

REVIEW

Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies

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ABSTRACT: Seagrass $\delta^{13}\text{C}$ values reported in the literature show variation over a range of approximately 20‰. A frequency histogram constructed on the basis of the collected data set shows a unimodal distribution, with values between -10 and -11 ‰ (relative to the PDB standard) found with the highest frequency. Seagrasses thus have stable carbon isotope signatures which are typically less depleted in ^{13}C than those of other groups of aquatic primary producers. This points to a characteristic set of physiological, anatomical and perhaps environmental features shared by all seagrasses. A significant negative correlation exists between seagrass $\delta^{13}\text{C}$ values and geographical latitude of the sampling location, indicating that from tropical to temperate regions seagrasses tend to become more ^{13}C depleted. The physiological and environmental causes of variability are discussed. The most relevant environmental factors inducing variation in seagrass $\delta^{13}\text{C}$ values appear to be, in order of decreasing importance, source carbon, irradiance and temperature. The integrated information reflected in the natural $\delta^{13}\text{C}$ values of seagrasses can be used in support of the reconstruction of environmentally related growth dynamics on the scale of individual shoots and that of entire populations. In addition, ^{13}C may be used experimentally as a tracer of carbon flow in short term carbon allocation and production studies.

KEY WORDS: Stable carbon isotopes · Seagrasses · Isotope signatures · Variability

INTRODUCTION

Seagrasses are the only descendants of terrestrial angiosperms which have been able to invade the marine environment (Den Hartog 1970, Dring 1982). They can be found in marine shallow waters all over the world at tropical and temperate latitudes; the geographical distribution of some species even extending to polar areas (Den Hartog 1970, Kuo & McComb 1989). In recent decades, scientific attention to these plants has strongly increased, coinciding with the growing awareness that seagrass meadows may have conspicuous impacts on the stability and morphogenesis of the coastal zone (e.g. Scoffin 1970, Harlin et al. 1982, Hemminga & Nieuwenhuize 1990), and that they

fulfill important ecological roles in the coastal environment, such as in providing food and shelter to many organisms (Bell & Pollard 1989, Howard et al. 1989, Heck et al. 1995). However, there is still a considerable deficit in knowledge on the functioning of these plants in comparison to terrestrial and freshwater plants.

It has become apparent that the analysis of the stable carbon isotope composition of plants is a useful tool in understanding physiological processes and the response of plants to varying environmental conditions. In addition, studies of the natural abundance of stable carbon isotopes in plants and in organisms at higher trophic levels has contributed to the understanding of the food web in various ecosystems (Peterson & Fry 1987, Farquhar et al. 1989, Ehleringer & Osmond 1989, Raven 1992, Lajtha & Marshall 1994). The above, however, so far hardly pertains to seagrasses. There is a substantial (and growing) body of data on stable car-

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bon isotope signatures in seagrasses, but the data are scattered over studies carried out with different purposes. At the current state-of-the-art, usually only a beginning of an ecological interpretation has been possible.

In this paper we review the presently available literature data on stable carbon isotope ratios in seagrasses, with particular emphasis on the factors relevant for the observed variation of isotope signatures. Subsequently, we address the potential of stable carbon isotopes for studies of seagrass ecology.

Throughout the paper we will use the familiar δ notation whenever isotopic compositions are given, which indicates the deviation (‰) of the isotopic composition of the sample from a standard. In this review all δ values given are relative to the limestone PeeDee Belemnite (PDB) standard. We are aware of the fact that the International Union of Pure and Applied Chemistry (IUPAC) recently has recommended the reporting of carbon isotopic ratios relative to Vienna PeeDee Belemnite (V-PDB) (IUPAC 1994, Limnology & Oceanography 1995). However, as the data we review in the present paper all were given relative to the PDB standard, we have opted to discuss the data relative to the same standard.

VARIABILITY

The range of natural $\delta^{13}\text{C}$ values in seagrasses is substantial, as has been evident since McMillan et al. (1980) published their paper on isotopic variability in seagrasses. Table 1 gives an overview of the natural variation in $\delta^{13}\text{C}$ values which has been reported up to now, derived from 31 studies carried out worldwide. The values were determined in 48 different species, i.e. nearly all seagrass species are present in the data set. The extremes of the range, i.e. a value of -3.0‰ reported for *Syringodium filiforme* at the high end of the scale, and a value of -23.8‰ for *Halophila beccarii* at the low end, have not changed since McMillan et al. (1980) published their study, but many data falling within this range have been reported since then.

The data set forming the basis of Table 1 was used to construct a frequency histogram of $\delta^{13}\text{C}$ values (Fig. 1). The data are not clustered in distinct groups but show a unimodal distribution; values between -10.0 and -11.0‰ are found with the highest frequency. Even the most negative values show an only comparatively limited depletion of the ^{13}C isotope compared to other aquatic primary producers, i.e. freshwater macrophytes (values extending to -50‰ ; Keeley & Sandquist 1992), marine macroalgae (values extending to -34‰ ; Fischer & Wiencke 1989, Raven et al. 1995), and plank-

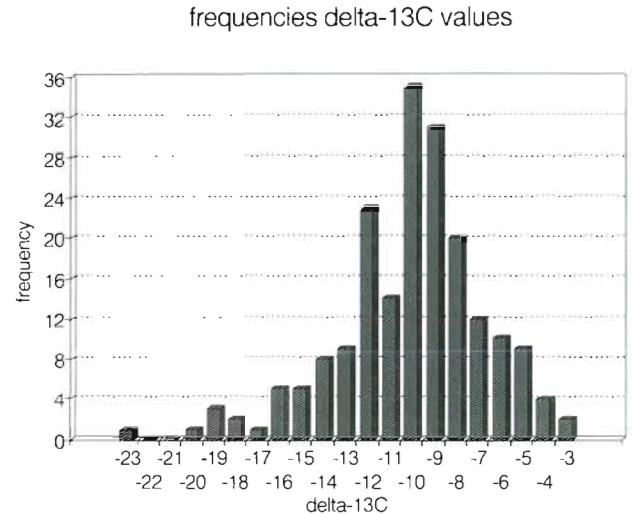


Fig. 1 Frequency histogram of seagrass $\delta^{13}\text{C}$ values as reported in the literature. The data in the figure are taken from the references indicated in Table 1

tonic freshwater and marine algae (minimum values of approximately -46 and -32‰ , respectively; France 1995).

