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δ^{13}C is a signature of light availability and photosynthesis in seagrass

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Abstract

We explored the role of light-saturated (carbon-limited) photosynthesis on δ^{13}C of turtlegrass (*Thalassia testudinum* Banks ex König) populations from the clear, blue waters of the Great Bahama Bank and the turbid, green waters of Florida Bay using field observations and radiative transfer models. Consistent with numerous previous observations, leaf δ^{13}C decreased significantly with water depth in both regions. However the δ^{13}C for Bahamas turtlegrass was 3‰ heavier than that for Florida Bay turtlegrass at equivalent depths, and broadband irradiance explained even less of the δ^{13}C variations than depth. Instead, leaf δ^{13}C showed a stronger relationship to the fraction of the day that photosynthesis of the intact plant canopy was carbon-limited. When the Bahamas and Florida Bay δ^{13}C values were related to the fraction of the day that photosynthesis was carbon-limited, the variations in leaf δ^{13}C observed for Florida and Bahamas populations collapsed into a single relationship that explained 65% of the variation in leaf δ^{13}C. Consequently, turtlegrass from the Bahamas was isotopically heavier than Florida Bay populations growing at equivalent depths because they were more carbon-limited (= light-saturated) for a larger fraction of the day. The ability to predict turtlegrass δ^{13}C from the daily period of carbon-limited photosynthesis provides a mechanistic link to fundamental relationships between light and photosynthesis that can transcend geographic differences in depth and water-column optical properties, and may permit leaf δ^{13}C to provide a robust indicator of recent photosynthetic performance and plant survival in response to changing environmental conditions.

Photoassimilatory discrimination among stable carbon isotopes (^{12}C and ^{13}C) is well-documented in numerous phototrophic systems (Smith et al. 1976; Roeske and O'Leary 1984; Raven et al. 1995). The resulting variations in carbon isotope composition among terrestrial phototrophs have been attributed to taxon-specific photosynthetic pathways (e.g., C_3, C_4, crassulacean acid metabolism or CAM) and a variety of environmental conditions including water relations, temperature, and atmospheric CO_2 concentration (O'Leary 1981). Carbon isotope fractionation in aquatic phototrophs has also been related to water motion, exposure to air, light, and the existence of carbon concentrating mechanisms that effectively utilize bicarbonate (HCO_3^-) as a source of inorganic carbon for photosynthesis (Cooper and McRoy 1988; Yoshioka 1997; Raven et al. 2002). Although reported environmental and temporal variations in δ^{13}C within taxa rarely exceed 5‰, variations among taxa can exceed 10‰. The frequent dominance of taxonomy over environment allows carbon isotopic composition to be a useful tracer of energy flow and trophic relations in many natural ecosystems (Haines 1976).

All aquatic phototrophs are depleted in δ^{13}C relative to dissolved inorganic carbon (C_i) in surface seawater (~ 1–2‰), because Ribulose Bisphosphate Carboxylase Oxygenase (Rubisco) discriminates against ^{13}C. Seagrasses (submerged marine vascular plants), however, are notably enriched in ^{13}C ($-14‰ \leq \delta^{13}C \leq -5‰$) relative to most, but not all, phototrophs ($-25‰ \leq \delta^{13}C \leq -15‰$), including many marine algae that derive much of their photosynthetic C_i from bicarbonate (HCO_3^-; Hemminga and Mateo 1996). The remarkably heavy isotope signature of seagrasses was originally thought to be indicative of C_4 or CAM physiology, but ^{14}C labeling patterns were shown to be dominated by C_3 intermediates, not malate or aspartate (Andrews and Abel 1979). Following demonstrations that seagrass photosynthesis was reliant on HCO_3^- transport (Larkum et al. 1989; Larkum and James 1996), the heavy isotopic signatures were interpreted as providing evidence for the role of HCO_3^- as a source of C_i (Raven et al. 2002).

In addition to the heavy isotopic signature relative to other C_3 marine phototrophs, many seagrasses exhibit unusually large variations in stable carbon isotope composition across time and space within taxa (Hemminga and Mateo 1996). Both field (Campbell and Fourqurean 2009) and lab (Grice et al. 1996) studies suggest that seagrass leaves become isotopically heavier with increased light availability. However, the primacy of light in controlling the variation in seagrass δ^{13}C has not been firmly established because the relationship between δ^{13}C and light availability proxies (i.e., depth, light attenuation, etc.) are variable across time and space, even within a single species, and the predictive power of such relationships is often weak ($r^2 < 0.50$; Campbell and Fourqurean 2009). Unfortunately, these proxies do not accurately account for differences in water-column optical properties, spectral shifts in irradiance and plant canopy architecture that can affect light penetration through the water column, and its absorption by seagrass leaves to drive photosynthesis. Consequently, the spatial and temporal variability in seagrass δ^{13}C values has been explained by other mechanisms, including isotopic shifts in available C_i resulting from light-induced HCO_3^- utilization, differences in water temperature, internal recycling of respiratory CO_2, remineralization of dissolved

