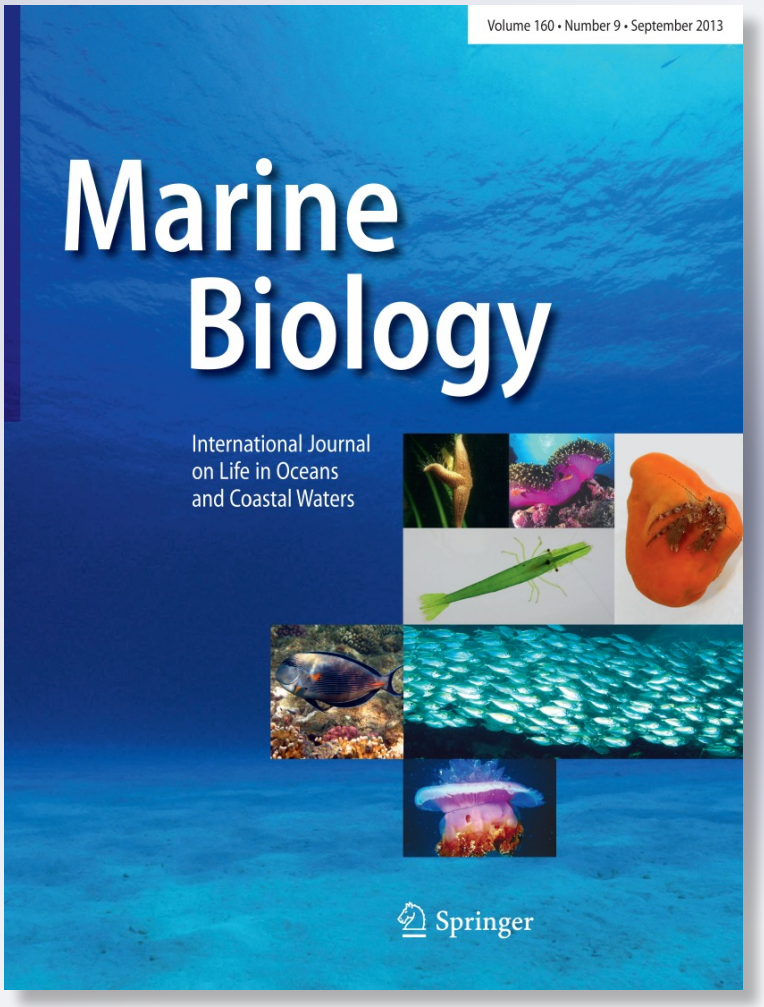
*Food sources of macrobenthos in an estuarine seagrass habitat (*Zostera noltii*) as revealed by dual stable isotope signatures*

Anna-Maria Vafeiadou, Patrick Materatski, Helena Adão, Marleen De Troch & Tom Moens

Marine Biology
International Journal on Life in Oceans and Coastal Waters

ISSN 0025-3162
Volume 160
Number 9

Mar Biol (2013) 160:2517-2523
DOI 10.1007/s00227-013-2238-0



Springer

Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Springer

Author's personal copy

Mar Biol (2013) 160:2517–2523
DOI 10.1007/s00227-013-2238-0

SHORT NOTE

Food sources of macrobenthos in an estuarine seagrass habitat (*Zostera noltii*) as revealed by dual stable isotope signatures

Anna-Maria Vafeiadou · Patrick Materatski · Helena Adão · Marleen De Troch · Tom Moens

Received: 14 November 2012 / Accepted: 3 April 2013 / Published online: 12 April 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Stable carbon and nitrogen isotope analysis was used to examine the resources and position of macrobenthos in an estuarine seagrass food web in two sampling moments, during summer and winter. The contribution of each food source to the carbon requirements of consumers was estimated by a mixing model. The used carbon sources were largely seagrass associated, although seagrass tissues were utilized by only few species, and equally contributed to microphytobenthos and suspended particulate organic matter. Based on isotopic data, Lucinidae bivalves have an alternative trophic pathway via symbiosis with chemoautotrophic bacteria. Resource utilization inside and adjacent to seagrass beds did not differ significantly, implying that seagrass-associated inputs extend well beyond the borders of the vegetation patches.

Communicated by F. Bulleri.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-013-2238-0) contains supplementary material, which is available to authorized users.

A.-M. Vafeiadou (✉) · M. De Troch · T. Moens
Marine Biology Section, Department of Biology, Ghent University, Krijgslaan 281/S8, 9000 Ghent, Belgium
e-mail: am.vafeiadou@gmail.com

P. Materatski
School of Sciences and Technology, University of Évora, NemaLab c/o IMAR, Apartado 94, 7002-554 Évora, Portugal

H. Adão
School of Sciences and Technology, University of Évora, CO-CIEMAR c/o NemaLab, Apartado 94, 7002-554 Évora, Portugal

Introduction

Food webs in estuarine ecosystems are characterized by the presence of diverse resources and high macrobenthic diversity (Deegan and Garritt 1997). Macrofauna rely on various carbon sources implying different competitive interactions (Herman et al. 2000) and often exhibit opportunistic feeding behaviour related to changes in habitat and food availability (Deegan and Garritt 1997; Stocks and Grassle 2001).