An interesting picture also emerges when the relation between seagrass $\delta^{13}\text{C}$ values and the corresponding geographical latitude of the sampling sites is considered. A moderate, but significant, negative correlation exists ($p < 0.001$) between $\delta^{13}\text{C}$ values and sampling latitudes (Fig. 2), indicating that from tropical

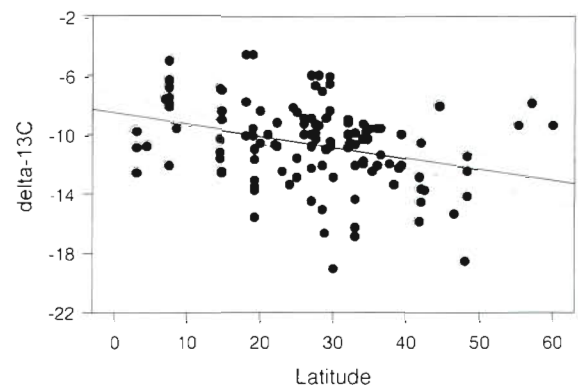


Fig. 2. Relation between seagrass $\delta^{13}\text{C}$ values and geographical latitude. Data are taken from the references mentioned in Table 1. The fitted line ($r = -0.32$, $p < 0.001$) is described by the equation: $\delta^{13}\text{C} = -8.532 - 0.0749 \text{ latitude}$. Dotted lines indicate 95% confidence intervals. If several observations on a species were made at the same location, the average value is used. Observations that were influenced by the proximity of mangroves (if explicitly stated), were not included. Latitudes corresponding to the reported $\delta^{13}\text{C}$ value were either obtained directly from the publications, or taken from geographical maps

Table 1. Seagrass $\delta^{13}\text{C}$ variability. The list of 104 $\delta^{13}\text{C}$ values for seagrasses given by McMillan et al. (1980) has been updated with 91 new observations. Species have been arranged following den Hartog (1970) as adapted in Kuo & McComb (1989). *Values as quoted in McMillan et al. (1980) from various authors. References: 1, Craig (1953); 2, Parker (1964); 3, Smith & Epstein (1971); 4, Smith et al. (1976); 5, Black & Bender (1976); 6, Benedict & Scott (1976); 7, Parker (1976); 8, Doohan & Newcomb (1976); 9, Fry (1977); 10, Thayer et al. (1978); 11, McConnaughey & McRoy (1979); 12, Andrews & Abel (1979); 13, Beer & Waisel (1979); 14, Fry & Parker (1979); 15, Beer et al. (1980a); 16, Benedict et al. (1980); 17, McMillan et al. (1980); 18, Fry et al. (1982); (19, Fry et al. (1983); 20, Stephenson et al. (1984); 21, Fry (1984); 22, Zieman et al. (1984); 23, Stephenson et al. (1986); 24, Harrigan et al. (1989); 25, Cooper (1989); 26, Cooper & DeNiro (1989); 27, Lin et al. (1991); 28, Durako & Hall (1992); 29, Hemminga et al. (1994); 30, Raven et al. (1995); 31, Yamamuro et al. (1995)

Family and genus	Species	Mean	n	SD	Min.	Max.	Range	Refs.
Hydrocharitaceae								
<i>Enhalus</i>	<i>acorooides</i> (L. f.) Royle	-5.8	2	1.2	-6.7	-4.9	1.8	17
<i>Thalassia</i>	<i>hemprichii</i> (Ehrenb.) Aschers.	-6.9	6	1.2	-8.1	-5.2	2.9	17,31
<i>Thalassia</i>	<i>testudinum</i> Banks ex König	-10.9	31	2.0	-16.3	-7.3	9.0	3, 6, 7, 12, 14, 15, 17, 18, 22, 24, 27, 28
<i>Halophila</i>	<i>decipiens</i> Ostenfeld	-10.2	11	1.7	-13.2	-7.5	5.7	12, 17
<i>Halophila</i>	<i>ovalis</i> (R. Br.) Hook. f.	-10.0	7	3.6	-15.5	-6.4	9.1	8, 12, 17
<i>Halophila</i>	<i>minor</i> (Zoll.) den Hartog	-8.3	2	1.3	-9.2	-7.4	1.8	5
<i>Halophila</i>	<i>hawaiiiana</i> Doty & Stone	-9.1	2	1.1	-9.9	-8.3	1.6	17
<i>Halophila</i>	<i>stipulacea</i> (Forsk.) Aschers.	-8.7	4	2.9	-11.7	-5.9	5.8	15, 17
<i>Halophila</i>	<i>johnsonii</i> Eiseman	-9.8	1					17
<i>Halophila</i>	<i>beccarii</i> Aschers.	-20.4	2	4.8	-23.8	-17.0	6.8	17
<i>Halophila</i>	<i>spinulosa</i> (R. Br.) Aschers.	-12.2	3	2.8	-14.5	-9.1	5.4	17
<i>Halophila</i>	<i>tricostata</i> Greenway	-20.8	1					17
<i>Halophila</i>	<i>engelmanni</i> Aschers.	-12.2	4	1.5	-14.7	-10.9	3.8	17*
Posidoniaceae								
<i>Posidonia</i>	<i>oceanica</i> (L.) Delile	-11.9	3	4.1	-16.4	-8.3	8.1	26
<i>Posidonia</i>	<i>angustifolia</i> Cambridge & Kuo	-9.5	2	0.4	-9.8	-9.2	0.6	17, 30
<i>Posidonia</i>	<i>australis</i> Hook. f.	-9.9	3	1.8	-11.9	-11.8	0.1	17, 30
<i>Posidonia</i>	<i>sinuosa</i> Cambridge & Kuo	-9.9	1					17, 30
<i>Posidonia</i>	<i>ostenfeldii</i> den Hartog	-12.0	1					17
Cymodoceaceae								
<i>Syringodium</i>	<i>isoetifolium</i> (Aschers.) Dandy	-6.0	3	2.4	-8.3	-3.6	4.7	13, 17
<i>Syringodium</i>	<i>filiforme</i> Kütz.	-5.9	15	1.8	-9.5	-3.0	6.5	4, 9, 14, 17*, 18, 21, 22
<i>Halodule</i>	<i>pinifolia</i> (Miki) den Hartog	-12.0	1					17
<i>Halodule</i>	<i>uninervis</i> (Forsk.) Aschers.	-11.2	3	2.9	-13.0	-7.8	5.2	12, 13, 17
<i>Halodule</i>	<i>wrightii</i> Aschers.	-11.5	11	1.9	-15.0	-8.5	6.5	3, 14, 16, 17, 18, 24
<i>Cymodocea</i>	<i>angustata</i> Ostenfeld	-10.7	1					17
<i>Cymodocea</i>	<i>rotundata</i> Ehrenb. & Hempr.	-8.1	2	1.1	-8.9	-7.4	1.5	8, 17
<i>Cymodocea</i>	<i>nodosa</i> (Ucria) Aschers.	-9.3	4	0.4	-9.7	-9.5	0.2	15, 17
<i>Cymodocea</i>	<i>serrulata</i> (R. Br.) Aschers. & Magnus	-10.7	3	2.3	-12.4	-8.0	4.4	8, 12, 17
<i>Amphibolis</i>	<i>antarctica</i> (Labill.) Sonder & Aschers.	-12.2	3	0.3	-12.5	-11.9	0.6	17, 30
<i>Amphibolis</i>	<i>griffithii</i> (J.M. Black) den Hartog	-16.2	1					17
<i>Thalassodendron</i>	<i>ciliatum</i> (Forsk.) den Hartog	-12.4	9	4.5	-19.7	-5.3	14.4	15, 17, 29
<i>Thalassodendron</i>	<i>pachyrhizum</i> den Hartog	-16.6	1					17
Zosteraceae								
<i>Zostera</i>	<i>marina</i> L.	-9.2	17	1.7	-12.4	-6.0	6.4	10, 17, 20, 25
<i>Zostera</i>	<i>caulescens</i> Miki	-9.7	1					17
<i>Zostera</i>	<i>asiatica</i> Miki	-12.8	1					17
<i>Zostera</i>	<i>capricorni</i> Aschers.	-10.8	2	2.3	-12.4	-9.2	3.2	12, 17
<i>Zostera</i>	<i>mucronata</i> den Hartog	-9.9	1					17
<i>Zostera</i>	<i>muelleri</i> Irmisch ex Aschers.	-10.6	1					17
<i>Zostera</i>	<i>capensis</i> Setchell	-15.8	2	4.6	-19.0	-12.5	6.5	17
<i>Zostera</i>	<i>novazelandica</i> Setchell	-9.6	1					17
<i>Zostera</i>	<i>noltii</i> Hornem.	-11.6	2	0.5	-12.0	-11.3	0.7	17
<i>Zostera</i>	<i>japonica</i> Aschers. & Graebn.	-12.4	1					17
<i>Zostera</i>	<i>americana</i> den Hartog	-15.3	1					17
<i>Heterozostera</i>	<i>tasmanica</i> (Martens ex Aschers.) den Hartog	-11.7	3	0.8	-12.2	-10.8	1.4	17, 30
<i>Phyllospadix</i>	<i>torreyi</i> S. Watson	-13.1	3	2.2	-14.3	-10.5	3.8	3, 17
<i>Phyllospadix</i>	<i>scouleri</i> Hook.	-12.8	4	1.3	-14.4	-11.4	3.0	17
<i>Phyllospadix</i>	<i>serrulatus</i> Rupr. ex Aschers.	-15.4	3	2.7	-18.5	-13.6	4.9	17
<i>Phyllospadix</i>	<i>iwatensis</i> Makino	-15.8	1					17
<i>Phyllospadix</i>	<i>japonicus</i> Makino	-16.8	1					17
All species		-11.5	48	3.2	-20.8	-5.8	15	
All observations		-10.5	195	3.3	-23.8	-3.0	20.8	

