1 **Specific growth rate as a determinant of the carbon isotope composition**
2 **of the temperate seagrass *Zostera marina***

3
4 RUNNING HEADING: Specific growth rate determines seagrass $\delta^{13}\text{C}$

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Abstract

The seasonal variability of specific growth rate and the carbon stable isotope ratio ($\delta^{13}\text{C}$) of leaf blades ($\delta^{13}\text{C}_{\text{leaf}}$) of a temperate seagrass, *Zostera marina* (within 10 days old) were measured simultaneously, together with the $\delta^{13}\text{C}$ of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) at three sites in the semi-closed Akkeshi estuary system, northeastern Japan, in June, September, and November 2004. The $\delta^{13}\text{C}_{\text{leaf}}$ ranged from -16.2 to -6.3‰ and decreased from summer to winter. The simultaneous measurement of the $\delta^{13}\text{C}_{\text{leaf}}$, growth rate, and morphological parameters (mean leaf length and width, mean number of leaves per shoot, and sheath length) of the seagrass and $\delta^{13}\text{C}_{\text{DIC}}$ in the surrounding water allowed us to compare directly the $\delta^{13}\text{C}_{\text{leaf}}$ and specific growth rate of seagrass. The difference in the $\delta^{13}\text{C}$ of seagrass leaves relative to the source DIC ($\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$) was the least negative (-11 to -7‰) in June at all three sites and became more negative (-17 to -8‰) as the specific growth rate decreased. This positive correlation between $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ and specific growth rate can be used to diagnose the growth of seagrasses. $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ changed by $-1.7 \pm 0.2\text{‰}$ when the leaf specific growth rate decreased by $1\% \text{ d}^{-1}$.

KEYWORDS: Akkeshi, morphology, seagrass, specific growth, stable isotope, *Zostera marina*

1. Introduction

To diagnose the health of seagrass ecosystems, it is necessary to monitor the spatiotemporal variability of seagrass productivity. The carbon stable isotope ratios ($\delta^{13}\text{C}$) of seagrass leaf blades ($\delta^{13}\text{C}_{\text{leaf}}$) can be used to estimate the growth rate because there are spatial and temporal relationships between $\delta^{13}\text{C}_{\text{leaf}}$ and productivity: $\delta^{13}\text{C}_{\text{leaf}}$ is high in summer and low in winter (Anderson and Fourqurean, 2003; Vizzini et al., 2003; Fourqurean et al., 2005; Papadimitriou et al., 2005) and is high in shallow sites and low in deep sites (Cooper and DeNiro, 1989; Lepoint et al., 2003). Experimentally, Grice et al. (1996) found a positive correlation between the productivity ($\text{g m}^{-2} \text{d}^{-1}$) of four seagrass species and their $\delta^{13}\text{C}_{\text{leaf}}$ using irradiance-adjusted cultivation with a shading technique in the laboratory. However, no previous studies of seagrass have measured the growth rate directly in the field simultaneously with $\delta^{13}\text{C}_{\text{leaf}}$. Therefore, we examined this relationship *in situ*.

For phytoplankton, the specific growth rate (cell growth rate per number of cells per unit time) is usually used to analyze the relationship between growth rate and $\delta^{13}\text{C}$ (Laws et al., 1995; Korb et al., 1996; Riebesell et al., 2000; Sato et al., 2006). The difference in $\delta^{13}\text{C}$ between the substrate dissolved inorganic carbon (DIC) and phytoplankton biomass decreases consistently as the specific growth rate increases (Goericke et al., 1994) because the transport of DIC increasingly becomes the major rate-limiting step of the carbon assimilation process and the effect of isotope fractionation by photosynthetic reactions becomes less apparent as the growth rate increases. A similar mechanism is thought to be at work for seagrasses. We defined the specific growth rate of seagrass as the rate of increase of the photosynthetic tissue, i.e., leaf area, relative to the entire photosynthetic tissue per shoot per day. The growth rate of a seagrass species will

1 vary considerably, even among meadows within a bay. Moreover, seagrass
2 morphological traits such as leaf blade length or shoot weight change plastically within a
3 species according to environmental conditions (Krause-Jensen et al., 2000; Tanaka and
4 Nakaoka 2004). Therefore, both morphological traits and growth rates must be measured
5 to evaluate the seagrass specific growth rate.

