

NOTE

Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources

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ABSTRACT: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured in benthic invertebrates and food sources collected in the salt marsh of the Aiguillon Bay, France. The results showed that, although *Spartina anglica* was dominant, this marine phanerogame did not contribute significantly to the carbon and nitrogen requirements of the invertebrates considered in this study. In fact, *Macoma balthica*, *Scrobicularia plana* and *Mytilus edulis* preferentially fed on a mixture of benthic diatoms and marine phytoplankton, but in different proportions, as was estimated through isotope mixing models. However, this isotopic data set revealed complex trophic relationships for the nematode community and for the crab *Carcinus maenas* which did not allow accurate isotopic determination of food sources.

KEY WORDS: Carbon and nitrogen isotope ratios · Muddy salt marsh · *Spartina anglica* · Benthic diatoms · Benthic invertebrates

Coastal environments are generally characterised by a large number of locally produced and allochthonous potential food sources for benthic consumers. The diversity in origin of organic matter makes it difficult to determine which are the most important food sources and to quantitatively estimate their proportions in animal diets. The different food sources contribute to an important detrital organic matter pool (Pocklington & Tan 1987, Mann 1988), and may be used differently by

different animals due to their digestibility or because of hydrology that influences their production and availability to consumers (Schwinghamer et al. 1983, Monbet 1992). For example, in salt marshes vascular plants are less digestible than organic matter derived from algae, but may be used as a food source when they are decomposed by bacteria (Tenore 1983, Newell & Langdon 1986, Mann 1988).

The analysis of stable isotope ratios of carbon and nitrogen of particulate organic matter (POM) in coastal environments has received increasing interest. This method allows the determination of different sources of organic matter in the POM pool and its exchange due to trophic interactions (Fry & Sherr 1984, Deegan et al. 1990). A recent study, using stable isotopes ratios, has pointed out the preferential utilisation of local organic matter sources by benthic invertebrates in coastal marshes (Deegan & Garritt 1997). However, allochthonous organic matter may increase the number of sources that can be used by local invertebrates. Particularly in estuarine habitats, significant incorporation of terrestrial organic matter by bivalve molluscs may occur in upper estuarine reaches or during a period of high freshwater discharge (Incze et al. 1982, Riera & Richard 1996). Once the main food sources of a consumer have been identified, a quantitative assessment of the proportions of the different sources in the diet can be calculated through the isotopic mixing model provided that their stable isotope ratios differ sufficiently (Dunton & Schell 1987, Gleason & Wellington 1988).

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The aim of this study was to investigate the role of the dominant local primary producers in a salt marsh (i.e. *Spartina anglica* and benthic diatoms) as a food source for the main benthic invertebrates through the use of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. In addition, on the basis of this data set this study addresses the question of whether isotopic mixing models can be used to estimate the proportions of food sources in the diet of benthic invertebrates.

Material and methods. Sample collection and preparation: Aiguillon Bay is centrally located on the Atlantic coast of France and contains an intertidal muddy salt marsh of about 50 km². Filter-feeding bivalves are the main component of the animal biomass in this bay, since it represents one of the largest areas for mussel culture in France (Dardignac-Corbeil & Prou 1995). In addition to oceanic inputs, Aiguillon Bay receives freshwater mainly from the river La Sèvre Niortaise (maximum 100 m³ s⁻¹ during winter). Parts of the muddy intertidal sediments support dense populations of the marine phanerogame *Spartina anglica* (Mahé 1994). Sampling was performed in April 1998 in a muddy salt marsh near the mouth of the river La Sèvre Niortaise, which was colonized by *Spartina anglica*. Water samples were collected by pumping (10 l) from about 20 cm below the water surface at the sampling site. POM for isotope analysis was collected by filtration on precombusted Whatman GF/F filters within 2 h after collection. Subsequently, the filters were acidified (10% HCl) in order to remove carbonates, quickly rinsed with distilled water and then lyophilized. At low tide, sediment samples were taken in the salt marsh by scraping the upper 1 cm of mud over a total surface area of approximately 1 m². In the laboratory, sediment was freeze-dried and ground to powder using a pestle and mortar. Freeze-dried sediment samples were analysed for organic carbon and nitrogen stable isotopes after acidification according to the procedure of Nieuwenhuize et al. (1994).