to temperate regions seagrass $\delta^{13}\text{C}$ values tend to become more negative.

In the following sections we will address aspects of the physiology and the natural environment of seagrasses relevant to the distribution and variation in their $\delta^{13}\text{C}$ values.

PHYSIOLOGICAL CAUSES OF VARIABILITY

C_3 versus C_4 characteristics

The isotope signatures of terrestrial plants predominantly fall in 2 distinct non-overlapping ranges related to the carbon fixing pathway used. Plants with the C_3 pathway of photosynthesis which fix inorganic carbon (CO_2) to ribulose-1,5-bisphosphate by ribulose-1,5-bisphosphate carboxylase (Rubisco) have $\delta^{13}\text{C}$ values from approximately -35 to -20‰ , the typical value of terrestrial C_3 plants being around -28‰ (with atmospheric CO_2 at -7.8‰ as source; Smith & Epstein 1971, Boutton 1991). These negative values are determined by the relatively strong isotopic discrimination which can be exerted by Rubisco (29 to 30‰ with respect to the source CO_2 ; Roeske & O'Leary 1984, Guy et al. 1987). In contrast, $\delta^{13}\text{C}$ values of C_4 plants which fix inorganic carbon (CO_2 hydrated to HCO_3^-) to oxaloacetate range from -15 to -9‰ (Ehleringer & Osmond 1989). The primary carboxylase involved in these plants, phosphoenolpyruvate carboxylase (PEP carboxylase), shows an only limited discrimination against the heavy carbon isotope (O'Leary et al. 1981, Guy et al. 1989). Although the vast majority of seagrass isotope signatures fall in the range typical of C_4 plants, the current belief is that seagrasses in general have the C_3 type photosynthetic metabolism. This point of view is mainly based on the results obtained in ^{14}C pulse-labeling experiments, which give insight into the pattern of organic compounds formed after photosynthetic CO_2 incorporation. These studies have shown that nearly all seagrasses investigated (belonging to 8 different genera) show C_3 -like characteristics (Andrews & Abel 1979, Beer & Waisel 1979, Beer et al. 1980a, Benedict et al. 1980, Beer & Wetzel 1982), notwithstanding the fact that PEP carboxylase activity has been found in appreciable quantities in several seagrasses (Beer et al. 1980b). Pulse chase studies, however, showed that C_4 compounds accumulated in the tissues and apparently did not provide CO_2 to the second carboxylating step by Rubisco as occurs in terrestrial C_4 plants (Beer 1989).

Insufficient data are presently available to assess the significance of PEP carboxylase and other comparably functioning enzymes for carbon assimilation in seagrasses. Studies carried out in phytoplankton and

macroalgae show that even when inorganic carbon is mainly assimilated via Rubisco, other carboxylases may contribute to carbon fixation (Raven et al. 1990, Descolas-Gros & Oriol 1992). Perhaps these enzymes only play a significant role in carbon fixation in seagrasses during specific conditions. In the nitrogen-limited C_3 freshwater microalga *Selenastrum minutum* it was found that during nitrogen assimilation, discrimination against ^{13}C was greatly reduced (Guy et al. 1989). The authors attributed this to the increased importance of carbon fixation by PEP carboxylases, even exceeding carbon fixation by Rubisco under these conditions.

Discrimination affected by diffusion versus carboxylation

The CO_2 assimilated by terrestrial plants enters the photosynthetic cells after diffusion of the CO_2 from the air boundary layer surrounding the leaves through the stomatal pores. It has been widely noted that, besides fractionation caused by the carboxylation process, diffusive limitations may affect the isotope signature of the photosynthetic products. Several models have been developed to express isotopic discrimination; the general model of Farquhar et al. (1982, 1989) for discrimination in leaves of C_3 plants has gained wide acceptance:

$$\Delta = a \frac{p_a - p_i}{p_a} + b \frac{p_i}{p_a} \quad (1)$$

where Δ indicates the carbon isotope discrimination by the plant relative to the source carbon, a is the fractionation occurring due to diffusion of CO_2 in air through the stomatal pore, b the fractionation due to (Rubisco) carboxylation, and p_a and p_i are the ambient and intercellular (respectively) partial pressures of CO_2 .

The formula expresses the relative importance of isotopic discrimination associated with diffusion and with enzymatic carboxylation, in relation to ambient and internal concentrations of CO_2 . If p_i is small relative to the ambient partial pressure of CO_2 (which would occur when stomatal conductance is small in relation to the capacity for CO_2 fixation), Δ would mainly be determined by differential diffusion of the ^{13}C and ^{12}C in the gas phase through the stomatal pore (a , -4.4‰ , Craig 1954).

The characteristically depleted $\delta^{13}\text{C}$ values of C_3 plants indicate that diffusion normally does not severely limit the carbon supply to terrestrial plants. The situation in aquatic plants, however, is quite different. Diffusion rates of CO_2 in water are much slower than in air, being different by 4 orders of magnitude.