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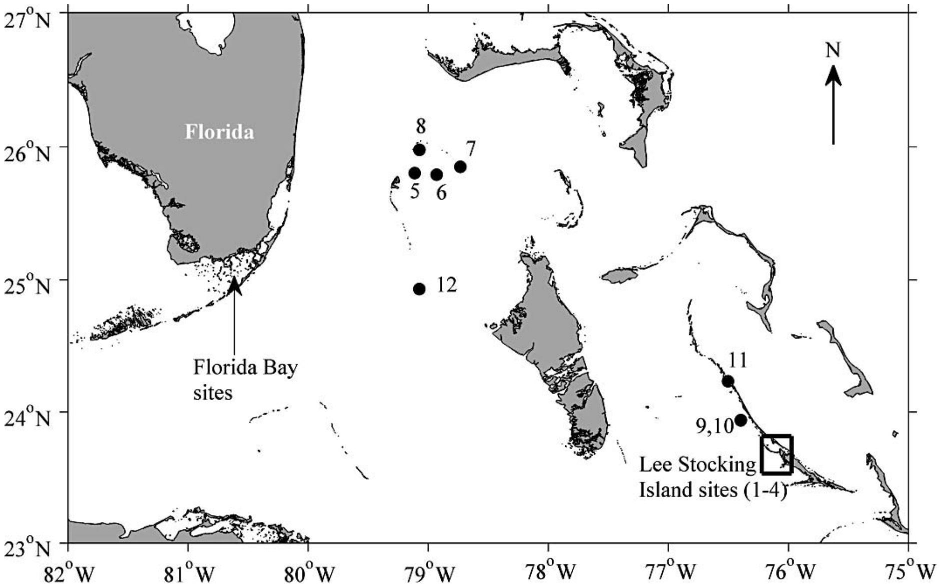

Fig. 1. Map of the Great Bahama Bank and the Florida Bay showing the general areas of seagrass populations examined in this study. Great Bahama Bank sites we examined in 2004 are shown in solid black circles. Sampling sites around Lee Stocking Island (Bahamas) and Florida Bay were reported in previous studies (Hu and Burdige 2007; Campbell and Fourqurean 2009; Burdige et al. 2010).

organic matter (DOM), and dissolution of sedimentary carbonate (Hemminga and Mateo 1996; Raven et al. 2002; Campbell and Fourqurean 2009).

Assimilatory demand for CO_2 (driven by, e.g., high light) can decrease isotopic discrimination by marine macrophytes (De Carvalho et al. 2009) with some exceptions (e.g., Marconi et al. 2011), and the observed variation in carbon isotope signature of many seagrasses may originate from photosynthetic carbon limitation in the modern ocean (Jiang et al. 2010 and references cited therein) in the high-light environments to which these plants are restricted (Duarte 1991). Here we explore the simple hypothesis that the daily period of light-saturated (and, therefore, carbon-limited) photosynthesis controls the spatial variation in isotope discrimination of turtlegrass (*Thalassia testudinum* Banks ex König) populations across the clear, blue-light–dominated waters of the Great Bahama Bank to the turbid, green-light–dominated waters of the Florida Bay.

Methods

Sampling sites—Turtlegrass shoots were collected in the summer of 2003 near Lee Stocking Island (Exumas Archipelago) and in spring of 2004 from meadows scattered across the Great Bahama Bank from the Bimini Islands through the Exumas Archipelago (Fig. 1). Water temperatures were 26°C to 29°C (mean = 27.5°C ± 1.7°C) in summer 2003 and 22°C to 24°C (mean = 23.7°C ± 1.0°C) in spring 2004. Turtlegrass was the predominant seagrass at most of the sites, with varying densities of manateegrass (*Syringodium filiforme*) and shoalgrass (*Halodule beaudetti* syn *H. wrightii*) also present (Table 1).

Water samples were collected 10 cm to 20 cm above the seagrass canopy at the time of seagrass collection and analyzed for C_i concentration using a coulometer (Dickson et al. 2007) with an uncertainty of $< 2\%$ (Hu and Burdige 2007). The $\delta^{13}C$ of the C_i was determined by gas-chromatography isotope ratio mass spectrometry with an uncertainty of $\sim 0.2‰$ (Hu and Burdige 2007).

Seagrass meadow characterization—Shoot density was determined at each site by direct counts of all shoots within 20 randomly located 0.05-m^2 quadrats. One shoot was collected from each quadrat for detailed measurement of shoot morphology. Lengths of all leaves on each shoot were measured to the nearest mm with a plastic meter tape. Leaf widths were measured to the nearest 0.01 mm using a digital caliper. Shoot-specific leaf area (L_s) was calculated as the sum of the one-sided area (length × width) of all leaves on each shoot. Shoots were separated from the root and rhizome tissues and frozen at $-20°C$ for later stable carbon isotope analysis.