At low tide, live and dead leaves and twigs of *Spartina anglica* were collected by hand. We also collected the phanerogame *Plantago maritima* in the salt marsh and the bladder wrack *Fucus vesiculosus* on artificial rocks bordering the marsh. These samples were cleaned of their epibionts, washed with 10% HCl to remove carbonates and rinsed with distilled water. Subsequently, the samples were freeze-dried and ground to powder using a pestle and mortar.

Spartina anglica and mats of benthic diatoms were common in the salt marsh. Benthic diatoms were collected in the marsh at low tide by scraping the surface of the sediment and extracted according to a method slightly modified from that of Couch (1989). The sediment containing the diatoms was put into flat trays to form a 1 cm thick layer. A nylon screen (63 μm mesh)

was put on top of the sediment and this was covered with a 5 mm thick layer of combusted silica powder (60 to 210 μm). The trays were illuminated for 1 to 3 h, until the first dense brown mats appeared at the surface (usually 0.5 to 2 h). Meanwhile, the silica powder was kept moist by spraying filtered (GF/F) seawater from the sampling site. The top 2 mm of the silica powder was removed, and sieved over a 63 μm mesh nylon screen in order to separate the diatoms from the remaining silica powder and nematodes or copepods. The diatoms were then collected on precombusted GF/F filters, washed with 10% HCl, quickly rinsed with distilled water and subsequently freeze-dried.

The tellinacean bivalves *Macoma balthica* and *Scrobicularia plana* and the green crab *Carcinus maenas* were collected in the salt marsh at low tide. The blue mussel *Mytilus edulis* was collected on the artificial rocks bordering the marsh. In the laboratory all individuals were cleaned of epibionts and incubated overnight in filtered seawater from the sampling site to allow evacuation of gut contents. The specimens were then killed by freezing. After dissection from the shell (molluscs) or from the entire cuticle (crabs), the tissue was acidified (10% HCl) to remove any carbonate debris and rinsed with distilled water. The material was subsequently freeze-dried and ground to powder using a pestle and mortar.

Nematodes were collected in the salt marsh at low tide by scraping the upper 2 cm of muddy sediments where most of the meiofauna (i.e. harpacticoids and nematodes) occurred (Coull & Bell 1979). Nematodes were extracted from 3 l of sediment. In the laboratory, meiofauna was concentrated by sieving the sediment cautiously through a 500 μm sieve and subsequently through a 125 μm sieve in order to remove macrofauna, large particles, and to decrease the sample volume (Couch 1988). The fraction retained on the 125 μm sieve was used for the extraction of nematodes. Nematodes were isolated due to their negative phototropism, following the procedure of Couch (1988), modified by Riera et al. (1996). Although this method did not give a quantitative extraction of all nematodes, it did not require the use of chemicals that could interfere with the analyses. Following extraction, the nematodes were starved for 12 h in 0.7 μm filtered seawater, during which time the gut contents were cleared, and then rinsed cautiously prior to further removal of any remaining contaminants, which was done manually, using a dissecting microscope. Nematodes were finally collected on precombusted Whatman GF/F filters (80 to 100 pooled individuals), briefly rinsed with distilled water and kept frozen until analysis.

Stable isotope analysis: Carbon and nitrogen isotope ratios were determined using a Fisons CN analyser

Table 1. Carbon and nitrogen stable isotope ratios (mean \pm SD) for SOM, POM, primary producers and benthic invertebrates in Aiguillon Bay, France. n: number of analysed samples

Sample	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Organic matter sources			
SOM	3	-20.6 ± 0.5	6.4 ± 0.4
Suspended POM	3	-22.4 ± 0.0	5.0 ± 1.2
Benthic diatoms	4	-14.4 ± 0.2	4.5 ± 0.3
Living <i>Spartina anglica</i>	3	-13.5 ± 0.2	4.4 ± 0.0
Detrital <i>Spartina anglica</i>	3	-14.2 ± 0.2	6.4 ± 0.0
<i>Plantago maritima</i>	3	-24.9 ± 0.1	9.4 ± 0.0
<i>Fucus vesiculosus</i>	3	-19.6 ± 0.1	5.7 ± 0.0
Bentic invertebrates			
Nematodes	7	-16.2 ± 1.7	7.5 ± 1.1
<i>Mytilus edulis</i>	5	-17.4 ± 0.2	9.4 ± 0.4
<i>Macoma balthica</i>	5	-14.9 ± 0.7	8.5 ± 0.2
<i>Scrobicularia plana</i>	2	-15.6 ± 0.4	9.0 ± 0.3
<i>Carcinus maenas</i>	2	-14.3 ± 0.0	10.4 ± 1.2

coupled, via a Finnigan con-flo 2 interface, to a Finnigan Delta S mass spectrometer.

