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ORIGINAL PAPER

# Diurnal variation in chlorophyll fluorescence of *Thalassia testudinum* seedlings in response to controlled salinity and light conditions

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**Abstract** Diurnal variability in chlorophyll fluorescence caused by dynamic irradiance conditions is an important issue when using pulse amplitude modulation fluorometry to measure physiological conditions of plants at the landscape scale. We examined the use of slopes and y-intercepts of diurnal effective photochemical efficiency of photosystem II (PSII) ($\Delta F/F_m{}'$) versus photosynthetically active radiation (PAR) regressions in addition to direct measurements of maximum photochemical efficiencies of PSII ($F_v/F_m$) values to assess physiological status of *Thalassia testudinum* seedlings in a controlled mesocosm study. Seedlings were exposed to two light treatments (full sun and 50–70 % light reduction) and three salinity treatments (20, 35, and 50). Measurements were taken at 0600, 0900, 1200, 1500, 1800, and 2100 hours in order to assess the diurnal variation in photochemical efficiency of PSII and PAR, with measurements at 2100 providing $F_v/F_m$. Results indicated significant effects of light and salinity on regression y-intercepts and measured $F_v/F_m$ values. Shaded seedlings had higher values for both parameters, suggesting low-light acclimation. The highest salinity treatment resulted in significant reductions for both parameters, suggesting stress. Stress was also indicated by significant reductions in both seedling leaf growth and mean differences between seedling leaves and media osmolalities in the hypersaline treatments (152.0 ± 26.4 vs. 630 ± 40.2 mmol kg$^{-1}$ for the control treatments). Slopes of $\Delta F/F_m{}'$ versus PAR significantly differed with varying light treatments, with full sun seedlings exhibiting shallower slopes than shaded seedlings, indicating higher efficiency of dissipation of excess energy (photoprotection). These experimental results confirm field data suggesting that diurnal $\Delta F/F_m{}'$ versus PAR regressions are responsive to changes in the physiological status of *T. testudinum* and that the y-intercepts of diurnal regressions may be used as a proxy for $F_v/F_m$.

## Introduction

*Thalassia testudinum* Banks ex König (Turtle grass) is the dominant seagrass species in Florida Bay, a shallow subtropical estuary in south Florida, USA (Zieman 1982; Zieman et al. 1989). A massive die-off of *T. testudinum* occurred in Florida Bay over the period from 1987 to 1990 (Robblee et al. 1991). The die-off resulted in the loss of 4,000 hectares and damage to an additional 23,000 hectares of *Thalassia*-dominated habitat (Robblee et al. 1991). Hypersalinity, disease, lack of hurricanes, and anoxic conditions are some of the hypothesized causes of this die-off event (Robblee et al. 1991; Durako and Kuss 1994; Borum et al. 2005). Due to the massive input of dead plant material, the die-off was followed by widespread algal blooms that initiated during the fall of 1991 and persisted for several years. The turbid water conditions that were created from these algal blooms and resuspended sediments from the loss of seagrass cover during the die-off resulted in additional losses of seagrasses, especially in the western portion of Florida Bay (Durako et al. 2002). Because seagrasses serve many important functions in coastal ecosystems, these drastic changes threatened the water quality, sport fishing, and nursery capabilities of Florida Bay (Robblee et al. 1991). In response to concerns

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for continued widespread losses of seagrasses across the bay, the Fish Habitat Assessment Program (FHAP) was established in 1995 to monitor the distribution and abundance of seagrasses and macroalgae within ten basins distributed across the bay (Durako et al. 2002).

In 2001, FHAP began monitoring variations in photosynthetic efficiency of *T. testudinum* using pulse amplitude modulated (PAM) fluorometry (Durako and Kunzelman 2002). The photosynthetic efficiency of a plant is largely dependent upon the quantity and quality of light that it is able to utilize for photosynthesis, balanced with its responses to excess irradiance such as downregulation and photoprotection (Ralph and Gademann 2005; Belshe et al. 2007). The proportion of light energy that is utilized by photosystem II (PSII), which is considered the most sensitive structure to stress in the photosynthetic electron transport system (Becker et al. 1990), depends largely on the light-harvesting complexes, the oxidation state of PSII reactions centers and electron transport chain, the connectivity of PSII, and the ratio of PSII to PSI, among other factors. Absorbed photons may initiate linear electron transfer, be emitted as fluorescence or be dissipated as heat (known as non-photochemical quenching, Kirk 1994). Active fluorescence methods use a weak, non-actinic measuring light and apply a brief (0.8 s) saturating light pulse to close all PSII photosynthetic reaction centers allowing the assessment of the relative difference between minimum ($F_o$ for dark acclimated or $F$ for light-acclimated samples) and maximum ($F_m$ if dark acclimated or $F_m'$ if light acclimated) fluorescence, which is an indicator of photophysiological state (Schreiber 1983, 2004). PAM fluorometry has become a very attractive way of determining photosynthetic health of plants since it is able to identify changes in a plant's photophysiological state before morphological changes or mortality occur (Ralph et al. 2007a). In addition, measurements are quick, nondestructive, and do not require enclosures (Ralph and Gademann 2005).

Effective photochemical efficiency of PSII ($F_m' - F/F_m' = \Delta F/F_m'$), where $F$ is the minimum chlorophyll fluorescence at non-actinic irradiance levels, and $F_m'$ is the maximum fluorescence after the fluorometer's saturating pulse, is one of the primary parameters measured using a PAM fluorometer. This parameter provides an estimate of the efficiency by which absorbed light energy is linearly transported through PSII under ambient light conditions. Values of $\Delta F/F_m'$ decrease as irradiance increases because more reaction centers become reduced, more electrons accumulate at PSII, and excess light energy is utilized for non-photochemical quenching (Falkowski and Raven 2007). Because $\Delta F/F_m'$ is sensitive to changes in irradiance, these values depend on the plant's light history prior to the fluorometer's saturating pulse (Schreiber 2004).

Maximum photochemical efficiency of PSII is calculated as $F_v/F_m$, where $F_v = F_m - F_o$, and is obtained from PAM fluorometer measurements of dark-acclimated plants in which photosynthesis has stopped and all reaction centers are open. The saturating pulse from the PAM fluorometer will close all the reaction centers and results in a much greater fluorescence emission in these plants. Generally, $F_v/F_m$ values are higher than $\Delta F/F_m'$ values because non-photochemical quenching should theoretically not be present in dark-acclimated leaves (Ralph and Gademann 2005). In the past, $F_v/F_m$ has been used to describe a plant's PSII optimal photochemical efficiency (Kromkamp and Forster 2003) and is also used as an indicator of stress (Beer et al. 2001); a reduction in $F_v/F_m$ is linked to more reaction centers being closed, and therefore the sink for light energy becomes limited (Ralph and Gademann 2005).

Diurnal variability in chlorophyll fluorescence parameters caused by dynamic irradiance conditions has become an issue when using PAM fluorometry to measure seagrass physiological condition at the landscape scale. This is especially apparent in shallow ecosystems such as Florida Bay, where seagrasses and other submerged aquatic vegetation (SAV) are exposed to a large range of irradiances throughout the day (Durako and Kunzelman 2002; Belshe et al. 2007). Other factors such as changes in water depth due to tides, wave focusing, and cloud patterns also result in changes in irradiance during the day (Falkowski et al. 1990). While these variations are natural, they have made it difficult to accurately determine the photosynthetic health of seagrasses across Florida Bay when point measurements are used and sampling occurs throughout the day (Durako and Kunzelman 2002). Belshe et al. (2007) observed diurnal variability even in rapid light curve (RLC) parameters, despite using a wide range of photosynthetically active radiation (PAR) exposures for each RLC and they suggested that accurate irradiance measurements and light history effects on seagrasses need to be taken into account when interpreting PAM data.

Recently, Durako (2012) suggested the use of slopes and y-intercepts of diurnal $\Delta F/F_m'$ versus PAR regressions rather than absolute $\Delta F/F_m'$ values may allow the assessment of the physiological status of *T. testudinum* when landscape scale sampling is utilized. This is due to the diurnally based regression coefficients being less sensitive to changing irradiances than are individual $\Delta F/F_m'$ values. The slopes of these regressions reflect the amount of absorbed light energy used for photosynthesis as irradiance changes, with excess energy dissipated as heat (e.g., non-photochemical quenching, which may be linked to the xanthophyll cycle). Increased magnitude of the negative slopes may represent decreased ability to dissipate excess energy (i.e., low-light acclimated or stressed). The y-intercepts of these regressions (photochemical efficiency at PAR = 0) may provide an estimate of the maximum photochemical efficiency of these plants, with high values reflecting high maximum quantum efficiency and very little non-photochemical quenching (shade-acclimated). Examining the 2006–2009 data set from the Southern Florida Fish Habitat Assessment Program (FHAP-SF), Durako (2012) observed relatively steeper slopes and higher y-intercept values during years of relatively low benthic irradiances, which correlated with increased phytoplankton blooms in Florida Bay (Boyer et al. 2009). Slopes and y-intercepts decreased during years after the blooms decreased and water clarity and irradiances increased. Durako (2012) suggested that high values of either parameter may represent shade-acclimated or stressed seagrasses that have lower photoprotection capacity to excess light. Shallower slopes may represent healthier or sun-acclimated seagrasses, which have low efficiency but high photosynthetic and photoprotective capacities (Demmig-Adams et al. 1999, Durako 2012). While the diurnal regression method may be applicable to the FHAP PAM data, it has yet to be tested under controlled conditions.

