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Variation in δ^{13}C values for the seagrass *Thalassia testudinum* and its relations to mangrove carbon

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ABSTRACT

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Carbon isotope ratios ($^{13}C/^{12}C$) were measured for the leaves of the seagrass *Thalassia testudinum* Banks ex König and carbonates of shells collected at the seagrass beds from seven sites along the coast of southern Florida, U.S.A. The δ^{13}C values of seagrass leaves ranged from −7.3 to −16.3‰ among different study sites, with a significantly lower mean value for seagrass leaves from those sites near mangrove forests (−12.8 ± 1.1‰) than those far from mangrove forests (−8.3 ± 0.9‰; $P < 0.05$). Furthermore, seagrass leaves from a shallow water area had significantly lower δ^{13}C values than those found in a deep water area ($P < 0.01$). There was no significant variation in δ^{13}C values between young and mature leaves ($P = 0.59$) or between the tip and base of a leaf blade ($P = 0.46$). Carbonates of shells also showed a significantly lower mean δ^{13}C value in the mangrove areas (−2.3 ± 0.6‰) than in the non-mangrove areas (0.6 ± 0.3‰; $P < 0.025$). In addition, the δ^{13}C values of seagrass leaves were significantly correlated with those of shell carbonates (δ^{13}C seagrass leaf $= -9.1 + 1.3$ δ^{13}C shell carbonate ($R^2 = 0.83$, $P < 0.01$)). These results indicated that the input of carbon dioxide from the mineralization of mangrove detritus caused the variation in carbon isotope ratios of seagrass leaves among different sites in this study.

INTRODUCTION

Variation in δ^{13}C values of vascular plants is high, ranging from −6 to −34‰ (Smith and Epstein, 1971). For terrestrial plants, the differences in carbon isotope ratios among species are primarily caused by the differences in the biochemical properties of the carboxylation enzymes of photosynthesis. The δ^{13}C values of C_3 plants range from −24 to −34‰, and those of C_4 plants range from −6 to −13‰, while those of CAM plants range from −10 to −22‰ (Bender, 1971; Smith and Epstein, 1971; Benedict, 1978; O'Leary, 1981). The carbon isotope ratios of seagrass species also exhibit a wide range in their values (Benedict et al., 1980; McMillan et al., 1980;

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McMillan and Smith, 1982; Cooper and DeNiro, 1989). For example, $\delta^{13}C$ values for 49 species in 12 seagrass genera ranged from −3.0 to −23.8‰ (McMillan et al., 1980). Even among individuals within a given species, variation in carbon isotope ratios is still quite obvious (McMillan and Smith, 1982; Cooper and DeNiro, 1989). For aquatic plants, however, differences in enzymatic discrimination against $^{13}CO_2$ and $^{12}CO_2$ between different photosynthetic pathways do not explain the variability in $\delta^{13}C$ values (Sternberg et al., 1984; Keeley et al., 1986). Variation in $\delta^{13}C$ values in seagrasses may indicate the influences of the following factors: (1) carbon sources; (2) differential use of bicarbonate and dissolved CO_2; (3) seasonal variability associated with biochemical processes; and (4) light intensity (Smith et al., 1976; Andrews and Abel, 1977; Thayer et al., 1978; Osmond et al., 1981; McMillan and Smith, 1982; Stephenson et al., 1984; Cooper and DeNiro, 1989).

