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Diurnal light curves and landscape-scale variation in photosynthetic characteristics of *Thalassia testudinum* in Florida Bay

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Abstract

When using pulse-amplitude modulated (PAM) fluorometry to measure landscape-scale photosynthetic characteristics, diurnal variations in fluorescence during sampling may confound the assessment of the physiological condition. In this study, two photophysiological assessment techniques: Diurnal Yield and Diurnal Rapid Light Curve (RLC) were investigated in an attempt to incorporate the temporal and spatial scales of sampling into a physiological assessment of *Thalassia testudinum* in Florida Bay. Photosynthesis–irradiance (P–E) curves were calculated using both methods and the ability of each to predict the relationship between relative electron transport rates and irradiance was assessed. Both methods had limitations in providing consistent estimates of photosynthetic efficiency or capacity. The Diurnal Yield method produced unrealistically high predictions of photosynthetic capacity (relative electron transport rate ($rETR_{max}$), 417–1715) and saturation irradiance (I_k, 1045–4681 μmol photons $m^{-2} s^{-1}$). In contrast, the Diurnal RLC method generally produced predictions of $rETR_{max}$ (100–200) and I_k (300–500 μmol photons $m^{-2} s^{-1}$) which were similar to average values calculated from each day's RLCs. The Diurnal RLC method was unable to predict photosynthetic efficiency (α) only when ambient irradiances were continuously $>I_k$ during the sampling period. We believe that with sampling modifications in high-light or shallow environments, such as starting sampling earlier in the morning, extending sampling later in the day, or using the average α from each day's RLCs, that the Diurnal RLC method can produce representative estimates of $rETR_{max}$, α, and I_k, providing a method to characterize seagrass photosynthesis at the landscape-level. The Diurnal RLC method does not negate Diurnal variation but it produces a curve that incorporates the changing ambient light environment into the assessment of seagrass physiological status.

Keywords: PAM fluorometry; Photosynthesis; Diurnal variation; Seagrasses; *Thalassia testudinum*

1. Introduction

Pulse-amplitude modulated (PAM) fluorometry provides almost instantaneous *in situ* measurements of a variety of photosynthetic characteristics, under ambient conditions. With the development of an underwater fluorometer, Diving PAM (Walz, Germany), it is now possible to study photosynthesis of aquatic organisms, such as seagrasses, without the use of gas-exchange enclosures (Beer et al., 1998). The measurement of chlorophyll fluorescence, emitted from photosystem II (PSII), provides insight into changes in photochemistry, and permits the study of effects of varying environmental conditions on photosynthetic reactions (White and Critchley, 1999; Schreiber, 2004; Ralph and Gademann, 2005; Ralph et al., 2007). PAM fluorometry is also an attractive assessment tool because it is rapid, non-destructive, and can provide in-depth, quantitative physiological information about an organism.

In an initial study incorporating PAM fluorometry into a landscape-scale assessment of seagrass condition in Florida Bay, significant diurnal variation in chlorophyll fluorescence was detected (Durako and Kunzelman, 2002). The variability of the physiological signal was evident as significant negative slopes in regressions of effective and maximum (5 min dark adapted) quantum yields against ambient irradiance or time of day. Florida Bay is a subtropical lagoonal estuary, thus, resident organisms are exposed to relatively large ranges of irradiances and temperatures throughout the day. Photosynthesis can rapidly respond to changes in the light environment (MacIntyre

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et al., 2000) and the sensitivity of PAM fluorometry in detecting these rapid changes may result in highly variable physiological signals when assessing the physiological condition of seagrasses at the landscape scale. Belshe et al. (2007) further observed that rapid light curve (RLC) derived parameters (α and relative electron transport rate ($rETR_{max}$)) also exhibited diurnal variability, but the magnitude, direction and significance of the variations were inconsistent among basins and years. Statistical testing was unable to determine which time of the day was best suited for assessing seagrass photophysiological status. Belshe et al. (2007) concluded that when performing ecosystem-level assessments, sampling the entire spatial scale of interest provides more representative information than using only a time-restricted subsample, but that diurnal variation has to be accounted for in spatial comparisons.

