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ORIGINAL PAPER

Food web analysis in intertidal *Zostera marina* and *Zostera noltii* communities in winter and summer

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Abstract The food web of two intertidal seagrass (*Zostera marina* and *Zostera noltii*) beds that may be influenced by the seasonal variation in food source abundance was studied in winter and in summer with δ^{13}C and δ^{15}N analysis. In spite of high relative variation of abundance of main primary producers at the two sites, the food web did not vary between winter and summer. The δ^{13}C range of primary producers was wide. *Zostera* leaves, the most ^{13}C-enriched source, were not consumed directly by grazers. Deposit and filter feeders have a similar δ^{13}C and could use a mix of suspended and sedimented organic particulate matter, largely composed of detritus from macroalgae to seagrass. This trophic pathway allows the local incorporation of the high biomass produced by seagrasses. The wide δ^{15}N range of predators was linked either to a large variety from omnivore to carnivore predators or to the also wide ranges of δ^{15}N of primary consumers.

Introduction

Seagrass beds constitute complex ecosystems in shallow coastal waters in regard to both diversity and abundance of organisms (Heck et al. 1995). Seagrasses only represent one of many primary producers in these ecosystems in which benthic and epiphytic micro- and macroalgae and phytoplankton also significantly contribute to total biomass (McRoy and McMillan 1977). These systems are highly productive (Duarte and Cebrian 1996) but the fate of primary production remains a matter of study. Studies from different parts of the world reported important export of detrital materials from seagrass beds to adjacent areas (Stephenson et al. 1986; Duarte 2002; Schaal et al. 2008), but the intensity of fluxes is difficult to quantify. The transfer of matter from primary producers to higher trophic levels in temperate seagrass bed has been largely investigated in subtidal areas using stable isotopes (e.g. Stephenson et al. 1986; Jaschinski et al. 2008; Jephson et al. 2008). This technique has been shown to be relevant to study several aspects of seagrass ecosystem functioning (see Lepoint et al. 2004 for review). In particular, studies based on this technique revealed a direct consumption of either *Zostera* fresh leaves (Stephenson et al. 1986; Jephson et al. 2008) or epiphytic algae (e.g. Jaschinski et al. 2008), benthic microalgae (Baeta et al. 2009; Lebreton et al. 2011) and macroalgae through the detrital material pool (e.g. McConnaughey and McRoy 1979; Kharlamenko et al. 2001) while phytoplankton seemed to have a minor trophic contribution (Jaschinski et al. 2008).

The relative abundance of co-occuring primary producers is known to vary temporally and spatially in *Zostera* beds. Because of the apparent plasticity in benthic consumer diets and their ability to opportunistically make use of food available in their surroundings, the

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relative density and distribution of different food sources are also likely to be important in determining dietary composition. This challenges conclusions of food web studies based on a single sampling. Considering the potential temporal variability of food web functioning in these ecosystems, it is therefore of paramount importance to understand the significance of each potential food source.

In this context, the aims of this study were to (1) highlight the main trophic pathways characterizing the food webs of two intertidal seagrass (*Z. marina* and *Z. noltii*) communities and (2) to study the impact of the seasonal variability of primary producer abundance on the food web structure and functioning of these communities.

Materials and methods

Study site

This study was carried out in two temperate intertidal seagrass beds in a mega-tidal system near Roscoff (Western English Channel, France, Fig. 1). The *Z. marina* (48°N44.299, 3°W58.390) and *Z. noltii* (48°N41.735, 3°W57.653) beds are located below 1.80 and 3.30 m (above chart datum, lowest astronomical tide), respectively, and can emerge for several hours during low water spring tides. The sediment was sandy (median grain size: 329 and 260 μm in *Z. marina* and *Z. noltii* sites, respectively), and the fine grain part (<63 μm) was negligible (<2.1%) in both sites.

Sample collection

The sampling was carried out during spring tide in February and August 2008 in the two *Zostera* beds. The most abundant food sources (organic matter, algae and seagrass) and the most common consumers (endofauna, epifauna and fishes) were sampled during low tide at each sampling site. Benthic microalgae were not collected because of the lack of collecting methods appropriate to low benthic microalgae biomass in sandy beds. At the *Z. noltii* site, Ouisse et al. (2010) have measured high Chlorophyll *a* content (133.70 mg Chl*a* m^{-2}) in sediment but the associated primary production remained very low, highlighting the low benthic microalgae biomass at the site. All samples of macroalgae, seagrass leaves and macrozoobenthic organisms were collected by hand, whereas fishes were collected with a fishnet (mesh size 1 cm) in *Zostera* beds. In the *Z. noltii* bed, few fishes have been sampled due to the presence of large drifting macroalgae. 20 L sea water were collected at ca 50 cm depth at each site for suspended particulate organic matter (POM of the site, POM_S) and more than 5 km offshore (in order to avoid the presence of macrophyte detritus in the water column) for marine POM (POM_M). Sedimented Organic Matter (SOM) was collected by scraping the upper two centimetres of sediment at low tide in each site.

Sample processing

In the laboratory, macroalgae and seagrass leaves with epiphytes were cleaned in filtered (0.45 μm) seawater in