Seagrass $\delta^{13}C$ analysis—Several thawed shoots from each station, containing 2–3 leaves each, were soaked in 1 mol L^{-1} HCl at room temperature for 30 min to dissolve attached carbonate, and then rinsed with deionized water to remove excess acid and salt. Shoots were pooled within each station to average across variations in $\delta^{13}C$ resulting from differences in leaf age or position along the leaf axis, oven-dried at 60°C overnight, and ground to a powder. Preliminary analysis of leaf samples revealed no significant effects of acid treatment or oven drying on $\delta^{13}C$ values. The powdered leaf samples were combusted at high temperature in O_2 using an automated nitrogen and carbon elemental

Table 1. Station depths and turtlegrass morphologies at the Great Bahamas Bank sites used to parameterize the *GrassLight* model of radiative transfer and photosynthesis of submerged aquatic vegetation. Definition of acronyms: TOC = top of canopy, LAI = leaf area index. Stations are shown in Fig. 1 and described in more detail in previous studies (Hu and Burdige 2007; Burdige et al. 2010).

Variables	Station											
	1	2	3	4	5	6	7	8	9	10	11	12
Bottom depth (m)	8.40	3.90	3.00	2.10	5.40	6.00	10.50	9.50	5.60	5.30	4.20	6.70
Canopy height (m)	0.31	0.25	0.13	0.21	0.36	0.13	0.35	0.25	0.15	0.20	0.23	0.18
TOC depth (m)	8.09	3.65	2.87	1.89	5.04	5.87	10.15	9.25	5.45	5.10	3.97	6.52
Leaf area per shoot (m^2)	0.00255	0.00273	0.00132	0.00246	0.00538	0.00293	0.00485	0.00337	0.00094	0.00270	0.00264	0.00326
Shoot density (shoots m^{-2})	588	514	248	301	277	327	96	664	101	727	281	332
Turtlegrass LAI	1.50	1.40	0.33	0.74	1.22	0.96	0.47	2.24	0.10	1.96	0.74	1.08

analyzer attached to an isotope ratio mass spectrometer. All samples were analyzed in triplicate, δ^{13}C values were calculated relative to the PeeDee belemnite (PDB) standard, and analytical uncertainty (1σ) was 0.2‰ (Hu 2007).

Florida Bay turtlegrass δ^{13}*C*—Turtlegrass δ^{13}C vs. depth data for Florida Bay sites were taken from the upper middle panel of fig. 6 in Campbell and Fourqurean (2009). Because we used their globally averaged shoot density (400 shoots m^{-2}) and shoot size (0.007 m^2 shoot^{-1}), multiple δ^{13}C estimates were averaged to produce a mean value for each depth. Consequently, the number of observations was reduced from > 74 in the original figure to 31 used in the comparative analysis presented here.

Optical properties of the Bahamas Bank and Florida Bay waters—The incident spectral irradiance (in water) at the sea surface was computed using the radiative transfer model *Hydrolight* Ver 5.0 (Mobley 1989) for local noon on the spring equinox at 24°N (Fig. 2A). The downwelling spectral irradiance propagated to the top of each turtlegrass canopy was calculated from the surface irradiance according to the Lambert–Beer Law using average vertical attenuation coefficient for downwelling irradiance [$K_d(\lambda)$] calculated from spectral irradiances measured using the diver-operated benthic biooptical spectroradiometer, a radiometrically calibrated spectroradiometer system (Hydro-Optics, Biology & Instrumentation Laboratories HydroRAD-3) mounted on a diver-operated portable frame system (Zimmerman 2003; Fig. 2B). Broadband irradiance (i.e., photosynthetically active radiation or PAR) at local solar noon (E_{PAR}) was calculated by integrating the spectral downwelling irradiance [$E_d(\lambda)$] over the visible portion of the spectrum $E_{PAR} = \int_{\lambda=400\text{ nm}}^{700\text{ nm}} E_d(\lambda)$. The irradiance absorbed by the submerged seagrass canopy, and the resulting photosynthesis, were calculated for each station (Bahamas) and depth class (Florida) using *GrassLight* Ver 1.56, a two-flow radiative transfer model of submarine irradiance distribution and photosynthesis for submerged aquatic vegetation (Zimmerman 2003). Water depth (to the seafloor), canopy height above the sea floor, shoot leaf area, and shoot density measured at each station (Table 1) were used to parameterize the model, which integrated the physiological performance of individual leaves into a population-level (i.e., whole canopy) response. Two Bahamas stations (Sites 5 and 7; Table 1) used to analyze the relationships between δ^{13}C, depth, and E_{PAR} were composed of mixed species (e.g., turtlegrass and manateegrass). They were excluded from the model calculations of δ^{13}C vs. carbon-limited photosynthesis because the architectural assumptions of the *GrassLight* model are currently unable to accommodate mixed-species assemblages. *GrassLight* calculations performed for turtlegrass from Florida Bay assumed a constant density of 400 shoots m^{-2} and a mean leaf area (= plant size) of 0.007 m^2 shoot^{-1} for all observations (Campbell and Fourqurean 2009), rather than tuning them individually for each population.