Data are expressed in the standard δ unit notation where

$$\delta X = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 10^3$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ for carbon and ${}^{15}\text{N}/{}^{14}\text{N}$ for nitrogen, and reported relative to the Vienna Pee Dee Belemnite standard (PDB) for carbon and to air N_2 for nitrogen. Average reproducibilities based on replicate measurements for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were about 0.1 and 0.13‰, respectively.

Results and discussion. Isotopic characterisation of SOM, POM and potential organic matter sources for invertebrates: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) for suspended POM, sedimented organic matter (SOM), primary producers and benthic invertebrates are summarized in Table 1. POM $\delta^{15}\text{N}$ was 5.0 ± 1.2 ‰, close to $\delta^{15}\text{N}$ of 5.9‰ for estuarine POM reported for the Tay estuary (Thornton & Mc Manus 1994). SOM $\delta^{15}\text{N}$ was 6.4 ± 0.4 ‰, similar to $\delta^{15}\text{N}$ values reported previously for other coastal intertidal sediments (Couch 1989, Owens & Law 1989, Riera et al. 1996). In the dual isotope plot, marsh SOM is found in the area in which it was predicted to be when it is derived from the main local primary producers. This indicated little

contribution of allochthonous sources to the SOM pool, while the isotopic composition of the suspended POM seemed to be more influenced by terrestrial organic matter (Fig. 1).

Mean $\delta^{13}\text{C}$ values of -14.4 ± 0.2 , -13.5 ± 0.2 and -14.2 ± 0.2 ‰ were measured for benthic diatoms and live and detrital *Spartina anglica*, respectively. The $\delta^{13}\text{C}$ for benthic diatoms were close to the $\delta^{13}\text{C}$ reported for benthic diatoms in mudflats of Marennes-Oléron Bay, France (Riera et al. 1996). The $\delta^{15}\text{N}$ for benthic diatoms (4.5 ± 0.3 ‰) were comparable to $\delta^{15}\text{N}$ values of benthic diatoms inhabiting mudflats of Marennes-Oléron Bay, France (4.1 to 6.9‰) (Riera 1998), but less depleted than the 3.9‰ reported by Couch (1989) for benthic diatoms in a salt marsh of the North Inlet Estuary (South Carolina). A mean $\delta^{15}\text{N}$ value of 6.4 ± 0.05 ‰ was measured in detrital