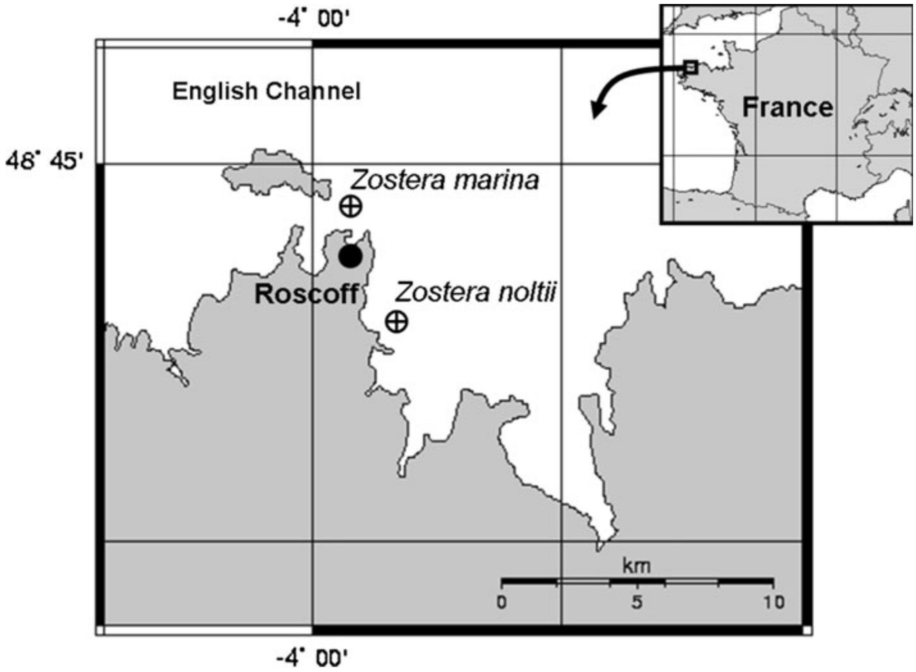

Fig. 1 Western English Channel, France. Location of the two sampling sites: the *Z. marina* and the *Z. noltii* beds

order to remove detrital fragments and attached animals. Leaves were carefully scraped with a thin brush (Dauby and Poulicek 1995) and removed epiphytes were then cautiously re-suspended in filtered seawater. Both POM and epiphytes were pre-filtered on a 63-µm mesh to remove zooplankton, meiofauna organisms and large detritus. The cleaned POM and epiphyte samples were then filtered on pre-combusted (450°C, 4 h) Whatman GF/F filters. All macrozoobenthic individuals were starved (12 h in 0.45 µm filtered seawater) to clear their guts. The flesh of molluscs and crustaceans was separated from their shell or calcareous external skeletons. Whole animals (small specimens) or muscle tissue (large specimens and fishes) were analysed.

All samples (POM, SOM, *Zostera* spp. leaves, epiphytes, macroalgae and consumers) were quickly acidified (1 M HCl) in order to remove carbonates, rinsed with distilled water and dried (60°C, 48 h). All samples were then ground to powder using a mortar and pestle before being frozen at −32°C for preservation pending stable isotope analyses (number of replicates for stable isotope analyses was indicated in Tables 1 and 2).

Stable isotope analyses

Carbon and nitrogen isotope ratios were determined using a Flash EA CN analyser coupled with a Finnigan Delta Plus mass spectrometer, via a Finnigan Con-Flo III interface. Data were expressed in conventional δ unit notation

$$\delta X(\%) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1\right] \times 10^3$$

with X is ^{13}C or ^{15}N and R is ^{13}C/^{12}C ratio for carbon or ^{15}N/^{14}N ratio for nitrogen.

The ^{13}C and ^{15}N abundances were calculated in relation to the certified reference materials Vienna-Pee Dee Belemnite-limestone (V-PDB) and atmospheric di-nitrogen (N_2). The V-PDB and N_2 at air-scales were achieved using in-house protein standards, calibrated against NBS-19 and IAEA N3 reference materials. The standard deviation of repeated measurements of δ^{13}C and δ^{15}N values of the laboratory standard was 0.10% versus V-PDB and 0.05% versus at air, respectively.

The mean isotopic composition of the consumers' diet can be estimated by assuming a mean trophic enrichment of 1% in δ^{13}C (DeNiro and Epstein 1978; Rau et al. 1983). For δ^{15}N, the trophic enrichment depends on the trophic level of organisms: a mean trophic enrichment of 3.4% was used for carnivores (McCutchan et al. 2003; Vanderklift and Ponsard 2003), whereas 2.5% was preferred for primary consumers (Vander Zanden and Rasmussen 2001).

Primary producer biomass and organic matter in the sediment

In February and August 2008, primary producer biomass in three samples (cores of 0.071 m^2) was determined in *Z. marina* and *Z. noltii* beds. *Rhodophyta*, *Phaeophyta*, *Chlorophyta* and seagrass (seagrass leaves and epiphytes) were dried separately at 60°C up to a constant weight. On additional samples, *Zostera* leaves and epiphytes were separated (see above for method) and dried at 60°C up to a constant weight. Epiphyte biomass per area unit was finally calculated from the ratio epiphyte-seagrass biomass and the total seagrass-plus-epiphyte biomass per area unit. Carbon part of Dry Weight (DW) of each primary producer was estimated from CHN analysis and then used to express biomass as gC m^{-2} using a carbon part of DW of 43.5% for *Z. noltii* leaves, 40.1% for *Z. marina* leaves, 33.7% for perennial macroalgae (small red and brown algae) and 35.2% for ephemeral macroalgae (*Enteromorpha* spp.).

Organic matter content in sediment was estimated in spring and autumn 2008 in *Z. marina* and *Z. noltii* beds. At each sampling date, six sediment cores (2.9 cm^2, 1 cm depth) were sampled (three at the upper and three at the lower level of the *Zostera* bed). Samples were dried at 60°C up to a constant weight (W_d), combusted for 6 h (520°C) and weighed again (W_c). The percentage of organic matter in the sediment (OM_{sed}) was calculated using the following formula

$$OM_{\text{sed}} = 100 \times \frac{(W_d - W_c)}{W_d}$$

Data and statistical analyses

Sixty-three and 52 species at *Z. marina* site and 24 and 39 species at *Z. noltii* site have been collected in February and August, respectively. For statistical analyses on isotopic ratios, macrozoobenthic organisms were merged in four trophic groups, grazers, deposit feeders, filter feeders and predators, according to literature.

Any difference on the range of the trophic groups has been highlighted using all consumers rather than only common consumers of the two sampling dates at each site. Only the common sources and consumers of the two sampling dates were then used for further analyses at each site.

As most outputs did not satisfy the conditions of normality, differences between sampling dates for organic matter content in sediment and isotopic ratios of potential primary food sources (organic matter, seagrass leaves, macroalgae and epiphytes) and trophic groups were analysed using non-parametric Mann–Whitney U tests.