Seagrass beds contribute to estuarine ecosystem functioning by supporting high biodiversity and more complex food webs than bare sediments (Boström and Mattila 1999). They provide a variety of microhabitats and food, including seagrass leaves and roots, detritus and other associated carbon sources; that is, epiphytes, suspended particulate organic matter (SPOM) trapped in the canopy, and epibenthic and other microalgae in the sediments (Moncreiff and Sullivan 2001).

Although several studies have focused on food utilization by macrobenthos in seagrass beds (Lepoint et al. 2000; Kharlamenko et al. 2001; Moncreiff and Sullivan 2001; Baeta et al. 2009; Carlier et al. 2009; Lebreton et al. 2011; Ouisse et al. 2012), information about the relative importance of resources is still inconclusive (but see Sarà 2006, 2007). This study aims to document food web structure and elucidate the contribution of potential carbon sources to macrofauna diets in an estuarine seagrass habitat, using stable carbon and nitrogen isotopes. We address the following research questions: (1) Do seagrass-associated sources contribute substantially to the diet of macrobenthos? If so, we would expect differences in resource utilization in the seagrass bed vs adjacent unvegetated sediments. (2) Is there temporal variation in resource utilization by macrofauna?

Author's personal copy

Materials and methods

Sampling area and design

Sampling was located in the Mira estuary, SW Portugal (37°40′N, 8°40′W) (see Fig. 1B in Adão et al. 2009), a small mesotidal system with semidiurnal tides (amplitude 1–3 m during neap and spring tides, respectively). It has a single channel, 5–10 m deep and up to 400 m wide, which allows tidal influence up to 40 km upstream. Due to the limited and seasonally varying freshwater input, the lower section of the estuary has a dominant marine signature.

Sampling was conducted at neap low tide in summer (22/6/2010) and winter (7/2/2011), at two sites (A, ca. 1.5 km from the mouth of the estuary, and B, 2 km more upstream) in intertidal *Zostera noltii* (Hornem.) beds. Samples were collected from two stations (1, 2) at each site, one inside the seagrass bed (A1 and B1), the other in the adjacent bare sediment (A2 and B2). Seagrass vegetation was relatively sparse and significantly less dense (>50 %) in February 2011 than in June 2010.

Sampling and sample treatment

Macrofauna was collected with a core (141 cm^2) to 30 cm depth and sieved over a 0.5-mm mesh. The most abundant species were live-sorted, identified and at least three individuals, where possible, were incubated for 4–5 h in filtered habitat water to allow evacuation of gut contents, then stored frozen (−20 °C).

Animals with hard exoskeletons or shells were first dissected. Muscle tissue was used (Yokoyama et al. 2005), except for small organisms which were treated in toto. Samples were divided in two subsamples, one of which was pre-treated with dilute (10 %) HCl to remove carbonates, the other not receiving acid treatment to avoid effects of acidification on $\delta^{15}N$ (Bunn et al. 1995; Mateo et al. 2008; Vafeiadou et al. in press). All samples were oven-dried (60 °C) and transferred into aluminium cups (6.5 × 8 mm, Elemental Microanalysis Ltd.), pre-combusted for 4 h at 550 °C to remove organic contamination.

Three replicate sediment cores (upper 6 cm) were collected for bulk organic matter analysis. Seagrass was collected by hand and separated in fresh leaves, roots and (partly) decomposed tissue (3 replicates each), then oven-dried (60 °C) for 48 h. Epiphytes were collected by scraping the surface of fresh seagrass leaves with a cover glass. Microphytobenthos (MPB) was collected based on active migration to light through lens tissue (Eaton and Moss 1966). MPB samples were obtained in February and June 2012, 1 year later than, but at very similar sampling moments as the other samples. SPOM was collected by filtration of 1.5 L of seawater over pre-combusted Whatman GF/F filters. Epiphytic biofilms, MPB and filters were stored frozen prior to further analysis.

Sediments and seagrass materials were first ground to a homogeneous powder. Acidified and non-acidified subsamples were prepared as for macrobenthos. Filters with SPOM were cut in half, one half being acidified for 24 h under HCl vapour, the other not. Epiphytic biofilms and MPB were acidified since insufficient biomass was available for subsampling. All source samples were prepared in pre-combusted silver cups (8 × 5 mm, Elemental Microanalysis Ltd.).

Stable carbon and nitrogen isotope analyses

Isotopic analyses were performed using a ThermoFinnigan Flash 1112 elemental analyser (EA) coupled on-line via a Conflo III interface to a ThermoFinnigan Delta Plus XL isotope ratio mass spectrometer (IRMS). Stable isotope ratios are expressed relative to the conventional standards in units of parts per thousand, according to the formula:

$$\delta X = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right) \times 10^3 \text{ppt}$$

where X is ^{13}C or ^{15}N and R the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. As external lab standards, we used CH-6 (sucrose) and N1 (ammonium sulphate) from the International Atomic Energy Agency, with $\delta^{13}C$ and $\delta^{15}N$ values of −10.4 and +0.4 ‰, respectively.