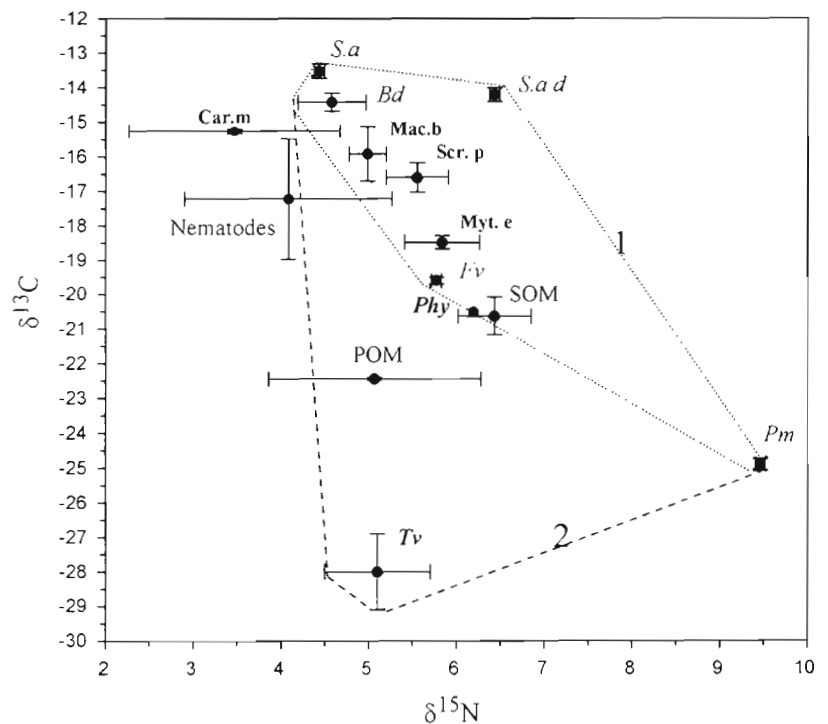


Fig. 1. $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ of benthic invertebrates corrected for trophic fractionation, compared to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of POM, SOM and the main organic matter sources in Aiguillon Bay. Primary producers: Sa = *S. anglica*, Sad = detrital *Spartina anglica*, Bd = benthic diatoms, Pm = *Plantago maritima*, Tv = terrestrial vegetation, Phy = phytoplankton. Primary consumers: Nematodes, Myt.e = *Mytilus edulis*, Mac.b = *Macoma balthica*, Scr.p = *Scrobicularia plana*. Secondary consumer: Car.m = *Carcinus maenas*. 1 and 3.5‰ were subtracted from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values respectively of primary consumers to correct for trophic fractionation. 2 and 7‰ were subtracted from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values respectively of *Carcinus maenas*. Dashed lines encompass the areas where isotopic values for consumers (corrected for trophic fractionation) are expected when their feeding consists of a mix of the main primary producers locally produced alone (1) or including allochthonous sources (2)

S. anglica, which was higher than the $\delta^{15}\text{N}$ of the corresponding living plant ($4.4 \pm 0.05\text{‰}$) and the $\delta^{15}\text{N}$ of 2.5‰ reported for detrital *S. alterniflora* (see Currin et al. 1995 for a review). The $\delta^{15}\text{N}$ for *Fucus vesiculosus* was slightly lower with $5.7 \pm 0.05\text{‰}$ than the $\delta^{15}\text{N}$ of 8.1‰ reported by Mayer et al. (1988). In comparison with other primary producers, *Plantago maritima* showed a higher $\delta^{15}\text{N}$ of 9.4‰ and a lower $\delta^{13}\text{C}$ of $-24.9 \pm 0.1\text{‰}$, which indicated the presence of the C3 pathway of photosynthesis (Fry & Sherr 1984). In this study, the main sources of organic matter had reasonably distinct $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, which allowed their use to imply food sources for the invertebrates considered. Particularly, a large difference in $\delta^{15}\text{N}$ was observed between live and dead *S. anglica* while $\delta^{13}\text{C}$ values were similar. Previous studies reported that $\delta^{15}\text{N}$ for detritus derived from *S. alterniflora* resulted largely from nitrogen sequestered from the environment by bacteria as well as from adsorbed external nitrogenous compounds (Couch 1989, Currin et al. 1995). Variation in $\delta^{15}\text{N}$ in detrital *S. anglica* might have been due to the uptake of external nitrogen by bacteria associated with *S. anglica* detritus (Rice 1982, Whites & Howes 1994).

Nematodes revealed a $\delta^{13}\text{C}$ value of $-16.2 \pm 1.7\text{‰}$, similar to the $\delta^{13}\text{C}$ reported from Marennes-Oléron Bay (Riera et al. 1996). The corresponding $\delta^{15}\text{N}$ ($7.5 \pm 1.1\text{‰}$) was in between the $\delta^{15}\text{N}$ of 9.1‰ observed in Marennes-Oléron Bay and the $\delta^{15}\text{N}$ of 5.3‰ for nematodes in a *Spartina* salt marsh in the United States (Couch 1989). The $\delta^{15}\text{N}$ values of bivalves increased from *Macoma balthica* ($8.5 \pm 0.2\text{‰}$) via *Scrobicularia plana* ($9.0 \pm 0.3\text{‰}$) to *Mytilus edulis* ($9.4 \pm 0.4\text{‰}$). The corresponding $\delta^{13}\text{C}$ decreased from *M. balthica* ($-14.9 \pm 0.7\text{‰}$), via *S. plana* ($-15.6 \pm 0.4\text{‰}$) to *M. edulis* ($-17.4 \pm 0.2\text{‰}$). *Carcinus maenas* had the highest values of $\delta^{15}\text{N}$ ($10.4 \pm 1.2\text{‰}$) and $\delta^{13}\text{C}$ ($-14.3 \pm 0.0\text{‰}$), which suggested a more carnivorous mode of life for this species.