The mixed model SIAR v 4.0 (stable isotope analysis in R) of Parnell et al. (2010) was used to estimate the likely

Table 1 $\delta^{15}N$ and $\delta^{13}C$ (mean ± SD) of sources and consumers sampled in February and August 2008 in *Z. marina* bed

Code	Group/species	TG	February			August		
			N	$\delta^{15}N$ (%)	$\delta^{13}C$ (%)	*N*	$\delta^{15}N$ (%)	$\delta^{13}C$ (%)
Polychaeta								
1	*Cirriformia tentaculata*	DF	3	8.5 ± 0.4	−17.5 ± 0.3	3	7.0 ± 0.4	−17.8 ± 0.4
2	*Arenicola marina*	DF	2	9.2	−16.4	1	9.8	−17.3
3	*Glycera tridactyla*	P	2	13.0	−16.6	3	11.9 ± 0.2	−16.7 ± 0.2
4	*Lanice conchilega*	FF	2	10.2	−17.9	2	8.1	−18.7
5	*Lumbrineris* sp.	P	3	11.0 ± 0.0	−17.2 ± 1.0	3	8.9 ± 0.3	−16.9 ± 0.5
6	*Megalomma vesiculosum*	FF	3	8.0 ± 0.4	−18.3 ± 0.3	3	8.2 ± 0.4	−18.1 ± 0.3
7	*Notomastus latericeus*	DF	3	8.9 ± 1.6	−17.3 ± 0.2	3	5.4 ± 0.1	−17.4 ± 0.6
8	*Perinereis cultrifera*	DF	3	9.2 ± 0.3	−18.6 ± 0.8	3	7.6 ± 0.5	−18.0 ± 0.1
9	*Phyllodoce* sp.	P	1	11.0	−17.3	2	10.7	−16.6
Mollusca								
10	*Aplysia punctata*	G	2	7.4	−23.7	3	7.7 ± 0.3	−20.0 ± 0.3
11	*Dosinia exoleta*	FF	3	9.8 ± 0.3	−19.1 ± 0.2	2	8.5	−18.6
12	*Ensis ensis*	FF	1	7.8	−18.0	1	7.0	−17.9
13	*Gibbula cineraria*	G	3	9.9 ± 0.1	−14.9 ± 1.5	3	9.0 ± 0.5	−16.5 ± 0.2
14	*Gibbula pennanti*	G	3	9.3 ± 0.4	−16.2 ± 0.1	3	9.3 ± 0.3	−16.0 ± 0.4
15	*Jujubinus striatus*	G	3	9.6 ± 0.1	−7.4 ± 0.8	2	9.1	−11.5
16	*Loripes lucinalis*	S	3	−1.1 ± 0.6	−28.4 ± 0.6	3	2.2 ± 0.2	−27.6 ± 0.6
17	*Lucinoma borealis*	S	3	1.8 ± 0.5	−28.1 ± 0.1	2	0.7	−25.8
18	*Nassarius reticulatus*	P	3	12.0 ± 0.3	−15.4 ± 0.3	3	12.2 ± 0.2	−14.2 ± 0.6
19	*Tricolia pullus*	G	3	9.8 ± 0.2	−14.2 ± 0.2	3	10.5 ± 0.5	−17.7 ± 0.4
Echinodermata								
20	*Ophiura ophiura*	P	1	9.0	−15.2	1	7.9	−15.2
Cnidaria								
21	*Anaemonia viridis*	P	3	10.2 ± 0.2	−16.9 ± 0.5	3	9.2 ± 0.1	−16.3 ± 0.3
22	*Cereus pedunculatus*	P	3	9.2 ± 0.6	−16.0 ± 0.4	3	10.2 ± 0.1	−15.3 ± 0.1
Crustacea								
23	*Carcinus maenas*	P	3	12.7 ± 0.3	−15.6 ± 0.3	2	10.6	−15.7
24	*Crangon crangon*	P	3	12.8 ± 0.3	−14.9 ± 0.5	3	11.3 ± 0.3	−14.1 ± 0.0
25	*Hippolyte inermis*	P	2	11.1	−16.2	1	9.3	−16.4
26	*Hippolyte varians*	P	1	10.1	−16.2	1	8.1	−15.9
27	*Liocarcinus navigator*	P	3	10.9 ± 0.5	−15.2 ± 0.9	1	7.8	−16.1
28	*Pagurus bernhardus*	P	3	9.3 ± 0.3	−15.8 ± 0.9	3	7.9 ± 0.1	−16.1 ± 0.1
29	*Palaemon serratus*	P	3	12.4 ± 0.5	−15.9 ± 0.6	3	12.0 ± 0.0	−15.8 ± 0.0
Chordata								
30	*Callionymus lyra*	P	1	13.0	−16.6	2	11.5	−15.7
31	*Ctenolabrus rupestris*	P	1	13.3	−17.1	1	11.9	−17.9
32	*Entelurus aequoreus*	P	3	10.5 ± 0.3	−19.1 ± 0.2	2	9.9	−15.6
33	*Labrus bergylta*	P	3	13.3 ± 0.1	−15.4 ± 1.0	3	12.7 ± 0.3	−15.7 ± 0.3
34	*Taurulus bubalis*	P	2	14.3	−15.1	3	12.3 ± 0.6	−15.8 ± 0.1
Macrophytes								
Cho	*Chondrus crispus*		3	6.6 ± 0.3	−18.9 ± 0.4	3	5.0 ± 0.3	−17.7 ± 0.1
Ent	*Eneromorpha* spp.		3	5.3 ± 0.3	−17.4 ± 0.3	3	5.3 ± 0.4	−14.4 ± 0.4
Him	*Himantalia elongata*		3	4.4 ± 0.5	−15.3 ± 0.2	3	4.8 ± 0.1	−13.7 ± 0.1
Gra	*Gracilaria gracillis*		3	4.8 ± 0.3	−21.8 ± 0.4	3	6.1 ± 0.1	−18.7 ± 0.3
Zmo	*Zostera marina* (old leaves)		3	4.4 ± 0.4	−12.1 ± 0.2	3	5.4 ± 0.3	−12.7 ± 0.4