Data analysis

We compared $\delta^{13}C$ and $\delta^{15}N$ ratios of macrofauna: (1) between vegetated (i.e. A1 and B1) and unvegetated stations (i.e. A2 and B2) and (2) between June and February. Comparisons used paired Student's t tests on pairs of isotopic ratios of the abundant species in both stations and/or months. Normality and homoscedasticity of data were tested using Shapiro–Wilk's and Levene's tests, respectively. No data transformation was required to meet these assumptions. We first assessed whether particular carbon sources had the same δ-values at both sampling times and sites, using the non-parametric Mann–Whitney U test. All statistical analyses were performed using Statistica 7.1 software (StatSoft).

A Bayesian stable isotope mixing model (Parnell et al. 2010) in SIAR 4.1.3 was applied to estimate the contribution of each source to macrobenthos diets. Data were carbon and nitrogen isotopic ratios per replicate sample of each species, excluding Lucinidae sp. based on its much depleted ^{13}C isotopic ratios (see results and discussion), and mean ± standard deviation (SD) of carbon and nitrogen isotopic ratios per source. Each species was considered at a certain trophic level according to its $\delta^{15}N$ composition (Vander Zanden and Rasmussen 2001). Mean ± SD

Author's personal copy

trophic enrichment factors of 1 ± 1.2 ‰ for $\delta^{13}C$ and 2.5 ± 2.5 ‰ for $\delta^{15}N$ were applied for each trophic step (Vander Zanden and Rasmussen 2001; Ouisse et al. 2012). Considering that bulk SOM is a mixture of several sources, we excluded it from the mixing model. To reduce SIAR pitfalls, we ran SIAR several times with different potential sources per consumer, fitting within available knowledge on their feeding mode (Fry 2013). We also ran SIAR to identify the contribution of each carbon source to the SOM pool, with the carbon isotopic ratios of all replicate samples of each source as input data, assuming zero fractionation. Admittedly, isotopic signatures of different SOM components can change during diagenesis (Benner et al. 1991; Altabet 1996) or decomposition (Cloern et al. 2002), as also shown by our data on detrital vs fresh seagrass material. Hence, the isotopic composition of the SOM pool is not a mere reflection of the different inputs. Nevertheless, including it in the SIAR would generally substantially and artificially reduce the estimated contributions of sources with intermediate isotopic signatures.

Results and discussion

Stable isotopic composition of potential food sources and their contributions to the SOM pool

Detritus was more depleted than fresh seagrass. Bulk SOM had $\delta^{13}C$ values closer to MPB (Fig. 1). As in Ouisse et al. (2012), $\delta^{13}C$ of epiphytes were intermediate between seagrass detritus and SPOM and very close to MPB signatures (Fig. 1). MPB were also more depleted in ^{13}C than typical for temperate estuarine MPB (Herman et al. 2000; Moens et al. 2002), albeit even lower values have been reported for sandy beach MPB (Maria et al. 2011). SPOM was the most ^{13}C-depleted source with $\delta^{13}C$ in the range of published values (Baeta et al. 2009). Source isotopic data did not show significant differences between months ($p > 0.05$ in all), except for MPB (for $\delta^{13}C$: $U = 0$; $Z = -2.7$, $N_1 = 4$, $N_2 = 6$, $p = 0.006$ and for $\delta^{15}N$: $U = 0$; $Z = 3$, $N_1 = 4$, $N_2 = 6$, $p = 0.003$), in accordance with Baeta et al. (2009) in the Mondego estuary (Portugal). Thus, seagrass detritus had the highest contribution to SOM in June (ca. 0.2–0.3), while epiphytes, MPB and SPOM contributed almost equally (c.a. 0.05–0.2; SM1). In February, the contribution of seagrass detritus decreased (ca. 0–0.1), whereas those of epiphytes and SPOM increased (SM1) as a result of the sparser seagrass vegetation.

Food web analysis of macrobenthos in *Z. noltii* beds

Macrofauna mean $\delta^{13}C$ ranged from −27.9 to −15.1 ‰ for Lucinidae sp. and Idoteidae sp., respectively, and mean $\delta^{15}N$ from −3.1 to 12.6 ‰ for Lucinidae sp. and Glyceridae sp., respectively (Table 1).

We initially performed several SIAR runs, each with different selections of carbon sources for each consumer taking into account available information on its feeding ecology. However, to avoid undue subjectivity and to reduce artefacts (especially biased contributions of sources with intermediate carbon isotopic ratios), we included all candidate sources with the exceptions of SOM (see above) and living seagrass tissue. The latter was included only for specific macrofauna based on their isotopic data (see Table 1) and on the literature. For instance, Idoteidae and *Hydrobia ulvae* (Pennant, 1777) can graze on fresh seagrass leaves (Philippart 1995; Sturaro et al. 2010), while the crab *Carcinus maenas* (Linnaeus, 1758) has a more complex feeding behaviour, including grazing on plant material, predation on other macrofauna, and even cannibalism (Ropes 1968; Moksnes 2004).

Seagrass detritus had a high contribution to the diet of the bivalves *Cerastoderma edule* (Linnaeus, 1758) and *Scrobicularia plana* (da Costa, 1778), the crab *C. maenas*, and the polychaete Nereidae sp. at the vegetated stations in June, with MPB being the second most important source; epiphytes also contributed to the carbon ration of the first two species. Seagrass leaves, detritus and MPB had almost equal contributions to the diet of the gastropod *H. ulvae*. MPB and SPOM were the primary sources for the polychaetes Capitellidae sp. and Cirratulidae sp.1. Results for the unvegetated stations were similar, except for much lower contributions of MPB and SPOM to the diets of *S. plana* and *H. ulvae* (Table 1).