Hyper- and hypo-salinities negatively affect the health of seagrasses in Florida Bay (Robblee et al. 1991; Durako et al. 1994; Kahn and Durako 2006; Koch et al. 2007a). The primary cause of hypersalinity in Florida Bay is linked to reduced freshwater input from the Everglades watershed as a result of drainage canals, levees, and pumps built in the late nineteenth century for flood control and agriculture (Steinman et al. 2002). Hypersaline conditions are thought to have been a primary contributor to the die-off of *T. testudinum* in the late 1980s (Robblee et al. 1991). Plans for releasing greater amounts of freshwater from the Everglades will result in decreased salinity of Florida Bay, causing unknown changes and possible stresses on *T. testudinum*, a marine seagrass. Changes in the hydrology of this region have also affected the levels of total nitrogen, phosphorous, sulfide, and pollutants including fecal bacteria in Florida Bay (Brand 2002). Increased freshwater from the land may carry higher levels of nutrients into the Bay, resulting in algal blooms. Recent phytoplankton blooms in northeast Florida Bay, believed to be the result of interactions between road construction activities and hurricane-related disturbances (Boyer et al. 2009), resulted in significant reductions in benthic irradiances and losses of *T. testudinum* (Hall and Durako 2011; Durako 2012). The blooms were thought to have been exacerbated and prolonged by large discharges of freshwater from the Everglades (Rudnick et al. 2006). Thus, bloom-related light reductions and salinity changes induce significant stress in *T. testudinum* and could result in yet another die-off of this foundation species.

The objectives of this research were (1) to compare regression slopes and y-intercepts of diurnal variation in effective photochemical efficiency of PSII versus PAR of *T. testudinum* seedlings in response to changes in light and salinity under controlled conditions, (2) to determine whether diurnal regression y-intercepts provide an estimate of maximum photochemical efficiency for *T. testudinum*, and (3) to observe whether PAM parameters of *T. testudinum* seedlings can recover from light and salinity stress once a stress is removed. Seedlings were examined for several reasons. First, adult short shoots of *T. testudinum* exhibit low transplant survival. In addition, the large size of *T. testudinum* experimental units containing adult shoots would not have allowed for replication in our experimental mesocosms; because of the monopodial clonal growth of this seagrass, the use of field-collected rhizome fragments could result in possible pseudoreplication. Seedlings represent independent experimental units and they are an important recruitment source in subtropical seagrass beds (Whitfield et al. 2004). The associated null hypotheses for our objectives were (1) slopes and intercepts of diurnal photochemical efficiency regressions will not change in response to irradiance and salinity variation, (2) regression intercepts will not differ from measured maximum photochemical efficiency, and (3) PAM parameters will not recover once light and salinity stress is removed.

## Materials and methods

### Sample collections

Seedlings of *T. testudinum* were collected from Key Biscayne, Florida (25.716° N 80.149° W) on August 14, 2010, from floating material or shoreline wrack. Seedlings were then placed in a cooler and shipped overnight to the University of North Carolina Wilmington, Center for Marine Science (UNCW/CMS), Wilmington, North Carolina. After arrival at CMS, the seedlings were immediately planted in six-celled plastic nursery pots (each cell 5 × 5 × 7 cm) containing aragonite shell hash and they were held in a holding vault (55 × 110 × 30 cm) with flow-through seawater (salinity 29–35). The Practical Salinity Scale (PSS) was used to determine salinity (UNESCO 1985). Seedlings were allowed to grow for 4 weeks in the vault prior to their placement in the experimental aquaria.

### Experimental design

Two six-celled pots were placed in each 38-l treatment aquarium. The experimental design consisted of two light treatments and three salinity treatments. In each aquarium, one pot received full sun (Sun), and one pot received a 50–70 % reduction in irradiance (Shade). The Shade treatment was accomplished by placing neutral density screen over a randomly selected half of each aquarium, covering the top and sides. Salinity treatment levels of each aquarium were 20 (hyposaline), 35 (control), and 50 (hypersaline). Four replicate aquaria per salinity treatment were used, resulting in twelve aquaria. Aquaria were placed outside within seawater-supplied fiberglass vaults (55 × 110 × 30 cm) located on the south side of CMS. The vaults acted as water baths to minimize daily water temperature fluctuations, and the aquaria were randomly arranged within the vaults (four aquaria per vault) to account for spatial differences. Water temperatures were monitored using Hobo temperature loggers (Onset®, Pocasset, MA USA) placed within replicate aquaria. Temperatures in the treatment aquaria during the 41-day experiment varied between 33.0 °C during the day in September and 15.7 °C during the night in October. The temperature loggers indicated that among-aquaria temperature variation was not significant ($P > 0.05$; data not shown). Artificial seawater was used for all salinity treatments, and the salinity of the aquaria was checked daily using a conductivity meter (YSI Model 80) and adjusted using either de-ionized (DI) water or Instant Ocean© salts to maintain the appropriate salinity treatment.

Beginning on September 13, 2010, all seedlings were acclimated to the control salinity (35) under full sun in the experimental aquaria for 7 days (pre-treatment). Following this pre-treatment period, screens were placed over the shaded treatment seedlings, and either de-ionized (DI) water or Instant Ocean© salts were added to the appropriate aquaria to decrease or increase the salinity by 2 per day until target salinities were reached 8 days later (changing). The gradual change in salinity allowed seedlings time to acclimate to the changes in salinity (Kahn and Durako 2006; Koch et al. 2007b). Seedlings were kept at target salinities for 17 days (target) until equilibrium in diurnal photochemical efficiency versus photosynthetically active radiation (PAR) was reached among all treatments, as determined by comparisons of regression slopes with analysis of covariance (data not shown). Following equilibrium, salinities were brought back to the control salinity over the course of 8 days by adding DI water or Instant Ocean© salt. At this time, shade cloths were removed and seedlings were kept at the control salinities for 6 days and monitored for recovery (recovery).

## Chlorophyll fluorescence

During the pre-treatment, changing, treatment, and recovery periods, photosynthetic characteristics of *T. testudinum* seedlings were quantified daily using a Mini-PAM fluorometer (Waltz, Germany). Two seedlings (one seedling from each irradiance treatment) were haphazardly chosen from each salinity treatment aquarium ($n = 4$), and $\Delta F/F_m'$ measurements were taken from the middle of the rank 2 blade of each seedling shoot. A leaf clip was used to ensure constant distance (5 mm) between the optical fiber tip and seedling leaves. Measurements were taken from the same seedlings at 0600, 0900, 1200, 1500, 1800, and 2100 hours of every day in order to assess the diurnal variation in photochemical efficiencies. The fluorescence measurements at 0600 (pre-dawn) and 2100 (post-dusk) represented maximum photochemical efficiencies of PSII; however, these values did not differ significantly from each other (data not shown) and so only 2100-hour measurements are presented here.

Photosynthetically active radiation (PAR) at the surface of the water and underwater at plant level, in both the Sun and Shade treatments, was determined using underwater scalar quantum PAR sensors (LiCor LI-193S) attached to a LiCor LI-1400 data logger (Lincoln, NE). PAR measurements were logged every 15 min, and PAR values were assigned to the PAM measurement(s) with the closest time stamp. PAR data were not recorded for 2 days at the start of the pre-treatment period, 6 days at the start of the target period, 2 days at the end of the target period, or for 3 days at the start of the recovery period because of low battery voltages in the LiCor data logger, so regressions could not be calculated during these periods.

## Osmolality

Leaf osmolality was measured on the last day of the target period and at the conclusion of the recovery period using a Wescor VAPRO Vapor Pressure Osmometer 5520©. A time-delay vapor-point depression protocol was used (Murphy et al. 2003; Kahn and Durako 2006; Koch et al. 2007a, b). Three replicate rank 2 leaves were taken from each treatment aquarium and submerged in a 15-mL centrifuge tube containing saltwater of the appropriate treatment salinity. A 10-mm section of leaf tissue was cut from the middle of the leaf and blotted with a Kimwipe before being placed in the osmometer. Readings were taken after the sample had been in the chamber for 10 min. Osmolality was also measured for 10 μL of the salinity treatment media that was pipetted onto a sample disc. This allowed for comparison of the leaf tissue to its treatment salinity.