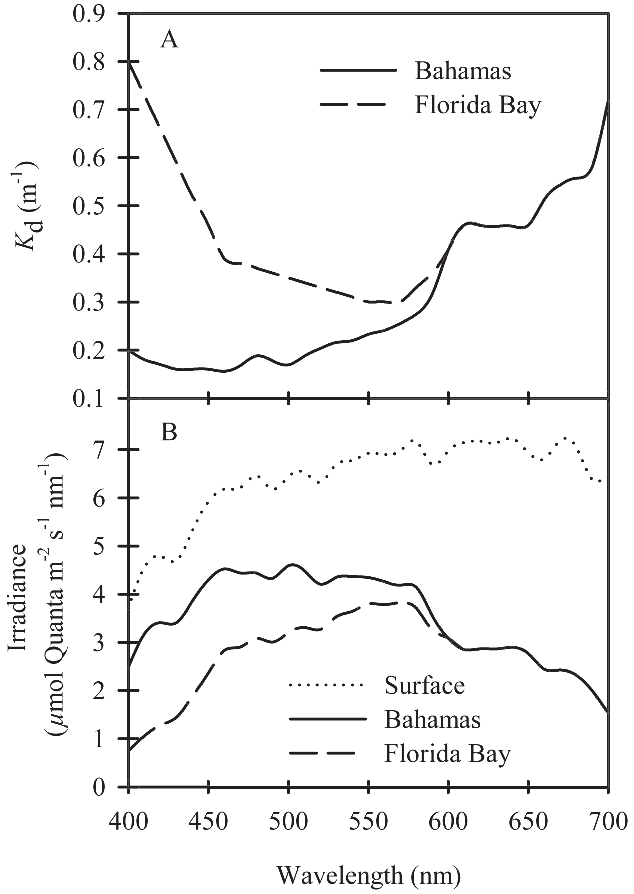

Fig. 2. (A) Mean diffuse attenuation coefficients for the Bahamas (solid line) and Florida Bay (dashed line) used to propagate in-water surface irradiance to the top of the turtlegrass canopies at different depths. (B) Spectral noon irradiance calculated by the radiative transfer model Hydrolight® for 24°N at the equinox for in water surface irradiance (dotted line), and 2 m below the surface for the Bahamas (solid line) and Florida Bay (dashed line).

Statistical analyses—Functional relationships between leaf δ^{13}C and potential driving variables (depth, E_{PAR}, and daily period of carbon-limited photosynthesis) were calculated using linear regression. Residuals of each regression analysis were subjected to one-sample Kolmogorov–Smirnov tests and found to conform to a standard normal distribution. Consequently, statistical significances of all regression coefficients were determined by regression ANOVA. Differences between the Bahamas and Florida Bay data sets were evaluated using Analysis of Covariance (ANCOVA).

Relationships between E_{PAR} and carbon-limited photosynthesis were fit to the exponential function (Webb et al. 1974) of

$$P_{\mathrm{C-lim}} = 1 - \exp\left(-\frac{E_{\mathrm{PAR}}}{E_{\mathrm{k}}}\right) \qquad (1)$$

using an iterative nonlinear regression procedure for Florida Bay and Bahamas populations separately. $P_{\mathrm{C\text{-}lim}}$ represents the daily period of carbon-limited photosynthesis (fraction of the day) and E_{k} represents the irradiance required to maximize $P_{\mathrm{C\text{-}lim}}$. Parameter uncertainties for this model were determined according to Zimmerman et al. (1987). Statistical significance of the difference in E_{k} between the two populations was determined using Student's *t*-test.

Results

The blue, oligotrophic waters of the Great Bahama Bank contain low concentrations of suspended or dissolved optically active materials, and light attenuation was dominated by the optical properties of the water itself (Fig. 2A). In contrast, the relatively high concentrations of colored DOM, phytoplankton, detritus, and sediment in Florida Bay (Mcpherson et al. 2011) resulted in higher mean values of $K_{\mathrm{d}}(\lambda)$ between 400 nm and 600 nm (Fig. 2A) relative to the Great Bahama Bank. This resulted in considerable differences in irradiances propagated to equivalent water depths between the two regions, particularly in the blue portion of the photosynthetically active spectrum (Fig. 2B).