In February, seagrass detritus had a high contribution to the diet of *S. plana* and insect larvae at the unvegetated stations, with epiphytes being their second most important source, while seagrass leaves and detritus contributed importantly to the diet of Idoteidae sp. SPOM was the primary source for all polychaetes at all stations; however, it contributed almost equally with seagrass detritus and epiphytes to the diet of Capitellidae sp. and Nephtyidae sp. (Table 1).

The slightly elevated $\delta^{15}N$ of *C. maenas* suggests that predation is part of its feeding ecology. Our $\delta^{13}C$ data point at *S. plana*, Mactridae sp., *H. ulvae*, and the polychaetes Glyceridae sp., Nephtyidae sp. and Nereidae sp. as candidate prey of the crab.

Despite the high nutritional value of epiphytes in temperate seagrass systems (Kitting et al. 1984; Lepoint et al. 2000), their role in *Z. noltii* beds is controversial due to their very low biomass, especially compared to that of benthic microalgae which is much higher in seagrass habitats and more constant throughout the year (Philippart 1995; Lebreton et al. 2009, 2011). Considering the relatively sparse seagrass vegetation in February, we suggest

Author's personal copy

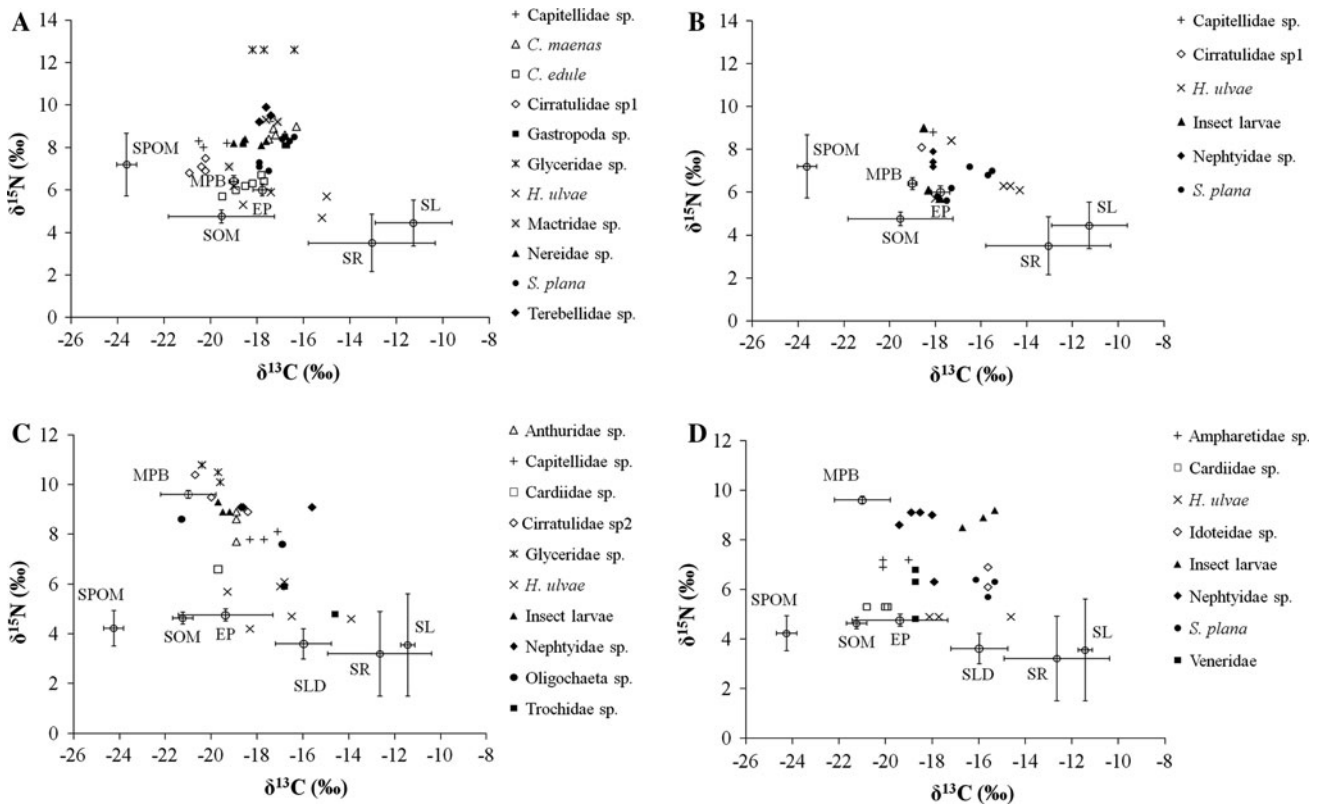


Fig. 1 $\delta^{13}C/\delta^{15}N$ plot of macrobenthos and their potential food sources at vegetated and unvegetated stations in June (**a**, **b**) and in February (**c**, **d**), respectively. Data of sources are mean values (±SD) of all replicates per type of material. (SL, SR and SLD state for seagrass fresh leaves, roots and leaves in decomposition, respectively, EP for epiphytes)

that the SIAR results may in part derive from the similar carbon isotopic ratios of MPB and epiphytes, which may have resulted in an overestimation of the contribution of one source at the expense of the other (Fry 2013).