The identification of food sources for benthic consumers (Fig. 1) considered: (1) a mean trophic enrichment in ^{13}C of 1‰ (DeNiro & Epstein 1978, Rau et al. 1983) and a mean trophic enrichment in ^{15}N of 3.5‰ (Minagawa & Wada 1984, Owens 1987) as a result of the assimilation of food, and (2) sources of organic matter locally produced and allochthonous sources. The main allochthonous food sources in the bay were terrestrial organic matter carried by the Sèvre Niortaise River and marine phytoplankton that entered the Aiguillon Bay through tidal currents. The most common terrestrial plants in the meadows and forests of the drainage basin of the Charente River were C3 plants and these had a mean $\delta^{13}\text{C}$ of $-28 \pm 1.1\text{‰}$ and $\delta^{15}\text{N}$ of $5.1 \pm 0.6\text{‰}$ (Riera & Richard 1996, Riera 1998). Recently, Fogel & Cifuentes (1993) reported a similar mean $\delta^{15}\text{N}$ of 4‰ for terrestrial plants. Isotopic values

for marine phytoplankton were not measured in this study. However, Richard et al. (1997) measured a $\delta^{13}\text{C}$ of -20.6‰ off Marennes-Oléron Bay close to the oceanic entrance of the Aiguillon Bay. Miyake & Wada (1967) and Wainright et al. (1998) reported $\delta^{15}\text{N}$ values for marine phytoplankton of 6‰ and of 6.6 to 6.7‰ , respectively.

Food sources of *Macoma balthica*, *Scrobicularia plana* and *Mytilus edulis*: A continuum of $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ values was found, starting with *M. balthica* via *S. plana* to *M. edulis* (Fig. 1). In this study, the contribution of ^{13}C -enriched sources of organic matter (i.e. benthic diatoms and live and dead *S. anglica*) to the benthic food web could not be distinguished by using the $\delta^{13}\text{C}$ values. The invertebrates might have used detrital organic matter derived from *Spartina anglica* as a food source. In fact, microbially mediated processes occurring during decomposition of eelgrass result in the production of detritus and associated bacteria, which are more nutritious and readily usable (Tenore et al. 1982). Experimental studies have shown that trophic mediation between vascular plants and bivalves can occur through bacteria associated with the decay of detrital refractory material (Crosby et al. 1990, Langdon & Newell 1990). Similarly, a bacterial trophic mediation has been hypothesised to explain the use of organic matter of terrestrial origin by oysters (Riera 1998). In the present study, $\delta^{15}\text{N}$ discriminated between live and dead *S. anglica* (Fig. 1), and these data suggested that detrital organic matter derived from this plant did not make an important contribution to the diet of these invertebrates.

However, benthic diatoms alone could not fully explain the isotopic composition of the invertebrates considered. It was unlikely that *Plantago maritima* and *Fucus vesiculosus* contributed significantly to the feeding of the invertebrates because they are not important as primary producers in this part of Aiguillon Bay compared with *Spartina anglica* (Riera pers. obs.). Moreover, although this marsh is directly influenced by river inputs, $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ indicated that terrestrial organic matter was also not incorporated into the tissues of the invertebrates (Fig. 1). In fact, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Macoma balthica*, *Scrobicularia plana* and *Mytilus edulis* could be mainly explained by a mixed diet of benthic diatoms and marine phytoplankton. This result is consistent with the hypothesis that living microalgae such as benthic diatoms and phytoplankton are easier to use and more nutritious for marine bivalves than detritus of vascular plants (Bayne et al. 1987, Crosby et al. 1989).