## Seedling morphology

At the initiation and completion of the experiment, the number of leaves as well as leaf width, leaf length, and area was measured for all seedlings. Shoot total leaf area was calculated as the sum of leaf areas for all individual blades on one shoot. Initial leaf measurements (leaf number, length, width, and area) did not vary significantly among seedlings in the different treatments ($P > 0.05$). Dry weights of leaf, seed, and root material were determined at the completion of the experiment by placing tissues in a drying oven set at 60 °C for 48 h, and weighing the separated material on a digital scale.

### Statistical analyses

Statistical analyses were completed using SAS 9.1, JMP7® and SigmaStat® for Windows. Mean $\Delta F/F_m'$ and PAR among days, light, and salinity treatments were compared using a repeated measures three-way ANOVA (salinity, light, and time) with a Tukey's test for pairwise comparisons when significant ($P < 0.05$) differences were detected. Because of the well-established relationship between increasing irradiance and decreasing $\Delta F/F_m'$ (Falkowski and Raven 2007) and the fact that we manipulated light as an experimental treatment variable, model I linear regressions were applied to the $\Delta F/F_m'$ versus PAR data (Sokal and Rohlf 1995). The regression approach assumes linearity in $\Delta F/F_m'$ versus PAR across the full range of daily irradiances, which vary among days and between light treatments. This assumption was tested by conducting ANCOVAs for the full sun treatments during the pre-treatment and target periods, which compared the regressions calculated using the full PAR data to those calculated for only PAR < 500 μmol quanta $m^{-2}$ $s^{-1}$. The ANCOVA results indicated that both slopes and intercepts were homogenous over the different irradiance ranges (data not shown). Regressions of $\Delta F/F_m'$ versus PAR were calculated for each day and treatment ($n = 16$, data not shown) and for pooled treatment periods (pre-treatment, changing, target, and recovery, Figs. 1, 2, 3 and 4). Treatment effects were assessed by comparing regression slopes, y-intercepts, and maximum photochemical efficiency values calculated for each day using a three-way ANOVA. We used ANOVA rather than ANCOVA for these analyses because we assumed, a priori, that the experimental manipulations would result in heterogeneity of slopes, which would preclude statistical comparisons of intercepts. Within treatments, calculated y-intercepts were compared to the measured maximum photochemical

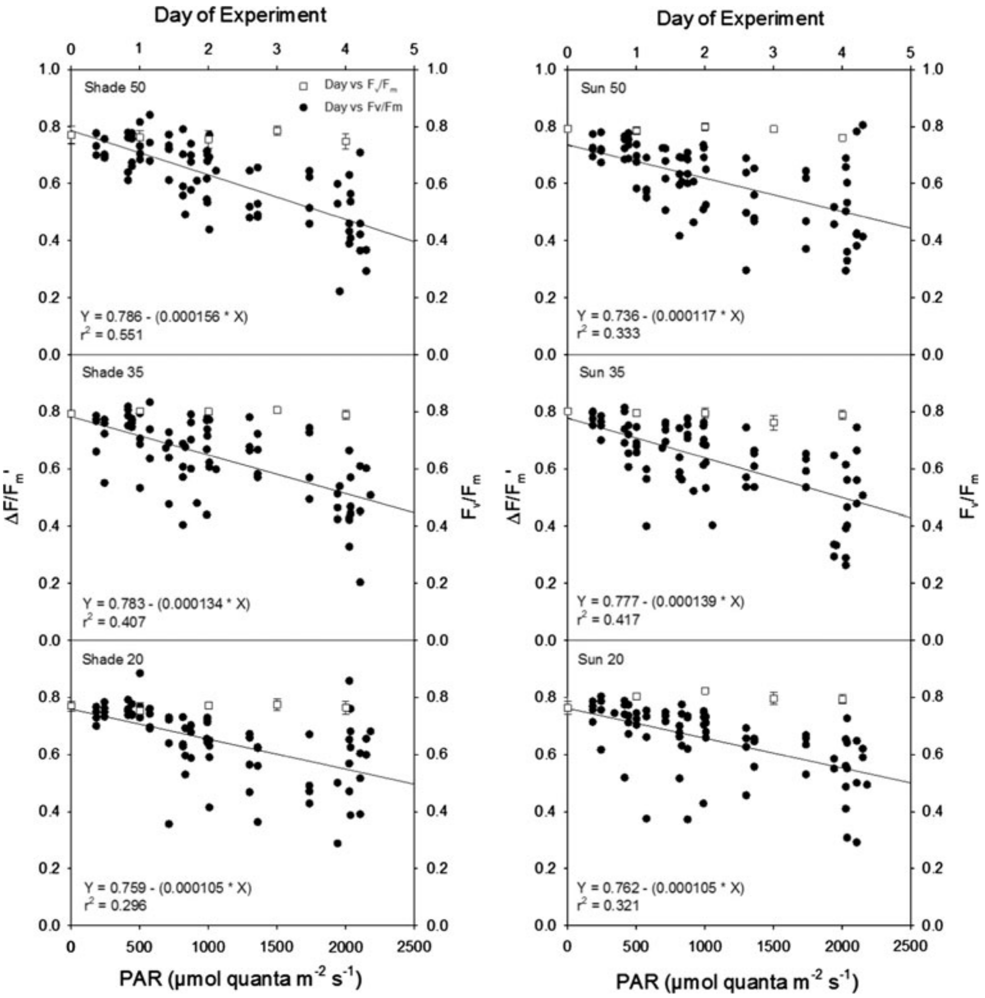

**Fig. 1** Regressions of effective quantum yield ($\Delta F/F_m'$) versus photosynthetically active radiation (PAR) during 5 days over the course of the 7-day pre-treatment period. White squares represent mean (±SE) dark-acclimated maximum quantum yield ($F_v/F_m$) measurements for each day of the pre-treatment period. Each graph represents measurements from aquaria that will be subjected to the experimental light and salinity treatments ($n = 76$)

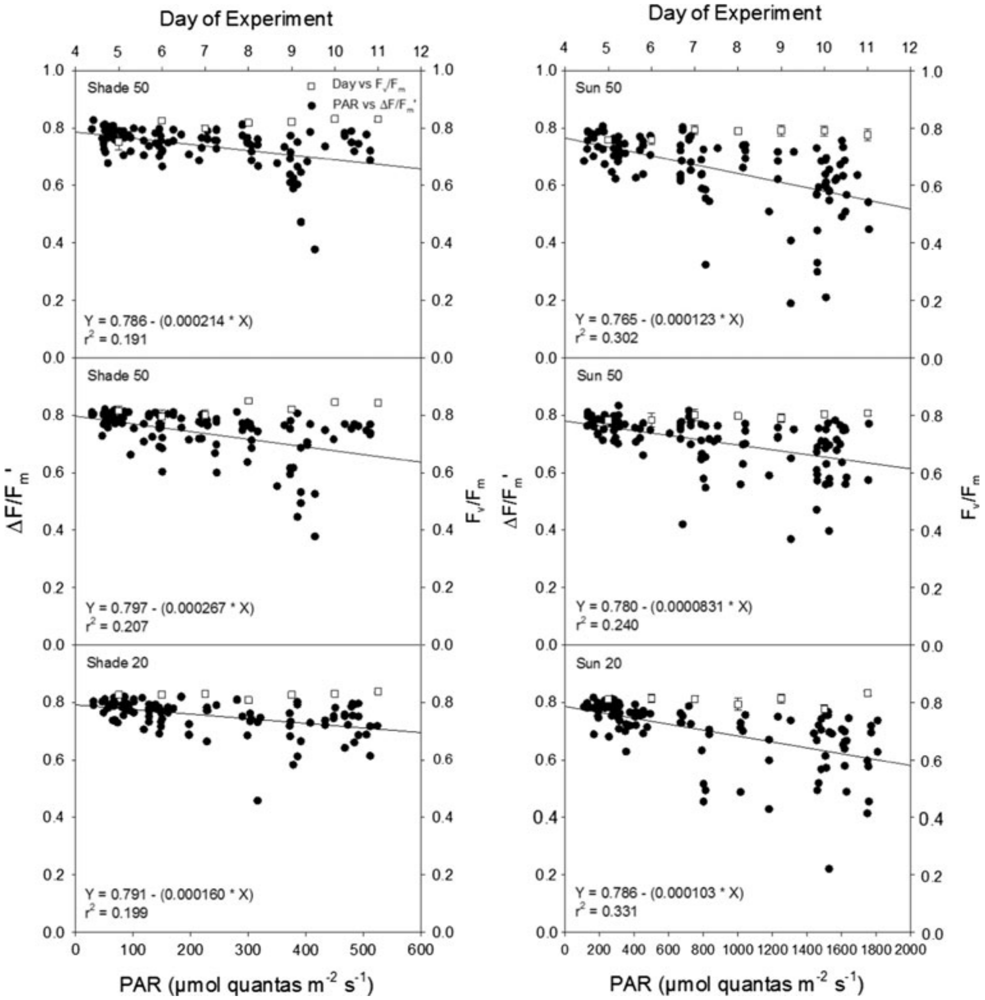

**Fig. 2** Regressions of effective quantum yield ($\Delta F/F_m'$) versus photosynthetically active radiation (PAR) over the course of the 7-day changing period. White squares represent mean (±SE) dark-acclimated maximum quantum yield ($F_v/F_m$) measurements for each day of the changing period. Each graph represents a light and salinity treatment combination. Note differing x-axis scales between the Shade and Sun treatment graphs ($n = 108$)

efficiencies (at 2100 hours) using Mann–Whitney *U* test. Normality of data was tested using the Shapiro–Wilk Goodness of Fit test. When data failed this test, the non-parametric Kruskal–Wallis and Wilcoxon signed rank tests were performed on individual treatment variability. All values are presented as mean ± standard error.