Microphytobenthos and sedimented phytoplankton are main food sources for epibenthic suspension feeders, grazers and deposit feeders (Herman et al. 2000; Lebreton et al. 2009, 2011). The increased contribution of SPOM—especially for polychaetes and insect larvae—together with its significant contribution to the SOM pool in February, indicates a shift from MPB to SPOM utilization from June to February. $\delta^{15}N$ placed consumers at four different trophic levels (Table 1). Glyceridae sp. is a top predator; other polychaetes (i.e. Nephtyidae sp., Nereidae sp.), oligochaetes, the crab species and Anthuridae sp. are intermediate predators. SIAR supports the expected suspension-feeding mode for Ampharetidae sp. and Cirratulidae spp. and a deposit-feeding strategy for Terebellidae sp., although their $\delta^{15}N$ ratios were unexpectedly high.

The isotopic composition of the Lucinidae sp. (mean $\delta^{13}C = -27.9 \pm 1.2$ ‰ and mean $\delta^{15}N = -3.1 \pm 1$ ‰) clearly indicates a deviant feeding ecology. The recorded δ-values are in line with those of Lucinidae from mangrove ($\delta^{13}C$ varying from −32 to −28 ‰ and $\delta^{15}N$ from −11 to +4 ‰, Bouillon et al. 2008) and deep-sea habitats ($\delta^{13}C \approx -30$ ‰ and $\delta^{15}N \approx +4$ ‰, Gulf of Cadiz, Rodrigues et al. 2010). These data confirm the idea that lucinid bivalves host chemoautotrophic sulphur-oxidizing bacteria and obtain a substantial part of their carbon from this symbiosis (Lebata and Primavera 2001; Duperron et al. 2007) and extend this finding for the first time to estuarine seagrass habitats.

Spatial and temporal variation of macrobenthos isotopic composition

There were no significant differences in $\delta^{13}C$ and $\delta^{15}N$ of consumers between vegetated and bare stations ($t_{10} = 2.44$, $p = 0.05$ and $t_{10} = 0.56$, $p = 0.585$, respectively) in both months; hence, our results support the idea that carbon inputs associated with seagrass beds in our study area extend well beyond the vegetation boundaries and contribute to the diet of macrobenthos in adjacent sediments (Heck et al. 2008).

Differences in $\delta^{13}C$ and $\delta^{15}N$ between sampling moments were not significant ($t_7 = 1.73$, $p = 0.133$ and $t_7 = 0.09$, $p = 0.926$, respectively), in agreement with Baeta et al. (2009) and Ouisse et al. (2012). While these

Author's personal copy

Table 1 Mean (±SD) carbon and nitrogen isotopic data of macrofauna and relative contributions of potential sources to their carbon requirements per sampling month, at vegetated and unvegetated stations

