

NOTE

Effects of light on the stable carbon isotope composition of the seagrass *Thalassia testudinum*

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ABSTRACT: Changes in the stable carbon isotope composition of *Thalassia testudinum* (turtle grass) leaves were measured in response to *in situ* light-reduction treatments in Tampa Bay, Florida, USA. Leaf $\delta^{13}\text{C}$ values of shaded *T. testudinum* were significantly lower than those of unshaded controls in both shallow (0.75 m below MLW) and deep (2 m below MLW) sites. Changes in leaf $\delta^{13}\text{C}$ were correlated with differences in the relative amount of light reaching the experimental treatments, and the magnitude of the responses increased between 1 and 3 mo after initiation of the shade treatments. Because of the close proximity of the experimental and control sites, the decrease in $\delta^{13}\text{C}$ in response to shading probably reflects a process (i.e. isotopic fractionation) effect rather than a source (i.e. dissolved inorganic carbon) effect. Greater isotopic fractionation in shaded *T. testudinum* may reflect reduced carbon demand and, hence, greater relative availability of carbon at lower irradiances. Thus, as light is reduced to levels that limit photosynthetic rates in *T. testudinum*, carbon appears to become non-limiting.

During photosynthetic carbon fixation, plants discriminate (i.e. fractionate) between ^{13}C and ^{12}C because of small dissimilarities in physical and chemical properties caused by the mass difference. Isotopic fractionation is useful for investigating the efficiency of CO_2 uptake associated with photosynthesis (Peterson & Fry 1987). In terrestrial plants, differences in $\delta^{13}\text{C}$ values, reflecting variation in isotopic fractionation, can be used to separate various photosynthetic pathways (e.g. C_3 vs C_4 ; Whelan et al. 1970). In aquatic systems, fractionation of the stable isotopes of carbon is more difficult to understand because of the importance of $\text{CO}_{2(\text{aq})}$ diffusion (O'Leary 1988). The relatively high

ratios of ^{13}C to ^{12}C characteristic of seagrasses ($\delta^{13}\text{C}$ values range from -3 to -19 ‰; McMillan 1980) are thought primarily to reflect physical (i.e. diffusion) constraints on the supply of carbon used in photosynthesis (Abel 1984). Seagrass $\delta^{13}\text{C}$ values can be affected by oxygen concentration, temperature, and light intensity (Benedict & Scott 1976, Smith et al. 1976, McMillan 1980, McMillan & Smith 1982, Cooper 1989, Cooper & DeNiro 1989), but most of these effects can ultimately be explained by their influence on $\text{CO}_{2(\text{aq})}$ availability (O'Leary 1988).

Leaf $\delta^{13}\text{C}$ values of the seagrass *Posidonia oceanica* (L.) Delile collected over a 38 m depth gradient decreased with depth (Cooper & DeNiro 1989). This decline was hypothesized to have been the result of a reduction in photosynthetic carbon demand. The basis for this is the assumption that if seagrass carbon supply is diffusion-limited under high-light conditions then a reduction in photosynthetic carbon requirement under lower-light conditions should decrease the importance of external carbon acquisition and allow for greater isotopic selectivity.

To test the above assumption experimentally, variations in leaf $\delta^{13}\text{C}$ values of short-shoots of *Thalassia testudinum* Banks ex König (turtle grass) were measured in response to *in situ* light reduction. The study was conducted in a *T. testudinum* bed located near the mouth of Tampa Bay, Florida, USA ($27^\circ 37.5' \text{N}$, $82^\circ 47.5' \text{W}$). Short-shoot density at this site decreases from the shallow to the deep regions of the bed, suggesting the deep edge may be light-limited (Hall et al. 1991). Three shade and 3 control plots were established on February 3, 1989, at both the shallow (0.75 m below MLW) and the deep (2 m below MLW) edges of the bed. Shading was accomplished by neutral-density