## Results

### Chlorophyll fluorescence

Figures 1, 2, 3, and 4 include data from all days pooled for each experimental period (pre-treatment, changing, target, and recovery). Date (i.e., day of experiment) was a significant source of variation within all four experimental periods for PAR (pre-treatment $H = 48.0$, $df = 4$, $P < 0.001$, changing $H = 48.4$, $df = 6$, $P < 0.001$, target $H = 45.6$, $df = 8$, $P < 0.001$, and recovery $H = 56.3$, $df = 2$, $P < 0.001$), and $\Delta F/F_m'$ (pre-treatment $H = 13.5$, $df = 4$, $P < 0.001$, changing $H = 67.0$, $df = 6$, $P < 0.001$, target $H = 18.9$, $df = 8$, $P < 0.05$, and recovery $H = 36.5$, $df = 2$, $P < 0.001$). There was no significant variation among aquaria for $\Delta F/F_m'$ or PAR during pre-treatment (Table 1). $\Delta F/F_m'$ did significantly vary by time of day, which reflected diurnal variation in irradiance (Table 1). During low irradiances of the evening and morning, $\Delta F/F_m'$ values were the highest, but not significantly different from each other; the afternoon $\Delta F/F_m'$ values were significantly lower with the noon $\Delta F/F_m'$ values being the lowest (when PAR > 2,000 μmol quanta $m^{-2}$ $s^{-1}$; $H = 13.5$, $df = 4$, $P < 0.001$). ANCOVA calculated for the $\Delta F/F_m'$ versus PAR regressions during pre-treatment (shown in Fig. 1) also indicated that both the 6 slopes ($F_s = 1.23$, $df = 5$, 446, $P > 0.05$) and 6 intercepts ($F_s = 1.77$, $df = 5$, 446, $P > 0.05$) were homogeneous.

There was a significant salinity effect during the changing period, but only for daily mean $\Delta F/F_m'$ (Table 1). The salinity 20 and 35 seedlings were not significantly different from each other, but their $\Delta F/F_m'$ values were significantly higher than the salinity 50 seedlings. There was also a significant light effect during the changing period for PAR and $\Delta F/F_m'$ (Table 1). Within each salinity treatment, the Shade treatments had significantly higher $\Delta F/F_m'$ ($H = 65.5$,

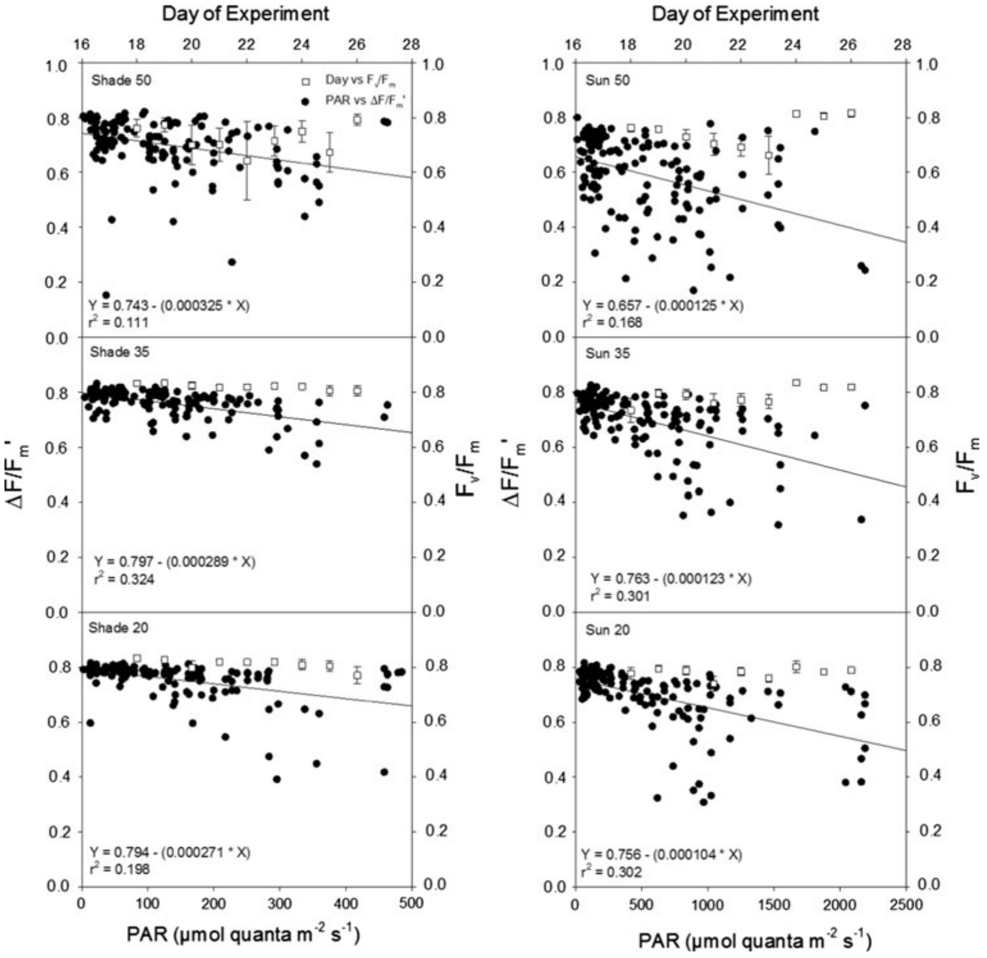

**Fig. 3** Regressions of effective quantum yield ($\Delta F/F_m'$) versus photosynthetically active radiation during 9 days over the course of the 17-day target period. White squares represent mean (±SE) dark-acclimated maximum quantum yield ($F_v/F_m$) measurements for each day of the target period. Each graph represents a light and salinity treatment combination. Note differing x-axis scales between the Shade and Sun treatment graphs ($n = 140$)

$df = 1$, $P < 0.001$), but significantly lower PAR values ($H = 245.0$, $df = 1$, $P < 0.001$; Fig. 2) than the Sun treatments. This same pattern was present during the target period ($H = 229.9$, $df = 1$, $P < 0.001$ for PAR and $H = 124.1$, $df = 1$, $P < 0.001$ for $\Delta F/F_m'$; Fig. 3).

Salinity also had an effect on $\Delta F/F_m'$ during the target period ($H = 104.4$, $df = 2$, $P < 0.001$). The salinity 20 and 35 seedlings had similar $\Delta F/F_m'$ values that were both significantly higher than those of the salinity 50 seedlings (Fig. 3). There were no significant differences among salinities for PAR values during the target period (Table 1).

During recovery, no significant residual salinity treatment effect was detected for any of the parameters (Table 1; Fig. 4). However, there was a significant residual light treatment effect, but only for $\Delta F/F_m'$ values, which were significantly higher for the Sun treatments compared to the Shade treatments ($H = 8.0$, $df = 1$, $P < 0.05$; Fig. 4).

Date was not a significant source of variation for the $\Delta F/F_m'$ versus PAR regression y-intercept and slope, or $F_v/F_m$ within any of the experimental periods, except changing. This was expected due to the temporally changing salinities within this experimental period. For the three periods that day of experiment was not significant (pre-treatment, target, and recovery), days were pooled for statistical analysis. During pre-treatment, $F_v/F_m$, slopes, and intercepts did not vary significantly among aquaria ($P > 0.05$; Table 2). Once shades were placed on the aquaria and salinities were changing, significant differences were detected. During the changing period, day of experiment and salinity were not significant for $F_v/F_m$, but Shade seedlings had significantly higher $F_v/F_m$ values than Sun seedlings ($H = 15.7$, $df = 1$, $P < 0.001$; Table 2). There was also a significant salinity and light interaction for $F_v/F_m$ ($H = 21.5$, $df = 3$, $P < 0.05$); Sun 50 seedlings had significantly lower $F_v/F_m$ values than seedlings in the other two Sun treatments (Table 2).

