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FA: Fatty acid
FAME: Fatty acid methyl ester
FIA: Flow injection analysis
LCFA: Long chain fatty acid
MTBE: Methyl *tert*-butyl ether
SPOM: Suspended particulate organic matter
SSOM: Sediment surface organic matter

ABSTRACT

A current predominant paradigm emphasizes the role of epiphytic algae for invertebrates in most of seagrass food webs. However, in some intertidal *Zostera noltii* beds, epiphyte biomass is very low compared to microphytobenthos and seagrass biomasses. We assessed the role of microphytobenthos in a temperate intertidal *Z. noltii* bed by combining stable isotope and fatty acid (FA) analyses on primary producers, composite sources — suspended particulate organic matter (SPOM) and sediment surface organic matter (SSOM) — and the main macrofaunal consumers. *Z. noltii* showed high $\delta^{13}C$ (-9.9‰) and high 18:2(*n*-6) and 18:3(*n*-3) contents. Microphytobenthos was slightly more ^{13}C-depleted (-15.4‰) and had high levels of diatom markers: 14:0, 16:1(*n*-7)c, 20:5(*n*-3). Low mean $\delta^{13}C$ (-22.0‰) and large amounts of diatom and bacteria (18:1(*n*-7)c) markers indicated that SPOM was mainly composed of a mixture of fresh and decayed pelagic diatoms. Higher mean $\delta^{13}C$ (-17.9 ‰) and high amounts of diatom FAs were found in SSOM, showing that microphytobenthic diatoms dominate. Very low percentages of 18:2(*n*-6) and 18:3(*n*-3) in consumers indicated a low contribution of *Z. noltii* material to their diets. Grazers, deposit and suspension-deposit feeders had $\delta^{13}C$ close to microphytobenthos and high levels of diatom FAs, confirming that microphytobenthos represented the main part of their diet. Lower $\delta^{13}C$ and higher amounts of flagellate FAs – 22:6(*n*-3) and 16:4(*n*-3) – in suspension feeders indicated that their diet resulted from a mixture of SPOM and microphytobenthos. These results demonstrate that invertebrates do not consume high amounts of seagrass and highlight the main role of benthic diatoms in this intertidal seagrass bed.

KEY WORDS

Food web - Intertidal seagrass bed - Microphytobenthos - *Zostera noltii* - Stable isotope ratio - Fatty acid profiles – European Atlantic coast

1. INTRODUCTION

Seagrass beds are widespread in shallow coastal waters (Green and Short, 2003), where they are considered to be one of the most productive ecosystems (Duarte and Chiscano, 1999). This high productivity is not due to angiosperm production alone, even though it can be very high (in average more than 1000 $gC.m^{-2}.year^{-1}$ according to Duarte

and Chiscano, 1999), since algae are known to contribute significantly to total system production (Daehnick et al., 1992; Moncreiff et al., 1992; Borowitzka et al., 2006). Epiphytic algae can thus represent up to 62 % (Wear et al., 1999, but see Borowitzka et al., 2006 for a review) and microphytobenthic algae can represent up to 54 % (Lebreton et al., 2009 and reference therein) of the seagrass bed production. Organic matter sources that support seagrass food webs are still debated and, depending on sites, different sources have been described as the main drivers of the seagrass food web (Mateo et al., 2006). The current predominant paradigm, based on the analysis of subtidal seagrass beds, asserts that diverse micro- or macroalgal epiphytes attached to seagrass leaves are the main food source for macrofaunal invertebrates (Kitting et al., 1984; Moncreiff and Sullivan, 2001; Borowitzka et al., 2006; Mateo et al., 2006; Jaschinski et al., 2008) because they can reach high biomass (Borowitzka et al., 2006) and may provide better quality nutrients compared to seagrasses (Cebrián, 1999).

However, in intertidal *Zostera noltii* beds, widespread along European Atlantic coasts (Green and Short, 2003), this paradigm might not hold because epiphyte biomass has been shown to be very low (between 0.001 and 0.20 % of the leaf biomass according to Hootsmans et al., 1993; Philippart, 1995; Schanz et al., 2002; Lebreton et al., 2009) compared to the large amount of seagrass or microphytobenthos biomass, which is sometimes equal to leaf biomass (Asmus and Asmus, 1985; Guarini et al., 1998; Lebreton et al., 2009). Microphytobenthos plays an important role in most intertidal habitats, particularly in bare mudflats (Riera and Richard, 1996; Choy et al., 2008) but little is known about its trophic role in seagrass beds (Boschker et al., 2000; Moncreiff and Sullivan, 2001; Jaschinski et al., 2008).

Discriminating microphytobenthos and its potential importance in such ecosystems is technically challenging because of the high diversity and variability (qualitatively and quantitatively) of the different food sources. Seagrass ecosystems are fueled by numerous primary producers and composite food sources (e.g. SPOM, SSOM) which are themselves mixtures of primary producers and/or their degraded forms (Mateo et al., 2006). These primary producers and composite food sources both undergo seasonal changes in their characteristics which affect their quality and availability for consumers (Duarte, 1989; Borowitzka et al., 2006). Moreover, metabolic processes can affect fresh organic matter properties during its assimilation or degradation, complexifying trophodynamics understanding (Benner et al., 1987). Natural ratios of stable isotopes and FAs profiles of primary consumers are known to be generally close to those of their main food sources

(Sargent and Whittle, 1981; Fry, 2006). Although natural ratios of stable isotopes have been shown to be useful to identify trophic relationships, they often lead to ambiguous conclusions in such complex systems because the δ values of sources frequently overlap and are highly variable (Fry et al., 1987), particularly for benthic producers. Moreover, stable isotopes do not allow a reliable discrimination of bacterial material, since bacteria have isotopic ratios close to their substrate (Abraham et al., 1998; Boschker et al., 2000) and because isolation of bacteria for stable isotope analyses are practically impossible. Thus, the analysis of food web functioning requires additional complementary information to better discriminate food sources. More recent ecological studies that use a combination of stable isotope ratios and FA profiles have demonstrated the usefulness of these two tracers to improve the understanding of complex trophic relationships (Kharlamenko et al., 2001; Nyssen et al., 2005; Jaschinski et al., 2008). In our case, FA profiles can improve discrimination between microphytobenthos and *Z. noltii* sources because they can be distinguished based on their specific FAs, particularly (*n*-3) polyunsaturated FAs and saturated long chain FAs (Kharlamenko et al., 2001; Jaschinski et al., 2008). Moreover, FAs clearly discriminate bacteria due to the high specificity of prokaryotic FAs.

The present study was undertaken to determine the role of microphytobenthos in an intertidal seagrass bed in Marennes-Oléron Bay (France). Stable isotope ratios and FA profiles of major primary producers were characterized and the origin of composite food sources was determined. The fate of these resources was studied on six macrofaunal species, largely representative of the total trophic fluxes since they account for more than 90% of macrofaunal biomass (Lebreton, 2009). The comparison of stable isotope ratios and FA profiles in food sources and in dominant macrofaunal consumers allowed for the identification of the main trophic fluxes and highlighted the role of benthic diatoms.

2. MATERIALS AND METHODS

2.1 Study area

The study was carried out in a *Z. noltii* meadow in Marennes-Oléron Bay, a semi-enclosed system along the French Atlantic coast (45°54’N, 1°12’W) (Fig. 1). This macrotidal bay (tidal range 0.9 - 6.5 m), located between Oléron Island and the mainland, receives continental water mainly from the Charente River, which flows out into the eastern side of the bay. The studied seagrass bed is located on the western side of the bay,

along Oléron Island, where the bay is more strongly influenced by offshore marine waters (Dechambenoy et al., 1977). The studied *Z. noltii* bed extends over 15 km along the shore and is 1.5 km wide in the upper part of the flat, limited in its lower part by extensive oyster farm structures (Guillaumont, 1991).

The sampling station in the seagrass bed was located at about 250 m seaward its upper limit (Fig. 1). Fluctuations in the biomass of the different benthic food sources (*Z. noltii* and its detrital matter, epiphytes and microphytobenthos) and of macrofaunal consumers were studied previously in 2006 (Lebreton, 2009; Lebreton et al., 2009). The major feature of this seagrass bed is that epiphytes are exclusively composed of diatoms from the genus *Cocconeis* and have very low biomass, three orders of magnitude lower than that of microphytobenthos (Lebreton et al., 2009), dominated by a large diversity of small pennate diatoms (Bogaczewicz-Adamczak, unpublished data). The macrofaunal community is largely dominated by grazers, mainly represented by *Hydrobia ulvae* (71% of the biomass) as in many other seagrass beds (Jacobs and Huisman, 1982; Asmus and Asmus, 1985; Schanz et al., 2002) and *Littorina littorea* (6% of the biomass). Suspension feeders *Tapes phillipinarum* and *Cerastoderma edule* are much less abundant and account for about 13% of the biomass. *Scrobicularia plana* and *Arenicola marina* represent on average 7 and 0.4 % of the biomass, respectively (Lebreton, 2009).

2.2 Sample collection and preparation for isotope and FA analyses

Food sources and consumers were sampled in winter and summer 2006 for stable isotopes analyses and in winter and summer 2007 for stable isotope and FA profile analyses. All food sources (except SPOM in 2006) were collected in the middle of each season and consumers at the end of each season to account for the dynamic nature of the marker signal integration within tissues of consumers. Numerous studies have shown that consumer isotope signature or FA composition reflects the average isotopic or FA composition of the diet assimilated over periods of weeks to months (Tieszen et al., 1983; Shin et al., 2008). A 1 month time lag between ingestion of sources and their integration in invertebrate consumers' tissues appeared as a good compromise taking into account the size of the macrofaunal species studied here but also possible variations of source isotopic compositions. In 2006, SPOM was biweekly sampled (3 to 5 replicates per sample) to study intraseasonal variations. Epiphytes were not sampled in winter due to the absence of *Z. noltii* leaves.