6 The $\delta^{13}\text{C}$ of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) affect the $\delta^{13}\text{C}_{\text{leaf}}$ as the carbon
7 source for primary production. Previous studies used a $\delta^{13}\text{C}_{\text{DIC}}$ measured at a single point
8 in time as the possible carbon source (Hemminga and Mateo, 1996; Papadimitriou et al.,
9 2005). However, the $\delta^{13}\text{C}_{\text{DIC}}$ may vary temporally, especially in river mouths, because of
10 the variable contribution of freshwater DIC, which has a low carbon stable isotope ratio,
11 caused mainly by the changing tide and variation in river discharge volume. Snapshot
12 $\delta^{13}\text{C}_{\text{DIC}}$ data collected during the sampling period may not represent the average $\delta^{13}\text{C}_{\text{DIC}}$
13 over the growth period of seagrass leaves. To address this, the averaged $\delta^{13}\text{C}_{\text{DIC}}$ was
14 estimated using a mixing model that incorporated the riverine and oceanic end-member
15 DIC. The model was based on assumed relationships between precipitation over the
16 watershed and river discharge volume and between precipitation and daily averaged
17 salinity.

18 We examined the hypothesis that $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ of *Zostera marina* L. was
19 significantly correlated with their growth rate in the field conditions. We measured the
20 $\delta^{13}\text{C}_{\text{leaf}}$, seagrass productivity and $\delta^{13}\text{C}_{\text{DIC}}$ simultaneously. Instead of a snapshot $\delta^{13}\text{C}_{\text{DIC}}$,
21 the averaged $\delta^{13}\text{C}_{\text{DIC}}$ was estimated to obtain a better correspondence with the growth
22 period of seagrass leaves. Then, $\delta^{13}\text{C}_{\text{leaf}}$ was calibrated as $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$. The specific
23 growth rate of the seagrass was used as the index of productivity.

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2. Materials and Methods

2 Study sites

The study was conducted at *Zostera marina* seagrass beds in Lake Akkeshi and Akkeshi Bay, northeastern Japan. Hasegawa et al. (2007) and Yamada et al. (2007) have described the environment of the sites in detail. Three sites were selected between the mouth of the Beganbeushi River and Akkeshi Bay, including the semi-closed estuary Lake Akkeshi: B (43°03.9' N, 144°52.3' E), C (43°02.0' N, 144°52.9' E), and F (43°00.3' N, 144°51.5' E). Of the rivers flowing into Lake Akkeshi, more than 98% of the water volume is attributed to the mainstream and branches of the Beganbeushi River (Mukai *et al.*, 2002). Sites B (80 cm in depth from mean sea level) and F (70 cm) were shallower than site C (150 cm). Lake Akkeshi, which contained sites B and C, is usually frozen over from December to March. In 2004, the monthly averaged surface water temperature in Akkeshi Bay measured at 10:00 was highest in August (19.1°C) and lowest in February (0.7°C; Nakamura *et al.*, 2005).

Morphological traits and growth rates of seagrass

The growth rate of *Zostera marina* was measured using the hole-punch method (Dennison and Alberte, 1982) at sites B, C, and F in June and September 2004. At each site, 30 randomly selected shoots were punched. After approximately 10 d, we gathered the shoots and measured the growth and morphological traits: leaf blade length, leaf blade width, number of leaves per shoot, and sheath length. The growth in November 2004 was estimated using sheath length following Gaeckle et al. (2006) because we had difficulty punching or collecting sufficient marked shoots due to bad weather. For this purpose, more than 20 shoots were collected at each site. Determination coefficient of regression

analysis between sheath length and the growth rate of *Z. marina* in June and September 2004 at three sites was significant: $\text{growth (cm}^2 \text{ d}^{-1} \text{ shoot}^{-1}) = 0.21 \times \text{sheath length (cm)}$ ($r^2 = 0.67$, $n = 92$, $F = 179.9$, $p < 0.001$).