δ^{13}C of turtlegrass leaves collected across the Great Bahama Bank ranged from −10.5‰ to −4.5‰ (mean = −6.3‰). The heaviest values were roughly 2‰ higher than those reported in the literature for turtlegrass growing in other locations (Hemminga and Mateo 1996; Fourqurean et al. 2005; Campbell and Fourqurean 2009). Consistent with numerous previous observations, water depth explained a statistically significant 56% of the variation in leaf δ^{13}C among Bahamas turtlegrass (Fig. 3A; regression ANOVA $F_{1,10} = 12.42$, $p = 0.006$, $r^2 = 0.56$). Additionally, the slope (-0.60 ± 0.15) of the δ^{13}C vs. depth relationship was statistically identical to the slope (-0.44 ± 0.06) for Florida Bay turtlegrass calculated from the data of (Campbell and Fourqurean 2009; Homogeneity of Slopes ANCOVA $F_{\mathrm{Location} \times \mathrm{Depth},1,19} = 1.19$, $p = 0.28$). However the δ^{13}C intercept at zero water depth for the Bahamas turtlegrass was 3‰ heavier than that for Florida Bay turtlegrass (Homogeneity of Slopes ANCOVA $F_{\mathrm{Location},1,39} = 9.58$, $p = 0.004$).

Water-column δ^{13}C values of $\mathrm{C_i}$ collected within the Bahamas turtlegrass meadows ranged from 1.0‰ to 2.2‰ (Burdige et al. 2010), and were similar to other values observed on the Great Bahama Bank (Patterson and Walter 1994; Swart et al. 2009), and in a tidal channel at the north end of Lee Stocking Island (Dill 1991). Further, the relatively small variation in δ^{13}C values of water-column $\mathrm{C_i}$ was not significantly correlated with either water depth or seagrass δ^{13}C values (Table 2). Thus, variation in the isotopic composition of source-water $\mathrm{C_i}$ could not explain the observed variation in turtlegrass leaf δ^{13}C, or the effect of water depth on turtlegrass leaf δ^{13}C across the Great Bahama Bank.

The relationship between turtlegrass δ^{13}C and E_{PAR} delivered to the top of the Bahamas turtlegrass canopies explained less of the variation in δ^{13}C than depth (Fig. 3B; $r^2 = 0.52$ vs. 0.56 for depth), although the relationship remained statistically significant (Regression ANOVA $F_{1,10} = 10.81$, $p = 0.008$, $r^2 = 0.52$). The slope of δ^{13}C vs. E_{PAR} for the Bahamas population could not be distinguished statistically from that for the Florida Bay population (Homogeneity of Slopes ANCOVA $F_{\mathrm{Location} \times E_{PAR},1,39} = 3.48$, $p = 0.07$) because of the low predictive value of the