Table 1 continued

Code	Group/species	TG	February			August		
			N	δ^{15}N (%)	δ^{13}C (%)	*N*	δ^{15}N (%)	δ^{13}C (%)
Zml	*Zostera marina* (leaves)		3	4.4 ± 0.3	−11.2 ± 0.5	3	5.6 ± 0.3	−11.0 ± 1.0
Ep	Epiphytes of *Z. marina*		2	7.2	−12.7	3	6.6 ± 0.2	−17.5 ± 0.7
Organic matter								
POM_M	Marine POM		3	6.7 ± 1.1	−23.5 ± 0.2	3	7.7 ± 0.1	−23.2 ± 0.1
POM_S	Site POM		3	6.5 ± 0.2	−22.2 ± 0.1	3	6.8 ± 0.2	−21.1 ± 0.0
SOM	SOM		3	3.0 ± 0.2	−17.2 ± 0.1	3	6.5 ± 0.4	−17.5 ± 0.5

According to the literature, *TG* corresponds to trophic groups: symbiosis *S*, grazers *G*, filter feeders *FF*, deposit feeders *DF* and predators *P*. *N* corresponds to the number of replicates

contribution of each of the potential food items to the diets of filter feeders in each site. Only SOM and POM of the site (POM_S) were used in the mixed model as an available source for the filter feeders. Mean trophic enrichment factors of 1 ± 1.2% for δ^{13}C and 2.5 ± 2.5% (Vander Zanden and Rasmussen 2001) for δ^{15}N were considered.

Results

Abundance and stable isotope ratios of sources

At the *Z. marina* site, the biomass (mean ± SD) of the main benthic primary producer, seagrass leaves, was 23.0 ± 6.7 gC m^{-2} in February and 80.0 ± 29.7 gC m^{-2} in August (Fig. 2a). Seagrass leaves represented more than 90% of the benthic primary producer biomass in February, while the biomass of red and brown macroalgae fixed on small rocks were low (9%). Epiphyte biomass and ephemeral green macroalgae (*Enteromorpha* spp.) biomass were negligible. In contrast, *Enteromorpha* spp. was the most abundant source in August (53%) and *Z. marina* leaves represented only 39% of the main primary producer biomass. The relative biomass of other macroalgae biomass did not vary (8%). The mean (±SD) organic matter content in the sediment was low and varied between 1.33 ± 0.16% in spring and 1.86 ± 0.51% in autumn.

At the *Z. noltii* site, the biomass of main benthic primary producers (mean ± SD) was 27.9 ± 13.8 gC m^{-2} in February and more than twofold higher in August (57.0 ± 22.1 gC m^{-2}, Fig. 2b). Macroalgae were present but scattered on little rocks and their biomass was negligible for both dates. *Z. noltii* leaves dominated the biomass of primary producers in February (64%) and August 2008 (94%) and epiphytes represented more than one-third of the main benthic primary producer biomass in February (35%). The mean organic matter content in the sediment was low (0.98 ± 0.29% in spring and 1.12 ± 0.19% in autumn).

At both sites, 9 sources were collected for stable isotope analyses in February and August (Tables 1, 2). Among them, *Zostera* spp. (leaves and old leaves, depending on the site) was the most ^{13}C-enriched whereas, the marine POM (POM_M) was the most ^{13}C-depleted (Figs. 3, 4).

At the *Z. marina* site, the δ^{13}C range displayed by primary producers was not different between the two dates (from −21.8 to −10.5% in February and from −18.7 to −10.2% in August, Fig. 3). The range of δ^{15}N displayed by macroalgae and epiphytes was low, their mean δ^{15}N values not varying between the two dates ($n = 14$, $P > 0.05$). The SOM was the most ^{15}N-depleted source in February (Table 1).

At the *Z. noltii* site, the range of δ^{13}C of macroalgae was twofold lower in February (3.0%) than in August (6.4%, Fig. 4). The δ^{15}N range of macroalgae was wide in February (3.6%) and narrow in August (0.8%). Neither δ^{13}C nor δ^{15}N of macroalgae varied between the 2 dates ($n = 12$, $P > 0.05$). The SOM was the most ^{15}N-enriched source in August (Table 2).

Finally, the δ^{13}C of epiphytes varied between February and August for both sites and epiphytes were clearly separated from *Zostera* leaves and macroalgae in either δ^{13}C or δ^{15}N in February but not from macroalgae in August.

Consumers stable isotope ratios

At the two sites, the molluscs *Loripes lucinalis* and *Lucinoma borealis* exhibited depleted δ^{13}C and δ^{15}N (Tables 1, 2). This did not reflect an utilization of much depleted sources but the presence of endosymbiontic bacteria in the gills of these bivalves which led to an important depletion in both δ^{15}N and δ^{13}C (Kiyashko et al. 1997). These species were thus excluded from the determination of main trophic pathways in the studied seagrass beds.

At the *Z. marina* site, δ^{13}C of grazers (5 species of molluscs) varied significantly between the 2 dates ($n = 14$, $P < 0.05$). Grazers displayed the widest range of δ^{13}C of consumers for both dates (16.3% in February and 8.4% in

Table 2 δ^{15}N and δ^{13}C (mean ± SD) of sources and consumers sampled in February and August 2008 in *Z. noltii* bed