Each inorganic carbon molecule must cross a non-turbulent boundary layer by diffusion before entering the leaves, as even in turbulent or rapidly flowing water an unstirred layer envelops the leaves (Dainty 1963). Smith & Walker (1980), in their review of the effects of unstirred layers on photosynthesis, demonstrated that the dependence of photosynthetic rate on CO_2 concentration by charophyte cells was perfectly fitted by the Hill-Whittingham equation, which holds for a first-order enzymatic reaction supplied with substrate by diffusion through a resistance. They concluded that the unstirred layers around aquatic plant cells and organs represent a major factor limiting their rate of photosynthesis under natural conditions. Consistent with their conclusion, Wheeler (1980) showed that the rate of photosynthetic oxygen production by the giant kelp *Macrocystis pyrifera* increased by 300% when water flow over the thalli increased from 0 to 4 cm s^{-1} .

The occurrence of epiphytes on the thalli and leaves of aquatic macrophytes is a further impediment to the diffusional entry of CO_2 , as it results in an increased thickness of the unstirred boundary layer. As a consequence, the resistance to diffusional entry of CO_2 may rise conspicuously (up to 11.5-fold; Sand-Jensen et al. 1985).

Accordingly, the characteristically high $\delta^{13}\text{C}$ values of seagrasses are often thought to reflect primarily diffusional constraints on the carbon supply to the plant tissues relative to the demand for carbon fixation, which limits the expression of discrimination by Rubisco against ^{13}C . It is noteworthy that recently France (1995), in a survey of stable carbon isotope signatures of benthic and planktonic algae, found that the average $\delta^{13}\text{C}$ value for marine phytoplankton was -22‰ compared to -17‰ for marine benthic algae, a difference that was ascribed to the smaller boundary layer thickness of planktonic algae as compared to benthic algae. However, there is considerable evidence, discussed in the next section, that the use of bicarbonate may alleviate the carbon limitation which would be imposed on seagrass plants if they depended on the diffusion of dissolved CO_2 alone.

Use of bicarbonate in addition to dissolved carbon dioxide

HCO_3^- in open marine waters generally has a $\delta^{13}\text{C}$ value of ca 0‰ , which is considerably higher than that of dissolved CO_2 (ca -9‰ ; Kroopnick 1985). Fixation of bicarbonate-derived inorganic carbon relative to assimilation of dissolved CO_2 thus leads to less ^{13}C -depleted tissues. The limited ^{13}C -depletion of seagrass tissues probably is caused, to some extent, by the assimilation of HCO_3^- instead of, or besides CO_2 . Par-

ticularly the $\delta^{13}\text{C}$ values higher than the $\delta^{13}\text{C}$ of CO_2 dissolved in seawater would be difficult to explain without the assimilation of HCO_3^- . The use of HCO_3^- by seagrasses has been demonstrated in a series of species (Beer et al. 1977, Beer & Waisel 1979, Sand-Jensen & Gordon 1984, Millhouse & Strother 1986), and this faculty thus may be the rule in seagrasses. As yet, it is not certain whether there are exceptions: research on *Thalassia testudinum* first seemed to indicate that this species only uses dissolved CO_2 as a carbon source (Benedict et al. 1980, Abel 1984), but a recent study, in contrast, gives evidence for the utilization of HCO_3^- (Durako 1993). The use of HCO_3^- in aquatic plants is widespread, in marine macroalgae (Maberly et al. 1992) and also in freshwater macrophytes, where approximately 50% of the species tested had this capacity (Bodner 1994). A feature that has come out of the research on these aquatic plants is the variation that may exist within the same species with respect to HCO_3^- use, and which may also be the basis for the above mentioned conflicting data on *Thalassia testudinum*. HCO_3^- utilization is not a rigid process with fixed properties, but a dynamic one tuned to the needs of the organism (Prins & Elzenga 1989). HCO_3^- affinity, for instance, is highly variable in response to growth conditions and may be induced by low CO_2 concentrations (Sand-Jensen & Gordon 1986). Light conditions which reduce the demand for inorganic carbon may also influence the capacity for HCO_3^- uptake. In marine rhodophytes, transfer to low light conditions resulted in a change in the characteristics of inorganic carbon uptake (the initial uptake slopes being lower), and in decreased $\delta^{13}\text{C}$ values (Kübler & Raven 1994, cf. Kübler & Raven 1995), likely resulting from repression of the ability for HCO_3^- use. The capacity for HCO_3^- use, furthermore, may even be spatially different within the same plant. In the freshwater macrophyte *Ranunculus peltatus*, for instance, the submersed leaves are able to use HCO_3^- , but the emergent leaves only assimilate CO_2 (Sand-Jensen et al. 1992). The underlying reason for the natural variation in HCO_3^- utilization may be the fact that it is energetically expensive (Raven & Lucas 1985, Prins & Elzenga 1989); hence, the maintenance of this ability is only advantageous when the supply of CO_2 is limiting.

It is interesting that it appears to be possible to divide marine macroalgae into users and non-users of HCO_3^- on the basis of their $\delta^{13}\text{C}$ value: values below -30‰ (particularly widespread in rhodophytes) indicate plants relying on diffusive entry of CO_2 only, whereas those plants which also use HCO_3^- have values higher than -25‰ (Maberly et al. 1992, Raven et al. 1995). If a similar division also held for seagrasses, the implication would be that seagrasses under natural conditions are always HCO_3^- users, as all samples from field col-

lections so far have yielded values well above -25‰ (Fig. 1). The question thus would be not so much whether seagrasses are able to use HCO_3^- as source of inorganic carbon, but whether there are situations in which seagrasses *only* rely on diffusive entry of dissolved CO_2 for their carbon supply.

The availability of HCO_3^- as an additional source of inorganic carbon will raise the inorganic carbon concentration at the site of carboxylation. According to the general Eq. (1) this would enhance the possibilities for discrimination by Rubisco. The situation, however, is probably less straightforward due to the variable degree of back-diffusion of inorganic carbon out of the plant cell. In terms of a chemical reaction chain the general rule is that when reactions go to completion (all substrate is converted to product) there is no opportunity for isotopic fractionation to occur. When a reversible equilibration occurs before a unidirectional reaction, isotopic fractionation depends on the back-flow of the intermediate product versus the flow to the end product (for a more detailed discussion, see Peterson & Fry 1987). Accordingly, Sharkey & Berry (1985) linked the ratio of carbon backflow to carbon inflow with the discrimination of Rubisco in their model of discrimination in algae with a HCO_3^- transport mechanism. Applied to the marine environment, with the $\delta^{13}\text{C}$ of HCO_3^- in the water being around zero, the $\delta^{13}\text{C}$ values of HCO_3^- using algae would be determined by the ratio of carbon inflow and outflow according to the following equation (Surif & Raven 1990):

$$\delta^{13}\text{C} = d_{\text{rub}} \frac{F_3}{F_1} \quad (2)$$

where d_{rub} is the kinetic discrimination by Rubisco, F_1 is the unidirectional influx of HCO_3^- into the cells, and F_3 the unidirectional efflux of CO_2 out of the cells.