Above-ground (leaves) and below-ground (roots) parts of *Z. noltii* were rinsed with tap water to remove detrital fragments. Coarse detrital matter from *Z. noltii* was collected for stable isotope analysis by sieving sediment on a 500 µm mesh sieve. Two types of detrital matter were collected by handpicking: fresh detrital matter, characterized by light brown colored pieces of rhizomes and roots, and degraded detrital matter, mostly composed of dark brown to black material. Samples were freeze-dried then ground to a fine and homogeneous powder using a ball mill. Samples were stored at -20°C for stable isotope analysis and at -80°C under a nitrogen atmosphere for FA analysis.

For SSOM analyses, surface sediment was sieved wet on a 315 µm mesh sieve, freeze-dried, ground using a mortar and pestle, and stored in the same manner as *Z. noltii* samples.

SPOM from surface water was sampled at mid-tide. A volume of seawater from 50 to 60 mL for stable isotopes and from 16 to 20 L for FA analyses was pre-filtered on a 200 µm mesh sieve to eliminate large zooplankton and detrital particles. Then water was filtered on precombusted Whatman GF/F glass fiber filters under moderate vacuum and stored in the same manner as other food sources.

The scraping methods generally used to collect epiphytes on seagrass leaves were not satisfactory owing to the very low biomass of epiphytes (Lebreton et al., 2009) and of the narrowness of *Z. noltii* leaves: from 0.5 to 2 mm width. The best separation method was found to be agitation (Cattaneo et al., 1995): leaves were first thoroughly rinsed with filtered seawater then vigorously shaken using a vortex mixer. The only epiphytes found on *Z. noltii* leaves in this seagrass bed were two species of epiphytic diatoms (*Cocconeis scutellum* and *Cocconeis placentula*) (Lebreton et al., 2009) so these diatoms were separated from organic and mineral detrital matter using a filtration method based on the narrow size spectrum of *Cocconeis spp*. diatoms (Round et al., 1990). The extracted solution was successively filtered on 48 and 10 µm mesh nets, then concentrated on 5 µm mesh polycarbonate membranes (Nucleopore, Whatman) under moderate vacuum. Membranes were carefully washed in filtered seawater, and epiphyte samples were concentrated by successive centrifugations (10 min., 1000 g), after checking the sample quality (state of cells, absence of detrital matter) under a microscope. Diatoms were then freeze-dried, homogenized and stored in the same manner as other food sources. Around 200 g of fresh leaves were necessary to obtain more than 50 mg of dry material, which was the minimal total mass for isotope and FAs analyses.

In the studied seagrass bed, mats of epipelic diatoms were very rare at the sediment surface. Nevertheless, surficial sediment was scraped and microphytobenthos was extracted in the laboratory following Riera and Richard (1996) and Herlory et al. (2007). Extracted samples were checked under a microscope for purity (samples exclusively composed by microalgae), then centrifuged, freeze-dried and stored in the same manner as other food sources.

Six dominant macrofaunal species (representing more than 90% of the total macrofaunal biomass) were sampled (Lebreton, 2009): two gastropod grazers (*H. ulvae* and *Littorina littorea*), two bivalve exclusive suspension feeders (*C. edule* and *T. phillipinarum*), one bivalve suspension-deposit feeder (*Scrobicularia plana*) and one annelid deposit feeder (*Arenicola marina*). These species were collected by sieving the sediment in the field and were kept alive for 36 hours in filtered seawater to allow gut content evacuation, before storage at -20°C or -80°C for stable isotope and FA analyses, respectively. Mollusks were removed from their shell, freeze-dried and ground using a ball mill. For stable isotope analyses, three entire individuals were analyzed (one small, one medium, one large) to be representative of the whole population, except for *H. ulvae*, which smallest individuals were pooled in groups of five individuals to obtain enough material. For FA analyses, 4 to 300 entire individuals per species, depending on their size, were pooled.

2.3 Stable isotope ratio analyses

When necessary, samples were acidified to remove carbonates: filters using HCl fumes, sediments using 1 $mol.L^{-1}$ HCl. Samples were then precisely weighed (± 0.001 mg) in a tin capsule for stable isotope analysis and were analyzed using an isotope ratio mass spectrometer (Isoprime Micromass, UK) coupled to an elemental analyzer (EuroVector EA 3024, Italy). Isotope values are expressed in the δ unit notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$) following the formula $\delta^{13}C$ or $\delta^{15}N = [(R_{sample/} R_{standard})-1]*10^3$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The analytical precision of the measurements was <0.15‰ and <0.2‰ for carbon and nitrogen, respectively.

2.4 Lipid extraction and FA analysis

Lipids were extracted from freeze-dried powders by a procedure modified from Folch et al. (1957). Samples were extracted 3 times with chloroform:methanol (1:2, 2:1 and 4:1, v/v). A volume of 1% NaCl solution was added and the mixture separated into 2 phases. The lower layer containing lipids was collected and water removed by addition of dry sodium sulfate. These extracts were stored in the dark at -26°C. Total lipids were quantified by flow injection analysis (FIA) using an HPLC system (Agilent Technologies) without column, coupled to an evaporative light-scattering detector (Polymer Laboratories).

Lipids were transmethylated by acid catalysis at 80°C for 2 h in H_2SO_4-methanol (4%, w/v) reagent (Christie, 1984) with addition of toluene (10%, v/v), to yield FA methyl esters (FAMEs). An internal standard (21:0) was added before transmethylation for the quantification of FAMEs. Water was added and FAMEs were extracted twice with a mixture of hexane:methyl *tert*-butyl ether (MTBE) 80:20 (v/v).

FAME purification was done in two steps. First, a Flash-LC was carried out on the HPLC fitted with a semi-preparative column (100 mm length * 10 mm ID) filled with a Puriflash Si-CN 60 µm phase (Interchim, France). A polarity gradient from 0.010 to 0.614, based on hexane:dichloromethane:methanol mixtures, allowed the separation of semi-polar compounds from non-polar compounds including FAMEs. Second, the HPLC was fitted with a semi-preparative HPLC column (250 mm length * 10 mm ID) filled with an Uptisphere Si-CN 5 µm phase (Interchim, France). A polarity gradient from 0.010 to 0.121, based on hexane:MTBE:acetone mixtures separated hydrocarbons and wax or sterol esters from FAMEs. The collected FAMEs were quantified by FIA as for total lipids before separating them by gas chromatography.

FA compositions were determined using a gas chromatograph (GC-6890N, Agilent Technologies) equipped with an automatic sampler and a J&W DB-23 capillary column (60 m × 0.25 mm ID × 0.25 µm film). Operating conditions were as follows: injector in split mode (1/20 to 1/40) at 240°C, 1 µL injected; detector FID at 260°C; carrier gas hydrogen in constant flow mode at an average linear velocity of 30 $cm.sec^{-1}$; linear temperature gradient from 100 to 240°C at 1°$C.min^{-1}$.

FAME identification was performed by comparing relative retention times with those of known standard mixtures: 37-FAME Mix, 26-BAME Mix, PUFA-2 and PUFA-3 (Supelco, Sigma Aldrich Chemicals). Equivalent chain lengths (Christie, 1988) were used as an aid in peak localization and identification. Each FAME area was corrected from the

corresponding FID response factor (Bannon et al., 1986) and from the difference in weight between the FAME and its corresponding free FA.

2.5 Data and statistical analyses

Isotope ratios of consumers and food sources were compared considering a trophic enrichment of 0.8‰ for $\delta^{13}C$ and of 2.5‰ for $\delta^{15}N$ (Vander Zanden and Rasmussen, 2001). FA results are expressed as the percent of each FA relative to the sum of all identified FAs. Only FAs with proportions higher than 0.004% were used in the data analysis. FA biomarkers were identified from published literature and, for trophic relation analysis, were associated with single groups of FA primary producer markers (vascular plants, diatoms, flagellates or bacteria) or considered as ubiquitous marker (Table 1). In order to enhance the discrimination between primary producers (*Z. noltii*, microphytobenthos, epiphytes), composite food sources (SPOM, SSOM) and consumers, $\delta^{13}C$ values were plotted versus values from each of these four groups of FA primary producer markers, as suggested by Nyssen et al. (2005).

Due to small sample size (replicate number almost always < 10) and non-independence of data within series, non-parametric procedures were used to achieve more robust statistics. Kruskal-Wallis tests were applied on isotopic data in order to compare the different food sources and consumers, and to test seasonal isotopic variations. These tests were followed by multiple comparisons of means using the pgirmess R package. Wilcoxon signed-ranks tests were used to identify differences in FA compositions of food sources and consumers among seasons. Hierarchical clustering analyses (Ward's method, Euclidean distance) were used to compare sources and consumers within seasons using the pvclust R package. Using this package, p-values were computed based on multiscale bootstrap resampling to determine the level of significance of identified clusters.

3. RESULTS

3.1 Stable isotope composition of food sources

Mean $\delta^{13}C$ of food sources ranged from -23.8 (SPOM, winter 2006) to -9.3‰ (roots, summer 2006) (Fig. 2A). Except for SPOM, temporal variations were low and, when statistical differences were observed, they do not allow detecting any seasonal pattern (Table 2). On an annual basis, some food sources exhibited large differences in their $\delta^{13}C$ values (Kruskal-Wallis test, $p < 0.001$, table 2). SPOM was always the most ^{13}C-depleted

except in summer 2007 (annual mean: -22.0‰) and was significantly different from *Z. noltii* material all year long (Table 2). In 2006, intraseasonal variations of SPOM $\delta^{13}C$ were observed in winter, with lower $\delta^{13}C$ at the end, but not in summer (Table 3). SPOM was much more ^{13}C-enriched in summer 2007 than in all other seasons, with values close to those of microphytobenthos. Alive or fresh detrital organic matter originating from *Z. noltii* showed the highest $\delta^{13}C$ values (from -10.4 to -9.7‰) but degraded detrital matter was slightly, but not significantly, more depleted in ^{13}C (annual mean = -12.8‰). SSOM (annual mean = -17.9‰) was significantly more depleted than *Z. noltii* leaves and roots. Microphytobenthos $\delta^{13}C$ values (annual mean = -15.4‰) were intermediate between those of SSOM and *Z. noltii* material and the $\delta^{13}C$ value of epiphytic diatoms (-12.8‰) was close to those of *Z. noltii* material.