Regression slopes were not significantly different among salinity treatments within each of the four experimental periods throughout the experiment (Table 2). However, there was a significant light treatment effect during changing ($H = 7.4$, $df = 1$, $P < 0.05$) and target ($H = 10.6$, $df = 1$, $P < 0.05$) periods; Shade seedlings had significantly steeper slopes than Sun seedlings (Table 2). Day of experiment was a significant source of variation for slope values only during the changing period ($H = 18.4$, $df = 5$, $P < 0.05$).

During the target period, a significant light effect was detected for $F_v/F_m$ ($H = 4.0$, $df = 1$, $P \leq 0.05$), slope

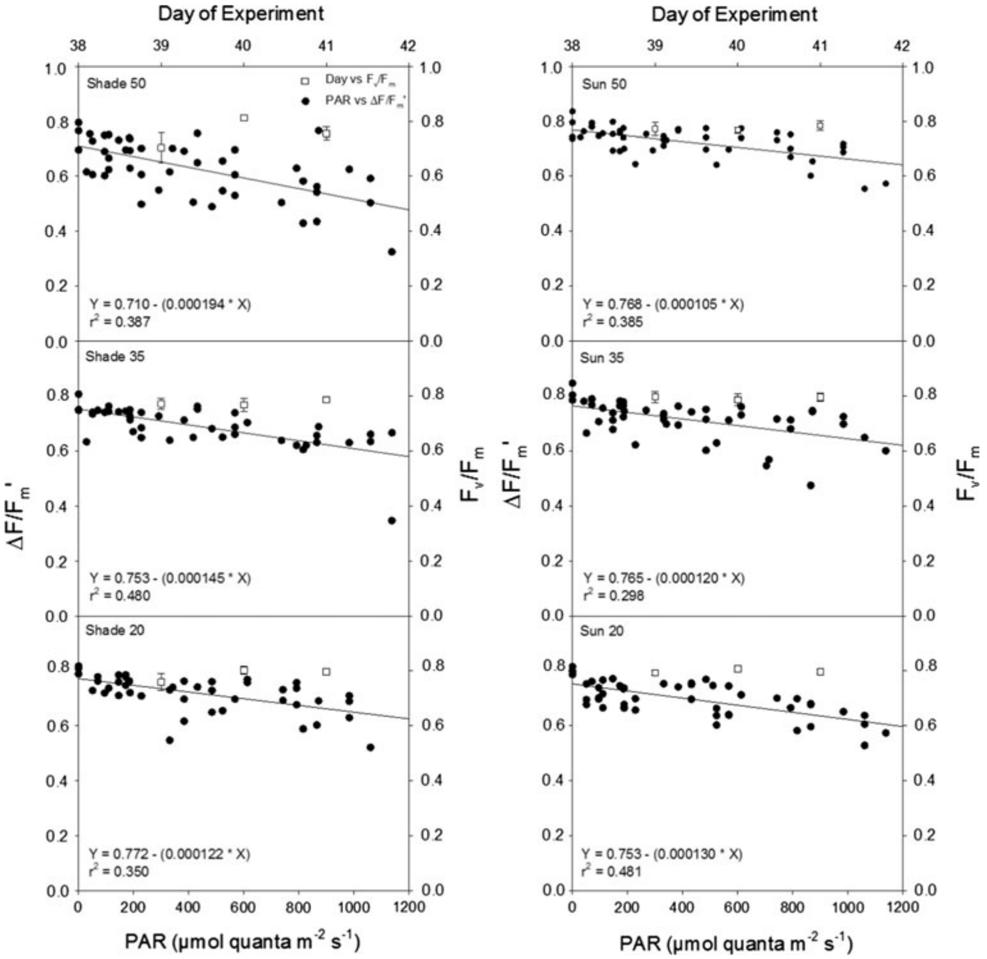

**Fig. 4** Regressions of effective quantum yield ($\Delta F/F_m{}'$) versus photosynthetically active radiation during the last 3 days of the 6-day recovery period. White squares represent mean (±SE) dark-acclimated maximum quantum yield ($F_v/F_m$) measurements for each day of the recovery period. Each graph represents a light and salinity treatment combination ($n = 48$)

($H = 10.6$, $df = 1$, $P < 0.01$), and y-intercept ($H = 8.2$, $df = 1$, $P < 0.01$). The Shade seedlings had significantly higher values than the Sun seedlings for all three of these parameters (Table 2). There was also a significant light and salinity interaction effect. The slopes of the Shade 20 seedlings were not significantly different from either the Shade 35 or 50 seedlings or the seedlings exposed to the three Sun salinity treatments (Table 2).

A significant salinity effect was detected for $F_v/F_m$ ($H = 37.0$, $df = 2$, $P < 0.001$) and y-intercept ($H = 22.7$, $df = 2$, $P < 0.001$) during the target period. Seedlings exposed to salinity 50 had significantly lower $F_v/F_m$ and regression intercepts than the salinity 20 and 35 treatments (Table 2). The interaction between light and salinity was also significant for $F_v/F_m$ ($H = 99.7$, $df = 3$, $P < 0.001$) and y-intercept ($H = 31.0$, $df = 3$, $P < 0.001$) during this period. Both parameters showed similar patterns with salinity 50 seedlings having the lowest values; the y-intercepts for the Sun 50 seedlings were also significantly lower than those for the Shade 50 seedlings (Table 2). During recovery, after the salinities had been returned to the control level (35) and shading was removed, neither the previous salinity nor light treatments had a significant effect on $F_v/F_m$, slope, or intercept, indicating full recovery (Table 2).

Y-intercepts from the $\Delta F/F_m{}'$ versus PAR regressions and measured $F_v/F_m$ values for the Sun 20 and 35 seedlings were not significantly different from each other within each of the four time periods (Table 2). In contrast, $F_v/F_m$ values were significantly higher than their y-intercept values in the Shade 20 and 50 treatments during the changing period, as well as in the Shade 35 and Sun 50 during the target period (Mann–Whitney or Student's *t* tests, $P < 0.05$ for all comparisons; Table 2). $F_v/F_m$ values were also significantly higher than y-intercept values during the changing period for the Shade 20 ($t = -6.4$, $P < 0.01$) and Shade 50 seedlings ($U = 8.0$, $P < 0.05$; Table 2). $F_v/F_m$ and y-intercepts of the Shade 35 seedlings were significantly different only during the target period ($U = 12.0$, $P < 0.05$; Table 2).

## Osmolality

Leaf tissue was consistently hyperosmotic compared to the media. Leaf osmolality did not vary significantly between the light treatments during target and recovery periods, so

**Table 1** Results from the Kruskal–Wallis tests comparing variation in $\Delta F/F_m'$ and PAR among experimental treatments during the four experimental periods

| Experimental period | Parameter | Factor | *n* | *df* | *H*-stat | *P* value |
|---|---|---|---|---|---|---|
| Pre-treatment | $\Delta F/F_m'$ | Light | 4 | 1 | 0.234 | 0.629 |
| | | Salinity | 4 | 2 | 4.927 | 0.085 |
| | | Time of day | 4 | 3 | 170.417 | **<0.001** |
| | PAR | Light | 4 | 1 | 0.005 | 0.942 |
| | | Salinity | 4 | 2 | 0.034 | 0.983 |
| | | Time of day | 4 | 3 | 312.940 | **<0.001** |
| Changing | $\Delta F/F_m'$ | Light | 4 | 1 | 65.466 | **<0.001** |
| | | Salinity | 4 | 2 | 14.845 | **0.001** |
| | | Time of day | 4 | 3 | 203.110 | **<0.001** |
| | PAR | Light | 4 | 1 | 245.017 | **<0.001** |
| | | Salinity | 4 | 2 | 0.183 | 0.912 |
| | | Time of day | 4 | 3 | 260.426 | **<0.001** |
| Target | $\Delta F/F_m'$ | Light | 4 | 1 | 124.055 | **<0.001** |
| | | Salinity | 4 | 2 | 104.380 | **<0.001** |
| | | Time of day | 4 | 3 | 183.516 | **<0.001** |
| | PAR | Light | 4 | 1 | 229.908 | **<0.001** |
| | | Salinity | 4 | 2 | 0.097 | 0.952 |
| | | Time of day | 4 | 3 | 413.556 | **<0.001** |
| Recovery | $\Delta F/F_m'$ | Light | 4 | 1 | 7.990 | **0.005** |
| | | Salinity | 4 | 2 | 2.641 | 0.267 |
| | | Time of day | 4 | 3 | 36.525 | **<0.001** |
| | PAR | Light | 4 | 1 | 0.0000 | 0.998 |
| | | Salinity | 4 | 2 | 0.086 | 0.958 |
| | | Time of day | 4 | 3 | 192.916 | **<0.001** |

*P* values in bold are significant ($\alpha = 0.05$)

these data were pooled. During the target period, osmolality of the salinity 35 and 50 seedlings did not differ significantly, but both were significantly higher than the salinity 20 seedlings ($F_{2,\ 15} = 28.0$, $P < 0.01$; Fig. 5). Differences between leaf and media osmolality in salinity 35 and 20 seedlings were very similar, maintaining a mean Δ value of 630.8 ± 40.2 mmol $\text{kg}^{-1}$. The leaf tissue and media Δ osmolality for both salinity 20 and 35 seedlings were significantly higher than the Δ value for the salinity 50 seedlings (152.0 ± 26.4 mmol $\text{kg}^{-1}$, $F_{2,\ 15} = 27.6$, $P < 0.001$; Fig. 5).