Thalassia testudinum Banks ex König, distributed broadly in tropical seagrass beds, is the dominant seagrass species in southern Florida (den Hartog, 1970; Zieman, 1982). The carbon isotope ratios for this seagrass species have been shown to exhibit a large variation (McMillan and Smith, 1982; Zieman and Fourqurean, 1985). In Florida Bay, the $\delta^{13}C$ values for leaves of this seagrass species ranged from −6.5 to −18.7‰ and it was speculated that mangrove-derived carbon contributes to this variation (Zieman and Fourqurean, 1985). In this study, we tested this hypothesis by measuring the carbon isotope ratios for *T. testudinum* leaves and carbonates of shells collected in the seagrass beds from different sites along the coast of southern Florida. The $\delta^{13}C$ value of shell carbonate has been shown to be determined by the isotopic composition of inorganic carbon in sea water (Lloyd, 1964). Thus, if the above hypothesis is correct, the $\delta^{13}C$ values of seagrass leaves and shell carbonates should covary and should be significantly lower at the sites near mangrove forests than those far from mangrove forests, because the respiratory CO_2 from the mineralization of mangrove detritus has a low $\delta^{13}C$ value (Zieman et al., 1984).

MATERIAL AND METHODS

Leaves of *T. testudinum* were collected from seven sites along the coast of southern Florida (Fig. 1) during the period from late 1988 to early 1989. Among these sites, Matheson Hammock, Manatee Bay, Virginia Key and John Pennekamp are four sites where seagrass beds are located close to mangrove forests, referred to here as "mangrove areas". At the other three sites (Bill Baggs, Islamorada and Indian Key), seagrass beds are far from mangrove forests. These sites are referred to as "non-mangrove-areas".

At each site, 15 mature leaves of the seagrass were collected randomly from the shallow area (with mean water depth of 0.5 m at mean high tide) of the

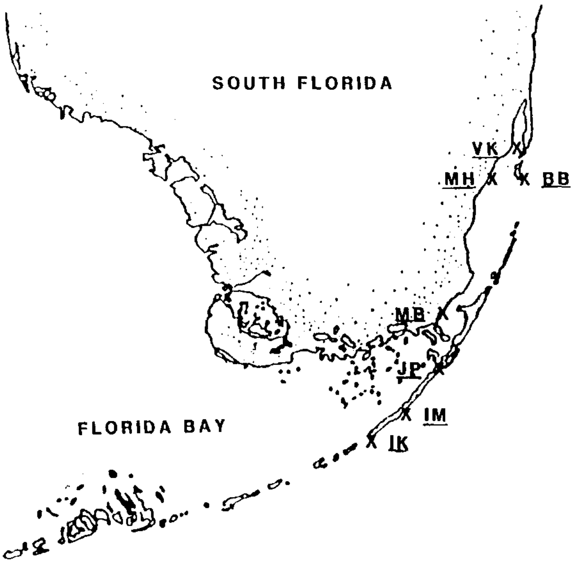

Fig. 1. Location map of southern Florida showing the sampling sites along the coast (Mangrove areas: MH, Matheson Hammock, MB, Manatee Bay; VK, Virginia Key; and JP, John Pennekamp. Non-mangrove areas: BB, Bill Baggs; IK, Indian Key; and IM, Islamorada).

seagrass bed, except for Matheson Hammock where each of 14 mature leaves were collected both from the shallow area (within 40 m of the shore, with a mean water depth of 0.5 m at mean high tide) and the deep area (between 40 and 80 m from the shore, with a mean water depth of 1.5 m at mean high tide). Another 12 young leaves of the seagrass were collected from the deep area at Matheson Hammock to compare the difference in $\delta^{13}C$ values between young and mature leaves. In addition, at least five species of mollusk shells were collected from the seagrass beds at each of the seven sites. The most common species of shells found in these areas were *Chione cancellata* (Linné), *Aequipecten irradians* (Lamarck), *Fasciolaria tulipa* (Linné), *Asterea americana* (Gmelin), *Melangina corona* (Gmelin) and *Natica canrena* (Linné).