Mean $\delta^{15}N$ of food sources ranged from 4.9 (leaves, winter 2006) to 10.5‰ (roots, summer 2007) and $\delta^{15}N$ values of *Z. noltii* material showed large standard deviations relative to $\delta^{13}C$ (Table 2). No seasonal pattern was observed even if some statistical differences could be observed between seasons (Table 2). On an annual basis, SSOM was more depleted in ^{15}N than *Z. noltii* material (Kruskal-Wallis test, $p = 0.012$).

3.2 Stable isotope composition of consumers

Mean $\delta^{13}C$ values of consumers ranged from -16.9 (*C. edule*, winter 2007) to -8.8‰ (*L. littorea*, summer 2007) (Fig. 2B). On an annual basis, grazers (*H. ulvae* and *L. littorea*) were the most ^{13}C-enriched ($\delta^{13}C$ ranging from -8.8 to -11.8‰). Grazers $\delta^{13}C$ were significantly different from these of suspension feeders (*C. edule* and *T. phillipinarum*), which were the most depleted organisms ($\delta^{13}C$ ranging from -16.9 to -13.9‰). $\delta^{13}C$ values of *L. littorea* were also higher than those of *A. marina* (deposit feeder) (Kruskal-Wallis test, $p < 0.001$). *S. plana* and *A. marina* showed $\delta^{13}C$ that were intermediate between suspension feeders and grazers: from -13.2 to -14.3‰ and from -12.6 to -14.0, respectively. All consumers except *A. marina* exhibited higher $\delta^{13}C$ values in summer than in winter, but no significant differences were observed. *L. littorea* and *C. edule* $\delta^{13}C$ presented large standard deviations (Fig. 2B, table 2).

Mean $\delta^{15}N$ values ranged from 7.6 (*T. philippinarum*, summer 2007) to 12.8‰ (*S. plana*, winter 2007) and, on an annual basis, *A. marina* presented $\delta^{15}N$ values higher than those of *H. ulvae*, *T. phillipinarum* and *C. edule* (Kruskal-Wallis test, $p < 0.001$). No clear seasonal pattern was observed for ^{15}N values (Table 2).

3.3 FA profiles of food sources

Eighty-one FAs were identified and used for food source data analysis. Within seasons, FA profiles of *Z. noltii* material were clearly distinguished from microphytobenthos, epiphytes, SSOM and SPOM (Fig.3).

Roots and leaves contained large quantities of two vascular plant FA markers in contrasting proportions (18:2(*n*-6) mean: leaves = 14.5%, roots = 35.3%; 18:3(*n*-3) mean: leaves = 47.7%, roots = 16.0%). *Z. noltii* was also enriched in some saturated long-chain FAs (LCFAs) such as 24:0 in roots (mean = 5.4%) and leaves (mean = 1.4%), as well as 26:0 (mean = 2.4%) and 28:0 (mean = 1.3%) in roots. Vascular plant FA markers thus represented, on an annual basis, 62.5% and 65.4% of total FAs in roots and leaves, respectively (Fig. 3). FAs profiles of *Z. noltii* roots and leaves were significantly different between winter and summer (Wilcoxon tests, leaves: $p < 0.0001$, roots: $p = 0.020$), with higher proportions of ubiquitous FAs in summer.

Microphytobenthos presented high proportions of diatom markers (mean = 34.8%), including large quantities of 20:5(*n*-3) (mean = 17.9%), 16:1(*n*-7)c (mean = 5.8%) and 14:0 (mean = 5.2%). Concentrations of typical flagellate FAs were also relatively high (mean = 6.9%), particularly those of 22:6(*n*-3) (mean = 1.9%) and 18:4(*n*-3) (mean = 1.1%) (Fig. 3). FAs profiles were different between winter and summer ($p = 0.020$) with a higher proportion of diatom FAs in winter.

FA composition in epiphytes was also characterized by a large proportion of diatom markers (21.2%), including 16:1(*n*-7)c (11.4%), 20:5(*n*-3) (3.3%) and 14:0 (3.9%). Percentages of flagellate FA markers were lower in epiphytes (3.7%) than in microphytobenthos (Fig. 3).

SSOM FAs profiles followed those of microphytobenthos at each season and were thus different between winter and summer ($p < 0.0001$). SSOM contained large proportions of diatom FA markers, with the highest percentage in winter (56.6%) compared to summer (33.6%). In contrast, proportions of vascular plant and bacterial FAs were higher in summer (vascular plants: summer = 6.2%, winter = 2.3%; bacteria: summer = 17.3%, winter = 6.2%).

SPOM profiles were dominated by ubiquitous FAs: 16:0, 18:0 and 18:1(*n*-9)c, representing on average 37.0% of the total (Fig. 3). High proportions of 16:1(*n*-7)c (mean = 7.7%), 20:5(*n*-3) (mean = 4.8%) and 14:0 (mean = 6.6%) FAs, all diatom markers, and large quantities of bacterial FAs (mean = 15.9%), including 5.3% of 18:1(*n*-7)c, were also

observed. Flagellate FA markers were rather low (5.3%). No difference of profile was observed between summer and winter ($p = 0.430$).

3.4 FA profiles of consumers

Seventy-height FAs were identified and used for consumer data analysis. Hierarchical clustering analyses allow discriminating 3 groups of consumers: 1. deposit feeders (*A. marina*), 2. suspension feeders (*T. phillipinarum* and *C. edule*) and 3. grazers and suspension-deposit feeder (*H. ulvae*, *L. littorea* and *S. plana*) (Fig. 4). Among seasons, FA profiles differed only for *C. edule* and *S. plana* (Wilcoxon-tests, *A. marina*: $p = 0.323$, *C. edule*: $p = 0.003$, *H. ulvae*: $p = 0.512$, *L. littorea*: $p = 0.482$, *S. plana*: $p = 0.045$, *T. philippinarum*: $p = 0.151$).

Both species of grazers were highly enriched in diatom markers (mean = 22.3%), including on average 15.1% of 20:5(*n*-3). Large proportions of flagellate markers were also observed (mean = 11.8%). Bacterial and vascular plant markers had low proportions, with on average 7.0 and 4.5% of total FAs, respectively.

S. plana FA composition was dominated by diatom markers (mean = 19.0%), particularly 20:5(*n*-3). Flagellate markers were the second most frequent marker (mean = 13.1%), followed by bacterial markers (mean = 7.1%) and vascular plant markers (mean = 1.8%).

Suspension feeders FA profiles were dominated by flagellate compounds, while percentages of diatom FAs were rather low (mean = 11.8%). Bacterial FA percentages were higher than in grazers and suspension-deposit feeders with on average 10.2% of total FAs, and the proportion of vascular FAs was very low, with percentages less than 2.0%.

A. marina had a characteristic FA profile in which bacterial FAs prevailed (mean = 23.9%), particularly the 18:1(*n*-7)c. Proportions of diatom markers (mean = 19.5%) were also high contrary to those of flagellate markers (mean = 10.0%). *A. marina* exhibited the lowest percentage of vascular plant markers (Fig. 4).

3.5 Two-dimensional analysis on food sources and consumers using $\delta^{13}C$ and FA profiles

Except for SPOM in summer 2007, $\delta^{13}C$ ratios discriminate relatively well among SPOM, SSOM, microphytobenthos and a fourth group composed of epiphytes and *Z. noltii* material. A great difference in FA composition occurred between *Z. noltii* material and all other food sources, including epiphytes, because *Z. noltii* has high quantities of vascular plant FAs while proportions of these FAs in other sources are very low (Fig. 5A).

In contrast, percentages of diatom marker FAs in every food sources were relatively high, except for *Z. noltii* material. Composite food sources (SPOM and SSOM) had also high proportions of diatom FAs, with the highest proportion in SSOM in winter 2007 (Fig. 5B). On all plots, SPOM and microphytobenthos samples from summer 2007 presented very similar composition. Relatively low percentages of flagellate and bacterial FAs were observed (Fig. 5C and 5D).

The low proportions of vascular plant FA markers in all consumers made it difficult to differentiate any particular consumer from the others (Fig. 6A). The highest proportion of vascular plant FA markers was observed in grazer tissues, particularly *L. littorea.* All consumers presented high proportions of diatom markers, except suspension feeders which were also slightly depleted in ^{13}C (Fig. 6B). Suspension feeders were relatively enriched in flagellate markers (Fig. 6C) whereas other consumers were depleted. *A marina* was very different from other species due to its enrichment in bacterial FAs.

4. DISCUSSION

4.1 Characterization of primary producers

Few isotope composition data of the microphytobenthos or epiphytic diatoms are available, probably due to technical difficulties in sampling. In this seagrass bed, isotope compositions of epiphytic diatoms are in the same range than those of Carlier et al. (2009). For microphytobenthos, values measured in the present study are well within the range of previous observations from other seagrass beds: from -18 to -11.5‰ (Dauby, 1995; Leduc et al., 2006; Kharlamenko et al., 2008) and from bare mudflats in Marennes-Oléron Bay (Riera and Richard, 1996; Malet et al., 2008). $\delta^{13}C$ values of *Z. noltii* roots, leaves and fresh detrital matter fractions were also similar to the values found in the literature for the same species (Boschker et al., 2000; Schaal et al., 2008).

Z. noltii roots and leaves, microphytobenthos and epiphytic diatoms are much better characterized by their $\delta^{13}C$ values and FA profiles than by their $\delta^{15}N$ values that largely overlap. However, $\delta^{13}C$ of *Z. noltii* material are sometimes close to those of epiphytes or microphytobenthos, particularly detrital fractions of *Zostera*, because phanerogam degradation leads to a ^{13}C-depletion likely due to higher levels of lignin which is ^{13}C-depleted (Benner et al., 1987). Nevertheless, alive or detrital *Z. noltii* material can be well distinguished from epiphytes and microphytobenthos by their FA profiles. Epiphytic diatoms and microphytobenthos present in fact large quantities of typical diatom FAs

such as 16:1(*n*-7)c, 14:0 and 20:5(*n*-3) (Viso and Marty, 1993; Dunstan et al., 1994; Bergé et al., 1995; Kharlamenko et al., 2008) and a very low proportion of FAs considered as markers of seagrass species. On the contrary, leaf and root FA profiles were typical of seagrass species with high levels of 18:2(*n*-6), 18:3(*n*-3) (Kharlamenko et al., 2001; Jaschinski et al., 2008) and of some LCFAs, particularly 24:0 in roots (Nichols et al., 1982; Khotimchenko, 1993; Viso et al., 1993).