* $\delta^{13}\text{C}$ (‰) = $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$; $R = ^{13}\text{C}/^{12}\text{C}$; standard is a Cretaceous belemnite from the Pee Dee Formation (PDB), South Carolina, USA, which is given an arbitrary $\delta^{13}\text{C}$ value of 0.0 ‰

nursery cloth attached to 1.5×1.5 m PVC frames held 0.5 m above the *T. testudinum* canopy. Screens were replaced every 1 to 2 wk to minimize additional light reduction due to fouling. Downwelling light at the top of the leaf canopy was measured using a LICOR LI-1000 quantum meter and a 4π sensor under both clean and fouled screens and in control plots whenever screens were changed. The reduction in ambient light relative to the shallow control (SC) plots was very similar for the shallow shade (SS) and deep control (DC) plots, averaging 42 and 37 %, respectively, compared to a 64 % relative reduction in light for the deep shade (DS) plots.

After experimental durations of 1 and 3 mo, 9 short-shoots were randomly harvested from within each experimental and control plot (27 short-shoots per treatment). Only the 1 m^2 areas in the center of the 2.25 m^2 experimental plots were sampled to avoid possible edge effects. Epiphytes were removed from leaves by gentle scraping, and short-shoots were separated into above-ground (green leaves) and below-ground fractions and were dried at 60°C until constant weight had been achieved. Dried samples from each experimental plot were pooled and then ground in a Wiley mill with a #40 mesh screen and stored in a desiccator until analyzed.

Triplicate leaf samples (6 to 15 mg) from each plot were prepared for stable carbon isotope analysis as described by Durako & Sackett (1991). All results are expressed in standard del notation relative to the international PDB standard. Stable carbon isotope data

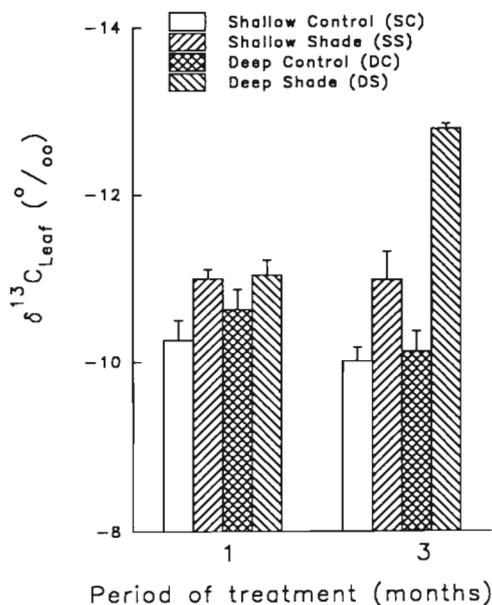


Fig. 1. *Thalassia testudinum*. Leaf $\delta^{13}\text{C}$ values (mean \pm SE) for short-shoots subjected to *in situ* light-reduction treatments after 1 and 3 mo

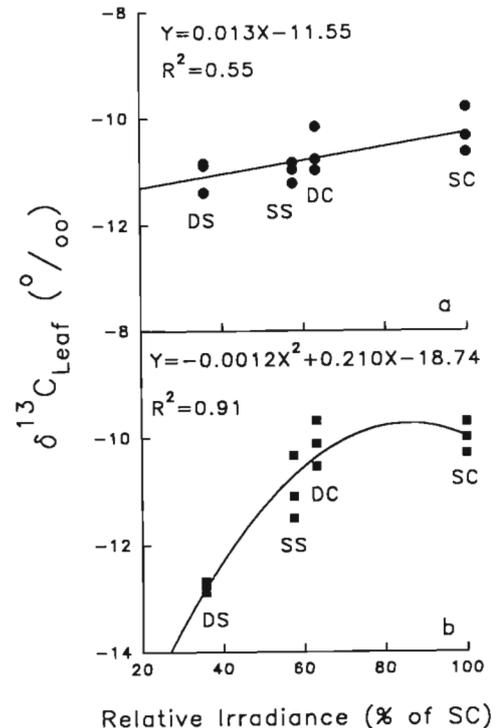


Fig. 2. *Thalassia testudinum*. Variation in leaf $\delta^{13}\text{C}$ values as a function of relative irradiance levels after (a) 1 mo treatment duration and (b) 3 mo treatment duration. Treatment abbreviations as in Fig. 1

($\delta^{13}\text{C}$) were log-transformed to satisfy the assumptions of normality and homogeneity of variance before being analyzed by Pearson product-moment correlation analyses, analysis of variance (ANOVA), and regression analyses ($p < 0.05$). Calculations were performed using Statistical Analysis Systems (Release 6.04) programs.