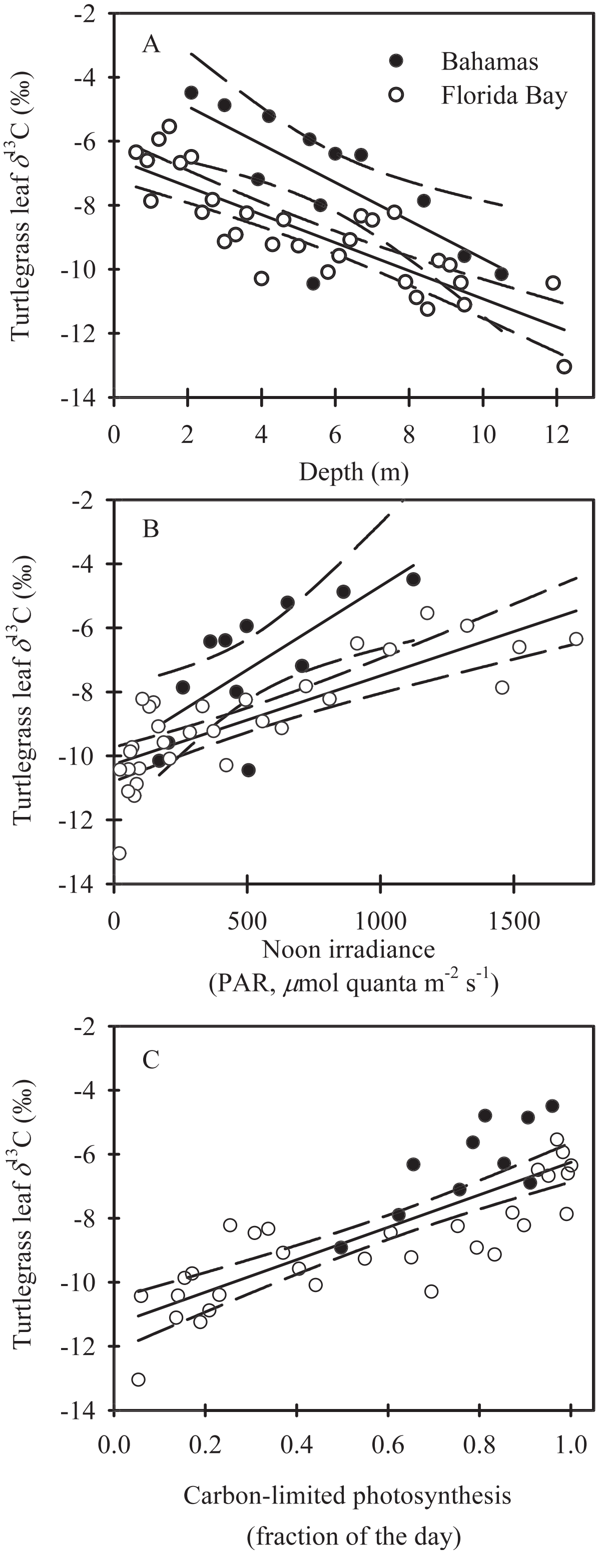

Fig. 3. (A) Relationship between water depth and δ^{13}C of turtlegrass leaves from the Great Bahama Bank and Florida Bay. Linear trends determined by least-squares regression are indicated by the solid lines. Dashed lines indicate 95% confidence intervals. (B) Relationship between noon irradiance (E_{PAR}) incident on the top of turtlegrass canopy and δ^{13}C of turtlegrass leaves. (C) Relationship between the daily period of carbon-limited (= light-saturated) photosynthesis and δ^{13}C of turtlegrass leaves.

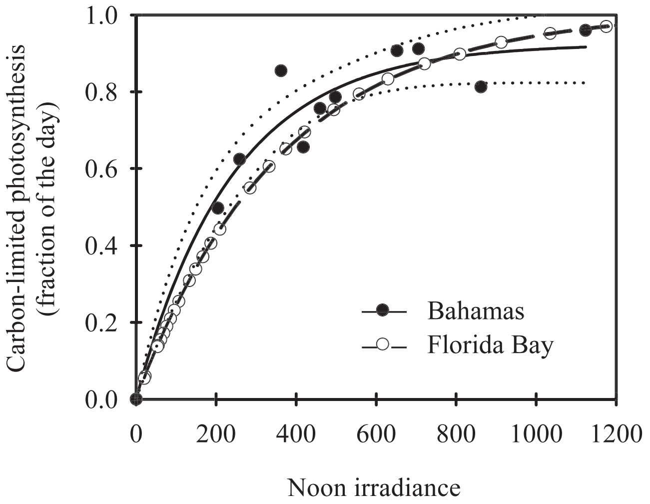

Fig. 4. Relationship between the daily period of carbon-limited photosynthesis and noon irradiance (E_{PAR}) incident on the top of the turtlegrass canopy for the Great Bahama Bank and Florida Bay. Nonlinear trends determined by least-squares regression are indicated by the solid lines (the fit of the Bahamas data is shown as the thick solid line). Dashed lines indicate 95% confidence intervals. There was no scatter in the Florida Bay relationship because all *GrassLight* simulations were performed here using the same shoot density and plant size parameters for all observations.

relationship. Although the isotopic signature of Bahamas seagrass leaves was no longer significantly heavier than leaves from Florida Bay when adjusted for E_{PAR} (Homogeneity of Slopes ANCOVA $F_{\mathrm{Location},1,39} = 0.17$, $p = 0.68$), the combined data set explained only 49% ($r^2 = 0.49$) of the variation in leaf δ^{13}C. Further, the residuals of the combined regression were not normally distributed, indicating residual systematic differences in δ^{13}C values between Bahamas and Florida Bay turtlegrass populations.

Leaf δ^{13}C showed a stronger relationship to the fraction of the day that photosynthesis of the intact plant canopy was carbon-limited (= light-saturated) than to either water depth or E_{PAR} delivered to the top of the canopy (Fig. 3C; regression ANOVA $F_{1,8} = 11.30$, $p = 0.001$, $r^2 = 0.59$). Further, the relationship between leaf δ^{13}C and the daily period of carbon-limited photosynthesis for Florida Bay populations also was highly significant (Regression ANOVA $F_{1,39} = 71.7$, $p < 0.001$, $r^2 = 0.67$), and statistically identical to the Bahamas relationship (Fig. 3C), despite using average density and plant size data here to parameterize the *Grasslight* model (Homogeneity of Slopes ANCOVA, $F_{\mathrm{Location} \times \mathrm{Clim},1,37} = 2.86$, $p = 0.10$, $F_{\mathrm{Location}} = 0.95$, $p = 0.33$). When the Bahamas and Florida Bay data were combined, the relationship between leaf δ^{13}C and daily period of carbon-limited photosynthesis collapsed into a single relationship across these two very optically different waters that explained 65% ($r^2 = 0.65$) of the variation in leaf δ^{13}C.