Code	Group/species	TG	February			August		
			N	δ^{15}N (%)	δ^{13}C (%)	*N*	δ^{15}N (%)	δ^{13}C (%)
Polychaeta								
1	*Lanice conchilega*	FF	2	9.5	−16.8	3	9.5 ± 0.4	−16.1 ± 0.7
2	*Megalomma vesiculosum*	FF	3	9.6 ± 0.1	−17.5 ± 0.4	3	9.3 ± 0.1	−17.3 ± 0.3
3	*Nephtys hombergii*	P	3	11.8 ± 0.3	−14.8 ± 0.3	3	11.3 ± 0.6	−13.9 ± 0.3
4	*Marphysa bellii*	P	3	11.7 ± 0.4	−15.4 ± 0.4	3	10.8 ± 0.5	−15.6 ± 1.2
5	*Glycera* sp.	P	1	12.9	−14.5	2	13.5	−15.0
Nemertea								
6	*Nemerte*		3	10.0 ± 0.6	−17.5 ± 1.1	3	9.4 ± 0.3	−19.1 ± 0.0
Mollusca								
7	*Gibbula pennanti*	G	3	10.7 ± 0.5	−14.7 ± 0.9	3	10.6 ± 0.6	−14.1 ± 0.5
8	*Gibbula cineraria*	G	3	10.2 ± 0.3	−14.2 ± 0.8	3	10.2 ± 0.0	−14.5 ± 0.7
9	*Nassarius reticulatus*	P	3	13.2 ± 0.0	−14.6 ± 0.2	3	13.5 ± 0.0	−14.5 ± 0.3
10	*Cerastoderma edula*	FF	1	7.5	−17.5	3	10.0 ± 0.5	−17.3 ± 0.2
11	*Venerupis decussatus*	FF	2	10.4	−17.1	3	9.5 ± 0.3	−17.1 ± 0.2
12	*Venerupis aurea*	FF	3	9.7 ± 0.3	−16.7 ± 0.1	1	8.9	−17.6
13	*Loripes lucinalis*	S	3	1.6 ± 0.1	−26.8 ± 0.2	3	0.8 ± 0.1	−26.0 ± 0.1
14	*Lutaria lutraria*	FF	2	9.8	−17.8	3	10.8 ± 0.1	−17.1 ± 0.1
Crustacea								
15	*Pagurus bernhardus*	P	3	10.0 ± 0.3	−15.3 ± 0.4	3	10.5 ± 0.5	−15.6 ± 1.3
Chordata								
16	*Pomatoschitus minutus*	P	3	14.7 ± 0.1	−13.9 ± 0.1	3	13.0 ± 0.4	−16.1 ± 0.3
Nematoda								
17	*Nematoda*		1	8.8	−19.0	1	7.4	−18.1
Macrophytes								
Ent	*Entermorpha* spp.		3	6.6 ± 0.4	−16.9 ± 0.2	3	6.6 ± 0.3	−15.5 ± 0.5
Fuc	*Fucus serratus*		3	4.6 ± 0.6	−18.0 ± 0.3	3	6.5 ± 0.3	−13.1 ± 0.0
Gig	*Gigartina acicularis*		3	7.7 ± 0.3	−19.9 ± 0.3	3	7.0 ± 0.4	−19.5 ± 0.6
Mas	*Mastocarpus stellatus*		3	8.2 ± 0.2	−18.1 ± 0.1	3	6.2 ± 0.5	−18.9 ± 0.7
Znl	*Zostera noltii* (leaves)		3	5.7 ± 0.2	−11.9 ± 0.4	3	7.4 ± 0.0	−10.3 ± 0.2
Ep	Epihytes of *Z. noltii*		3	5.5 ± 0.2	−14.3 ± 0.1	3	7.3 ± 0.0	−17.7 ± 0.2
Organic matter								
POM_M	Marine POM		3	6.7 ± 1.1	−23.5 ± 0.2	3	7.7 ± 0.1	−23.2 ± 0.1
POM_S	Site POM		3	7.6 ± 0.3	−22.2 ± 0.2	3	7.4 ± 0.3	−20.4 ± 0.1
SOM	SOM		3	5.3 ± 0.1	−17.8 ± 0.1	3	7.7 ± 0.1	−17.5 ± 0.2

According to the literature, *TG* corresponds to trophic groups: symbiosis *S*, grazers *G*, filter feeders *FF* and predators *P*. *N* corresponds to the number of replicates

August, Fig. 3). *Aplysia punctata* was the most ^{13}C-depleted consumer in February and August (−23.7 and −20.0%, respectively, see 10 in Fig. 3) and *Jujubinus striatus* was the most ^{13}C-enriched consumer in February and August (−7.4 and −11.5%, respectively, see 15 in Fig. 3). Mean stable carbon isotope ratios of deposit feeders (4 species of polychaetes, Table 1) varied between −18.6 and −16.4% in February and between −18.0 and −17.3% in August. δ^{15}N of deposit feeders varied significantly between the 2 dates ($n = 11$, $P = 0.004$). The mean δ^{13}C of filter feeders (2 species of polychaetes and 2 species of molluscs) varied from −19.1 to −17.9% in February and from −18.7 to −17.9% in August and their δ^{15}N between 7.8 and 10.2% in February and between 7.0 and 8.5% in August. Neither δ^{13}C nor δ^{15}N varied between the two dates ($n = 9$, $P > 0.05$). Among all 34 consumers, the predator trophic group was the most diversified (19 species among the six sampled phyla). Predator species displayed a wide range of δ^{13}C and δ^{15}N for both dates (Fig. 3). The fishes *Labrus bergylta* and *Taurulus bubalis*

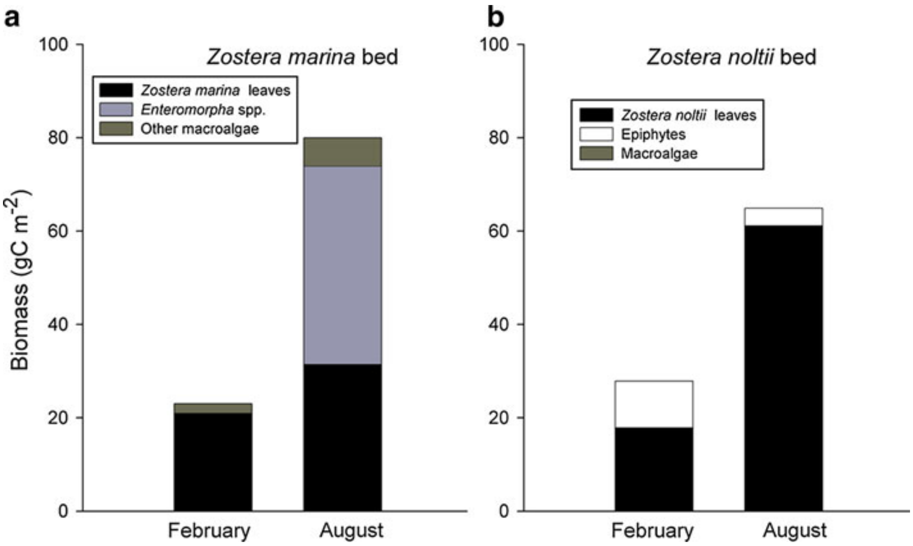

Fig. 2 Mean biomass (gC m^{-2}) of main benthic primary producers in the (**a**) *Z. marina* and (**b**) *Z. noltii* beds in February and August 2009

were the most N^{15}-enriched consumers in February and August, respectively (see 33 and 34 in Fig. 3).