The ratio F_3/F_1 (indicative for the 'leakiness' of the plant cells) has been calculated to be in the range 0.5 to 0.8 for a number of macroalgae with $\delta^{13}\text{C}$ values between circa -15 and -22‰ (Surif & Raven 1990, Maberly et al. 1992). Following the same calculation model (Eq. 2), for HCO_3^- using seagrasses with a $\delta^{13}\text{C}$ of -10‰ the ratio would be considerably smaller (0.3). We are not aware of studies specifically addressing the 'leakiness' of seagrasses in relation to carbon isotope signatures. In several papers, however, the lacunal system of the leaves has been proposed to act as a CO_2 trap which promotes recycling of the internal CO_2 pool, whether derived from respiration or from the ambient water (Cooper 1989, Abal et al. 1994, Grice et al. 1996). According to the authors, enhanced lacunal development in the leaves (such as may occur when plants grow in anoxic sediments or at increased light intensities) would lead to a greater use of the internal pool of CO_2 , and consequently, to less isotopic discrim-

Table 2. Relative volume of the leaf lacunal system (% of total leaf volume) and $\delta^{13}\text{C}$ values in seagrasses. Data on leaf lacunal volumes (%) are derived from Larkum et al. (1989). Stable carbon isotope signatures from the various species (means of 1 to 8 observations) are derived from the compiled literature data set (see Table 1)

Species	Relative lacunal volume	$\delta^{13}\text{C}$
<i>Halophila ovalis</i>	2.7	-10.0
<i>Posidonia ostenfeldii</i>	4.8	-12.0
<i>Amphibolis antarctica</i>	16.0	-12.2
<i>Halodule uninervis</i>	25.6	-11.2
<i>Posidonia australis</i>	27.9	-9.9
<i>Zostera capricorni</i>	45.7	-10.8

ination. The enhanced recycling ascribed to the presence of the lacunal system would be the equivalent of reducing the 'leakiness' of the plant cells, and, hence, the flux F_3 in Eq. (2). A comparison across species, however, does not support the importance of the lacunal system in determining the $\delta^{13}\text{C}$ values of seagrasses: although the relative volume of the system in the leaves of various seagrass species may differ by more than an order of magnitude, the isotope values established so far in these species are in a similar range (Table 2). Therefore, apart from studies more specifically addressing the role of the lacunal system in restricting the efflux of inorganic carbon from the leaves, future investigations should also be focussed on the possibility that physiological or anatomical barriers restricting efflux of inorganic carbon may exist in the photosynthetic cells themselves.

Uptake of inorganic carbon from air and soil

Intertidal populations of the genus *Zostera* appear to have the capacity to absorb CO_2 from the atmosphere during emersion at low tides. Studies on *Z. muelleri* (Clough & Attiwill 1980) and *Z. marina* and *Z. noltii* (Leuschner & Rees 1993) showed that these species were able to maintain considerable rates of net photosynthesis during low tide exposure, as could be derived from measurements of CO_2 uptake. The use of 'light' atmospheric CO_2 (-7.8‰) instead of HCO_3^- was given as the most likely reason for the relatively ^{13}C -depleted character of biomass produced by periodically air-exposed populations of *Phyllospadix torreyi*, *Phyllospadix serrulatus* and *Zostera marina* (Cooper & McRoy 1988, Cooper 1989). Perhaps more likely, the presumably decreased diffusive boundary layer thickness during emerged conditions leads to an increased supply of CO_2 to the photosynthetic cells, allowing enhanced discrimination against

the heavy isotope. The periodical availability of atmospheric CO_2 nonetheless appears to have an only limited effect on the stable carbon isotope signature: both in the aforementioned studies on seagrasses and in an analysis of emergent macroalgae (Surif & Raven 1990), tidal emersion appeared to lower the $\delta^{13}\text{C}$ value by only 1 to 4‰.

Another potential source of inorganic carbon is the sediment, where mineralization processes generate CO_2 . The elaborate system of lacunae in seagrasses which interconnects roots, rhizomes and leaves is a pathway for gas movements within the plant (Larkum et al. 1989). Via the lacunae, the photosynthetic tissues aboveground thus potentially may be provided with CO_2 taken up by the roots. Alternatively, CO_2 derived from the sediment could be transported with the acropetal water transport from roots to leaves (Pedersen & Sand-Jensen 1993). Indeed, Wetzel & Penhale (1979) showed that ^{14}C -label from HCO_3^- , which was added to the root compartment of various species of seagrasses, was largely recovered in the water of the leaf compartment, indicating upward transport; a comparatively small amount was retained in the leaf tissue itself. No other studies on the use of carbon taken up by seagrass roots appear to have been published so far. Uptake of soil inorganic carbon has been established in some freshwater plants, allowing them to grow in water of low inorganic carbon content (e.g. Richardson et al. 1984), and in unusual fern allies, which lack stomata (Keeley et al. 1984, Sternberg et al. 1985). However, the assimilation of soil carbon is clearly more the exception than the rule in rooting plants (Agami & Waisel 1986, Enoch & Olesen 1993), and its occurrence in seagrasses remains doubtful.

ENVIRONMENTAL CAUSES FOR VARIABILITY

Source carbon

The isotopic signature of the inorganic carbon available for photosynthesis is of major importance in determining plant $\delta^{13}\text{C}$ values. As mentioned above, the $\delta^{13}\text{C}$ values of dissolved CO_2 and HCO_3^- in open marine waters are usually around -9‰ and 0‰ , respectively (Kroopnick 1985). In estuarine and other coastal environments which receive land run-off, however, considerably lower $\delta^{13}\text{C}$ values can be expected. The inorganic carbon pool in these areas is influenced by the input of ^{13}C -depleted carbon derived from the decomposition of terrestrial organic matter. Such inputs may account for an important part of the variability observed in seagrass $\delta^{13}\text{C}$ values. The best documented examples of the effect of land run-off on seagrass $\delta^{13}\text{C}$ values are provided by studies of sea-