Longstaff et al. (2002) investigated the accuracy of PAM fluorometry versus O_2 evolution techniques in assessing diel variability *in situ* photosynthetic rates. They took measurements with an automated O_2 exchange apparatus, performed RLCs and obtained point measurements of effective quantum yield over a diel (20 h) period. By constructing traditional photosynthesis–irradiance (P–E) curves with O_2 measurements and fluorescence-based diel light curves using what they termed the 'Diel Yield' and 'Diel Rapid Light Curve' methods, they were able to generate comparable curves that spanned the entire temporal scale of sampling. They found that under certain conditions and with some limitations (mainly at higher irradiance levels) PAM fluorescence could accurately assess photosynthetic rates of the simple laminate algae, *Ulva lactuca* L. Here, we tested the application of the Diel Yield and Diel RLC methods (termed here Diurnal Yield and Diurnal RLC because our measurements were obtained during daylight hours) in order to incorporate time-of-day considerations in conducting large-scale physiological assessments. The objective was to discern the usefulness of the two diel light curve methods described by Longstaff et al. (2002) for overcoming methodological and logistical constraints (i.e., <15 min to complete sampling at each station) inherent with landscape-scale ecological assessment and also to determine the two methods' effectiveness in characterizing the physiological condition of the seagrass *Thalassia testudinum*.

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). As part of the Fish Habitat Assessment Program (see Durako et al., 2002 for more information on FHAP), 10 basins were sampled that lie within the borders of the Everglades National Park (ENP) (Table 1). The basins were chosen to represent the range of conditions within the bay. Each basin was divided into 27–33 tessellated hexagonal subunits, and one station was randomly chosen within each subunit. This resulted in 275–330 stations that were randomly sampled throughout the Bay (see Hackney and Durako, 2004 for a map of sampling stations). Florida Bay is approximately 2000 km^2 and the sampled basins range in size from 5.8 to 62.4 km^2 (Durako et al., 2002). As a result of the large sample area, stations must be sampled systematically in order to minimize station-to-station travel time, yet it still takes an entire day (~0800 to 1700 h) to sample each basin. Because of navigational and safety concerns, FHAP sampling in the Bay can only be conducted during daylight.

2.2. Sampling technique

Photosynthetic characteristics were measured using an underwater fluorometer, Diving PAM (Walz, Germany), in 2002 (13–23 May) and 2004 (20–31 May), during the spring FHAP sampling. 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 each station. A dark leaf clip (DIVING-LC) was attached to the middle of the rank 2 blade of each *T. testudinum* short shoot (Durako and Kunzelman, 2002). 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 for a reduction in instrument gain to achieve a higher signal to noise ratio. Each RLC was initiated within 2–5 s after attaching the leaf clip to minimize dark acclimation (i.e. quasi-darkness yield, Ralph and Gademann, 2005). Leaves were exposed to eight incremental steps of irradiance ranging from 90 to 2060 μmol photons $m^{-2} s^{-1}$ in 2002, and 5 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 (Φ_{PSII}) measurement ($\Delta F/F_m'$) was taken at the beginning of each curve, before light was applied, and at the end of each 5 s irradiance step, resulting in nine yield measurements for each RLC performed. Each Φ_{PSII} measurement was used to calculate the relative electron transport rate through photosystem II using the equation recommended by Beer et al. (2001):

$$rETR = \Phi_{PSII} \times PAR \times AF \times 0.5$$

where PAR is the light generated by the internal halogen lamp of the Diving PAM, AF is the fraction of light absorbed by the leaf, and 0.5 assumes that the photons absorbed are equally partitioned between PSII and PSI (Genty et al., 1989). Due to time limitations at each station (28–33 stations were sampled each day, allowing <15 min $station^{-1}$), it was not possible to measure leaf absorption; therefore, AF was assigned a value of 1 (Beer et al., 2001), and relative electron transport rates are presented.

2.3. Diurnal light curves

To assess changes in photosynthesis in response to changing ambient irradiances, two types of light curves were calculated using a modification of what Longstaff et al. (2002) termed the