The decaying process probably do not affect the vascular plant FAs in the same way: the LCFAs are very resistant to degradation (Nichols et al., 1982; Volkman et al., 1998) and can help identify decaying *Z. noltii* matter, especially in sediment. In contrast, 18:2(*n*-6) and 18:3(*n*-3) FAs are labile and degrade quickly (Nichols et al., 1982; Kharlamenko et al., 2001); they are therefore good markers of fresh *Z. noltii* material. These FA properties can consequently be used to characterize the presence of different decay stages during data analysis of composite food sources and consumers.

Epiphytic and microphytobenthic diatoms could probably be distinguished each other, due to their contrasting proportions of 16:1(*n*-7)c and of 20:5(*n*-3).

4.2 Discrimination and composition of composite food sources

The relative stability of stable isotope signatures and FA profiles of the primary producers throughout the year facilitated source identification using a dual-tracer approach. Nevertheless, some of them, such as *Z. noltii* (Auby and Labourg, 1996; Lebreton et al., 2009) or phytoplankton (Héral et al., 1987; Galois et al., 1996), exhibit large seasonal variations of their biomass, that can potentially affect the isotopic and FA composition of SPOM and SSOM.

Except in summer 2007, SPOM $\delta^{13}C$ were very similar to previous measurements in the same area (Malet et al., 2008). These signatures encompasses the full range of $\delta^{13}C$ between oceanic (-19.5 to -21.8‰) and estuarine SPOM values (-22.6 to -24.4‰) observed by Riera and Richard (1997) and classically encountered on an estuary-ocean continuum. This indicates that SPOM was composed by a mixture of material from both origins. Intraseasonal variation of $\delta^{13}C$ in winter is thus probably related with the more variable hydrodynamic conditions (Charente River floods, storms…). FA profiles indicate that SPOM was composed by a mixture of fresh and decaying diatoms (Galois et al., 1996) associated with bacteria, whereas flagellates remained low. There are thus relatively high levels of 20:5(*n*-3) and 16:1(*n*-7)c, which are markers of living diatoms

(Viso and Marty, 1993; Dunstan et al., 1994; Bergé et al., 1995), of 14:0, which presents a lower degradation rate than 16:1(*n*-7)c (Canuel and Martens, 1996), and of bacterial FAs (18:1(*n*-7)c). Even if 18:1(*n*-7)c has mainly a bacterial origin (Sargent et al., 1987; Volkman et al., 1998), it may also be present in different microalgae (Nichols *et al*., 1984; Volkman *et al*., 1989), suggesting that other groups than diatoms constitute also the phytoplanktonic assemblage. In summer 2007, there is evidence for high levels of resuspended microphytobenthos in SPOM samples because SPOM FA profile and $\delta^{13}C$ were very similar to those of microphytobenthos. This observation highlights the importance of high frequency samplings for a good monitoring of SPOM composition.

SSOM $\delta^{13}C$ values, intermediate between those of SPOM and those of benthic sources (i. e. *Zostera* material, microphytobenthos), showed that SPOM contributes significantly in SSOM composition. In seagrass beds, SPOM sedimentation may thus be enhanced because the seagrass canopy greatly weakens the hydrodynamism (Koch et al., 2006). SSOM FAs profiles, with high levels of diatom FAs and low levels of flagellate FA markers, demonstrate that SSOM was composed by a dominance of diatoms. This diatom material was present as living or fresh detrital matter since diatom FA markers are quickly degraded (Canuel and Martens, 1996). FA profiles of SSOM, which followed marked summer/winter difference of microphytobenthos FA profiles, confirmed that microphytobenthos largely contributes to SSOM composition. SSOM was thus mainly constituted by microphytobenthic diatoms, with a significant contribution of sedimented phytoplankton (Kharlamenko et al., 2008). Microphytobenthic diatoms thus can be available to deposit feeders and grazers but also to suspension feeders when resuspended in the water column.

These observations ruled out a possible contribution of *Z. noltii* organic matter or terrestrial organic matter (Copeman et al,. 2009) in SSOM since vascular plant FAs represented only a low percentage: 2.6 to 6.4% of the total, even when accounting for degradation-resistant LCFAs. This low contribution was unexpected considering the large amount of large *Z. noltii* organic matter in the sediment (Lebreton et al., 2009), that is probably deeply buried and thus locally much less available to consumers. This low contribution of seagrass organic matter and the dominance of diatom in SSOM composition has already been demonstrated at the same site (Vouvé, 2000) and in other seagrass beds (Boschker et al., 2000; Volkman et al., 2008).

4.3 Limited use of seagrass material by macrofaunal consumers

$\delta^{13}C$ values of deposit feeders, suspension-deposit feeders and grazers are in the range of those of *Z. noltii* fresh or degraded material. Thus, considering an isotopic enrichment from food to consumers of 0.8‰ (Vander Zanden and Rasmussen, 2001), *Z. noltii* material could appear as a potential food resource for these consumers and particularly for grazers when fresh. However, the very low percentages of vascular plant FAs in these consumers—with sum less than 6%—indicate that the contribution of fresh *Z. noltii* material was very low in their diets. Moreover, the low percentages of bacterial FAs and of LCFAs suggest also that degraded seagrass matter was not utilized via bacteria mediation. Even if the biomass of *Z. noltii* highly increased in summer, higher $\delta^{13}C$ values observed for grazers at that period are probably not related with a higher consumption of seagrass material since grazer FA profiles showed no increase of vascular plant and bacteria markers. This absence of temporal variation highlights the limited utilization of seagrass material by grazers. Nevertheless, grazers had the highest $\delta^{13}C$ values and the highest percentages of vascular plant FAs, demonstrating that these consumers are the most influenced by seagrass sources among all studied. These observations highlight the importance of trophic marker combination for complex food web analysis. In this case, a single stable isotope analysis would have been limited for a clear understanding of ecosystem trophodynamics.

A number of previous studies has emphasized the importance of fresh seagrass as the main food source for invertebrates (Valentine et al., 2000; Leduc et al., 2006; Schaal et al., 2008) but many others have demonstrated that this material is not consumed by most consumers (Kitting et al., 1984; Moncreiff and Sullivan, 2001; Borowitzka et al., 2006; Mateo et al., 2006; Jaschinski et al., 2008), and our observations confirmed this last hypothesis. This low utilization by invertebrate consumers can be explained by low nutritional quality of seagrass material that is poorly assimilated, has a low nitrogen content (Cebrián, 1999), high levels of lignin and tannins and can synthesize phenols, which consumers generally avoid (Harrison, 1982). Moreover, even though large amounts of *Z. noltii* detrital matter are stored in the sediment (Lebreton et al., 2009), this material is relatively unavailable for consumers because it is trapped in deep sediment layers, such as previously demonstrated in SSOM composition analyses.

4.4 Influence of phytoplanktonic material

Large amounts of flagellate FAs in suspension feeder profiles, particularly 22:6(*n*-3) and 16:4(*n*-3), confirmed the influence of SPOM in their diet, as shown in other seagrass meadows (Kharlamenko et al., 2001; Jaschinski et al., 2008). Abundances of flagellates can be high in the water column (Fenchel, 1988; Galois et al., 1996) and are generally very low in sediment (Moorthi and Berninger, 2006; Pascal et al., 2009), suggesting that flagellates come from phytoplankton. In our SPOM samples, low percentages of flagellate FAs were observed, that may be linked to either the scarcity of flagellate cells compared with those of diatoms or bacteria, or to sampling carried out between the short successions of flagellate blooms observed in Marennes-Oléron Bay (Héral et al., 1987). It is worth noting that the stable isotope ratios and FA profiles of suspension feeders were not affected by the strong temporal variation in the quality and quantity of SPOM (Héral et al., 1987; Galois et al., 1996), probably due to the consumption of microphytobenthos resuspended by waves and tidal currents, which stabilizes consumer tissue compositions.

4.5 Importance of diatoms in the seagrass food web

Considering trophic enrichment, $\delta^{13}C$ values of suspension-deposit feeders, deposit feeders and suspension feeders suggested that these consumers utilize microphytobenthos and SSOM as main food sources. We previously demonstrated that SSOM originates mostly from microphytobenthic diatoms (see section 4.2), reinforcing the role of this resource in these consumer diets. FA profiles showed a large influence of diatoms in diets of grazers, suspension-deposit feeders and deposit feeders. The combination of trophic markers for this study allows thus to determine that diatoms constitute the main part of the diet of all studied consumers, whichever their feeding types.

The high proportions of diatom markers in *S. plana* profiles and the $\delta^{13}C$ close to microphytobenthos showed that this suspension-deposit feeder feeds mainly as a deposit feeder all year long on this area. Diatom FA percentages, particularly of 20:5(*n*-3), are higher than those observed by Kharlamenko et al. (2001) in another typical suspension-deposit feeder (*Macoma incongrua*) from the Novgorodskaya Bight, where microphytobenthos biomass is considered to be very low. On the contrary, high microphytobenthos biomass was observed all year long in the Marennes-Oléron seagrass bed, explaining this higher contribution.

$\delta^{13}C$ values and high FA percentages of diatom markers also demonstrated the high influence of microphytobenthos in *A. marina* diet. Nevertheless, large amounts of

bacterial FAs and relatively higher $\delta^{15}N$ clearly discriminated it from other consumers. This deposit feeder generally has large amounts of bacteria in its digestive tract, which is considered as a form of gardening (Grossmann and Reichardt, 1991). *A. marina* has probably a diet based on these bacteria, which themselves use likely microphytobenthos as a substrate. Relatively higher $\delta^{15}N$ observed on *A. marina* are thus possibly related with this bacterial activity, known to increase ^{15}N concentration due to isotopic fractionation (Dijkstra et al., 2008).