After collection, leaf samples were washed several times with distilled water and any visible epiphyte was removed. The samples were dried in an oven at 50–60°C. About 3 mg of sample was taken from either the blade tip or the base of each leaf for all leaf samples, except for the five leaves collected at Matheson Hammock. For these five leaves, both the tip and base of the leaf blade were sampled for comparison of the difference in $\delta^{13}C$ value between the tip and the base of a leaf blade. The sample was then burned in the presence of cupric oxide, copper and silver foil at 800°C in a vacuum-sealed quartz glass tube. The CO_2 generated was purified and analyzed on a PRISM mass spectrometer (VG). All values are reported in parts per thousand (‰) relative to the PDB standard, where $\delta^{13}C$ (‰) = ($R_{sample}/R_{standard} - 1$) × 1000, and $R = {}^{13}C/{}^{12}C$ of sample or standard CO_2 respectively. The precision of analysis for the multiple samples was ±0.2‰ (SD). The shell samples were cleaned thoroughly with 5.3% sodium hypochlorite to remove any organic matter, then ground into a fine powder. About 5 mg portions of each sample were reacted with 100% H_3PO_4 at 50°C in a vacuum system. The CO_2 produced was purified and analyzed as previously.

RESULTS

Carbon isotope ratios for the leaves of *T. testudinum* ranged from −7.3 to −16.3‰ among different sites in southern Florida (Table 1), within the range of −6.5 to −18.7‰ reported by Zieman and Fourqurean (1985) for the same species in Florida Bay. The mean $\delta^{13}C$ value for seagrass leaves in mangrove areas (−12.8±1.1‰) was significantly lower than that in non-mangrove areas (−8.3±0.9‰; $P<0.05$, df=6). Leaves collected from the shallow area (with mean water depth of 0.5 m) also showed significantly lower $\delta^{13}C$ values than those from the deep area (with mean water depth of 1.5 m) at Matheson Hammock ($P<0.01$, df 26). However, there was no significant variation in $\delta^{13}C$ values either between seagrass leaves of different ages or between different parts of seagrass leaves (Table 2). At Matheson Hammock,

TABLE 1

Carbon isotope ratios (mean±SE) for leaves of *Thalassia testudinum* at different sites in southern Florida

Study site	n	$\delta^{13}C$ (‰)
Mangrove areas		
Matheson Hammock (S)[1]	14	−16.3±0.6
Matheson Hammock (D)	14	−14.4±0.2
Manatee Bay (S)	15	−11.8±0.2
John Pennekamp (S)	15	−11.5[2]
Virginia Key (S)	15	−10.0±0.5
Average ($n=5$)		−12.8±1.1
Non-mangrove areas		
Bill Baggs (S)	15	−10.0±0.4
Islamorada (S)	15	−7.6[2]
Indian Key (S)	15	−7.3[2]
Average ($n=3$)		−8.3±0.9

[1] S, from a shallow area with mean water depth of 0.5 m at mean high tide; D, from a deep area with mean water depth of 1.5 m at mean high tide.

[2] At these sites, equal amounts of each of the 15 leaves were mixed and analyzed together.

TABLE 2

Comparison in $\delta^{13}C$ values (mean ± SE) between different leaf ages and between different leaf parts of *Thalassia testudinum* at Matheson Hammock, southern Florida

Leaf type	*n*	$\delta^{13}C$ (‰)
Age		
Young	12	-14.5 ± 0.3
Mature	14	-14.4 ± 0.2
T statistic		0.544
Significance		$P = 0.59$
Part		
Blade tip	5	-14.8 ± 0.4
Blade base	5	-15.2 ± 0.3
T statistic		0.768
Significance		$P = 0.46$

TABLE 3

Carbon isotope ratios (mean ± SE) for carbonates of shells in *Thalassia testudinum* seagrass beds along the coast of southern Florida

Study site	*n*	$\delta^{13}C$ (‰)
Mangrove areas		
Matheson Hammock	6	-3.7 ± 0.3
Manatee Bay	5	-2.8 ± 0.7
Virginia Key	6	-1.7 ± 0.2
John Pennekamp	5	-0.8[1]
Average ($n = 4$)		-2.3 ± 0.6
Non-mangrove areas		
Bill Baggs	6	0.0 ± 0.3
Islamorada	5	$+1.0$[1]
Indian Key	5	$+0.9$[1]
Average ($n = 3$)		$+0.6 \pm 0.3$

[1] At these sites, equal amounts of each of the five shell carbonate samples were mixed and analyzed together.

the mean $\delta^{13}C$ value for young leaves was almost the same as that for mature leaves ($P = 0.59$, df = 24). Similarly, the $\delta^{13}C$ value of the blade tip was not significantly different from that of the blade base of seagrass leaf ($P = 0.46$, df = 8).