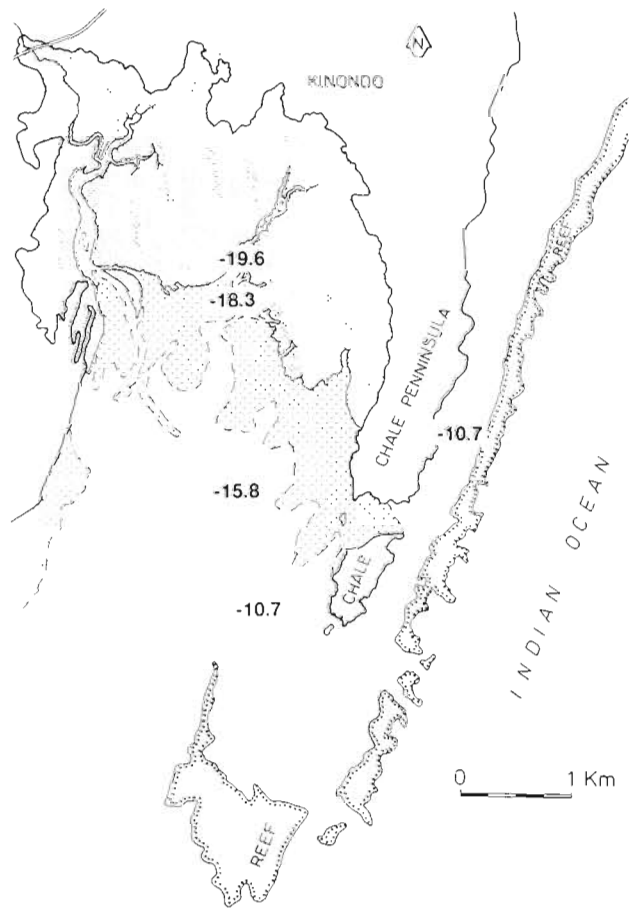


Fig. 3. Map of Gazi Bay (Kenya), showing variation of $\delta^{13}\text{C}$ values (means of 4 pooled leaf samples) of *Thalassodendron ciliatum* in relation to distance to the mangrove forest (heavy stippling). Light stippling indicates intertidal flats. Data from Hemminga et al. (1994)

grass meadows adjacent to mangrove forests. The $\delta^{13}\text{C}$ values of mangroves are generally in the range -30 to -24‰ (Rodelli et al. 1984, Rao et al. 1994), i.e. in the typical range of C_3 plants. Mineralization of mangrove litter will result in the formation of CO_2 with a $\delta^{13}\text{C}$ value close to the $\delta^{13}\text{C}$ values of living mangrove biomass, as little change in the isotopic composition seems to occur during senescence and decomposition (Zieman et al. 1984, Rao et al. 1994). This ^{13}C -depleted inorganic carbon, dissolved in the tidal water inundating the mangrove forest and subsequently captured by adjacent seagrass meadows, may result in comparatively ^{13}C -depleted seagrass isotope signatures. Data of Hemminga et al. (1994) show that seagrass $\delta^{13}\text{C}$ values rapidly become more negative (by as much as 9‰) with decreasing distance from a mangrove forest (Fig. 3). Other authors have also reported low values in mangrove areas compared to non-mangrove areas (Zieman et al. 1984, Fleming et al. 1990, Lin et al.

1991). Indeed, the most negative value reported for a seagrass until now (-23.8‰) was found in *Halophila beccarii* collected in a mangrove creek (McMillan et al. 1980). The magnitude of the mangrove input into the tidal water inundating the mangrove is subject to seasonal variations and tidal patterns (Rezende et al. 1990), but the effects of these phenomena on seagrass $\delta^{13}\text{C}$ values is not known.

Important riverine inputs of terrestrial materials can also affect the isotopic ratio of estuarine seagrasses as shown by Simenstad & Wissmar (1985). $\delta^{13}\text{C}$ values of *Zostera marina* were lower in meadows on an estuarine tidal flat than in the ones occurring in marine littoral areas. The difference in samples from both environments was higher in winter than in summer (ca 6‰ and 3‰ , respectively), probably reflecting a higher riverine discharge into the estuary during winter time.

Irradiance

It should be noted that in both models of discrimination formalized in Eqs. (1) & (2), the balance between inorganic carbon supply and inorganic carbon use can be expected to have a profound influence on the isotopic discrimination. In Eq. (1), the fixation of inorganic carbon will lower p_i relative to p_a , and in Eq. (2), back-diffusion of CO_2 (F_3) will be limited by fixation of this compound during photosynthesis. Hence, both models predict decreased isotopic fractionation during carboxylation by Rubisco under conditions of enhanced photosynthesis and growth. Evidence that light affects the $\delta^{13}\text{C}$ values of aquatic plants has been provided repeatedly. Wefer & Killingley (1986) found that specimens of the macroalga *Halimeda incrassata* growing in shallow waters were less depleted in ^{13}C than the ones growing in deeper waters. Fischer & Wiencke (1989) found a similar pattern in Antarctic algae growing along a bathymetric gradient. They observed that species growing in deep waters with low growth rates had values of about -30‰ while fast-growing algae occurring at shallower depths had much higher values (ca -10‰). Laboratory experiments with several species subsequently showed strong ^{13}C enrichments (10 to 25‰) with increasing photon flux rates (Wiencke & Fischer 1990). Furthermore, the $\delta^{13}\text{C}$ values of the rhodophyte *Palmaria palmata* showed a positive, linear correlation with irradiance in the range of 5 to $125 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, increasing from -18.4 to -12.5‰ (Kübler & Raven 1995).

Data on seagrasses also indicate that increasing irradiance leads to diminished isotopic discrimination. Cooper & DeNiro (1989) reported decreasing $\delta^{13}\text{C}$ values in entire leaves of *Posidonia oceanica* with increas-

ing depths, from -11‰ at 5 m depth to -16.4‰ at 35 m depth. No other factor but light attenuation could adequately explain these differences. The relation between $\delta^{13}\text{C}$ values and depth was most pronounced when the distal parts of the leaves were considered, but was lost for the leaf parts closest to the meristem. This was attributed to interference with the shading effect of the canopy. ^{13}C -depletion was also observed in response to experimental light reduction in beds of *Thalassia testudinum* (Durako & Hall 1992) and in mesocosmos experiments with *Zostera capricorni* (Abal et al. 1994). Most convincingly, Grice et al. (1996) recently reported that leaves of 5 seagrass species each showed increased $\delta^{13}\text{C}$ values when grown in seawater aquaria at higher light intensities. The $\delta^{13}\text{C}$ values, furthermore, were significantly correlated with productivity rates of the seagrasses.

The straightforward relation between light and stable carbon isotope signature in seagrasses which emerges from the currently published data, may prove to be more complicated when additional information becomes available. A reversed response of $\delta^{13}\text{C}$ to light intensity, for instance, has recently been described in 2 phytoplankton species grown in laboratory batch cultures (Thompson & Calvert 1995). Phytoplankton species may be able to increase the CO_2 concentration at the carboxylation site of Rubisco by actively taking up HCO_3^- when necessary (Raven & Johnston 1991). Thompson & Calvert (1995) pointed out that if uptake increased as a function of increasing irradiance but the carboxylation rate would not increase to a similar extent, the elevated levels of CO_2 at the carboxylation site could lead to intensified isotopic discrimination.

Isotopic discrimination may also be affected by a complex interaction between light intensity and daylength. The Antarctic/cold temperate macroalgal species *Adenocystis utricularis* showed no significant variation in $\delta^{13}\text{C}$ values in the irradiance range of 10 to $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ at a light/dark cycle of 6:18 h, whereas at a 18:6 h light/dark cycle $\delta^{13}\text{C}$ increased from ca -20 to -15‰ (Wiencke & Fischer 1990). Thompson & Calvert (1994), in a study of the diatom *Thalassiosira pseudonana* cultured in different light/dark cycles, found that discrimination was highest when the algae were cultured at cycles of 16:8 and 20:4 h. Furthermore, they found a highly significant interaction between daylength and light intensity associated with ca 4‰ of the variation in ^{13}C discrimination by the diatom. Differences in the activity ratios of the various carboxylases may be involved in these phenomena: Cosper (1982) and Descolas-Gros & Oriol (1992) found that in phytoplankton both daylength and irradiance intensity influenced the proportion of carbon fixed by β -carboxylation relative to that fixed by Rubisco carboxylation.