$\delta^{13}\text{C}$ of seagrass leaf blades

The $\delta^{13}\text{C}_{\text{leaf}}$ was measured at sites B, C, and F in June, September, and November 2004. At each site and on each occasion, two *Z. marina* shoots were collected. From each leaf blade on a shoot, several pieces 5 cm long were sampled. The age of each leaf piece was determined from the growth rate and morphological traits based on Hamamoto and Mukai (1999). Leaf pieces that were < 10 days old were then selected, except for site B in November (17.1 and 18.6 d for each shoot), for which the growth rate was low compared to the other sites and occasions. Epiphytes were removed gently from the seagrass leaves using a soft toothbrush. The seagrass samples for stable isotope analysis were stored at -20°C

The pieces of seagrass leaves were dried at 60°C for 24 h. The dried leaves were exposed to the vapor of 12 N HCl for 10 h to remove inorganic carbon. The excess HCl was then removed from the leaves in a vacuum, and the leaves were ground into fine powder. The $\delta^{13}\text{C}$ were determined using an elemental analyzer connected on-line to an isotope-ratio mass spectrometer (Thermo-electron, FLASH EA-Conflo III-DELTA^{plus} XP).

The variation in the $\delta^{13}\text{C}_{\text{leaf}}$ was tested using mixed-model, hierarchical analysis of variance (ANOVA; at a significance level of $\alpha = 0.05$) among the three sites, three occasions, and two shoots (Table 1), where site and season were adopted as fixed factors, shoot was the nested-block (random) factor, and $\delta^{13}\text{C}_{\text{leaf}}$ was the dependent variable. Prior

1 to ANOVA, the heterogeneity of variance was tested using Cochran's *C*. When a
2 significant interaction was detected by the ANOVA, multiple comparison of means was
3 conducted among nine levels (three sites times three seasons) using Scheffe's *F* test (at a
4 significance level of $\alpha = 0.05$).

6 **Estimating average $\delta^{13}\text{C}_{\text{DIC}}$ during the period when the sample leaf pieces formed**

7 The salinity at sites B and C was measured using a data-logged salinity meter
8 (Compact CT, Alec Electron, Kobe, Japan) in September 2005. The snapshot salinity at
9 average tide level was measured using a manual refractometer (S/Mill, ATAGO, Itabashi,
10 Japan) in November 2004 and June, September, and November 2005.

11 To measure the DIC concentration, surface water samples were collected in
12 30-mL serum vials at five sites along the river (site 1, 43°08.0' N, 144°51.2' E, site 2,
13 43°06.4' N, 144°53.5' E, site 3, 43°07.5' N, 144°47.2' E, site 4, 43°05.8' N, 144°51.7' E,
14 site 5, 43°04.6' N, 144°50.3' E,) and four sites in the lake and bay (sites B, C, E, and F) in
15 September 2005. Immediately, 0.2 mL of saturated HgCl_2 solution was added, and the
16 vials were sealed with butyl-rubber septa without trapping air bubbles inside. Surface
17 water samples for $\delta^{13}\text{C}_{\text{DIC}}$ were collected in the same manner in September and
18 November 2004 and June and September 2005.

19 DIC concentration was measured using a TOC 5000 (Shimadzu). The $\delta^{13}\text{C}_{\text{DIC}}$ was
20 determined according to Miyajima et al. (1995) using gas
21 chromatography-combustion/isotope ratio mass spectrometry (GC-C-IRMS;
22 Thermo-electron, GC6890-Combustion III -DELTA^{plus} XP).

23 The average $\delta^{13}\text{C}_{\text{DIC}}$ for the growth periods was estimated using the snapshot
24 $\delta^{13}\text{C}_{\text{DIC}}$, salinity, and some assumptions concerning the relationships among the

1 precipitation, salinity, and $\delta^{13}\text{C}_{\text{DIC}}$ at the study sites. The seagrass leaf meristem is located
2 at the base of the sheath (Dennison, 1990). Because we sampled the leaf blade parts above
3 the sheath, we need to consider the time required for the sample leaf piece to grow
4 through the sheath. We assumed that it took approximately 20 days for this to occur, and
5 the age of the sample leaf blade was within 10 d. Hence, the average salinity during the 30
6 d before sampling was estimated from the precipitation data.