Leaf osmolality at the conclusion of the recovery period, when all media were at salinity 35, was higher for the salinity 20 seedlings than during the target period, due to the increase in media salinity. However, the salinity 20 osmolality values were still significantly lower than those for the salinity 35 and 50 seedlings ($F_{2,\ 15} = 5.3$, $P < 0.05$; Fig. 5), which were not significantly different from each other. Leaf tissue and media Δ osmolality values were also significantly lower in the salinity 20 seedlings (408.3 ± 30.8 mmol $\text{kg}^{-1}$) compared to those in the salinity 35 and 50 treatments (630.0 ± 44.0 mmol $\text{kg}^{-1}$, $F_{2,\ 15} = 5.3$, $P < 0.05$), which were again not significantly different from each other (Fig. 5).

## Morphology

Final leaf measurements were significantly higher than initial leaf measurements for most treatments, indicating net growth (Table 3). Seedlings in the salinity 20 and 35 treatments showed significant increases in leaf width ($H = 57.0$, $df = 1$, $P < 0.001$, and $H = 52.8$, $df = 1$, $P < 0.001$, respectively) and total shoot area ($F_{1,\ 54} = 16.9$, $P < 0.001$, and $F_{1,\ 54} = 16.8$, $P < 0.001$, respectively); salinity 35 seedlings also had a significant increase in leaf length ($H = 14.0$, $df = 1$, $P < 0.001$). Salinity 50 seedlings were the only seedlings to show significant decreases in leaf morphology, with total shoot area and number of leaves per seedling being significantly lower than their initial measurements ($F_{1,\ 54} = 4.5$, $P < 0.05$, and $H = 7.1$, $df = 1$, $P < 0.01$, respectively). Final leaf measurements did not vary significantly between light treatments so these data were pooled. Final leaf length, leaf width, and total shoot area were significantly different among salinity treatments ($F_{2,\ 73} = 5.0$, $P < 0.01$, $H = 32.6$, $df = 2$, $P < 0.001$, and $H = 9.9$, $df = 2$, $P < 0.01$, respectively; Table 3). Salinity 20 and 35 seedlings did not vary significantly from each other, but values for these two treatments were significantly higher than values for the salinity 50 seedlings. Final seedling leaf numbers did not vary significantly among treatments.

## Tissue dry weights

Dry weights of tissues (leaves, roots, and seeds) were not significantly affected by salinity treatments, so these data were pooled. Light was also not a significant factor for leaf (0.032 ± 0.004 vs. 0.030 ± 0.005 g dwt for Shade vs. Sun, respectively) and root (0.023 ± 0.005 vs. 0.090 ± 0.072 g dwt for Shade vs. Sun, respectively) tissue. However, a significant light effect was detected for seeds, with Shade treatment seedlings having significantly lighter seeds (0.101 ± 0.010 g dwt) than Sun treatment seedlings (0.132 ± 0.009 g dwt) at the conclusion of the experiment ($H = 3.9$, $df = 1$, $P < 0.05$).

**Table 2** Mean (±SE) slopes and y-intercepts of $\Delta F/F_m'$ versus PAR regressions and $F_v/F_m$ values measured at 2100 hours with days in each experimental period pooled for each light and salinity treatment combination

| Period | Parameter | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | Shade 20 | Shade 35 | Shade 50 | Sun 20 | Sun 35 | Sun 50 |
| Pre-treatment | Slope | 0.00012<br>(2.61E-05) | 0.00013<br>(2.46E-05) | 0.00016<br>(1.16E-05) | 0.00011<br>(1.09E-05) | 0.00015<br>(2.01E-05) | 0.00013<br>(2.48E-05) |
| | y-intercept | 0.765<br>(0.0256) | 0.784<br>(0.027) | 0.792<br>(0.008) | 0.767<br>(0.009) | 0.800<br>(0.024) | 0.7500<br>(0.017) |
| | $F_v/F_m$ | 0.767<br>(0.004) | 0.798<br>(0.003) | 0.764<br>(0.006) | 0.796<br>(0.010) | 0.789<br>(0.007) | 0.7858<br>(0.006) |
| Changing | Slope | 0.00016 A<br>(2.75E-05) | 0.00025 A<br>(7.39E-05) | 0.00020 A<br>(6.60E-05) | 0.00010 B<br>(1.75E-05) | 0.00007 B<br>(2.04E-05) | 0.00012 B<br>(1.66E-05) |
| | y-intercept | 0.790<br>(0.005) | 0.791<br>(0.012) | 0.782<br>(0.009) | 0.783<br>(0.014) | 0.772<br>(0.018) | 0.761<br>(0.012) |
| | $F_v/F_m$ | 0.827 A*<br>(0.004) | 0.825 A<br>(0.008) | 0.8111 A*<br>(0.011) | 0.807 A<br>(0.007) | 0.795 B<br>(0.004) | 0.780 C<br>(0.006) |
| Target | Slope | 0.00026 AB<br>(8.88E-05) | 0.00030 A<br>(4.49E-05) | 0.00032 A<br>(9.39E-05) | 0.00014 B<br>(2.77E-05) | 0.00014 B<br>(2.02E-05) | 0.00016 B<br>(3.05E-05) |
| | y-intercept | 0.795 A<br>(0.011) | 0.798 A<br>(0.007) | 0.744 C<br>(0.014) | 0.769 B<br>(0.010) | 0.772 B<br>(0.010) | 0.667 D<br>(0.014) |
| | $F_v/F_m$ | 0.811 A<br>(0.006) | 0.819 A*<br>(0.003) | 0.723 C<br>(0.016) | 0.780 B<br>(0.006) | 0.787 B<br>(0.011) | 0.749 C*<br>(0.019) |
| Recovery | Slope | 0.00014<br>(2.29E-05) | 0.00015<br>(7.23E-06) | 0.00022<br>(4.17E-05) | 0.00013<br>(1.13E-05) | 0.00012<br>(4.70E-05) | 0.00010<br>(1.03E-05) |
| | y-intercept | 0.774<br>(0.017) | 0.753<br>(0.012) | 0.711<br>(0.010) | 0.753<br>(0.010) | 0.756<br>(0.023) | 0.768<br>(0.002) |
| | $F_v/F_m$ | 0.786<br>(0.014) | 0.775<br>(0.006) | 0.757<br>(0.031) | 0.799<br>(0.004) | 0.793<br>(0.003) | 0.775<br>(0.005) |

Letters represent values of treatment combinations that are significantly different from each other, and asterisks indicate the treatments where y-intercepts and maximum quantum yields were significantly different from each other

## Discussion

### Chlorophyll fluorescence

Slopes from diurnal $\Delta F/F_m'$ versus PAR regressions significantly increased in response to the reduced light treatments in this mesocosm study, suggesting a change in photoacclimation in our *T. testudinum* seedlings. During the target period, the light effect was reduced in the salinity 20 treatment, which had slopes not significantly higher than any of the Sun treatments. These results support the suggestion by Durako (2012) that slopes of diurnal $\Delta F/F_m'$ versus PAR regressions are responsive to changes in physiological status of *T. testudinum*. In addition, despite the diurnal regression y-intercepts and measured $F_v/F_m$ values being significantly different from each other in some of the more stressful treatments (Shade 20 and 50), both of these parameters responded similarly to light and salinity, increasing with reduced light and decreasing under hypersaline conditions. This suggests that diurnal regression y-intercepts may be used as proxies for $F_v/F_m$ for *T. testudinum* when maximum photochemical efficiencies cannot be directly measured.