Temperature

In Fig. 2 we showed that a negative correlation exists between the $\delta^{13}\text{C}$ values of seagrasses and their corresponding geographical sampling latitude. Rau et al. (1989) found a similar relation for plankton in the South Atlantic Ocean and Weddell Sea. The authors attributed the highly significant decrease of $\delta^{13}\text{C}$ values with increasing latitude to the effect of temperature on CO_2 solubility and, hence, concentration in seawater. They calculated the CO_2 concentration in seawater ($\text{CO}_{2(\text{aq})}$) by considering the temperature-sensitive CO_2 solubility described by the equation:

$$\text{CO}_{2(\text{aq})} = a \times p\text{CO}_2$$

where a is the CO_2 solubility constant, which is a function of both salinity and temperature, and $p\text{CO}_2$ is the atmospheric partial pressure of CO_2 at sea level. $\text{CO}_{2(\text{aq})}$ increases with decreasing temperatures, being ca 2.5 times higher at 60°S than near the equator. According to the authors, the positive relationship between carbon availability and carbon isotope discrimination presumed in current models explains the lighter carbon composition of Antarctic plankton. However, laboratory experiments with phytoplankton cultures failed to reproduce such variations, although much wider ranges of $\text{CO}_{2(\text{aq})}$ were used (Thompson & Calvert 1994 and references therein, Thompson & Calvert 1995). Furthermore, macroalgae grown at a temperature range of 0 to 25°C did not show continuous isotope changes (Wiencke & Fischer 1990).

Lower temperatures, in principle, would tend to increase the $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC) in the seawater as ^{13}C -fractionation between atmospheric CO_2 and DIC increases with falling temperatures (ca 1‰ per 10°C ; Mook et al. 1974, Zhang et al. 1995). This tendency is not consistent with the latitudinal change in isotope signatures found in seagrasses and, obviously, cannot offer an explanation for it.

To the best of our knowledge, no specific information concerning temperature effects on the carbon isotopic composition of seagrasses is presently available, and therefore the observed relation between stable carbon isotope signatures and latitude cannot be explained with certainty. However, Durako & Sackett (1993) observed that *Thalassia testudinum* conspicuously reacted to experimentally manipulated levels of dissolved CO_2 , fractionation of the stable carbon isotopes increasing as a function of $\text{CO}_{2(\text{aq})}$. The effect of temperature on CO_2 availability in seawater thus seems to offer presently the most plausible explanation for the latitudinal trend in seagrass $\delta^{13}\text{C}$ values, but gradients in irradiance and daylength may also be involved. Irrespective of its cause, the relationship shown in Fig. 2 suggests that the balance between carbon availability

and carbon fixation in seagrasses shifts with geographical latitude, with a higher proportion of the inorganic carbon supplied to the carboxylation site fixed at lower geographical latitudes.

APPLICATIONS OF STABLE CARBON ISOTOPES IN SEAGRASS ECOLOGY

The limited use of stable carbon isotopes in studies of seagrass ecology is perhaps not surprising considering the fact that a number of factors potentially may cause variability in $\delta^{13}\text{C}$ values, whereas the impact of these factors often is ill-defined. A better understanding of this complex matter is certainly a prerequisite for further development of stable carbon isotope analysis as a tool in the study of seagrass ecology. Nonetheless, possibilities for applications already exist. In the following sections, the established application of stable carbon isotopes in food web studies is briefly addressed along with potential applications whose contours are emerging from the now available information.

Carbon flow in food webs

The $\delta^{13}\text{C}$ value of animals reflects the organic matter they assimilate, which has made stable carbon isotopes a valuable tool in the study of trophic relations. The pioneering work of Haines (1976a, b), who used stable carbon isotopes to elucidate the carbon flow in the salt marsh food web, was followed by a series of papers addressing trophic relations in seagrass ecosystems. Some of these studies indicated the use of seagrass derived carbon by fauna in the seagrass system (Fry & Parker 1979, McConnaughey & McRoy 1979, Fry et al. 1983, Harrigan et al. 1989), whereas others did not (Thayer et al. 1978, Fry 1984). As has frequently been pointed out, the use of isotope signatures to trace carbon flow is dependent upon there being clear and consistent differences in the isotopic composition of the various classes of primary producers. These conditions are not always met in seagrass systems. Fry et al. (1982), who studied Caribbean seagrass meadows, for instance, found that the isotopic values of a large proportion of the algal species sampled were in the same range as the dominant seagrass species, *Thalassia testudinum*; this prevented assessment of the role of this seagrass in the local food web on the basis of $\delta^{13}\text{C}$ only. Furthermore, the source carbon may show seasonal variation in its isotopic signature (Smith & Epstein 1971, Simenstad & Wissmar 1985). The scanty information available suggests that seasonal variation in seagrass $\delta^{13}\text{C}$ values may be rather limited (1 to 4‰ ; Thayer et al. 1978, Fry 1984, Stephenson et al. 1984),

but data collected in other aquatic ecosystems show much more variation (Boon & Bunn 1994), and preclude convenient assumptions that could be made. In addition, as discussed above, the isotopic signature of seagrasses may vary locally in response to environmental factors such as the depth-associated light gradient (2 to 6‰; Cooper & DeNiro 1989, Durako & Hall 1992). Obviously, the use of stable carbon isotopes for the study of carbon flow in food webs has its own pitfalls and limitations. The analysis of other stable isotopes besides carbon (nitrogen, sulphur) can significantly improve the possibilities of unravelling trophic relations (Peterson & Howarth 1987), but appears not to have been attempted in seagrass systems.

Temporal changes in seagrass functioning

As has been described by Tomlinson (1974) and more recently by Duarte et al. (1994), seagrasses grow by the reiterative development of 3 types of modules: rhizome internodes (which lead to extension of the plant in vertical or horizontal directions), leaves and roots. Leaves develop from meristems at the rhizome apices. After leaf senescence and subsequent loss, the insertion points of the leaves on the rhizome remain identifiable as scars (nodes). Due to seasonally changing environmental conditions, the internodes along rhizomes often display regular patterns of alternating waves of short and long internodal lengths (Duarte et al. 1994). In a species such as *Posidonia oceanica*, the leaf sheaths remain attached to the rhizome long after the leaf blades have been fallen from the plant. Sheath thickness in this species also shows seasonal variation (Boudouresque et al. 1983). The seasonal signals detected in internodal lengths or in sheath thickness allows absolute dating of rhizome pieces of many seagrass species, up to several decades BP. The rhizomes thus present a fascinating historical archive allowing the present-day researcher to draw conclusions on e.g. primary productivity and aspects of population dynamics in past years (Duarte et al. 1994, Pergent-Martini & Pergent 1994). Stable carbon isotope data on rhizome time series may provide additional information on seagrass functioning. For instance, in *Thalassodendron ciliatum* growing in a Kenyan lagoon we observed that the first-formed (often 3 to 5 yr old) parts of the vertical rhizomes bearing the leaf clusters had more depleted $\delta^{13}\text{C}$ values (ca -13‰) than the parts formed in subsequent years (ca -10‰) (Slim & Hemminga unpubl.). These data suggest that growth of young shoots is light-limited (the reduced carbon demand relative to the carbon supply allowing a higher level of isotopic discrimination), and explain the extended length of the first (oldest) internodes which serve to bring the young