7 The residence time of precipitation was determined from the size and geological
8 condition of the drainage area. We determined how many days we needed to sum
9 precipitation (Japan Meteorological Agency 2006) over the watershed to obtain the best
10 correlation with the actual discharge at the mouth of the Bikanbeushi River (Mukai *et al.*
11 2002), the major freshwater source for the study sites. Determination coefficient of
12 regression analysis was highest between discharge of the Bikanbeushi River and the sum
13 of the precipitation over the watershed for 5 d before the water volume measurement ($r^2 =$
14 0.57, $n = 32$, $F = 40.4$, $p < 0.001$).

15 The average salinity at sites B and C was estimated using the regression analysis
16 between the sum of the precipitation and the daily-averaged salinity. The averaged
17 salinity at the sites showed a negative linear correlation with the amount of precipitation
18 over the watershed during 5 d before the salinity measurement: site B ($r^2 = 0.67$, $n = 8$, F
19 $= 12.4$, $p < 0.012$) and site C ($r^2 = 0.49$, $n = 15$, $F = 12.7$, $p < 0.003$). These data show that
20 the river discharge and freshwater delivery to sites B and C could be predicted from the
21 precipitation over the watershed integrated for the previous 5 d.

22 The $\delta^{13}\text{C}_{\text{DIC}}$ of the water surrounding the seagrass beds is thought to be explained
23 by the mixing of the riverine and oceanic end-member DIC (Equation (1)).

$$\delta^{13}\text{C}_{\text{siteX}} = \delta^{13}\text{C}_{\text{ocean}} * \frac{C_{\text{ocean}} * R_{\text{siteX}}}{(C_{\text{ocean}} * R_{\text{siteX}}) + (C_{\text{river}} * (1 - R_{\text{siteX}}))} + \delta^{13}\text{C}_{\text{river}} * \frac{C_{\text{river}} * (1 - R_{\text{siteX}})}{(C_{\text{ocean}} * R_{\text{siteX}}) + (C_{\text{river}} * (1 - R_{\text{siteX}}))} \quad (1)$$

where $\delta^{13}\text{C}_{\text{siteX}}$ represents the $\delta^{13}\text{C}_{\text{DIC}}$ (‰) at site X, C_{ocean} is the concentration of DIC (μM) for the oceanic end-member, C_{river} is the concentration of DIC (μM) for the riverine end-member, and $R_{\text{siteX}} = \frac{\text{salinity at siteX}}{\text{salinity of oceanic endmember}}$.

The salinity of the oceanic end-member was assumed to be 32.5 based on direct observations and a previous report (Fisheries Research Agency [FRA] 2006). The oceanic and riverine end-member $\delta^{13}\text{C}_{\text{DIC}}$ were assumed to be identical to those measured at sites F and 1, respectively, and the averaged value for the three occasions was used. Unfortunately, we did not determine the $\delta^{13}\text{C}_{\text{DIC}}$ for these sites in June 2004. We substituted the values for June 2005 when the monthly accumulated precipitation and average tide level were almost identical: 49 and 45 mm and 187.6 and 189.3 cm from the base point, respectively (Japan Meteorological Agency 2006). The end-member DIC concentrations were assumed to be constant and identical to the DIC concentrations measured at sites F and 1 in September 2005 throughout the study period.

Comparison of $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ and the specific growth rate of *Z. marina*

To eliminate the possible effect of the substrate DIC, the averaged $\delta^{13}\text{C}_{\text{DIC}}$ at each site was subtracted from $\delta^{13}\text{C}_{\text{leaf}}$, and the difference was assigned as $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$. The regression between the specific growth rate of the seagrass and $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ was tested to quantify their relationship. We defined the seagrass specific growth rate as the increase in photosynthetic tissue, i.e., leaf area, relative to the total photosynthetic tissue per shoot per day.