At the *Z. noltii* bed, 15 species (2 grazers, 7 filter feeders and 6 predators) and 2 groups of species (nematodes and nemertes which trophic group could not be specified) have been collected in February and August (Table 2). The 2 grazers (the molluscs *Gibbula pennanti* and *Gibbula cineraria*) had a similar mean δ^{13}C and δ^{15}N (see 7 and 8 in Fig. 4). Filter feeders (polychaetes and molluscs) displayed a narrow range of δ^{13}C (from -17.5 to -16.8% in February and from -17.6 to -16.1% in August). Their mean δ^{15}N varied between 7.5 and 10.4% in February and between 8.9 and 10.8% in August (Fig. 4). Predators (3 polychaetes, 1 mollusc, 1 crustacean and 1 chordate) displayed a low range of mean δ^{13}C values (1.4% in February and 1.6% in August), whereas the range of mean δ^{15}N value was wide (4.7% in February and 3.0% in August). The mean δ^{13}C and δ^{15}N of each trophic groups did not vary between dates ($n = 6$, $P > 0.05$).

Using mixing model, the two sources equally contributed to the diet of the two filter feeders at the *Z. marina* site (Fig. 5) while the contribution of SOM was higher (about 70%) at the *Z. noltii* site (Fig. 6).

Discussion

Isotopic characterization of sources in the Zostera food web

The δ^{13}C of *Zostera* leaves fell in the range reported by Hemminga and Mateo (1996) for seagrass. In addition, the δ^{13}C of old *Z. marina* leaves was gently more ^{13}C depleted (<1%) compared to fresh tissue for both dates, as previously measured by Kharlamenko et al. (2001) and Hoshika et al. (2006) in the Sea of Japan. The macroalgae were distributed over a wide range of δ^{13}C values, typical of temperate coastal environments (Fredriksen 2003; Bode et al. 2006; Jephson et al. 2008; Kang et al. 2008) and overlapped the δ^{13}C values of epiphytes in August. In contrast, the differences of δ^{15}N among co-occurring macrophytes (seagrass, macroalgae and epiphytes) were low and the values observed here are in accordance with the absence of anthropogenic or freshwater nitrogen sources in both sites which would have resulted in ^{15}N enrichment in primary producers (e.g. Baeta et al. 2009).

Marine POM (POM_M) δ^{13}C is typical of POM dominated by marine phytoplankton, as already measured in the Western English Channel (Schaal et al. 2010). The POM of the site (POM_S) was less ^{13}C depleted, and this might reveal the presence of suspended detritus mixed with phytoplankton (Kaehler et al. 2000).

The organic matter in the sediment (SOM) can derive from various marine sources (mean δ^{13}C of all the primary producers). First, benthic microalgae were not sampled but their δ^{13}C and δ^{15}N presented in the literature are in the range of the ones of the SOM (Riera and Hubas 2003; Jaschinski et al. 2008). In addition, seagrass leaves can greatly modify the hydrodynamic processes enhancing the sedimentation of suspended particulate matter (Kock et al. 2006) and decreasing the export of matter from seagrass bed to neighbouring environment. The SOM in seagrass beds results therefore from a mixture of sources of various origins. Here, the isotopic values found at both sites suggested that the SOM pool was composed of a mixture of benthic microalgae, macroalgae (wide range of values), seagrass detritus (^{13}C-enriched) and sedimented phytoplankton (^{13}C-depleted).

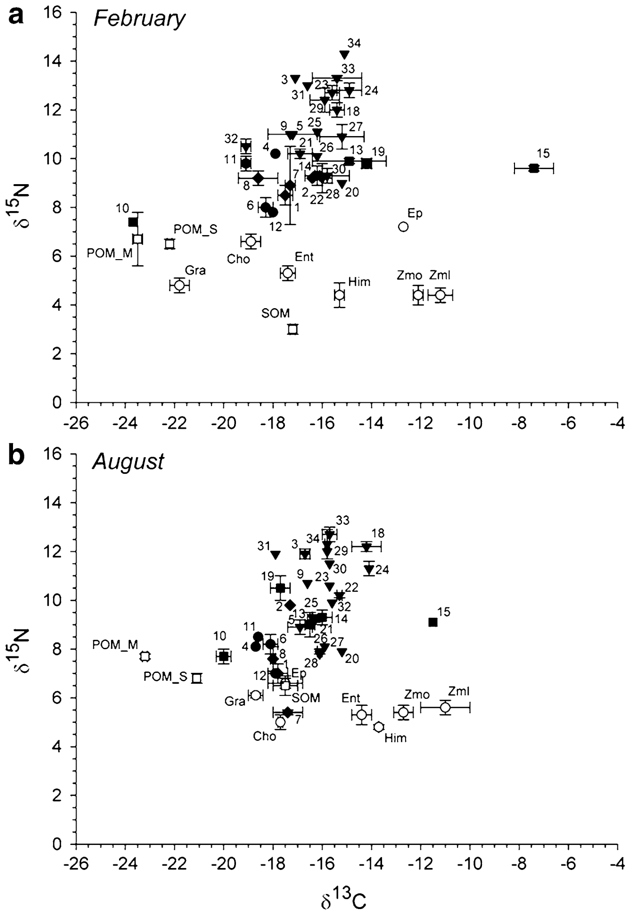

Fig. 3 Mean (±SD) δ^{15}N versus δ^{13}C of potential sources and consumers sampled in (**a**) February and (**b**) August 2008 in the *Z. marina* bed. *Legends* for consumers (*closed symbol* and number) and sources (*open symbol*) are given in the Table 1. *Loripes lucinalis* (16) and *L. borealis* (17) are not represented on the *figure*. *Symbol shape* corresponds to trophic group: grazers (*square symbol*), filter feeders (*circle symbol*), deposit feeders (*diamond symbol*) and predators (*triangle symbol*)