Carbonates from mollusk shells collected at the seagrass beds also showed much lower $\delta^{13}C$ values in the mangrove areas than those in the non-mangrove areas ($P < 0.025$, df = 5; Table 3). The mean value for four study sites in mangrove areas was about 3‰ more negative than that for three study sites in non-mangrove areas. Furthermore, the $\delta^{13}C$ values of seagrass leaves were significantly correlated with those of shell carbonates (Fig. 2), with the

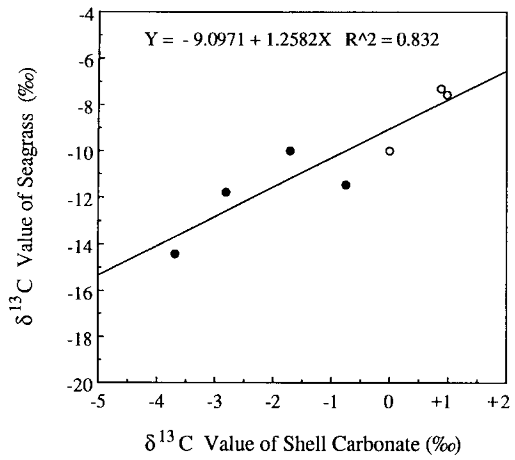

Fig. 2. Correlation between $\delta^{13}C$ values of *Thalassia testudinum* leaves and those of shell carbonates collected at the seagrass beds along the coast of southern Florida (closed symbols are from mangrove areas and open symbols are from non-mangrove areas).

regression equation: $\delta^{13}C$ seagrass leaf $= -9.1 + 1.3$ $\delta^{13}C$ shell carbonate ($R^2 = 0.83, P < 0.01$). The $\delta^{13}C$ values of seagrass leaves increased from −14.4 to −7.3‰ with a concomitant increase in the $\delta^{13}C$ values of shell carbonates from −3.7 to +1.0‰.

DISCUSSION

The most important factor determining the $\delta^{13}C$ values of seagrass is the isotopic composition of the inorganic carbon source in the environment (Smith et al., 1976; Benedict et al., 1980; Osmond et al., 1981). Possible main carbon sources for seagrasses include bicarbonate, dissolved CO_2 in sea water, and respiratory CO_2 from seagrasses and mangroves through the decomposition and mineralization of their organic detritus. Bicarbonate in ocean water usually has a $\delta^{13}C$ value around zero (Hoefs, 1987). For dissolved CO_2 in sea water in isotopic equilibrium with bicarbonate, the $\delta^{13}C$ value is about −6.8‰ (Smith et al., 1976). The carbon isotope ratios for respiratory CO_2 from the mineralization of seagrass and mangrove detritus are close to their original substrate (Zieman et al., 1984), i.e. about −10.0‰ for *T. testudinum* (McMillan and Smith, 1982) and −26 to −27‰ for mangrove species in southern Florida. (Zieman and Fourqurean, 1985). In addition, there is a −1.2‰ fractionation of CO_2 by ribulose biphosphate carboxylase and the Calvin cycle in seagrasses (Benedict et al., 1980). Thus, if seagrasses do not incorporate any respiratory CO_2, their carbon isotope ratios should be close to −8.0‰ ($-6.8 + (-1.2) = -8.0$), as in all non-mangrove sites studied here with the average $\delta^{13}C$ value of −8.3‰ (Table 1). Thus, lower values of carbon isotope ratios for seagrass leaves in mangrove areas may indicate that there is a significant source of respiratory CO_2 entering these areas and seagrasses incorporate this isotopically lighter carbon during photosynthesis and/or that there are physiological effects in the seagrass beds near mangrove forests.