Diet of suspension feeders (*T. phillipinarum*, *C. edule*) is also influenced by microphytobenthic diatoms, which are probably resuspended in the water column at high tide. Tides and waves make microphytobenthos available to suspension feeders, such as previously described on the east side of the Marennes-Oléron Bay, where epipelic diatoms contributed to the main part of suspension feeders' diets (Riera and Richard, 1996). Nevertheless the relatively low proportions of diatom markers in FA profiles indicated that diatoms have a lower influence on these consumer diets compared to others. The large standard deviations observed on *C. edule* $\delta^{13}C$ were due to the wide size range of individuals analyzed, since in this bay a clear change in cocckle diet with age has been observed (Kang et al., 1999).

Considering that seagrass material represented a small part of grazer diets (see section 4.3), $\delta^{13}C$ indicated that they were probably mostly based on microphytobenthic or epiphytic diatoms. The high percentages of diatom FAs confirmed that diatoms constitute the main part of grazers' diets and the balance between the high proportions of 20:5(*n*-3) and the low percentages of 16:1(*n*-7)c suggests that diatoms have mainly a microphytobenthic origin.

4.6 Epiphytic *vs.* microphytobenthic diatoms

Some arguments are in favour of a higher role of microphytobenthos rather than epiphytes. Firstly, epiphytes are almost absent in winter due to the lack of seagrass leaves whereas microphytobenthos biomass is constant throughout the year (Lebreton et al., 2009). An exclusive or a large utilization of epiphytes by grazers in summer would lead to a large variation of $\delta^{13}C$ between this season and winter, when epiphytes are absent, and our observations showed only a moderate enrichment of $\delta^{13}C$. Moreover, proportions of 20:5(*n*-3) (high in microphytobenthos) and of 16:1(*n*-7)c (high in epiphytes) remain constant among seasons for these grazers, with high proportions of 20:5(*n*-3). Even if

epiphytes are probably used by grazers as a food resource in summer, this suggests that epiphytes have a minor role in food fluxes in comparison with microphytobenthos.

Secondly, in the studied seagrass bed, the only epiphytes species found on *Zostera* leaves are *Cocconeis* diatoms which biomass are three orders of magnitude lower than microphytobenthos biomass (Lebreton et al., 2009). Considering that the productivity of epiphytic and microphytobenthic diatoms are relatively close (see Coleman and Burkholder (1995), Raniello et al. (2007), Lebreton (2009) for epiphytes and MacIntyre et al. (1996), Guarini et al. (2000) for microphytobenthos), productions of these sources are directly related to their biomass and should be also in a large disproportion. A rough calculation, based on the highest exponential growth rates found in literature for *Cocconeis* culture (1.03 d^{-1}) (Raniello et al., 2007) and the highest biomass observed on the Marennes-Oléron seagrass bed 3.97 mg $C.m^{-2}$ (Lebreton et al., 2009), shows that the potential production of epiphytic diatoms is around 4 mg $C.m^{-2}.d^{-1}$, while the ingestion of diatoms by the main grazer, *H. ulvae*, can be estimated to be 37 times more, based on a mean *H. ulvae* biomass of 4 000 $mg.m^{-2}$ (Lebreton, 2009) and an ingestion rate of 0.04 mg $C_{food\ source}$.mg $C_{consumer}^{-1}.m^{-2}.d^{-1}$ (Baird et al., 2007). On the contrary, microphytobenthos biomass is always very high, ranging from 5.7 to 14.5 g $C. m^{-2}$, with values close to the biomass of *Zostera* leaves (Lebreton et al., 2009) and its production, even it is less available to grazers, appears to be a much more important resources for invertebrates than epiphytes.

The importance of microphytobenthos for grazers was already noticed in the Gulf of Mexico (Moncreiff and Sullivan, 2001), in the Baltic (Jaschinski et al., 2008) and the Mediterranean Seas (Dauby, 1995; Lepoint et al., 2000), but only as a complementary source to epiphytic diatoms. On the contrary, Kharlamenko et al. (2001) consider that the main diet of gastropod grazers in Novgorodskaya Bay is based on epiphytes; however, the microphytobenthos was not quantified in their study and it is thus not possible to conclude on the actual importance of this food source. Very few studies have really quantified the actual contributions of different sources, probably because the microphytobenthos is difficult to isolate.

In the intertidal seagrass bed studied here, the importance of the microphytobenthos compared to epiphytes was more easily highlighted, due to the very low biomass – and potential production – of epiphytes. Microphytobenthos appears to be the main trophic resource for four consumers having different feeding types (grazers, deposit feeders,

suspension-deposit feeders) and accounts for a large portion of the diet in the two suspension feeders. The role of microphytobenthos is therefore of paramount importance in this seagrass bed food web: the predominance of benthic microalgae in macrofaunal diets is probably due to their higher nutritional quality and assimilation rates compared to those of vascular plants (Cebrián, 1999) and to their constant biomass throughout the year (Auby and Labourg, 1996; Lebreton et al., 2009).

5. CONCLUSION

As the six consumer species analyzed dominate the macrofauna (representing more than 90% of the total biomass), the present study illustrates the general trophodynamics between producers and macrofaunal consumers in this intertidal seagrass bed. We showed that pelagic, epiphytic or microphytobenthic diatoms represent the main part of these consumers' diets. Among these food sources, microphytobenthos appears to be the main trophic resource in consumer diets, which could be explained by its high biomass, its constant availability and its high nutritional quality. The role of microphytobenthos nevertheless remains poorly understood in most seagrass beds and may be largely underestimated, even when seagrass leaves have a large epiphyte load, and it deserves a better consideration in future studies of seagrass trophic webs.

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FIGURE CAPTIONS

Fig. 1. Position of the sampling station in the *Zostera noltii* seagrass in Marennes Oléron Bay.

Fig. 2. $\delta^{13}C$ (mean ± SD) of food sources (Fig. 2A) and consumers (Fig. 2B) from winter 2006 to summer 2007 in Marennes-Oléron seagrass bed. SSOM: Surface sediment organic matter; SPOM: Suspended particulate organic matter. In order to distinguish error bars, $\delta^{13}C$ were temporally shifted ahead 3 days.

Fig. 3. FA composition (relative percentages) of food sources in winter (Fig. 3A) and summer 2007 (Fig. 3B) in Marennes-Oléron seagrass bed. FAs are grouped according to their marker groups (see Materials and Methods). Only FAs with proportions ≥5% are given and asterisks represent sum of other FAs for each marker group. Hierarchical clustering (Ward's method, Euclidean distance) are showed for each season. p-values from multiscale boostrap resampling are showed under each node and identified clusters ($p < 0.050$) are highlighted with grey rectangles.

Fig. 4. FA composition (relative percentages) of consumers in winter (Fig. 4A) and summer 2007 (Fig. 4B) in Marennes-Oléron seagrass bed. FAs are grouped according to their marker groups (see Materials and Methods). Only FAs with proportions ≥5% are given and asterisks represent sum of other FAs for each marker group. Hierarchical clustering (Ward's method, Euclidean distance) are showed for each season. p-values from multiscale boostrap resampling are showed under each node and identified clusters ($p < 0.050$) are highlighted with grey rectangles.

Fig. 5. Plots of mean $\delta^{13}C$ (‰) and marker FA composition (relative percentages) of food sources in Marennes-Oléron seagrass bed. FAs were classified according to their marker groups (see Materials and Methods): A: vascular plants, B: diatoms, C: flagellates, D: bacteria. SSOM: Surface sediment organic matter; SPOM: Suspended particulate organic matter.

Fig. 6. Plots of mean $\delta^{13}C$ (‰) and marker FA composition (relative percentages) for consumers in Marennes-Oléron seagrass bed sampled in winter and summer 2007. FAs were classified according to their biomarker properties (see Materials and Methods): A: vascular plants, B: diatoms, C: flagellates, D: bacteria.

TABLES

Table 1. Fatty acids used as markers of food sources in the studied *Zostera noltii* seagrass bed. References: 1: Sargent and Whittle, 1981; 2: Bergé et al., 1995; 3: Rajendran et al., 1993; 4: Wannigama et al., 1981; 5: Kharlamenko et al., 2001; 6: Meziane and Tsuchiya, 2000; 7: Sargent et al., 1987; 8: Viso et al., 1993; 9: Volkman et al., 1989; 10: Viso and Marty, 1993; 11: Dunstan et al., 1994; 12: Renaud et al., 1999; 13: Volkman et al., 1998; 14: Ramos et al., 2003; 15: Volkman et al., 1980; 16: Galois et al., 1996.

Group	Fatty acid		
	Saturated	Monoenoic	Polyenoic
Bacteria	13:0 (3, 15)	15:1(*n*-5)c (3, 15)	
	15:0 (3, 15)	15:1(*n*-7)c (3, 15)	
	17:0 (7)	17:1(*n*-5)c (3, 15)	
	19:0 (7)	17:1(*n*-7)c (3, 15)	
	23:0 (3, 6, 15)	17:1(*n*-8)c (3, 7, 15)	
	25:0 (3, 15)	18:1(*n*-7)c (7, 13)	
	Iso 14:0 (3, 7, 15)	19:1(*n*-5)c (3, 15)	
	Iso 15:0 (3, 7, 15)	19:1(*n*-7)c (3, 15)	
	Iso 16:0 (3, 7, 15)	19:1(*n*-8)c (3, 15)	
	Iso 17:0 (3, 7, 15)	19:1(*n*-9)c (3, 15)	
	Iso 19:0 (3, 7, 15)		
	Anteiso 15:0 (3, 7, 15)		
	Anteiso 17:0 (7, 15)		
Diatoms	14:0 (1, 9, 10, 11, 14)	16:1(*n*-7)c (2, 9, 11, 14)	16:2(*n*-4) (9, 11)
	16:0 (1, 7, 13)		16:2(*n*-7) (9)
			16:3(*n*-4) (2, 9, 11)
			16:4(*n*-1) (2, 9, 11, 14)
			18:2(*n*-7) (11)
			20:5(*n*-3) (1, 10, 11, 14)
Flagellates			16:2(*n*-6) (9)
			16:3(*n*-3) (9, 10)
			16:4(*n*-3) (9, 10, 12)
			18:4(*n*-3) (7, 9, 12, 14)
			18:5(*n*-3) (7, 9)
			22:5(*n*-3) (16)
			22:6(*n*-3) (1, 14)
Vascular plants	22:0 (8)		18:2(*n*-6) (5, 6, 8)
	24:0 (4, 8)		18:3(*n*-3) (5, 8, 10)
	26:0 (4, 8)		
	28:0 (4, 8)		
Ubiquitous	16:0 (1, 7, 13)		
	18:0 (1, 7, 13)		
		18:1(*n*-9)c (1, 7)	

Table 2. Annual mean, minimum and maximum mean of stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) of food sources and main consumers (‰, mean ± SD), summary of Kruskal-Wallis tests and comparisons of means between sampling seasons (W: winter, S: summer). Replicate numbers are in brackets. Sampling season is stated for minimum and maximum values. Consumer feeding types: DF: Deposit feeder, G: Grazer, SDF: Suspension-deposit feeder, SF: Suspension feeder.