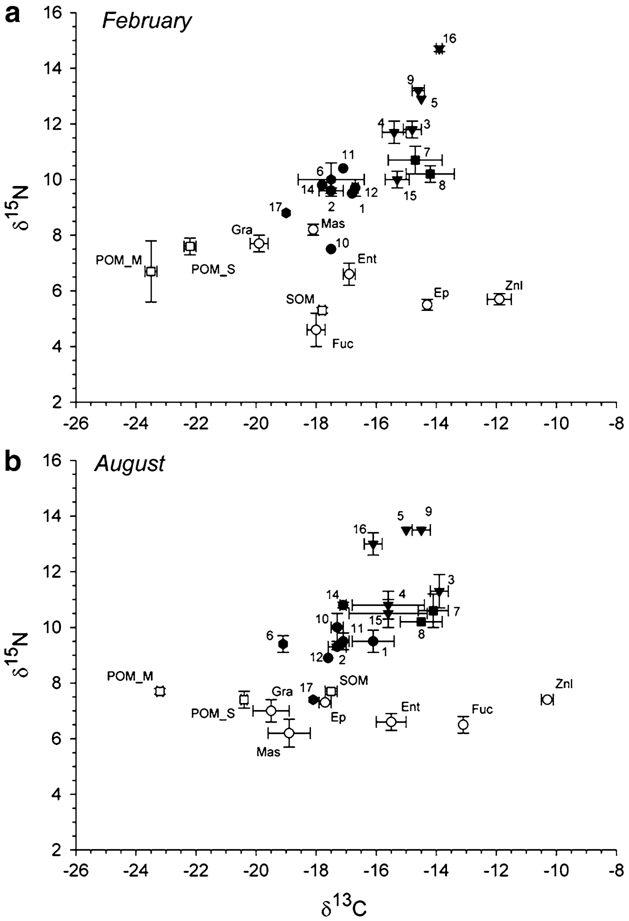

Fig. 4 Mean (±SD) δ^{15}N versus δ^{13}C of potential sources and consumers sampled in (**a**) February and (**b**) August 2008 in the *Z. noltii* bed. *Legends* for consumers (*closed symbol* and number) and *sources* (*open symbol*) are given in the Table 2. *Loripes lucinalis* (13) is not represented on the *figure*. *Symbol shape* corresponds to trophic group: grazers (*square symbol*), filter feeders (*circle symbol*) and predators (*triangle symbol*)

Major trophic pathways in Zostera beds

In the *Z. marina* bed, the five species of grazer exhibited a wide range of δ^{13}C. Among them, *J. striatus* seemed to be the only one able to directly use *Zostera* leaves or epiphytes as a source of carbon. However, its δ^{15}N value rather indicated a preferentially use of epiphytic algae. Seagrass contains a large proportion of indigestible material (lignin) and their phenolic compounds are known to deter herbivory (Harrison 1982). *Jujubinus striatus* could actually graze epiphytic algae as experimentally shown by Hily et al. (2004). In the *Z. noltii* bed, the δ^{13}C and δ^{15}N values of the two species of grazer also indicated a use of macroalgae or epiphytes. Then, from these results, the resource of the grazers are likely to derive more from macroalgae or epiphytes than from seagrass, as already concluded in other temperate seagrass beds (e.g. McConnaughey and McRoy 1979; Stephenson et al. 1986; Jaschinski et al. 2008). Therefore, it can be assumed that *Z. marina* and *Z. noltii* primarily provide habitat and shelter for grazers, whereas food is mainly supplied indirectly by providing space for attached epiphytes. Grazers play a major role in the food source availability for the others trophic groups (Hily et al. 2004). Some of the material removed from *Z. marina* or *Z. noltii* leaves by grazers but not eaten is dropped to the sediment floor (SOM) and thus available for the macrodetritivores and decomposers.

The isotopic values of potential sources for deposit feeders in the *Z. marina* bed were close to SOM for both dates as expected. However, the more ^{15}N enriched values of deposit feeders in February suggested also the assimilation of a more ^{15}N enriched source than SOM like removed sources dropped to the sediment by grazers (see above). Potential sources for filter feeders were more ^{13}C enriched than POM and more ^{13}C depleted than SOM of

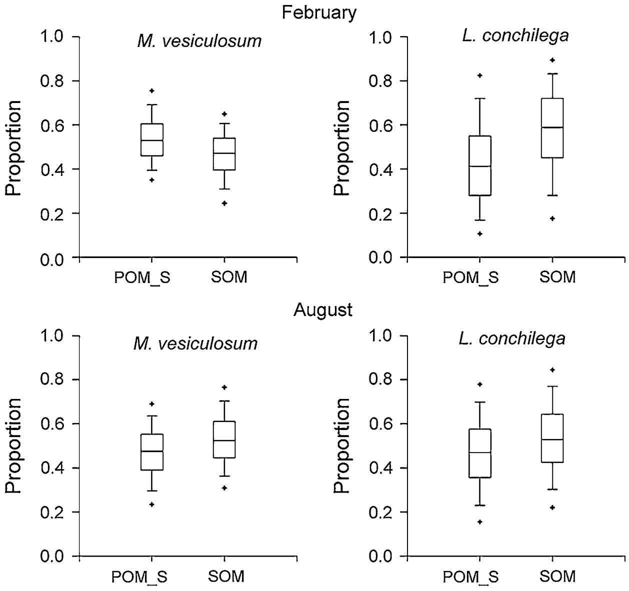

Fig. 5 Contribution of marine particulate organic matter of the site (POM_M) and SOM to the diet of the two deposit feeders: *Megalomma vesiculosum* (*M. vesiculosum*) and *Lanice conchilega* (*L. conchilega*) at the *Z. marina* site using mixed model of Parnell et al. (2010) in February and August. The proportions show the credibility intervals plotted at 95–5, 90–10, 75–25 and 50%