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2 **3. Results**

3 **Morphological traits and growth rates of seagrass**

4 The morphological traits of *Zostera marina* differed obviously among sites and
5 seasons (Table 2). At site B, which was located at the river mouth in shallow water (80
6 cm), the variation in the mean longest leaf blade length of a shoot (MSL) from June to
7 November was small (45.2–63.3 cm), and the mean leaf blade width (MLW) was always
8 smallest (0.4–0.6 cm) among the three study sites. At site C, which was located at the
9 center of the lake at a depth of 150 cm, the MSL showed large seasonal variation
10 (98.9–157.5 cm), with a maximum in September. Both MSL and MLW at site C were the
11 largest among the three study sites. September was also the peak season for the MSL
12 (108.1 cm) at the outer site F (water depth 70 cm). At site F, the MSL was lowest in June,
13 whereas it was lowest in November at the other two sites. MLW and MSL roughly
14 paralleled each other in all seasons at the study sites. The number of leaves per shoot
15 ranged from three to five, irrespective of site and season.

16 The growth rate (rate of leaf area increase) of the seagrass leaves at the three sites
17 (B, C, and F) was generally high in June and low in November, although the pattern of
18 decrease differed from site to site (Table 3). Large growth per shoot was observed at the
19 site with larger MSL.

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21 **$\delta^{13}\text{C}$ of seagrass leaf blades**

22 Large seasonal and spatial changes in $\delta^{13}\text{C}_{\text{leaf}}$ were observed (–16.2 to –6.3‰; Fig.
23 [1](#)). The variation in $\delta^{13}\text{C}_{\text{leaf}}$ among seasons was significant, with significant site $\times$ season
24 interaction (Table 4). The $\delta^{13}\text{C}_{\text{leaf}}$ was highest in June at all of the sites. It was lower in

1 November at sites B and F, whereas it was lowest in September at site C. The variation
2 among shoots was also significant.

3 4 **Estimating average $\delta^{13}\text{C}_{\text{DIC}}$ during the period when the sample leaf pieces formed**

5 The changes in salinity at the sites reflected the influence of river discharge. The
6 average and range of salinity (practical salinity unit) in September 2005 was 20.9
7 (3.6–30.7) at site B and 29.1 (21.3–32.4) at site C. Unfortunately, we did not have
8 continuous salinity monitoring data for the outer site (F), but snapshot samplings at the
9 site indicated that there was little variation in salinity (32–34). Site B experienced a large
10 effect of river discharge. A freshwater effect was also obvious at site C, but was less
11 extensive compared to that at site B.

12 The DIC concentration in September 2005 was low in the river sites (1–5) and
13 high in the ocean sites (B–F). The total DIC at the outermost site (F) was three times
14 higher than that of Bekanbeushi River sites 1 and 2. The $\delta^{13}\text{C}_{\text{DIC}}$ was obtained on four
15 occasions in 2004 and 2005 (Table 5). It was low at the river sites and ranged from –9.9 to
16 –13.6‰, except near the river mouth at sites 4 and 5. Among the ocean sites, the $\delta^{13}\text{C}_{\text{DIC}}$
17 was generally low at the river mouth site (B) and increased toward the outermost site (F),
18 where a $\delta^{13}\text{C}_{\text{DIC}}$ typical of surface ocean water (+0 to +2‰) was always observed,
19 suggesting a negligible effect of freshwater DIC.

20 In November 2004 and September 2005, when both the $\delta^{13}\text{C}_{\text{DIC}}$ and salinity were
21 measured at each site, both determination coefficients of regression analyses were high
22 (for November 2004, $r^2 > 0.98$, $n = 5$, $F = 146.6$, $p < 0.01$, for September 2005, $r^2 > 0.99$, n
23 $= 5$, $F = 1211.68$, $p < 0.001$).

The estimated average salinity at site B (avg. $\pm$ SD) was 22.1 ± 1.6 in June, 22.7 ± 2.0 in September, and 23.1 ± 1.1 in November. The respective values at site C were 29.3 ± 0.4 , 29.4 ± 0.4 , and 29.5 ± 0.2 . Using these parameters, the $\delta^{13}\text{C}_{\text{DIC}}$ at site B was estimated using the mixing model as -0.2‰ in June, 0.0‰ in September, and 0.0‰ in November; the respective values at site C were 1.0 , 1.0 , and 1.0‰ , and the average at site F was 1.3‰ for all seasons. The range of $\delta^{13}\text{C}_{\text{DIC}}$ was 1.5‰ .