The daily fraction of carbon-limited (= light-saturated) photosynthesis ($P_{\mathrm{C\text{-}lim}}$) calculated from *GrassLight* revealed saturating, nonlinear relationships when plotted against noon E_{PAR} delivered to the top of turtlegrass canopy for both Bahamas and Florida Bay turtlegrass, similar to the instantaneous photosynthesis vs. irradiance curves of individual leaves (Fig. 4). The resulting light-saturation term derived from the exponential curve for the Bahamas population ($E_k = 244 \pm 44$ μmol quanta m^{-2} s^{-1}) was significantly lower than that estimated for the Florida Bay population ($E_k = 370$ μmol quanta m^{-2} s^{-1}) without error (t-test, $t = 2.86$, df = 1,9, $p = 0.02$). Both estimates of E_k, however, were 5- to 10-fold above the irradiances typically required to saturate photosynthesis of individual leaves in the laboratory (Zimmerman 2003; Bodensteiner 2006), illustrating the effects of leaf orientation and self-shading on vertically integrated photosynthesis of the plant canopy as a whole (Zimmerman 2003).

The differences in the relationships of $P_{\mathrm{C\text{-}lim}}$ vs. E_{PAR} between the Bahamas and Florida Bay populations can be explained by spectral differences in the incident light field. The strongly blue-light environment of the Bahamas was more efficient at driving photosynthesis than the green-light environment of Florida Bay, and, consequently, a larger fraction of E_{PAR} in the Bahamas was actually absorbed by the photosynthetic pigments used for photosynthesis. This is known as photosynthetically utilized radiation, or PUR (Morel 1978). Although irradiances in the Bahamas and Florida Bay both decreased logarithmically in accordance with the Lambert–Beer Law (Fig. 5A), Bahamian turtlegrass was exposed to higher irradiances at equivalent water depths. Further, the fraction of PUR increased in the blue-light environment of the Bahamas and decreased in the green-light environment of Florida Bay. Consequently, turtlegrass from the Bahamas was isotopically heavier than Florida Bay populations growing at equivalent water depths because Bahamas seagrasses were more carbon-limited (= light-saturated) for a larger fraction of the day.

Discussion

The fraction of the day during which photosynthesis was carbon-limited proved to be a more robust predictor of the variation in δ^{13}C in turtlegrass populations across the Florida Bay and the Great Bahama Banks than either water depth or E_{PAR} reaching the top of the plant canopy. More importantly, the ability to explain 65% of the variation in turtlegrass δ^{13}C across both environments combined represents a considerable predictive improvement over water depth alone, which yielded different relationships in different locations, or incident E_{PAR}, which explained only 49% of variability in δ^{13}C, and did not fully eliminate site-specific differences in the relationship. Further, the ability to predict turtlegrass δ^{13}C from the daily period of carbon-limited photosynthesis provides a mechanistic linkage to fundamental relationships between incident spectral irradiance, light absorption by the intact plant canopy and photosynthesis (Zimmerman 2003, 2006) that can transcend geographic differences in water depth, water-column optical properties and submarine light environments. Turtlegrass δ^{13}C, however, remains persistently heavier than many other phototrophs even in low-light environments, because the regression between turtlegrass δ^{13}C and $P_{\mathrm{C\text{-}lim}}$ yielded a y-intercept of −11.3‰ ± 0.4‰ (Fig. 3C). Thus, photosynthetic reliance on HCO_3^- (James and Larkum 1996; Larkum and James 1996; Raven et al. 2002) may set a relatively heavy lower bound for seagrass δ^{13}C generally, while C_i limitation of photosynthesis under high light (Jiang et al. 2010, and references cited therein) can be invoked to explain the most of the variation in turtlegrass δ^{13}C across time and space.