	$\delta^{13}C$					$\delta^{15}N$				
	Annual mean	Min. mean	Max. mean	p-values	Comparisons of means	Annual mean	Min. mean	Max. mean	p-values	Comparisons of means
Sources	‰	‰	‰			‰	‰	‰		
Z. noltii: roots	-9.7 ± 0.4 (15)	-10.2 ± 0.1 (3) W 06	-9.3 ± 0.4 (6) S 06	0.018	W 06 < S 06	7.8 ± 1.9 (12)	6.4 ± 0.2 (3) W 06	10.5 ± 1.8 (3) S 07	0.023	W 06 < S 07
Z. noltii: leaves	-9.9 ± 0.7 (15)	-11.1 ± 0.2 (3) S 07	-9.4 ± 0.2 (6) S 06	0.015	S 06 > S 07	7.8 ± 2.2 (15)	4.9 ± 0.3 (3) W 06	9.4 ± 0.4 (3) S 07	0.020	W 06 < S 07
Z. noltii: fresh detrital matter	-10.4 ± 0.8 (9)	-11.0 ± 1.0 (3) S 07	-9.7 ± <0.1 (3) W 06	0.061	=	8.4 ± 1.8 (9)	6.5 ± 0.1 (3) W 06	10.3 ± 1.6 (3) S 07	0.061	W 06 < S 07
Z. noltii: degraded detrital matter	-12.8 ± 0.9 (9)	-13.2 ± 0.8 (3) S 06	-12.5 ± 0.7 (3) S 07	0.564	=	7.5 ± 0.8 (9)	6.9 ± 0.1 (3) W 06	8.5 ± 0.7 (3) W 07	0.055	=
Epiphytes	-12.8 ± <0.1 (3)	-	-	-	-	6.3 ± 0.3 (3)	-	-	-	-
Microphytobenthos	-15.4 ± 0.9 (10)	-15.5 ± 1.2 (6) W 07	-15.3 ± 0.2 (4) S 07	0.831	=	6.9 ± 0.1 (4)	-	-	-	-
SSOM	-17.9 ± 1.4 (9)	-18.6 ± 0.1 (2) S 07	-17.4 ± 1.9 (4) W 07	0.326	=	6.1 ± 1.0 (8)	5.6 ± 0.4 (3) W 07	6.8 ± 1.3 (3) S 06	0.096	=
SPOM	-22.0 ± 2.3 (26)	-23.8 ± 0.2 (3) W 06	-15.3 ± 0.4 (3) S 07	0.019	W 06 > S 07 W 07 > S 07	-	-	-	-	-
Consumers										
Cerastoderma edule (SF)	-15.7 ± 2.2 (15)	-16.9 ± 2.2 (3) W 07	-13.9 ± 0.3 (3) S 07	0.160	=	9.5 ± 0.7 (15)	8.7 ± 0.4 (3) S 07	10.5 ± 0.5 (3) W 07	0.013	W 07 > S 07
Tapes philippinarum (SF)	-15.4 ± 0.8 (10)	-15.7 ± 1.3 (3) W 06	-14.5 ± 0.1 (3) S 07	0.511	=	9.3 ± 0.8 (10)	8.6 ± 0.4 (3) S 07	10.1 ± 1.0 (3) W 07	0.306	=
Hydrobia ulvae (G)	-11.2 ± 0.8 (21)	-11.8 ± 0.3 (3) W 07	-10.1 ± 0.7 (3) S 07	0.033	=	9.6 ± 1.1 (21)	9.2 ± 0.4 (3) W 07	10.0 ± 1.0 (6) S 06	0.423	=
Littorina littorea (G)	-9.7 ± 1.8 (14)	-10.6 ± 2.0 (3) W 07	-8.8 ± 3.0 (3) S 07	0.642	=	10.4 ± 0.7 (15)	9.5 ± 0.7 (3) S 06	10.7 ± 0.4 (3) S 07	0.156	=
Scrobicularia plana (SDF)	-13.7 ± 0.5 (14)	-14.3 ± 0.4 (3) W 07	-13.2 ± 0.1 (3) S 07	0.046	S 06 < W 07	10.2 ± 1.6 (14)	8.7 ± 0.3 (3) S 07	12.8 ± 1.3 (3) W 07	0.015	W 07 > S 07
Arenicola marina (DF)	-13.6 ± 0.6 (7)	-14.0 ± 0.1 (3) S 06	-12.6 (1) S 07	0.073	=	11.3 ± 0.4 (7)	11.4 ± 0.6 (3) W 07	11.4 ± 0.6 (3) W 07	0.752	=

Table 3. Seasonal mean, minimum and maximum mean of $\delta^{13}C$ of SPOM (‰, mean ± SD) and summary of Kruskal-Wallis tests. Replicate numbers are in brackets.

	$\delta^{13}C$ (‰)			p-values
	Seasonal mean	Min. mean	Max. mean	
Winter 2006	-22.5 ± 1.6 (14)	-23.8 ± 0.2 (3)	-21.0 ± 0.1 (3)	0.028
Summer 2006	-22.5 ± 0.7 (16)	-22.9 ± 0.2 (3)	-21.4 ± 0.5 (4)	0.062
Winter 2007	-23.2 ± 1.5 (5)	-	-	-
Summer 2007	-15.3 ± 0.4 (3)	-	-	-