Class	Macrofauna species	*n*	TP	Mean $\delta^{13}C \pm SD$ (‰)	Mean $\delta^{15}N \pm SD$ (‰)	Relative contribution (CI = 95 %)				
						SL	SLD	EP	MPB	SPOM
June 2010 vegetated stations										
Bivalvia	*Cerastoderma edule*	6	2	−18.43 ± 0.69	6.22 ± 0.34	Nc	0.29–0.4	0.17–0.33	0.24–0.36	0.03–0.14
	Lucinidae sp.	6	Nc	−28.17 ± 1.07	−2.92 ± 0.38	Nc	Nc	Nc	Nc	Nc
	Mactridae sp.	3	3	−17.97 ± 1.1	8.53 ± 1.24	Nc	0.23–0.37	0.16–0.33	0.2–0.37	0.12–0.28
	Scrobicularia plana	6	3	−17.2 ± 0.66	7.75 ± 0.73	Nc	0.31–0.46	0.14–0.34	0.24–0.37	0.01–0.1
Malacostraca	*Carcinus maenas*	6	3	−17.07 ± 0.44	8.82 ± 0.36	0.08–0.19	0.18–0.32	0.09–0.25	0.22–0.34	0.12–0.24
Gastropoda	Gastropoda sp.	3	3	−16.77 ± 0.06	8.23 ± 0.23	Nc	0.29–0.43	0.21–0.39	0.15–0.31	0.02–0.15
	Hydrobia ulvae	5	2	−17.04 ± 1.87	5.56 ± 0.58	0.16–0.3	0.18–0.34	0.01–0.17	0.18–0.32	0.01–0.13
Polychaeta	Capitellidae sp.	3	3	−20.03 ± 0.64	8.17 ± 0.15	Nc	0.04–0.18	0.01–0.16	0.28–0.47	0.22–0.4
	Cirratulidae sp.1	4	2	−20.43 ± 0.33	7.08 ± 0.31	Nc	0.02–0.1	0.01–0.11	0.31–0.52	0.24–0.46
	Glyceridae sp.	3	4	−17.43 ± 0.06	12.6 ± 0.23	Nc	0.17–0.33	0.16–0.34	0.2–0.36	0.19–0.34
	Nereidae sp.	6	3	−18.35 ± 0.54	8.25 ± 0.1	Nc	0.24–0.37	0.06–0.22	0.3–0.46	0.17–0.21
	Terebellidae sp.	3	3	−17.63 ± 0.25	9.53 ± 0.35	Nc	0.23–0.37	0.2–0.36	0.18–0.33	0.13–0.28
June 2010 unvegetated stations										
Bivalvia	Lucinidae sp.	6	Nc	−27.85 ± 0.56	−3.35 ± 0.81	Nc	Nc	Nc	Nc	Nc
	Scrobicularia plana	6	2	−16.73 ± 0.99	6.43 ± 0.66	Nc	0.32–0.49	0.17–0.27	0.15–0.3	0–0.1
Gastropoda	*Hydrobia ulvae*	6	2	−16.1 ± 1.61	6.87 ± 1.21	0.22–0.32	0.18–0.32	0.1–0.27	0.1–0.25	0.02–0.14
Insecta	Insect larvae	3	2	−18.2 ± 0.36	6.93 ± 1.8	Nc	0.19–0.35	0.17–0.35	0.2–0.37	0.16–0.32
Polychaeta	Capitellidae sp.	1	3	−18.1	8.8	Nc	0.17–0.27	0.16–0.26	0.16–0.26	0.16–0.25
	Cirratulidae sp.1	1	3	−18.6	8.1	Nc	0.17–0.36	0.16–0.35	0.17–0.38	0.16–0.38
	Nephtyidae sp.	3	3	−18.13 ± 0.06	7.5 ± 0.36	Nc	0.21–0.36	0.15–0.35	0.22–0.38	0.13–0.31
February 2011 vegetated stations										
Bivalvia	Cardiidae sp.	1	2	−19.7	6.6	Nc	0.17–0.36	0.16–0.36	0.15–0.35	0.18–0.37
	Lucinidae sp.	3	Nc	−28.37 ± 1.63	−1.67 ± 0.42	Nc	Nc	Nc	Nc	Nc
Malacostraca	Anthuridae sp.	3	3	−18.87 ± 0.06	8.4 ± 0.62	Nc	0.18–0.34	0.17–0.36	0.01–0.17	0.28–0.41
Gastropoda	*Hydrobia ulvae*	6	2	−16.97 ± 1.84	5.2 ± 0.79	0.19–0.32	0.17–0.35	0.12–0.3	0–0.05	0.19–0.34
	Trochidae sp.	2	2	−15.7 ± 1.56	5.35 ± 0.78	0.2–0.35	0.17–0.33	0.13–0.3	0.01–0.18	0.06–0.24
Insecta	Insect larvae	3	3	−19.47 ± 0.25	9.03 ± 0.23	Nc	0.12–0.27	0.17–0.36	0.04–0.22	0.3–0.44
Oligochaeta	Oligochaeta sp.	2	3	−19.1 ± 3.11	8.1 ± 0.71	Nc	0.17–0.37	0.17–0.38	0.02–0.24	0.21–0.4
Polychaeta	Capitellidae sp.	3	3	−17.7 ± 0.6	7.9 ± 0.17	Nc	0.25–0.4	0.17–0.36	0.1–0.15	0.2–0.35
	Cirratulidae sp.2	3	3	−19.7 ± 1.18	9.6 ± 0.75	Nc	0.01–0.16	0.1–0.33	0–0.18	0.3–0.51
	Glyceridae sp.	4	4	−20.03 ± 0.43	10.55 ± 0.33	Nc	0–0.1	0.02–0.2	0–0.1	0.5–0.69
	Nephtyidae sp.	3	3	−17.63 ± 1.76	9.07 ± 0.06	Nc	0.24–0.38	0.2–0.37	0.03–0.19	0.2–0.36
February 2011 unvegetated stations										
Bivalvia	Cardiidae sp.	3	2	−20.23 ± 0.49	5.33 ± 0.06	Nc	0.14–0.29	0.16–0.35	0.01–0.17	0.28–0.44
	Lucinidae sp.	3	Nc	−26.7 ± 1.01	−4.43 ± 0.35	Nc	Nc	Nc	Nc	Nc
	Scrobicularia plana	3	2	−15.67 ± 0.4	6.13 ± 0.38	Nc	0.36–0.54	0.17–0.38	0–0.15	0.01–0.14
	Veneridae sp.	3	2	−18.7 ± 0.1	5.97 ± 1.04	Nc	0.25–0.38	0.17–0.36	0.02–0.16	0.2–0.35
Malacostraca	Idoteidae sp.	2	2	−15.6	6.5 ± 0.57	0.21–0.35	0.17–0.32	0.12–0.27	0.03–0.18	0.03–0.18
Gastropoda	*Hydrobia ulvae*	3	2	−16.80 ± 1.92	4.8 ± 0.1	0.18–0.31	0.16–0.33	0.13–0.3	0–0.12	0.14–0.28