To distinguish between these two possibilities, we compared the carbon isotope ratios of the shell carbonates collected from the seagrass beds in mangrove areas with those from the seagrass beds in non-mangrove areas. Carbon isotope ratios of shell carbonates have been considered a good indicator of the isotopic composition of inorganic carbon in seawater and can be used to define major environmental gradients (Lloyd, 1964). In an open ocean environment, the $\delta^{13}C$ value of shell carbonate should be close to the atmosphere–seawater equilibrium value of about 0‰ (Hoefs, 1987). In the three non-mangrove areas in this study, the $\delta^{13}C$ values of shell carbonates were similar to the predicted values (0.0–1.0‰, Table 3). However, the carbonates of shells from mangrove areas showed much lower $\delta^{13}C$ values (-3.7 to -0.8‰, Table 3). This shift in $\delta^{13}C$ values of shell carbonates is consistent with the hypothesis that the variation in $\delta^{13}C$ values of seagrass leaves is caused by shifts in isotopic composition of source carbon. Lower $\delta^{13}C$ values of shell carbonates from the seagrass beds in mangrove areas reflect the mixing of inorganic carbon in sea water with CO_2 produced from the decomposition of mangrove detritus. The positive correlation and a regression equation with a slope of about 1.0 was observed between $\delta^{13}C$ values of seagrass leaves and those of shell carbonates collected in respective seagrass beds (Fig. 2). This indicates that both the variation in $\delta^{13}C$ values of seagrass leaves and that for shell carbonates can be fully accounted for by the input of inorganic carbon having lower $\delta^{13}C$ values. Since the decrease in $\delta^{13}C$ values of carbonates and seagrasses was associated with the proximity to mangrove areas, we conclude that the isotopically lighter carbon derived from mangrove litter was the main factor influencing the $\delta^{13}C$ value of the inorganic carbon pool. The respiratory CO_2 from the decomposition and mineralization of mangrove detritus enters the water column in seagrass beds and changes the isotopic composition of the inorganic carbon pool, resulting in the variation in $\delta^{13}C$ values of the seagrass leaves in this study.

Osmond et al. (1981) proposed that the isotopic variation in aquatic plants could be the result of differential utilization of bicarbonate and dissolved CO_2 during photosynthesis. However, coastal waters are well buffered and usually have a pH value of about 8.0 throughout the year so that a constant proportion (about 98%) of total inorganic carbon in coastal waters would be in the form of bicarbonates (Stephenson et al., 1984). Thus, it is unlikely that the relative utilization of bicarbonate and dissolved CO_2 would change at different sites along the coast of southern Florida. Seasonal variability in carbon isotope ratios in this study was minimized by sampling over a short period (from late 1988 to early 1989). Also, there was no variation in $\delta^{13}C$ values between seagrass leaves of different ages (Table 2). Change in light intensity can be responsible for the variation in $\delta^{13}C$ values of some seagrass species. Higher light intensity could increase the photosynthetic rates, thus increasing the $\delta^{13}C$ values of seagrasses (Cooper and DeNiro, 1989). However, our results showed that the $\delta^{13}C$ values of seagrass leaves from a shallow water area with higher light intensity were much lower than those from a deep water area (Table 1). In addition, the $\delta^{13}C$ values for relatively well-illuminated blade tips were not significantly different from those for blade bases (Table 2). Thus the difference in light intensity cannot be responsible for the isotopic variation of the seagrass studied here.

From the discussion above, it can be concluded that, among a variety of potential factors controlling the isotopic variation of seagrass species, the input of respiratory CO_2 from the decomposition of mangrove detritus is the main factor determining the $\delta^{13}C$ values of the leaves of the seagrass *T. testudinum* in this study. Other factors such as differential use of bicarbonate and dissolved CO_2, seasonal changes associated with biochemical processes and difference in light intensity are not responsible for the variation in carbon isotope ratios of this seagrass species.

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