Diurnal variability in effective photochemical efficiency was observed over the course of this mesocosm experiment and has previously been observed in field studies of seagrasses (Durako and Kunzelman 2002; Ralph and Gademann 2005; Belshe et al. 2007), corals (Brown et al. 1999), and phytoplankton (Kurzbaum et al. 2010). Observations from this mesocosm study and previous field studies suggest that *T. testudinum* exhibits relatively rapid (hours to days) photoacclimatory responses to changes in available irradiance (Ralph et al. 1998; Major and Dunton 2002; Belshe et al. 2007). While PAR was relatively low in the mornings and afternoons, on cloudy days, and in the shaded treatments, effective photochemical efficiency was high during those times, indicating higher efficiency of light use in photosynthesis. These results are consistent with in situ observations, which have also shown effective photochemical efficiency to increase with reduced light (Beer et al. 2001; Durako and Kunzelman 2002; Silva and

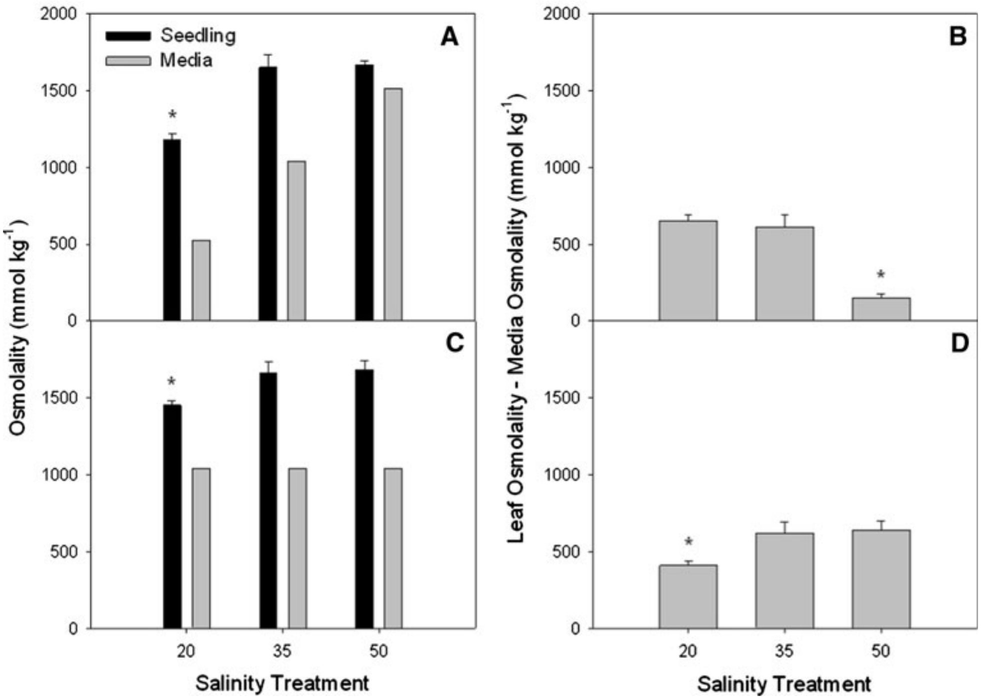

**Fig. 5** **a** Osmolality of seedling leaves and media, for all three salinity treatments at target salinities. **b** Difference between leaf osmolality and media osmolality, for all three salinity treatments at target salinities. **c** Osmolality of seedling leaves and media, for all three salinity treatments at the conclusion of the recovery period. **d** Difference between leaf osmolality and media osmolality, for all three salinity treatments at the conclusion of the recovery period. All values presented as mean ± SE. *Asterisks* represent salinity treatments that are significantly different ($n = 6$)

**Table 3** Mean (±SE) initial and final leaf length, leaf width, leaf number per shoot, and total leaf area per shoot for all three salinity treatments

| Time | Parameter | Treatment | | |
|---|---|---|---|---|
| | | 20 | 35 | 50 |
| Initial | Leaf length | 2.08 | 1.97 | 2.01 |
| | | (0.06) | (0.06) | (0.06) |
| | Leaf width | 0.33 | 0.34 | 0.34 |
| | | (0.003) | (0.004) | (0.004) |
| | Total leaf area | 2.61 | 2.53 | 2.59 |
| | | (0.11) | (0.12) | (0.14) |
| | Leaf number | 3.71 | 3.75 | 3.79 |
| | | (0.10) | (0.12) | (0.14) |
| Final | Leaf length | 2.34 A | 2.97 A* | 1.88 B |
| | | (0.21) | (0.28) | (0.19) |
| | Leaf width | 0.48 A* | 0.45 A* | 0.33 B |
| | | (0.02 | (0.01) | (0.01) |
| | Total leaf area | 4.12 A* | 4.38 A* | 1.84 B* |
| | | (0.67) | (0.86) | (0.21) |
| | Leaf number | 3.75 | 3.25 | 2.88* |
| | | (0.31) | (0.31) | (0.23) |

Letters represent final measurements that are significantly different among treatments. Asterisks represent final measurements that are significantly different from the initial measurements ($n = 6$)

Santos 2003; Ralph and Gademann 2005; Bite et al. 2007; Belshe et al. 2007).

PAM fluorescence responses to salinity indicate that $\Delta F/F_m'$ values may be valuable as stress indicators if compared at equal PAR values. In our study, $\Delta F/F_m'$ values were consistently lower in the hypersalinity (50) seedlings compared to the control (35) and hyposalinity (20) seedlings during the changing and target periods, suggesting greater sensitivity to hypersaline than hyposaline stress in *T. testudinum* seedlings. After salinities were returned to their control level, there was no significant difference in $\Delta F/F_m'$ among the salinity treatments, indicating *T. testudinum* seedlings can recover from short-term exposure to salinity stress once the stress is removed (Fernández-Torquemada and Sánchez-Lizaso 2005). In contrast, there was a persistent light effect during the recovery period. The screens were removed at the beginning of the recovery period, so PAR levels were similar between the two groups of seedlings for several days. However, $\Delta F/F_m'$ values were significantly higher in the Sun seedlings compared to the Shade seedlings. It appears that the low-light-acclimated Shade seedlings may have been more susceptible to photoinhibition or downregulation than the high-light-acclimated Sun seedlings. This could be due to reduced capacity for photoprotective responses (Demmig-Adams and Adams 1992; Levy et al. 2004), which would result in decreased effective photochemical efficiencies at high irradiances (Brown et al. 1999).

The trade-off between photosynthetic efficiency and photoprotection in sun- versus shade-acclimated plants has been previously documented using PAM fluorescence (Silva and Santos 2003), but never before using slopes and y-intercepts from diurnal $\Delta F/F_m'$ vs. PAR regressions. The significantly higher measured $F_v/F_m$ values and calculated y-intercepts from the diurnal $\Delta F/F_m'$ versus PAR regressions for the Shade seedlings compared to the Sun seedlings during the changing and target periods suggest that the Shade seedlings were low-light acclimated with increased maximum quantum efficiency, while the Sun seedlings had reduced photosynthetic efficiency (Gorbunov et al. 2001; Durako 2012). Regression slopes were also significantly steeper in the Shade treatments compared to the Sun treatments during the changing and target periods, suggesting that the Sun seedlings exhibited an increased ability to utilize higher irradiances and had higher photosynthetic capacities. In contrast, the Shade seedlings may have been more efficient at capturing light at lower irradiances, but exhibited lower efficiencies in using higher irradiances, which may indicate stress (Ralph and Gademann 2005).

Seedlings in the salinity 50 treatments had significantly lower $F_v/F_m$ and y-intercept values than the salinity 20 and 35 treatments during the target period, suggesting greater stress under hypersaline conditions. Maximum photochemical efficiencies decline in response to hypersalinity stress in seagrasses as a result of interrupted electron transport (Fernández-Torquemada and Sánchez-Lizaso 2005) and impaired photosystem function (Touchette 2007; Marín-Guirao et al. 2011). Healthy seagrasses typically have $F_v/F_m$ values between 0.73 and 0.83, with values below this range indicating physiological stress (Beer et al. 2001). The $F_v/F_m$ values and y-intercepts of both the Sun (0.74 ± 0.02 and 0.67 ± 0.01, respectively) and Shade (0.72 ± 0.02 and 0.74 ± 0.01, respectively) 50 seedlings were at or below the low end of this range and were significantly lower than the other treatments (0.77–0.82). The regression slopes and y-intercepts during the target period suggest that hypersalinity and reduced light may act synergistically to increase photosynthetic stress in the seedlings (Ralph et al. 1998).