Author's personal copy

Table 1 continued

Class	Macrofauna species	*n*	TP	Mean $\delta^{13}C \pm SD$ (‰)	Mean $\delta^{15}N \pm SD$ (‰)	Relative contribution (CI = 95 %)				
						SL	SLD	EP	MPB	SPOM
Insecta	Insect larvae	3	3	−15.93 ± 0.71	8.87 ± 0.35	Nc	0.32–0.5	0.2–0.39	0.02–0.15	0.03–0.17
Polychaeta	Ampharetidae sp.	3	2	−19.73 ± 0.64	7.1 ± 0.17	Nc	0.05–0.2	0.15–0.35	0.01–0.21	0.3–0.46
	Nephtyidae sp.	5	3	−18.54 ± 0.63	8.42 ± 1.2	Nc	0.22–0.35	0.16–0.34	0.01–0.12	0.3–0.42

n number of replicate samples, *TP* Trophic position, *CI* confidence intervals, *Nc* not considered

results demonstrate that macrobenthos in *Z. noltii* beds in southern Europe rely on the same food sources year-round, this does not necessarily imply constant contributions of different carbon sources across seasons. Indeed, our results showed that the contribution of carbon sources differed between sampling times for some species.

Conclusions

In spite of the relatively large variation of stable isotopic ratios for most macrofauna, there is evidence that seagrass-associated carbon sources, such as epiphytic microalgae and seagrass detritus, were equally important as MPB and SPOM for the diet of estuarine macrobenthos. Resource utilization inside and adjacent to seagrass patches was very similar across species, demonstrating that seagrass-associated inputs extend beyond the borders of the vegetation patches. This highlights the importance, direct and indirect, of seagrass vegetation to the macrobenthos. Furthermore, this study confirms for the first time the dependence of Lucinidae on symbiotic chemoautotrophic bacteria in estuarine seagrass environments.

Acknowledgments The authors acknowledge financial support from the Flemish Science Foundation FWO through project 3G.0192.09 and from the research council of Ghent University through projects BOF0110600002 and BOF-GOA 01GA1911 W. MDT is a postdoctoral researcher financed by the latter project. We are grateful to Prof. Dr. Frank Dehairs for access to the stable isotope facilities of the Department of Analytical and Environmental Chemistry of the Free University Brussels and to Michael Korntheuer and Leen Rymenans for technical assistance during stable isotope measurements. Two anonymous reviewers contributed to constructive remarks.

References

Adão H, Alves AS, Patrício J, Neto JM, Costa MJ, Marques JC (2009) Spatial distribution of subtidal Nematoda communities along the salinity gradient in southern European estuaries. Acta Oecol 35:287–300. doi:10.1016/j.actao.2008.11.007

Altabet MA (1996) Nitrogen and carbon isotopic tracers of the source and transformation of particles in the deep sea. SCOPE 57:155–184

Baeta A, Pinto R, Valiela I, Richard P, Niquil N, Marques JC (2009) $\delta^{15}N$ and $\delta^{13}C$ in the Mondego estuary food web: seasonal variation in producers and consumers. Mar Environ Res 67:109–116. doi:10.1016/j.marenvres.2008.11.005

Benner R, Fogel ML, Sprague EK (1991) Diagenesis of below ground biomass of *Spartina alterniflora* in salt marsh sediments. Limnol Oceanogr 36:1358–1374

Boström C, Mattila M (1999) The relative importance of food and shelter for seagrass associated invertebrates: a latitudinal comparison of habitat choice by isopod grazers. Oecologia 120:162–170. doi:10.1007/s004420050845

Bouillon S, Connolly R, Lee SY (2008) Organic matter exchange and cycling in mangrove ecosystems: recent insights from stable isotope studies. J Sea Res 59:44–58. doi:10.1016/j.seares.2007.05.001

Bunn SE, Loneragan NR, Kempster MA (1995) Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes. Limnol Oceanogr 40:622–625. doi:10.2307/2838177

Carlier A, Riera P, Amouroux JM, Bodiou JY, Desmalades M, Grémare A (2009) Spatial heterogeneity in the food web of a heavily modified Mediterranean coastal lagoon: stable isotope evidence. Aquat Biol 5:167–179. doi:10.3354/ab00147

Cloern JE, Canuel EA, Harris D (2002) Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnol Oceanogr 47:713–729. doi:10.4319/lo.2002.47.3.0713

Deegan LA, Garritt RH (1997) Evidence for spatial variability in estuarine food webs. Mar Ecol Prog Ser 147:31–47. doi:10.3354/meps147031

Duperron S, Fiala-Medioni A, Caprais JC, Olu K, Sibuet M (2007) Evidence for chemoautotrophic symbiosis in a Mediterranean cold seep clam (Bivalvia: Lucinidae): comparative sequence analysis of bacterial 16S rRNA, APS reductase and RubisCO genes. FEMS Microbiol Ecol 59:64–70. doi:10.1111/j.1574-6941.2006.00194.x

Eaton JW, Moss B (1966) The estimation of numbers and pigment content in epipelic algal populations. Limnol Oceanogr 11:584–595

Fry B (2013) Alternative approaches for solving underdetermined isotope mixing problems. Mar Ecol Prog Ser 472:1–13. doi:10.3354/meps10168

Heck KL Jr, Carruthers T, Duarte C et al (2008) Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems 11:1198–1210. doi:10.1007/s10021-008-9155-y