Comparison of $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ and the specific growth rate of *Z. marina*

Determination coefficient of regression analysis between $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ and the specific growth rate of *Z. marina* was significant ($r^2 = 0.39$; $n = 89$, $F = 56.7$, $p < 0.001$, Fig. 2). The $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ changed by $1.7 \pm 0.2\text{‰}$ when the leaf specific growth rate changed by $1\% \text{ d}^{-1}$. The $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ was less negative in June when the specific growth rate was high, whereas it was more negative when the specific growth rate was low.

4. Discussion

The simultaneous measurement of the $\delta^{13}\text{C}_{\text{leaf}}$, growth rate, and morphological parameters of *Z. marina* and $\delta^{13}\text{C}_{\text{DIC}}$ in the surrounding water allowed us to conduct an *in situ*, quantitative comparison of $\delta^{13}\text{C}_{\text{leaf}}$ and the specific growth rate of *Z. marina*. Our data revealed that $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ changed by $1.7 \pm 0.2\text{‰}$ when the leaf specific growth rate changed by $1\% \text{ d}^{-1}$. However, the determination coefficient was relatively weak ($r^2 = 0.39$) compared to previous laboratory studies (e.g., $r^2 = 0.86$: Grice *et al.* 1996).

The greatest outlier for $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ and the specific growth was site F in November (Fig. 3). Factors other than specific growth rate and $\delta^{13}\text{C}_{\text{DIC}}$ might be responsible at this site and occasion. Site F is the most open site, and the wind velocity is

1 rather strong in November. France and Holmquist (1997) demonstrated that a macroalga
2 in a flow-shielded habitat was ^{13}C -enriched relative to that in a high-water-motion
3 environment because the thick boundary layers restrict carbon exchange. At site F, a thin
4 boundary layer due to strong water movement might have caused the larger fractionation
5 resulting in the lower $\delta^{13}\text{C}$. When the November value for site F was excluded, the
6 determination coefficient of regression analysis was improved ($r^2 = 0.57$). In future, we
7 need to consider the variety of local environmental conditions.

8 As indicated by hierarchical ANOVA, the variation in $\delta^{13}\text{C}_{\text{leaf}}$ among shoots was
9 significant. Within a shoot, each leaf usually has a different growth rate (Gaeckle et al.,
10 2006). Similarly, within a genet, each shoot may have a different growth rate. The shoot
11 close to the horizontal meristem may have a higher growth rate compared to the older
12 shoot. Although the calculated age is similar, this possible difference in shoot age may
13 affect the $\delta^{13}\text{C}_{\text{leaf}}$. The growth rate and $\delta^{13}\text{C}_{\text{leaf}}$ were measured using different shoots in
14 this research. If these values were measured using the same sample, the determination
15 coefficient might improve.

16 There was a positive correlation between specific growth rate and $\delta^{13}\text{C}_{\text{leaf}}$. The
17 correlation was not as strong *in situ* as in laboratory studies, possibly because untargeted
18 factors such as the difference in water movement affect the $\delta^{13}\text{C}_{\text{leaf}}$. When the
19 contributions of these possible factors are evaluated in future studies, the growth rate
20 might be estimated from the $\delta^{13}\text{C}_{\text{leaf}}$ more precisely. Moreover, the relationship between
21 $\delta^{13}\text{C}$ and the growth rate might be used to reconstruct the growth history from a $\delta^{13}\text{C}$
22 analysis of the older parts of a shoot such as the older leaf parts or long-lived horizontal
23 rhizomes.

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15 **Figure legend**

17 | Fig. 1

18 | *Zostera marina*.

19 Seasonal changes in carbon stable isotope ratio of leaf blade at the three sites. Avg. $\pm$ S
20 D are shown. Different letters denote pairs of values where significant variations were d
21 etected by the multiple comparisons of means.

23 | Fig. 2 *Zostera marina*. Simple regression analysis between $\Delta\delta^{13}\text{C}_{\text{leaf-DIC}}$ and specific
24 growth rate, growth area (% d⁻¹) to whole leaf area.

