and to detect landscape scale trends in photosynthetic characteristics within the bay that are consistent with structural trends indicates that PAM fluorometry may be useful as a monitoring tool and can potentially detect signs of physiological stress before morphological changes take place. However, the results here, as well as those of Durako and Kunzelman (2002), clearly indicate that time of day (or irradiance pre-history) effects must be factored into the experimental design and interpretation of PAM fluorescence data. Parameters derived from rapid light curves were as sensitive as effective and maximum quantum yields to diurnal fluctuations, even though they are derived from measurements over a range of irradiances. Accurate physiological information can be masked by diurnal variations caused by the photosystems' dynamic response to changing environmental conditions. In ecosystems where the magnitude of changes are large and occur on much faster time scales than the ecosystem changes being measured, the resulting fluctuations may obscure the true physiological signal. Therefore, when using PAM fluorometers over large spatial and temporal scales, diurnal variability must be considered.

We were unable to discern which time of day was best suited for assessing the photophysiological status of *T. testudinum* in Florida Bay. One approach would be to restrict measurements to the morning before irradiance rises to the mid-day maximum, but after the extended dark period of night. This approach allows time for reactions centers to all be available and for repair of any photodamage. On the other hand, in order to detect the effects of stress, a better approach may be to do assessments during or after the high-light stress of mid-day. But to do this effectively, it is necessary to take some fluorescence measurements after an extended dark acclimation period to obtain a base line maximum quantum yield for comparison. When taking virtually simultaneous replicate measurements of effective yield over a diel cycle, Runcie and Durako (2004) found that measurements taken during mid-day exhibited the highest variability (~25% of the mean), which in turn decreased their ability to detect changes in efficiency. In certain seagrass species, calculated ETRs based on fluorescence measurements have a linear relationship with O_2 evolution, but for other species they only correlate at lower irradiances, with increasing discrepancies at higher irradiances (Beer et al., 1998b; Beer and Björk, 2000). Using PAM fluorometry at times or irradiances when it most closely correlates with oxygen evolution may provide a clearer physiological signal. When doing landscape scale assessments one must factor in both temporal and spatial variation. The time-restricted approaches presented above place limits on the spatial scale that can be examined. In order to evaluate the status of an ecosystem, it may still be preferable to comprehensively cover the entire spatial scale of sampling. Thus, evaluating mean daily characteristics and consequently using data from the entire spatial sample should provide the most representative scale-appropriate information.

Acknowledgments

This project was supported by the United States Geological Service Biological Resources Division, Everglades National Park, and the National Undersea Research Center. Additional support was provided by the University of North Carolina Wilmington Department of Biology and Marine Biology and the Center for Marine Science. The authors thank Jennifer Kunzelman and Brooke Landry from UNCW and Manuel Merello, Donna Berns, Kerri Fareq, and Dr. M. O. Hall from the Florida Marine Research Institute for their help and support in the field. **[SS]**

References

Beer, S., Björk, M., 2000. Measuring rates of photosynthesis of two tropical seagrasses by pulse-amplitude modulate (PAM) fluorometry. Aquat. Bot. 66, 69–76.

Beer, S., Ilan, M., Eshel, A., Weil, A., Brickner, I., 1998a. The use of pulse amplitude modulated (PAM) fluorometry for *in situ* measurements of photosynthesis in two Red Sea Faviid corals. Mar. Biol. 131, 607–612.

Beer, S., Vilenkin, B., Weil, A., Veste, M., Susel, L., Eshel, A., 1998b. Measuring photosynthesis in seagrasses by pulse amplitude modulated (PAM) fluorometry. Mar. Ecol., Prog. Ser. 174, 293–300.

Durako, M.J., Kunzelman, J.I., 2002. Photosynthetic characteristics of *Thalassia testudinum* measured in situ by pulse-amplitude modulated (PAM) fluorometry: methodological and scale-based considerations. Aquat. Bot. 73, 173–185.

Durako, M.J., Hall, M.O., Merello, M., 2002. Patterns of change in the seagrass dominated Florida Bay hydroscape. In: Porter, J.W., Porter, K.G. (Eds.), The Everglades, Florida Bay and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press, pp. 523–537.

Enríquez, S., Merino, M., Iglesias-Prieto, R., 2002. Variations in the photosynthetic performance along the leaves of the tropical seagrass *Thalassia testudinum*. Mar. Biol. 140, 891–900.

Fourqurean, J.W., Robblee, M.B., 1999. Florida Bay: a history of recent ecological changes. Estuaries 22, 345–357.

Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92.

Gorbunov, M.Y., Kolber, Z.S., Lesser, M.P., Falkowski, P.G., 2001. Photosynthesis and photoprotection in symbiotic corals. Limnol. Oceanogr. 46 (1), 75–85.

Hackney, J.W., Durako, M.J., 2004. Size-frequency patterns in morphometric characteristics of the seagrass *Thalassia testudinum* reflect environmental variability. Ecol. Indic. 4, 55–71.

Hackney, J.W., Durako, M.J., 2005. A hierarchical approach to the evaluation of variability in ecoindicators of the seagrass *Thalassia testudinum*. Estuarine Indicators. CRC Press, pp. 175–192.

Kirk, J.T.O., 1994. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press, Cambridge, England, p. 509.

MacIntyre, H.L., Kana, T.M., Greider, R.J., 2000. The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. Trends Plant Sci. 5 (1), 12–17.

Major, K.M., Dunton, K.H., 2002. Variations in light-harvesting characteristics of the seagrass, *Thalassia testudinum*: evidence for photoacclimation. J. Exp. Mar. Biol. Ecol. 275, 173–189.

Marshall, H.J., Geider, R.J., Flynn, K.J., 2000. A mechanistic model of photoinhibition. New Phytol. 145, 347–359.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51, 659–668.

Phlips, E.J., Lynch, T.C., Badylak, S., 1995. Chlorophyll *a*, tripton, color, and light availability in a shallow tropical inner-self lagoon, Florida Bay, USA. Mar. Ecol., Prog. Ser. 127, 223–234.

Platt, T., Gallegos, C.L., Harrison, W.G., 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. 38, 687–701.

Ralph, P.J., Gademann, R., 2005. Rapid light curves: a powerful tool for the assessment of photosynthetic activity. Aquat. Bot. 82, 222–237.

Ralph, P.J., Gademann, R., Dennison, W.C., 1998. In situ seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. Mar. Biol. 132, 367–373.

Runcie, J.W., Durako, M.J., 2004. Among-shoot variability and leaf-specific absorbance characteristics affect diel estimates of in situ electron transport of *Posidonia australis*. Aquat. Bot. 80, 209–220.

Taiz, L., Zieger, E., 1998. Plant Physiology, 2nd ed. Sinauer Associates Inc., Sunderland, Massachusetts.

White, A.J., Critchley, C., 1999. Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. Photosynth. Res. 59, 63–72.

Zieman, J.C., 1982. The Ecology of the Seagrasses of South Florida: A Community Profile. U.S. Fish and Wildlife Services, Washington, DC, p. 158.

Zieman, J.C., Fourqurean, J.W., Iverson, R.L., 1989. Distribution, abundance and productivity of seagrasses and macroalgae in Florida Bay. Bull. Mar. Sci. 44, 292–311.