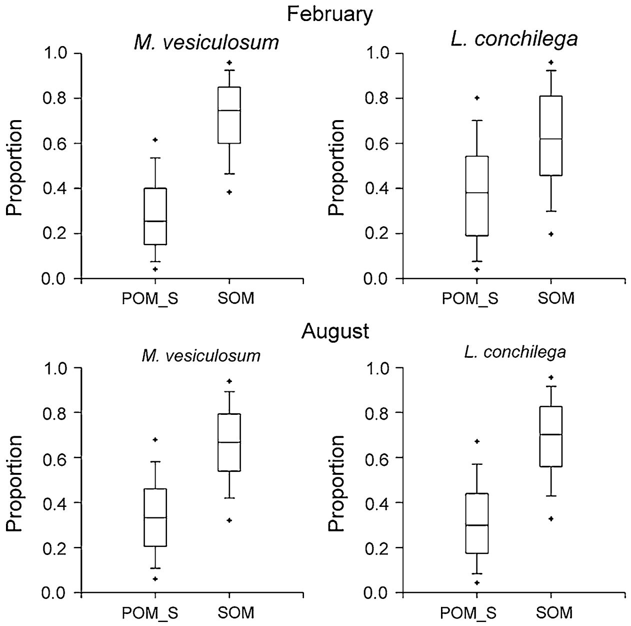

Fig. 6 Contribution of marine particulate organic matter of the site (POM_M) and SOM to the diet of the two deposit feeders: *Megalomma vesiculosum* (*M. vesiculosum*) and *Lanice conchilega* (*L. conchilega*) at the *Z. noltii* site using mixed model of Parnell et al. (2010) in February and August. The proportions show the credibility intervals plotted at 95–5, 90–10, 75–25 and 50%

both sites, suggesting an assimilation of SOM blended with POM. The proportion of each of them to the diet of the filter feeder species common to the two sites (*Megalomma vesiculosum* and *Lanice conchilega*) was estimated via a mixed model (Parnell et al. 2010). The contribution of the SOM and POM_S to the diet of the two filter feeders in both sites may reveal the importance of re-suspension events in the trophic functioning of these seagrass beds, as previously suggested by Lebreton et al. (2011) in the Marennes-Oleron basin. In addition, the δ^{13}C and δ^{15}N range of each trophic group (deposit and filter feeders) suggested that these consumers may display more or less selective ability while foraging in the sediment or in the water column, as already evidenced for filter feeder bivalves for example (Jorgensen 1996).

In the present study, the δ^{15}N range of predators was wide and overlapped those of primary consumers (deposit feeders, filter feeders and grazers) for both sites in February and August. This δ^{15}N range can be linked either to a large variety of predators, from omnivore to carnivore, or to the also wide δ^{15}N ranges of primary consumers and primary producers. The misleading consequences of wide δ^{15}N range of primary producers on the determination of trophic levels have recently been highlighted for rocky shore ecosystems nearby the present study area (Riera et al. 2009). In this context, it was impossible to differentiate each trophic level based on δ^{15}N as it has been currently done in seagrass beds (Lepoint et al. 2004 for review). *Z. marina* and *Z. noltii* beds are two complex habitats that can offer to preys protection from predators (Duarte 2002). The high diversity of predators found in this study suggests that this trophic group is likely to exert an important top-down control in the food web associated with the *Zostera* spp. meadow (Moksnes et al. 2008).

Comparison of summer versus winter situation

At the *Z. marina* site, the abundance of *Enteromorpha* spp. was low in winter and its role in the food web may be limited. In summer, the *Enteromorpha* spp. became the most abundant source, highly palatable, representing a potential source for grazers (Granado and Caballero 2001). Although, the *Enteromorpha* spp. can probably be consumed directly, this was not highlighted by the isotope analysis. The ^{13}C enrichment observed between February and August both on *Enteromorpha* spp. and one grazer (*A. punctata*) suggested the use of that source among others. In addition, the large *Enteromorpha* spp. development in summer enriched the organic matter pool in sediment (e.g. Riera and Hubas 2003; Ouisse et al. 2011) and likely contributed indirectly to the diet of deposit and filter feeders via the detritus pathway all over the year.

At the *Z. noltii* site, seagrass epiphytes biomass decreased strongly from winter to summer. In addition, the change in their associated δ^{13}C value suggested a qualitative change of the epiphyte algae community. In the literature, Jaschinski et al. (2008) concluded that epiphytes were one of the major carbon sources for grazers in *Z. marina* beds. In the present study, the epiphyte biomass strongly varied in *Z. noltii* bed between winter and summer and their contribution to higher trophic levels remained to be demonstrated during summer since their δ^{13}C and δ^{15}N were not clearly separated from the other macroalgae at this period. In fact, the low abundance of macroalgae and epiphytes and the low palatability of *Z. noltii* seem rather to confirm the importance of organic matter available in the sediment or in the water column for the benthic food web in the studied *Zostera* bed.

In conclusion, our results have furthermore emphasized the functional complexity of the food webs associated with seagrass beds in temperate coastal zones. Demersal and benthic compartments were coupled in the same trophic pathway, based on a mixture of sedimented and suspended particulate organic matter, originating from the different food sources of this ecosystem (including seagrass detritus). Although, macroalgae, seagrass and epiphytes varied in biomass and stable isotope ratios over the course of the year, the food webs displayed a striking seasonal stability. This supported the idea that the food web associated with *Z. marina*, and *Z. noltii* is mostly based on composite sources rather than pure food sources. The trophic structure of these *Zostera* beds differ from other *Zostera* spp. ecosystems previously investigated. As a consequence, it is impossible to generalize the ecosystem functioning to all intertidal *Zostera* spp. beds. Although, *Z. marina* and *Z. noltii* seemed to be of limited importance as fresh food sources for the food web, seagrass created substrate and decreased the flow enhancing the sedimentation of particles which can be used in the food web. In addition, detrital matter of seagrass is incorporated in the SOM pool and integrated in the food web. Finally, the high diversity of generalist predators, in particular in *Z. marina* bed, suggests an important top-down control of the seagrass community.

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