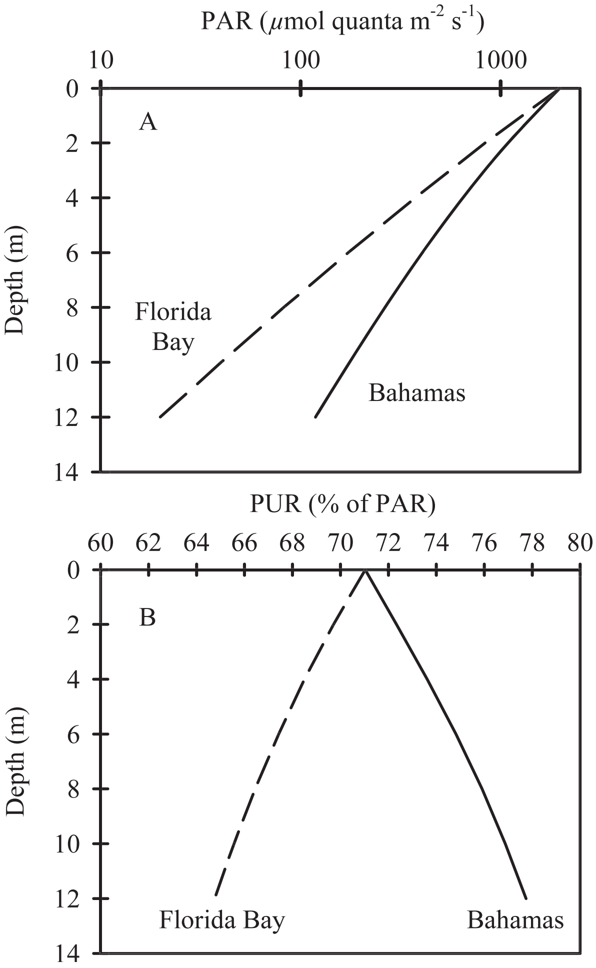

Fig. 5. (A) Vertical attenuation of E_{PAR} on the Great Bahama Bank (solid line) and Florida Bay (dashed line), resulting from local mean diffuse attenuation coefficients applied to the Lambert–Beer Law. (B) Vertical distribution of the fraction of E_{PAR} that was actually utilized by turtlegrass to drive photosynthesis on the Great Bahama Bank (solid line) and Florida Bay (dashed line), based in spectral irradiances calculated using local mean diffuse attenuation coefficients applied to the Lambert–Beer Law.

Some of the remaining variations in δ^{13}C may be attributable to other factors including temperature, isotopic composition of the C_i source, and water motion. However, turtlegrass δ^{13}C values at any given site in Florida Bay exhibited an average range of only 1.4‰ across a temperature range from 16°C to 29°C (Fourqurean et al. 2005). In addition, mean sea-surface temperatures of turtlegrass populations sampled across the Great Bahamas Bank showed no significant relation to the residual variation in

Table 2. Correlation analysis (p-values, we chose $p < 0.05$ as the criteria for significant correlation) of geochemical parameters with either *Thalassia* δ^{13}C values (average of leaf and root tissue) or water depth. BW represents bottom water.

	$\delta^{13}C_{leaf}$			Water depth		
	r	df	p	r	df	p
$\delta^{13}C_{Ci\text{-}BW}$	0.01	1,10	0.97	0.07	1,10	0.82
$\delta^{13}C_{added}$*	0.30	1,10	0.34	0.12	1,10	0.70
Bottom-water CO_2(aq)	0.48	1,10	0.12	0.44	1,10	0.14
Pore-water CO_2 (aq)†	0.29	1,10	0.37	0.37	1,10	0.24

* $\delta^{13}C_{added}$ is defined as the isotopic composition of the C_i added to the pore-water pool by sedimentary processes such as organic matter remineralization or sediment carbonate dissolution (Hu and Burdige 2007).

† Pore-water CO_2 (aq) is an average value for pore water collected below 5 cm in the sediments, where the majority of seagrass underground biomass is distributed (Burdige et al. 2008).

δ^{13}C after accounting for carbon-limited photosynthesis (Regression ANOVA $F_{1,10} = 0.182$, $p = 0.65$, $r^2 = 0.018$). The observed increase in turtlegrass δ^{13}C during warmer months in Florida Bay has also been attributed to metabolic dissolution of isotopically heavy carbonate sediments (0–2‰), resulting in the lacunal transport of isotopically heavy CO_2 from the root zone to the leaves, where it is fixed by photosynthesis (Fourqurean et al. 2005). However, carbonate dissolution coupled to sedimentary organic matter oxidation adds C_i both from heavy (carbonate sediments) and light sources (seagrass or planktonic organic matter; Hu and Burdige 2007). Thus, pore-water C_i is not entirely reflective of a sedimentary carbonate source. Furthermore, there was no significant correlation between δ^{13}C of the C_i added to the sediment pore waters by these processes, or pore-water CO_2 (aq) concentration, and either turtlegrass δ^{13}C or water depth (Table 2). Water flow may also have a significant effect on turtlegrass δ^{13}C by controlling the size of the diffusive boundary layer and delivery of C_i to the leaf surface (Koch 1993), but this remains an open question.

Seagrass ecosystems are an understudied, but potentially significant, global carbon sink (Duarte et al. 2010; Kennedy et al. 2010) being challenged world-wide by rapid anthropogenic changes to coastal environments that can be difficult, or even impossible to reverse (Orth et al. 2006). Unfortunately, seagrass loss is often the first indicator of a problem, and the development of nonlethal indicators of the status of these 'coastal canaries' has been elusive. The mechanistic linkage between leaf δ^{13}C and the daily fraction of carbon-limited (= light-saturated) photosynthesis presented here in Fig. 3C indicates that leaf δ^{13}C may provide a robust indicator of recent photosynthetic performance, whole-plant carbon balance, and plant survival that can be linked to light availability across regions with very different optical properties, such as the Great Bahama Bank and Florida Bay. In otherwise stable light environments, this relationship can be used to predict changes in δ^{13}C values in response to ocean acidification that should to reduce carbon-limitation of seagrass photosynthesis (Zimmerman et al. 1997; Invers et al. 2001; Hall-Spencer et al. 2008), thereby lowering leaf δ^{13}C values relative to current (or historic) values.

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