Figure

Tanaka et. al Fig.1

Figure

Table 1. Structure of mixed model analysis of variance for sites, seasons and shoots.

Source of variation	<i>F</i> value
Site	$MS_{site} / MS_{shoot(site*season)}$
Season	$MS_{season} / MS_{shoot(site*season)}$
Site * Season	$MS_{site*season} / MS_{shoot(site*season)}$
Shoot (Site * Season)	$MS_{shoot(site*season)} / MS_{error}$

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Table 2. Morphological traits of seagrasses in the three sites and the three occasions.

Avg. ± SD are shown. Numbers in parentheses are sample sizes.
In cases the sample sizes equal 2, the range was shown.

		Longest leaf blade length of a shoot (cm)		Leaf blade width (cm)		Leaf No. shoot ¹		Sheath length (cm)	
Site B	June	63.3 ± 10.6	(20)	0.6 ± 0.1	(96)	4.8 ± 0.7	(20)	16.7 ± 3.1	(20)
	Sep.	50.9 ± 9.0	(14)	0.6 ± 0.1	(65)	4.6 ± 0.5	(14)	12.8 ± 2.3	(14)
	Nov.	45.2 ± 12.9	(30)	0.4 ± 0.0	(30)	3.0 ± 0.0	(2)	11.0 ± 2.6	(30)
Site C	June	122.5 ± 28.3	(12)	1.0 ± 0.1	(54)	4.5 ± 0.5	(12)	45.3 ± 11.7	(12)
	Sep.	157.5 ± 2.5	(2)	1.0 ± 0.0	(2)	4.0 ± 0.0	(2)	60.0 ± 6.0	(2)
	Nov.	98.9 ± 19.0	(25)	0.9 ± 0.1	(25)	4.3 ± 0.8	(7)	25.9 ± 5.0	(25)
Site F	June	66.1 ± 12.8	(22)	0.7 ± 0.1	(109)	5.0 ± 0.7	(22)	16.9 ± 6.0	(22)
	Sep.	108.1 ± 28.1	(22)	0.7 ± 0.1	(96)	4.4 ± 0.6	(22)	26.0 ± 7.4	(22)
	Nov.	89.9 ± 24.4	(19)	0.6 ± 0.1	(19)	4.0 ± 0.0	(2)	19.2 ± 4.5	(19)

Table 3. Growth area (cm² shoot⁻¹ d⁻¹) of seagrass in the three sites and the three occasions.
Avg. ± SD are shown.

	June	Sep.	Nov.
Site B	5.2 ± 1.6	3.1 ± 1.3	2.3 ± 0.5
Site C	11.5 ± 3.4	4.8 ± 1.1	5.4 ± 1.1
Site F	5.3 ± 1.7	4.6 ± 1.4	4.0 ± 0.9

Table 4. Carbon stable isotope ratios of seagrass leaf blade.
Mixed model analysis of variance for sites, seasons and shoots.

Source of variation	df	MS	<i>F</i>	p
Site	2	9.06	2.41	0.15
Season	2	165.38	43.91	< 0.001
Site * Season	4	32.61	8.57	0.004
Shoot (Site * Season)	9	3.92	17.85	< 0.001
Error	77	0.22		

Table 5 Total DIC and $\delta^{13}\text{C}$ (‰) of DIC at the nine sites

	Total DIC (mM)	$\delta^{13}\text{C}_{\text{DIC}}$ (‰)			
	Sep. 2005	Sep. 2004	Nov. 2004	June 2005	Sep. 2005
Site (1)	0.56	-11.9	-10.4	-12.6	-12.4
Site (2)	0.57	-12.1	-9.9	-12.4	-11.4
Site (3)	1.07	-13.6	-13.3	-13.5	-13.2
Site (4)	1.06	-13.3	-6.2	-13.9	-13.3
Site (5)	1.10	-8.1	-5.5	-12.1	-12.6
Site B	1.46	-2.7	-1.9	2.3	-2.2
Site C	1.87	-0.8	-0.7	1.5	0.6
Site E	2.00	-0.2	0.0	0.9	0.7
Site F	2.03	1.3	0.6	2.1	1.1