Once the screens were removed during the recovery period, light and salinity effects were no longer detected in the $F_v/F_m$, slope or y-intercept PAM fluorescence characteristics, indicating that the seedlings had recovered, photosynthetically. Similar recovery responses have been demonstrated in *T. testudinum* adults. Both Kraemer and Hanisak (2000) and Czerny and Dunton (1995) measured growth rates and carbohydrate levels of *T. testudinum* leaves, while the shoots were shaded and then after the shading had been removed; they determined that *T. testudinum* was able to tolerate severe reductions in irradiance for intermediate durations of time. Our results support this finding, but for *T. testudinum* seedlings. Also similar to our *T. testudinum* seedlings, Fernández-Torquemada and Sánchez-Lizaso (2005) demonstrated the recovery of the seagrass *Posidonia oceanica* from hypersaline conditions once salinity was returned to control conditions.

y-intercepts from the diurnal $\Delta F/F_m'$ versus PAR regressions were hypothesized to not be significantly different from $F_v/F_m$ values. This was true over the whole course of the experiment for the Sun 20 and 35 (control) seedlings, suggesting that our hypothesis is true when seedlings are not stressed. Measured and estimated maximum photochemical efficiencies were significantly different from each other during either the changing or the target periods for the other treatment combinations. However, the response patterns to the treatments were always similar for both parameters.

## Osmolality

When the seedlings were at their target treatment salinities, osmolality of leaf tissue increased with increasing salinity, as has been previously observed (Murphy et al. 2003; Kahn and Durako 2006; Koch et al. 2007a, b). However, osmolality for the hypersalinity seedlings was not significantly different from the control salinity seedlings. This indicates that the seedlings exposed to hypersalinity did not make the necessary osmotic adjustments to maintain constant cell turgor, indicating osmotic stress (Ralph et al. 1998; Xia et al. 2004). The control and hyposalinity seedlings maintained a similar internal hyperosmolality relative to their environment (600–650 mmol kg$^{-1}$ difference). This mean Δ between tissue osmolality and media osmolality was similar to Δ values previously reported for *T. testudinum* seedlings (646 ± 108.22 mmol kg$^{-1}$) and hypothesized to be the optimal Δ osmolality between seagrass leaf tissue and media (Kahn and Durako 2006). The significantly lower Δ leaf–media osmolality for the hypersalinity treatment seedlings (152 mmol kg$^{-1}$) again suggests osmotic stress in this treatment, which was also indicated by reduced $F_v/F_m$.

Six days after salinities were returned to control levels (35), the hyposalinity treatment seedlings increased their internal osmolality along with media osmolality, but not back to those of the control seedlings. Perhaps, the osmolality of the hyposalinity seedlings would have returned to values closer to the control values if given more time, but in the 6-day recovery period used in this experiment, these seedlings did not fully re-adjust their internal osmolality. The Δ leaf–media osmolality of the hypersalinity treatment was not significantly different from the control salinity seedlings during the recovery period, but this was only due to the change in media osmolality rather than changing leaf tissue osmolality in the hypersalinity seedlings. Thus, it appears that the hypersaline treatment seedlings were still not fully able to osmoregulate during the recovery period.

Osmoregulation in *T. testudinum* is an energy-requiring process that seagrasses utilize to maintain cell turgor by producing osmolytes such as soluble carbohydrates, organic acids, and free amino acids (Jagels 1973; Touchette 2007). As a result of this process, plants may have to allocate energy for osmoregulation, sacrificing photosynthetic capacity (Xia et al. 2004). However, our seedlings still had seed material remaining that the conclusion of this experiment, so limited energy stores would not have been an issue. Osmotic stress due to hypersalinity also directly causes reduction in photosynthetic rates (Dawes et al. 1989). It is likely that ion imbalances in our hypersalinity seedlings, indicated by decreases in the leaf–media Δ osmolality, contributed to the declines in their photosynthetic efficiency (Touchette 2007).

### Morphology

Seedlings in the hypersaline treatments had significantly smaller leaves than the other two salinity treatment seedlings, at the end of the experiment. This was a result of no significant leaf elongation or widening over the course of the experiment, as well as a significant reduction in the number of leaves per shoot of these seedlings. Salinity stress often impacts plants by limiting photosynthetic rates, which may cause declines in growth rates (Parida et al. 2003). Extreme salinity conditions have been shown to result in lower biomass and growth rates of seagrasses (Fernández-Torquemada and Sánchez-Lizaso 2005; Herbert and Fourqurean 2009; Marín-Guirao et al. 2011). Specifically, Koch et al. (2007a, b) observed declining photosynthetic and growth rates in *T. testudinum* adult shoots as salinity increased, with salinities above 60 causing leaf growth rates to drop below 2 mm day$^{-1}$ (the threshold value for a normal leaf growth rate in seagrasses) and $F_v/F_m$ to drop below 0.70. The reduced growth observed in the hypersalinity seedlings in this study may reflect these seedlings' declining photosynthetic rates, as also indicated by our PAM fluorometry data.

### Tissue dry weight

Seed tissue weights of the shaded seedlings were significantly lower than the Sun seedlings suggesting that the shaded seedlings used more of their carbon stores from their seeds than the Sun seedlings. This may be a result of the light stress that lower irradiance placed on those seedlings. Seagrasses are known to respond to decreases in light by reducing their number of leaves per shoot (Ruiz and Romero 2001) and their total shoot mass (Dennison and Alberte 1985). These responses enable the plants to minimize demands for carbon and respiration while maximizing their exposure to light. Tropical seagrass species such as *T. testudinum* have relatively high minimum light requirements, and small decreases in light availability can cause significant declines in growth and photosynthetic health of these plants (Dennison et al. 1993; Ralph et al. 2007b). However, the use of carbohydrate reserves, such as those in the seeds of seedlings, can increase the tolerance of a plant to reduced light availability (Lee and Dunton 1997). This may be the reason why a significant light effect was not detected in our morphological measurements; the shaded seedlings were able to compensate for lower irradiance levels with extra stored carbon. Similar results have been observed in the seagrass *Posidonia sinuosa*, which is able to tolerate low-light availability at depths due to its ability to access stored carbohydrates (Collier et al. 2008).

Because the hypersalinity seedlings were significantly smaller at the end of the experiment than seedlings in the other two salinity treatments, we would have expected salinity to be a significant factor in controlling differences in dry weights, with the hypersaline treatment seedlings weighing significantly less than the others. However, no significant salinity differences were detected for dry tissue weights. This may have been due to the increased accumulation of osmolytes in leaf tissues of the hypersalinity seedlings, which may have increased their tissue dry weight. Increase in compatible solutes is a response of *T. testudinum* to increased salinity in order to remain hyperosmotic to its environment (Kahn and Durako 2006; Koch et al. 2007b).

## Conclusions

Our results show that diurnal $\Delta F/F_m'$ versus PAR regression slopes and y-intercepts are responsive to environmental changes and physiological status of *T. testudinum* seedlings. The data presented here provide experimental support for the conclusions of Durako (2012) that diurnal regression slopes and y-intercepts may better reflect seagrass condition than absolute effective photochemical efficiencies when measurements are taken over the large spatial and temporal scales of landscape-level assessments. Y-intercepts and measured $F_v/F_m$ also showed similar response patterns to our salinity and light treatments, during the target period of this mesocosm study. We suggest that the y-intercepts from diurnal $\Delta F/F_m'$ versus PAR regressions may serve as proxies for $F_v/F_m$ when logistics and safety prevent direct nighttime measurements of maximum photochemical efficiency values. However, the finding that regression y-intercepts were significantly different from measured $F_v/F_m$ only while stressful light or salinity treatments were in effect needs to be investigated further.

PAM fluorescence parameters indicated a significant light effect in our seedlings, suggesting a shift in photoacclimation from Sun to Shade acclimated. We suggest that no light effect was observed in morphological parameters due to the shaded seedlings having access to carbohydrate reserves in their seeds. PAM fluorescence parameters, growth, and osmolality data, all indicated that hypersalinity was more stressful than hyposalinity for *T. testudinum* seedlings. Kahn and Durako (2006) also observed declining health in seedlings placed in salinities of 50, but mortalities were only observed in salinity treatments of 60 and 70. Kahn and Durako (2006) concluded that the growth of seedlings was only affected by salinities outside a range of 20–40. Our findings concur with those of Kahn and Durako (2006) since seedlings in low salinities (20) exhibited PAM responses and growth not significantly different from seedlings in marine salinities (35). Koch et al. (2007a, b) reported that the tolerable salinity range for *T. testudinum* adults is 28–60, which suggests that adults may have a higher tolerance to hypersalinity conditions than seedlings. The salinity tolerance information presented here should be useful to managers in Southern Florida, who have already made moves to restore the Everglades and Florida Bay to its natural hydrology through the release of freshwater as part of the Comprehensive Everglades Restoration Plan (CERP 2001). Managers should be conservative and account for this narrower salinity tolerance of seedlings so that *T. testudinum* within Florida Bay may recruit sexually, rather than just clonally.

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