Use of isotopic mixing model: The quantitative proportion of the main food sources incorporated by consumers was based on a mixing model using $\delta^{13}\text{C}$ values. In fact, $\delta^{15}\text{N}$ is not as powerful as $\delta^{13}\text{C}$ in the

discrimination of food sources, because of the lower ranges of $\delta^{15}\text{N}$ values and overlapping of values for food sources and invertebrates, as shown previously in coastal ecosystems (Fry & Sherr 1984, Couch 1989). The model assumed a proportional assimilation of food and that the $\delta^{13}\text{C}$ value becomes 1‰ less in the diet (De Niro & Epstein 1978). The proportion of carbon assimilated by invertebrates from benthic diatoms and phytoplankton can be calculated using the equation:

$$\delta^{13}\text{C}_{\text{animal}} = P_b \delta^{13}\text{C}_b + P_p \delta^{13}\text{C}_p$$

with $P_b + P_p = 1$

where P is the proportion of benthic diatoms (b) and phytoplankton (p) that contribute to animal diet. To solve for P_b and P_p the mean carbon isotope values for benthic diatoms and phytoplankton were fractionated by 1‰.

The results indicated that *Macoma balthica* was preferentially a deposit feeder because this bivalve used a higher proportion of benthic diatoms (75.8%) compared to marine phytoplankton (24.2%). *Scrobicularia plana* also preferentially used benthic diatoms (64.5%), but behaved slightly more like suspension feeders, using a higher proportion of phytoplankton (35.5%). These values confirmed the suspension-deposit-feeding mode previously observed in feeding behaviour studies of *M. balthica* (Hummel 1985) and *S. plana* (Hughes 1969). However, this study suggests a slight difference in feeding modes between the 2 bivalves, although they were from the same habitat. The isotopic position of *Mytilus edulis* suggested that this suspensivore subsists primarily on phytoplankton (64.6%) but also consistently uses benthic diatoms (35.4%).

The examination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in this salt marsh showed that the diet of benthic consumers was not necessarily dependent on the dominant primary producers to which they were exposed. In fact, although *Spartina anglica* was largely available to the benthic invertebrates through detrital organic matter, other primary producers, autochthonous or allochthonous, which were more readily consumable (i.e. living microphytes) were preferentially used as food sources.

Food sources of nematodes and *Carcinus maenas*:

The $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ for nematodes and *C. maenas* did not match the values that would have been expected when their feeding consisted of a mix of the main sources to which they were exposed (Fig. 1). Although $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ suggested that benthic diatoms were part of the food of the nematodes, their contribution was less important than previously observed in the mudflat of Marennes-Oléron Bay. In this mudflat, benthic diatoms represented the main food source ingested (Montagna et al. 1995) and assimilated over a

sampling year (Riera et al. 1996) by nematodes. However, in Marennes-Oléron Bay vascular plants like *Spartina* are virtually absent. These results support the suggestion made by Couch (1989) that microphytobenthos cannot constitute the sole food source of nematodes in a *S. anglica* salt marsh. Meiobenthic fauna mainly consists of grazers, adapted to ingest microbial food items such as microalgae, bacteria and protozoa (Montagna 1995). Particularly, in detritus-based food webs, bacteria often appear to constitute the main food source for meiofauna (Gerlach 1978). In the salt marsh of Aiguillon Bay, large ranges in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of nematodes suggest a high diversity of food sources. This diversity could be explained assuming a nematode community which consists of a mixture of species from different feeding guilds (i.e. algivores, bacterivores and predators). This would be consistent with the classification of Weiser (1953). According to this hypothesis, the heterogeneity of feeding modes of different nematode species at different trophic levels would imply that the trophic enrichments (i.e. 1‰ for carbon and 3.5‰ for nitrogen) as shown in Fig. 1 are not valid for the whole nematode community. This result is consistent with the suggestion made by Bongers & Ferris (1999) that, when functional divergence within a nematode community occurs, identification to genus or species level should be necessary.

Furthermore, *Carcinus maenas* exhibited a high variability in $\delta^{15}\text{N}$, which could be explained by a high diversity of food sources and/or different trophic levels for the individuals considered. This result is in agreement with the omnivory feeding mode of *C. maenas*, which was evidenced by the patterns of digestive enzymes in the larvae (Kumlu & Jones 1997).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured in this salt marsh suggest complex trophic relationships for nematodes and *Carcinus maenas*. This did not allow the use of trophic enrichment coefficients and the use of isotopic mixing models to accurately estimate the proportional contributions of the main food sources for these invertebrates.

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