Shading resulted in a decrease in *Thalassia testudinum* leaf $\delta^{13}\text{C}$ at both shallow and deep plots (Fig. 1). After 1 mo, results of ANOVA indicated that shading had a significant effect on leaf $\delta^{13}\text{C}$ ($F = 7.95$, $df = 1,8$), but within treatments, comparisons between shallow- and deep-plot $\delta^{13}\text{C}$ values indicated no significant depth effects ($F = 1.03$, $df = 1,8$). However, after 3 mo, both shading and depth effects were significant ($F = 56.57$ and 14.13 , respectively, $df = 1,8$) and there was a significant interaction between the two ($F = 10.59$, $df = 1,8$).

A significant positive correlation ($0.01 < p < 0.05$ and $0.001 < p < 0.005$, after 1 and 3 mo respectively) was observed between relative irradiance levels and leaf $\delta^{13}\text{C}$ (Fig. 2). The change in leaf $\delta^{13}\text{C}$ values with respect to relative irradiance exhibited a linear relationship after 1 mo. Between Month 1 and Month 3, this relationship became curvilinear, and the response of leaf $\delta^{13}\text{C}$ to changes in relative irradiance signifi-

cantly increased ($F = 9.28$, $df = 3,16$). The increase in the response of leaf $\delta^{13}\text{C}$ to changes in light between Months 1 and 3 is probably a reflection of the leaf-turnover characteristics of *Thalassia testudinum*. Plastochrone intervals in this species range from about 14 to 16 d and short-shoots usually have from 3 to 5 leaf blades at any one time (Zieman 1982). This results in leaf turnover times of ca 60 d under ambient light regimes, although turnover times may be longer in shaded *T. testudinum* (Carlson & Acker 1985). Because of this, only about half of the leaf material harvested after 1 mo was produced under the experimental treatment conditions. After 3 mo of shading, all blade material should have been replaced.

Correlations between depth and plant $\delta^{13}\text{C}$ values have been observed in the green alga *Halimeda incrassata* (Ellis) Lamouroux (Wefer & Killingley 1986) and in the seagrass *Posidonia oceanica* (Cooper & DeNiro 1989). In both cases, changes in $\delta^{13}\text{C}$ were attributed to a light-intensity effect on isotopic fractionation. Increasing isotopic fractionation with light reduction may reflect decreasing carbon demand associated with lower photosynthetic rates (Farquhar 1983). Our study provides experimental evidence to support this hypothesis. Because of the close proximity of the experimental and control sites (all were within a 50×50 m area), uniformity in environmental characteristics and in the $\delta^{13}\text{C}$ of the seawater dissolved inorganic carbon (DIC) among the plots can be assumed. Accordingly, the decrease in *Thalassia testudinum* leaf $\delta^{13}\text{C}$ in response to reduced irradiance probably reflects a process (i.e. isotopic fractionation) effect rather than a source (i.e. $\delta^{13}\text{C}_{\text{DIC}}$ or $\delta^{13}\text{C}_{\text{CO}_2(\text{aq})}$) effect (Peterson & Fry 1987). Changes in isotopic fractionation associated with aquatic photosynthesis are generally dependent on carbon availability (e.g. diffusion) rather than on differences in enzymatic discrimination (Kerby & Raven 1985). A reduction in carbon demand should decrease the influence of diffusion-dependent processes controlling carbon availability and this may allow an increase in enzymatic discrimination against ^{13}C at lower irradiances.

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