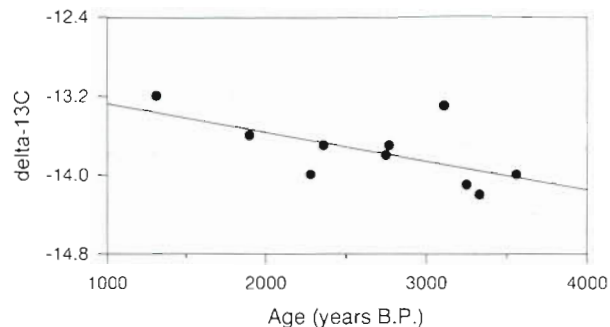


Fig. 4. $\delta^{13}\text{C}$ values of samples taken from a *Posidonia oceanica* matte (Medes Islands, Spain) as a function of their corresponding radiocarbon age (yr BP). The regression line fitted to the data ($r = 0.62$, $p = 0.054$) is described by the formula: $\delta^{13}\text{C} = -12.981 - 0.00029 \text{ age}$. Dotted lines indicate 95% confidence interval

leaf cluster rapidly to canopy height where high light conditions prevail.

Stable carbon isotope signatures may be informative over longer time scales as well. In the case of *Posidonia oceanica* it has been established that dead sheaths and rhizomes decompose very slowly in the sediment and may persist for millennia, forming a so-called 'matte' (Boudouresque et al. 1980, Romero et al. 1994, Mateo et al. 1996). Fig. 4 shows data from one of us (M.A.M.) on the $\delta^{13}\text{C}$ values of samples taken along a depth profile of such a 'matte' near the Medes Islands (Spain) in the Mediterranean. The absolute age of the samples was established by dendrocalibrated ^{14}C -radiocarbon dating. The samples cover a period of nearly 2500 yr, but their $\delta^{13}\text{C}$ values only differ by 1‰ or less. This uniformity in $\delta^{13}\text{C}$ values strongly suggests stable functioning (similar growth rates) of this *Posidonia* population over thousands of years. The very slight (but significant) increase in $\delta^{13}\text{C}$ values with decreasing age of the samples may point to a slow change in the meadow environment, e.g. seawater temperature or irradiance, affecting the balance between carbon supply and carbon use, or to preferential degradation of ^{13}C -rich organic compounds.

Use of ^{13}C as an experimental tracer

Plants can be exposed, both in the laboratory and in the field, to an environment enriched in ^{13}C -inorganic carbon. ^{13}C subsequently can be used as a tracer of carbon flow in the clonal structure of the plant. Earlier studies have shown the potential usefulness of ^{13}C for the assessment of carbon allocation in individual terrestrial plants (Mordacq et al. 1986, Svejcar et al. 1990). Recently, the proportional allocation of photosynthetically-fixed carbon to the roots and shoot sys-

tem of the salt marsh halophyte *Spartina anglica* was measured in the natural field situation after exposing leaves and stems briefly (1 h) to CO₂ enriched in ¹³C (Hemminga et al. 1996). Adaptations of this procedure may also be used in studies of seagrasses. Durako & Sackett (1991) perfused a seawater medium in cylinders containing seedlings of *Thalassia testudinum* with gaseous CO₂ strongly depleted in ¹³C ($\delta^{13}\text{C} = -49.6\text{‰}$); this resulted in plants with an overall $\delta^{13}\text{C}$ of -57.1‰ . Similarly, it can be expected that short-term perfusion of an ambient medium with ¹³C-enriched CO₂, or incubation with dissolved ¹³C-enriched bicarbonate, will lead to ¹³C-enrichment of growing plant structures. We expect that, in this way, it will be possible to study patterns in carbon allocation in seagrasses, for instance in relation to changing environmental conditions.

¹³C may possibly also be useful for short-term measurements of photosynthesis. Studies with phytoplankton have shown that there is a good agreement between primary productivity measurements carried out with the conventional ¹⁴C method and with ¹³C (Slawyk et al. 1984, Mousseau et al. 1995). The advantage of the use of ¹³C above that of ¹⁴C is obviously that its use requires no specific safety precautions or permission, and, in principle, can be used without environmental risks. The investigator, however, should be aware of the discrimination against ¹³C, which in certain situations, e.g. when rates of incorporation in specific organic molecules are studied, could complicate the interpretation of the results. A second, more general, caveat with respect to the use of ¹³C as an experimental tracer is that such work should be clearly spatially separated from studies on natural isotopic abundance (both in the field and in the laboratory) to avoid erroneous sample values.

FINAL REMARKS

As we have shown in this review, the range of seagrass $\delta^{13}\text{C}$ values is substantial, but considerably smaller than that of other aquatic macrophytes. In comparison to freshwater plants the difference will be caused by the fact that the source inorganic carbon in the marine environment, in general, is less variable than in lakes and streams. Why the range is less in comparison to marine macroalgae can only be a matter of speculation; it may be related to the widespread use of HCO₃⁻ as a source of inorganic carbon along with dissolved CO₂. Equally intriguing is the fact that seagrasses typically have a high $\delta^{13}\text{C}$ of -10‰ . This clearly points to a characteristic set of determining physiological, anatomical and perhaps environmental features shared by all seagrasses. If we assume that the balance between carbon supply and carbon fixation is

a key element in the process of isotopic fractionation, it suggests that inorganic carbon taken up from the environment normally is fixed to a relatively large extent, restricting discrimination by Rubisco. It is as yet doubtful whether the lacunal system is an anatomical feature relevant in this respect. Obviously, elucidating the discriminating properties of seagrasses will go hand in hand with clarification of functionally important aspects of carbon use and dynamics in these marine plants.

Based on the now available information, the 3 most significant environmental factors inducing variation in seagrass $\delta^{13}\text{C}$ values appear to be, in order of decreasing importance: source carbon, irradiance and temperature. However, other factors which may also induce variation, because they are expected to influence the balance between carbon availability and carbon fixation rates (e.g. ambient water flow and nutrient levels, Abel & Drew 1989), have not been specifically investigated until now.

The various environmental factors that influence the stable carbon isotope signature in seagrasses can be considered both a limitation and an asset for studies of seagrass ecology. Spatial and temporal variability in seagrass $\delta^{13}\text{C}$ values restricts, for instance, the use in food web studies. On the other hand, as we have illustrated, the integrated information reflected in seagrass stable carbon isotope signatures can be used in support of the reconstruction of environmentally related growth dynamics on the scale of individual shoots or on that of entire populations.

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