FIGURES

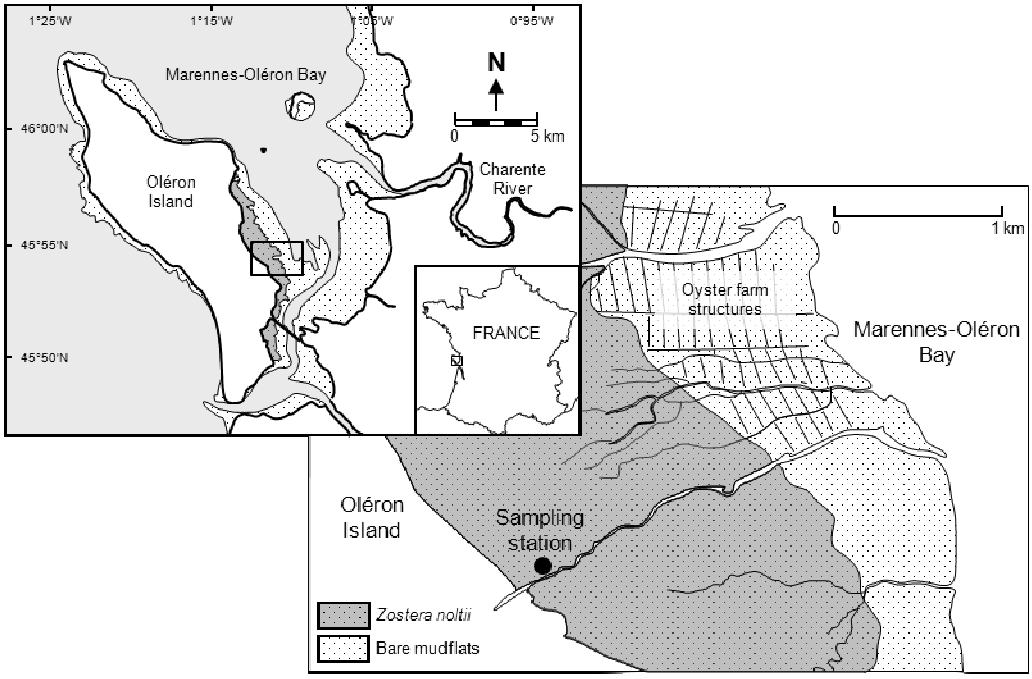

Fig. 1

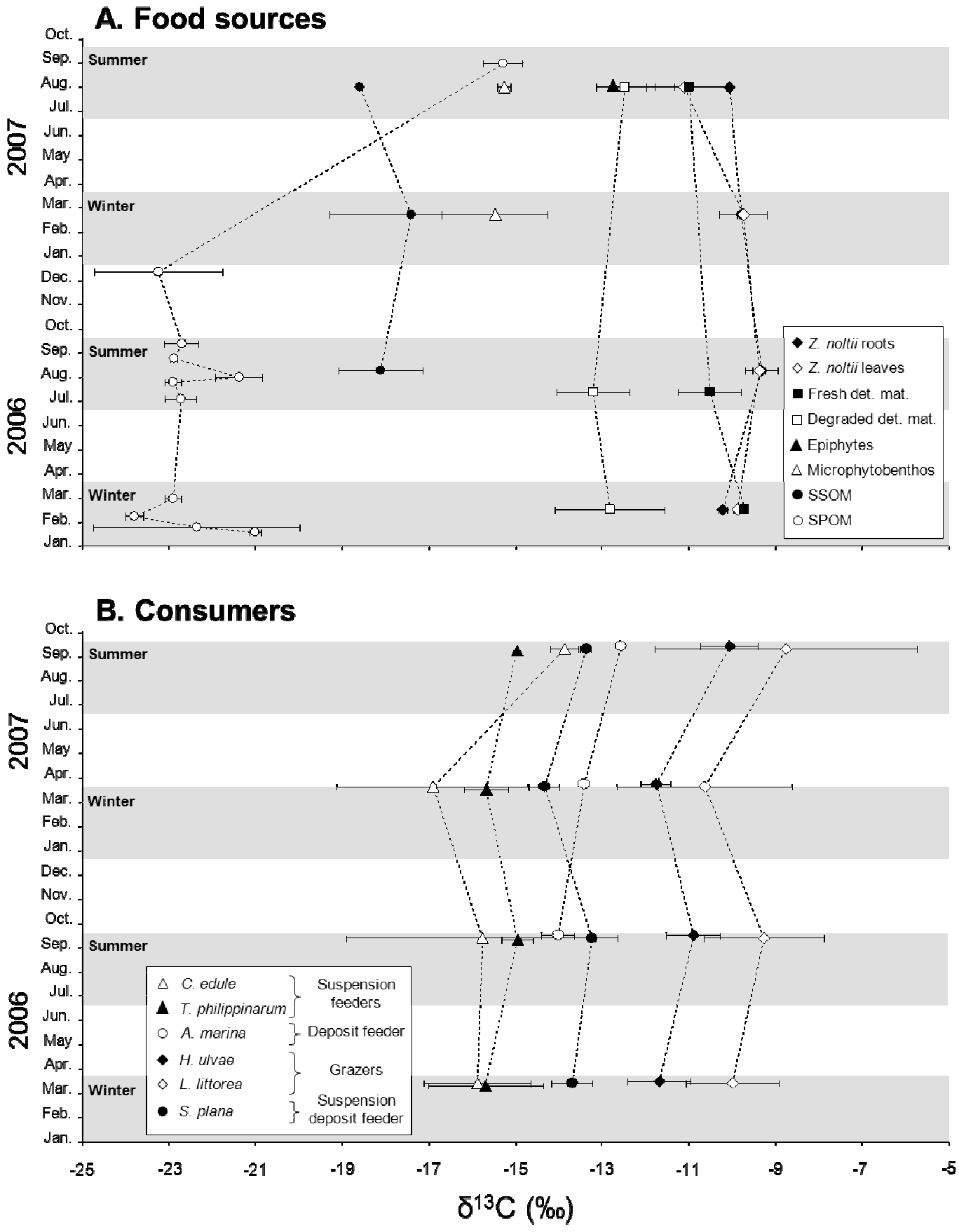
A. Food sources
Oct.
Sep.
Aug.
Jul.
Jun.
May
Apr.
Mar.
Feb.
Jan.
Dec.
Nov.
Oct.
Sep.
Aug.
Jul.
Jun.
May
Apr.
Mar.
Feb.
Jan.
2007
2006
Summer
Winter
Summer
Winter
Z. noltii roots
Z. noltii leaves
Fresh det. mat.
Degraded det. mat.
Epiphytes
Microphytobenthos
SSOM
SPOM
B. Consumers
Oct.
Sep.
Aug.
Jul.
Jun.
May
Apr.
Mar.
Feb.
Jan.
Dec.
Nov.
Oct.
Sep.
Aug.
Jul.
Jun.
May
Apr.
Mar.
Feb.
Jan.
2007
2006
Summer
Winter
Summer
Winter
C. edule
T. philippinarum
Suspension feeders
A. marina
Deposit feeder
H. ulvae
L. littorea
Grazers
S. plana
Suspension deposit feeder
-25
-23
-21
-19
-17
-15
-13
-11
-9
-7
-5
δ13C (‰)

Fig. 2

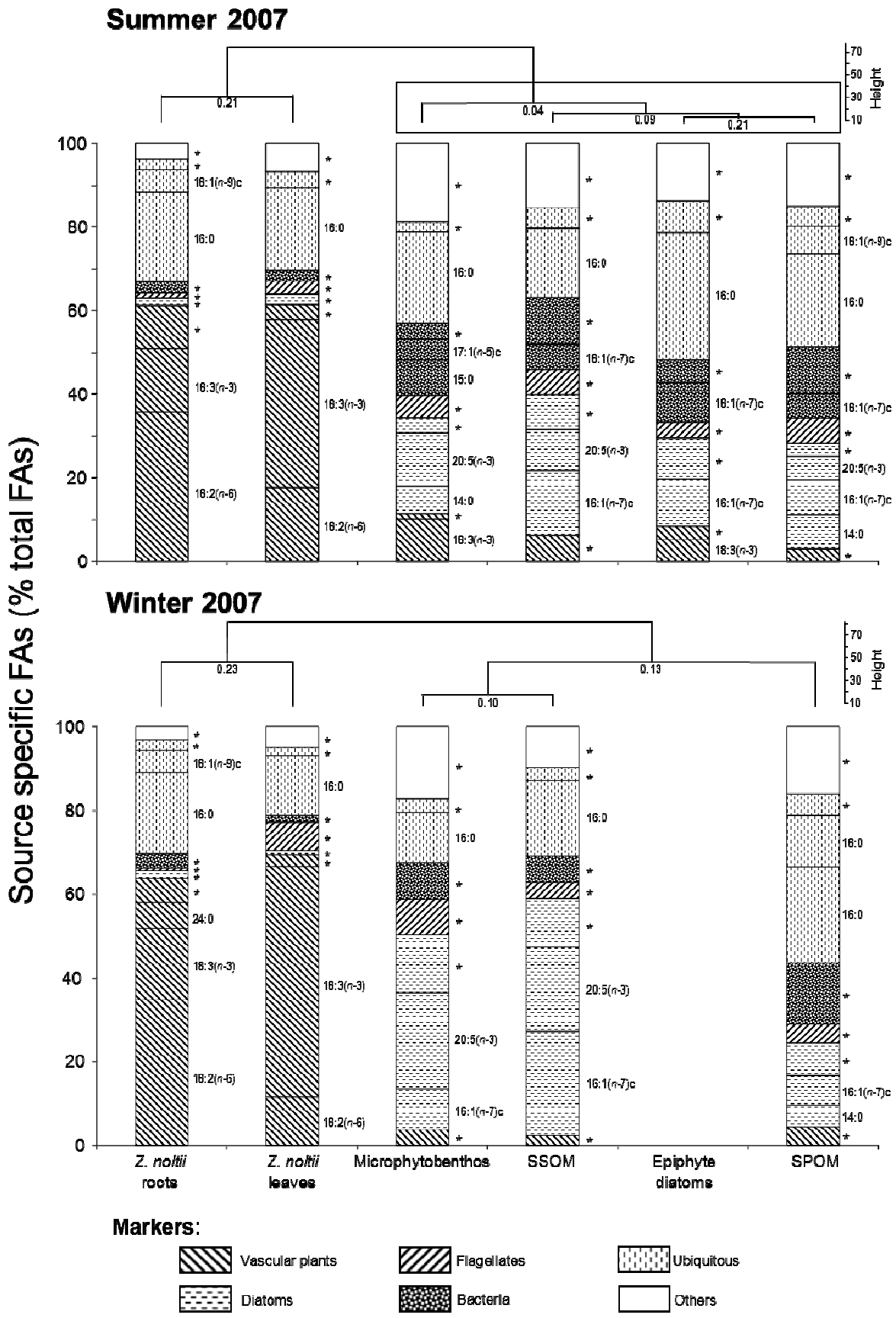
Summer 2007
Winter 2007
Source specific FAs (% total FAs)
Height
70
50
30
10
0.21
0.04
0.09
0.21
0.23
0.13
0.10
16:1(n-9)c
16:0
16:3(n-3)
16:2(n-6)
17:1(n-5)c
15:0
20:5(n-3)
14:0
18:3(n-3)
16:1(n-7)c
16:1(n-7)c
18:3(n-3)
24:0
Z. noltii roots
Z. noltii leaves
Microphytobenthos
SSOM
Epiphyte diatoms
SPOM
Markers:
Vascular plants
Flagellates
Ubiquitous
Diatoms
Bacteria
Others

Fig. 3

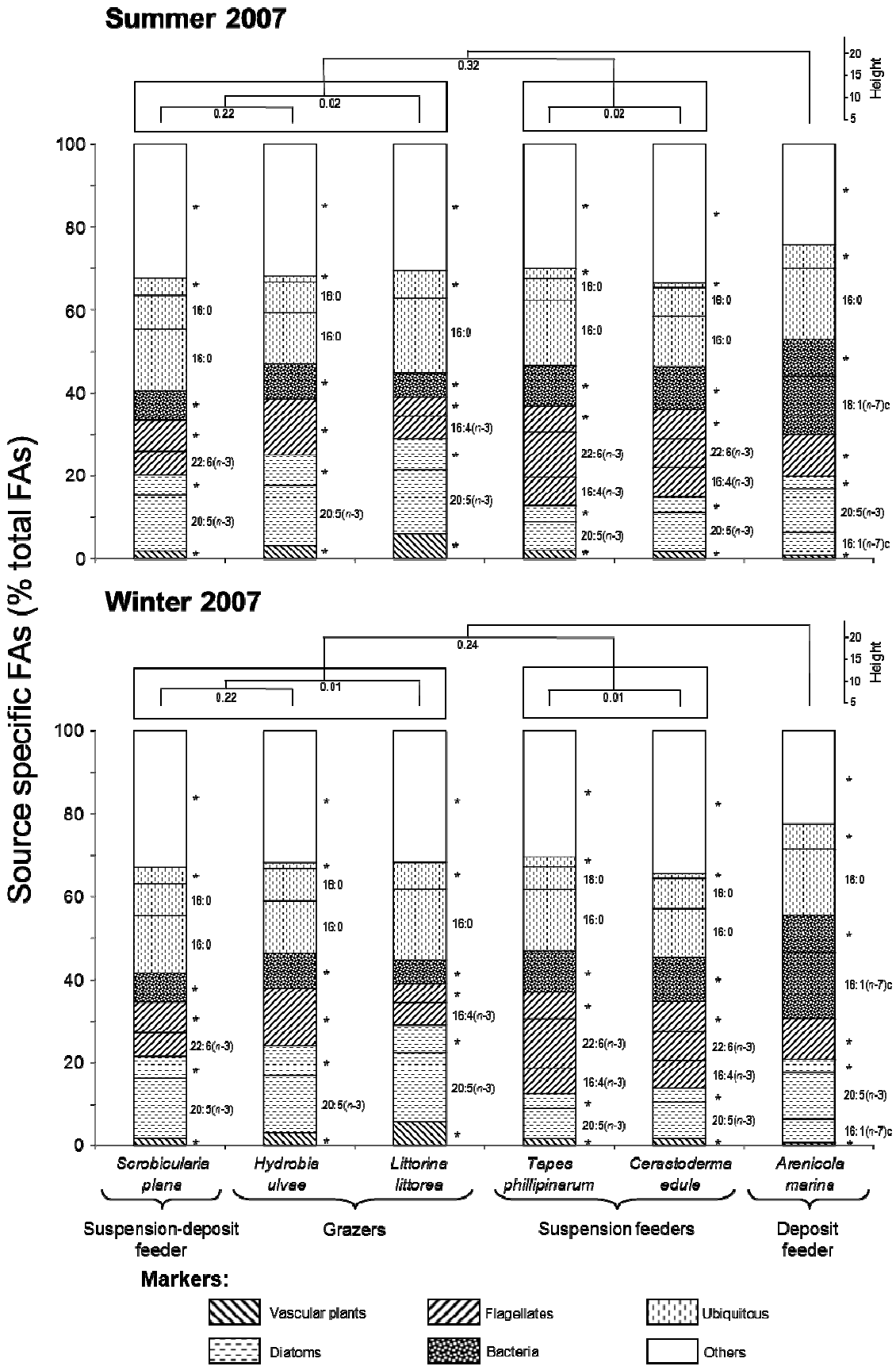
Summer 2007
Winter 2007
Source specific FAs (% total FAs)
Height
0.32
0.22
0.02
0.24
0.01
16:0
22:6(n-3)
20:5(n-3)
16:4(n-3)
18:1(n-7)c
16:1(n-7)c
Scrobicularia plana
Hydrobia ulvae
Littorina littorea
Tapes phillipinarum
Cerastoderma edule
Arenicola marina
Suspension-deposit feeder
Grazers
Suspension feeders
Deposit feeder
Markers:
Vascular plants
Flagellates
Ubiquitous
Diatoms
Bacteria
Others