Herman PMJ, Middelburg JJ, Widdows J, Lucas CH, Heip CHR (2000) Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. Mar Ecol Prog Ser 204:79–92

Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001) Identification of food sources of invertebrates from the seagrass

Author's personal copy

Zostera marina community using carbon and sulphur stable isotope ratio and fatty acid analyses. Mar Ecol Prog Ser 220:103–117. doi:10.3354/meps220103

Kitting CL, Fry B, Morgan MD (1984) Detection of inconspicuous epiphytic algae supporting food webs in seagrass meadows. Oecologia 62:145–149. doi:10.1007/BF00379006

Lebata MJHL, Primavera JH (2001) Gill structure, anatomy and habitat of *Anodontia edulenta*: evidence of endosymbiosis. J Shellfish Res 20:1273–1278

Lebreton B, Richard P, Radenac G, Bordes M, Bréret M, Arnaud C, Mornet F, Blanchard GF (2009) Are epiphytes a significant component of intertidal *Zostera noltii* beds? Aquat Bot 91:82–90. doi:10.1016/j.aquabot.2009.03.003

Lebreton B, Richard P, Galois R, Radenac G, Pfléger C, Guillou G, Mornet F, Blanchard GF (2011) Trophic importance of diatoms in an intertidal *Zostera noltii* seagrass bed: evidence from stable isotope and fatty acid analyses. Estuar Coast Shelf Sci 92:140–153. doi:10.1016/j.ecss.2010.12.027

Lepoint G, Nyssen F, Gobert S, Dauby P, Bouquegneau JM (2000) Relative impact of a seagrass bed and its adjacent epilithic algal community in consumer diets. Mar Biol 136:513–518. doi:10.1007/s002270050711

Maria TF, De Troch M, Vanaverbeke J, Esteves AM, Vanreusel A (2011) Use of benthic vs planktonic organic matter by sandy-beach organisms: a food tracing experiment with ^{13}C labeled diatoms. J Exp Mar Biol Ecol 407:309–314. doi:10.1016/j.jembe.2011.06.028

Mateo MA, Serrano O, Serrano L, Michener RH (2008) Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. Oecologia 157:105–115. doi:10.1007/s00442-008-1052-8

Moens T, Luyten C, Middelburg JJ, Herman PMJ, Vincx M (2002) Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. Mar Ecol Prog Ser 234:127–137. doi:10.3354/meps234127

Moksnes PO (2004) Interference competition for space in nursery habitats: density dependent effects on growth and dispersal in juvenile shore crabs *Carcinus maenas*. Mar Ecol Prog Ser 281:181–191. doi:10.3354/meps281181

Moncreiff CA, Sullivan MJ (2001) Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. Mar Ecol Prog Ser 215:93–106. doi:10.3354/meps215093

Ouisse V, Riera P, Migné A, Leroux C, Davoult D (2012) Food web analysis in intertidal *Zostera marina* and *Zostera noltii* communities in winter and summer. Mar Biol 159:165–175. doi:10.1007/s00227-011-1796-2

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5:e9672. doi:10.1371/journal.pone.0009672

Philippart CJM (1995) Effect of periphyton grazing by *Hydrobia ulvae* on the growth of *Zostera noltii* on a tidal flat in the Dutch Wadden Sea. Mar Biol 122:431–437. doi:10.1007/BF00350876

Rodrigues CF, Webster G, Cunha MR, Duperron S, Weightman AJ (2010) Chemosynthetic bacteria found in Bivalvia species from mud volcanoes of the Gulf of Cadiz. FEMS Microbiol Ecol 73:486–499. doi:10.1111/j.1574-6941.2010.00913.x

Ropes JW (1968) The feeding habits of the green crab, *Carcinus maenas* (L.). Fish Bull 67:183–203

Sarà G (2006) Hydrodynamic effect on the origin and quality of organic matter for bivalves: an integrated isotopic, biochemical and transplant study. Mar Ecol Prog Ser 328:65–73. doi:10.3354/meps328065

Sarà G (2007) Sedimentary and POM: mixed sources for *Cerastoderma glaucum* in a Mediterranean shallow pond. Aquat Living Resour 20:271–277. doi:10.1051/alr:2007040

Stocks KI, Grassle JF (2001) Effects of microalgae and food limitation on the recolonization of benthic macrofauna into in situ saltmarsh–pond mesocosms. Mar Ecol Prog Ser 221:93–104. doi:10.3354/meps221093

Sturaro N, Caut S, Gobert S, Bouquegneau JM, Lepoint G (2010) Trophic diversity of idoteids (Crustacea, Isopoda) inhabiting the *Posidonia oceanica* litter. Mar Biol 157:237–247. doi:10.1007/s00227-009-1311-1

Vafeiadou AM, Adão H, De Troch M, Moens T (in press) Sample acidification effects on carbon and nitrogen stable isotope ratios of macrofauna from a *Zostera noltii* bed. Mar Freshwater Res

Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

Yokoyama H, Tamaki A, Harada K, Shimoda K, Koyama K, Ishihi Y (2005) Variability of diet-tissue isotopic fractionation in estuarine macrobenthos. Mar Ecol Prog Ser 296:115–128. doi:10.3354/meps296115