Fig. 4

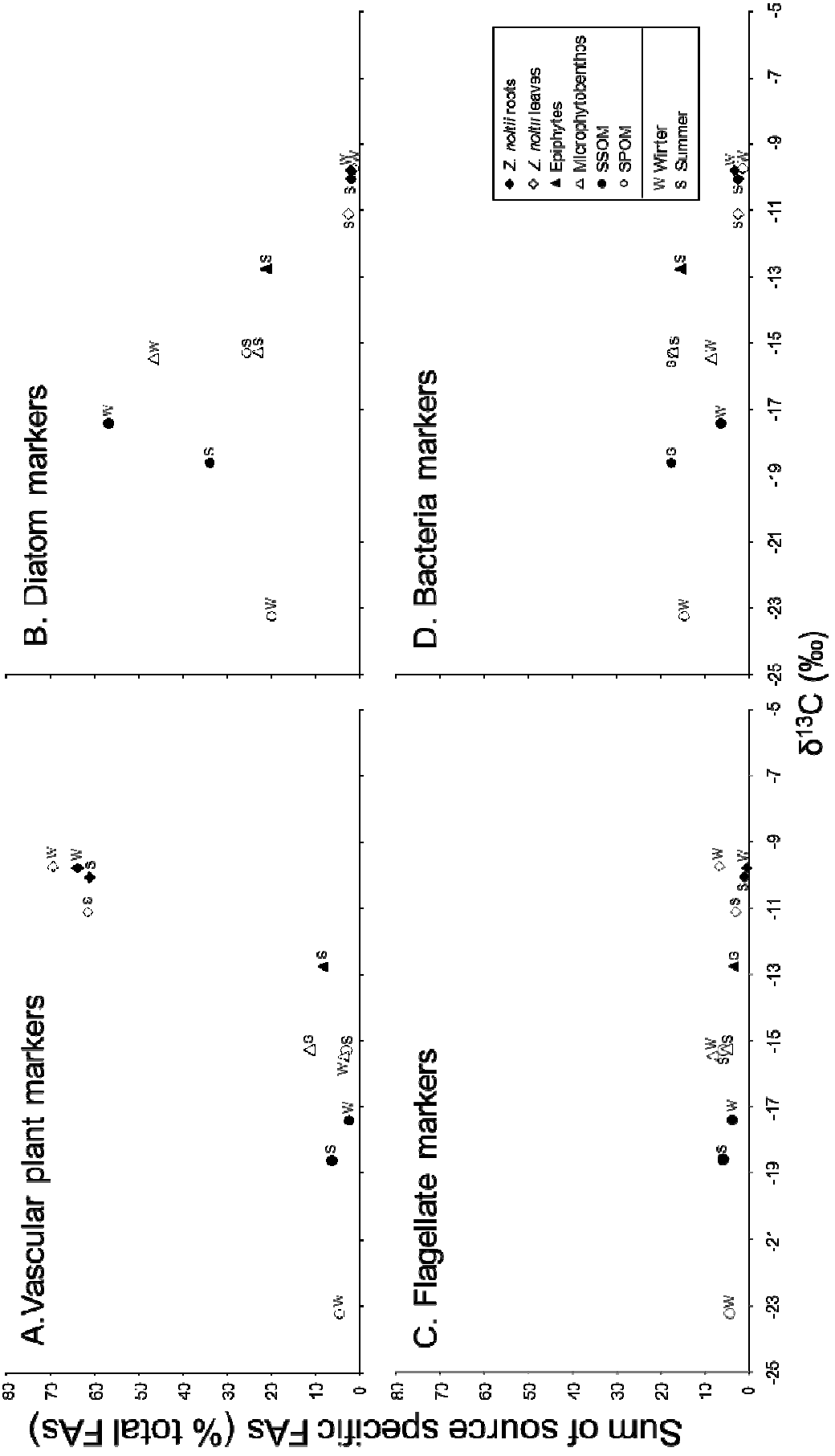
A.Vascular plant markers
B. Diatom markers
C. Flagellate markers
D. Bacteria markers
Sum of source specific FAs (% total FAs)
δ13C (‰)
Z. noltii roots
Z. noltii leaves
Epiphytes
Microphytobenthos
SSOM
SPOM
W Winter
S Summer

Fig. 5

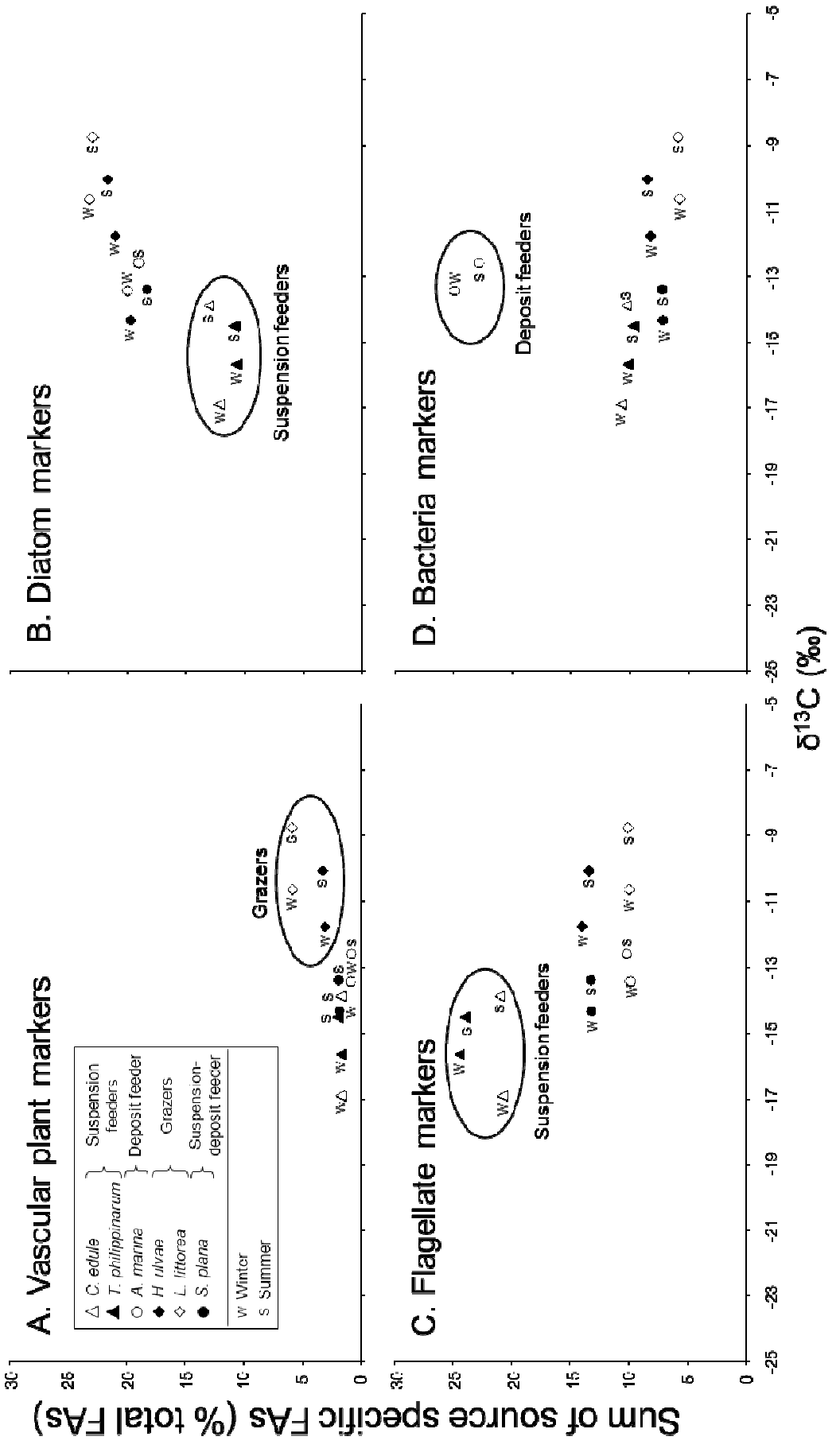
A. Vascular plant markers
B. Diatom markers
C. Flagellate markers
D. Bacteria markers
Sum of source specific FAs (% total FAs)
δ13C (‰)
C. edule
T. philippinarum
A. marina
H. ulvae
L. littorea
S. plana
Suspension feeders
Deposit feeder
Grazers
Suspension-deposit feeder
W Winter
S Summer
Grazers
Suspension feeders
Deposit feeders

Fig. 6