δ^{15}N of organic matter sources and benthic invertebrates along an estuarine gradient in Marennes-Oléron Bay (France): implications for the study of trophic structure

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ABSTRACT: This study examined the δ^{15}N of suspended and sedimented particulate organic matter, primary sources of organic matter and benthic invertebrates along an estuarine gradient in Marennes-Oléron Bay, France. Particular emphasis was given to the use of δ^{15}N as a tracer of the origin of organic matter and as a means of determining the benthic food web structure in estuarine environments. δ^{15}N values indicated that there was direct utilisation of benthic diatoms as a food source by oyster Crassostrea gigas near intertidal mudflats but suggested trophic mediation between terrestrial detritus and oysters in the upper estuarine reaches. δ^{15}N data showed that the trophic position of oysters may vary in the estuarine bay. The δ^{15}N values for the other invertebrates investigated revealed that apparent discrepancies may occur concerning the correspondence between δ^{15}N, trophic level and feeding mode of invertebrates in estuarine ecosystems. In fact, these results suggest that δ^{15}N can be a useful tool to characterise trophic transfers and to establish an isotopic food web model, provided that one considers the different feeding habitats along the estuarine gradient.

KEY WORDS: Nitrogen isotope ratio - Particulate organic matter - Crassostrea gigas - Benthic food web - Estuary - Marennes-Oléron Bay

INTRODUCTION

In coastal environments, the use of δ^{15}N can complement other isotopic data (e.g. carbon and/or sulfur) for the determination of food sources but it can also contribute to understanding the mechanisms involved in trophic transfers of organic matter and the trophic structure of the food web. The trophic mediation between a food source and a consumer cannot be clearly detected on the basis of δ^{13}C analyses, because δ^{13}C only shows an average enrichment of 1% per trophic level (Rau et al. 1983, Fry & Sherr 1984); the enrichment in δ^{13}C allows a direct correspondence between a food source and its consumer to be established (Fry & Sherr 1984) but the enrichment is not large enough for following the trophic mediation between primary production at the base of the food chain and the animal of interest. However, δ^{15}N has been used successfully to investigate the trophic structure within aquatic ecosystems, since an increase in δ^{15}N by about 3.5%o per trophic level occurs as nitrogen is transferred (Minagawa & Wada 1984, Wada et al. 1987). Due to this trophic level effect for nitrogen isotopes, δ^{15}N values in marine and coastal ecosystems can be interpreted as a function of both food sources and trophic level. In an Arctic marine food web, Hobson & Welch (1992) have shown an increase in δ^{15}N from 5.4–9.4% in primary producers to 11.1–21.1% in predatory vertebrates.

The diversity of primary producers and the importance of the detrital organic matter pool as a food...
source in estuarine environments (Mann 1988) make tracing the trophic links in these habitats more complicated. Along an estuarine gradient within Marennes-Oleron Bay, France, a δ¹³C study has been performed to determine food sources of the oyster Crassostrea gigas (Riera 1995). The results showed that the main food sources for oysters included terrestrial inputs in the upper reaches of the estuary, benthic diatoms at the river mouth of the estuary and phytoplankton in the oceanic part of the bay (Riera & Richard 1996). However, although δ¹³C was effective for determining the food sources of oysters, the mechanisms by which the organic matter was transferred into oyster tissues could not be discerned from these data. In particular, the possibility that trophic mediation occurs between oysters and benthic diatoms at the river mouth or between oysters and terrestrially derived organic matter in the upper estuary was only hypothesized on the basis of previous results (see Riera & Richard 1996).

The aim of the present study was to investigate the natural nitrogen isotopic composition of organic matter in order to provide an overall description of δ¹⁵N in primary sources of organic matter and benthic invertebrates along an estuarine gradient in Marennes-Oleron Bay. Further, the trophic structure of the benthic food web was examined. Particularly, the study addressed the questions of trophic mediation for oysters (Crassostrea gigas) and the correspondence between δ¹⁵N, trophic level and feeding mode for benthic estuarine invertebrates.

**MATERIAL AND METHODS**

The estuarine bay of Marennes-Oleron is shallow (average depth 4 m) and is located on the Atlantic coast of France (Fig. 1). The bay is protected by 2 islands, Ré Island to the north and Oleron Island to the west. The bay has an area of approximately 196 km² including extensive intertidal mudflats (112 km²), and it is surrounded by wide areas (110 km²) of salt marshes. Oceanic water enters the bay through the north entrance and moves south with a residence time of 5 to 11 d (Bacher 1989). Freshwater flows into the bay mainly from the Charente River, which drains 10⁴ km² of agricultural and forested land, with minor inputs of industrial or sewage effluents. Water from the Gironde Estuary can enter the bay through the north entrance during periods of high river discharge. The Gironde River flows into the ocean 40 km south of the bay. Occasionally, at high tides water from the Gironde enters the bay through the south entrance (Dechambeau et al. 1977). The salt marshes lining the bay of Marennes-Oleron are entirely managed and consist of oyster ponds without any natural marsh-plant vegetation, and there are no marshes along the Charente Estuary itself.

Oyster sampling locations included 3 sites along the Charente Estuary (Port-Neuf, Fort Lupin, Les Palles) and 2 marine littoral sites at the northwestern end of Oleron Island and Ré Island (Chassiron and Les Baleines). The 2 upper estuarine sites, Port-Neuf, 8 km from the mouth of the estuary, and Fort Lupin, 4 km from the mouth, are dominated by muddy sediments. The third estuarine site, Les Palles, is a rocky reef right at the mouth of the estuary, with some patches of macroalgae (mainly Fucus sp.) and is surrounded by extensive mudflats. The marine sites (Chassiron and Les Baleines) are rocky reefs without any mud and are almost entirely covered by diverse macroalgae.

Particulate organic matter (POM), sedimented organic matter (SOM), organic matter sources and oysters were sampled within the period May 1992 to October 1993. Samples of POM were collected at different dates within the same period from the different stations, except at Port-Neuf, but included the riverine station at St-Savinien. For each sampling, 20 l of water was pumped from about 50 cm under the water surface at high tide ± 1 h. POM for isotope analyses was

![Fig. 1. Sampling sites in Marennes-Oleron Bay. Dashed line shows the limit of the intertidal mudflats and land areas are shaded.](image-url)
obtained by filtration of the water on precombusted Whatman GF/F fiber glass filters under moderate vacuum within 2 h after collection. Samples were freeze-dried and kept frozen until analysis. Sediment samples were taken at Fort Lupin and Les Palles at low tides by scraping the upper 1 cm of mud, for a total surface of approximately 1 m². In the laboratory, sediment was freeze-dried, ground using a mortar and pestle and acidified with 1 M HCl to remove any inorganic carbon. These samples were not rinsed, to prevent any loss of dissolved organics. They were dried overnight at 50°C under a slight vacuum to evaporate the acid. Once dried, the sediment was mixed with Milli-Q water, freeze-dried, ground again to a fine powder and kept frozen (−80°C) until analysis. Benthic diatoms were sampled on the large intertidal mudflat at Les Palles and extracted using a method of Couch (1989) slightly modified by Riera & Richard (1996). The sediment was spread on flat trays to form a 1 cm thick layer. A nylon screen (63 µm mesh) was laid upon the sediment surface and covered with a 5 mm thick layer of combusted silica powder (60 to 210 µm). The trays were held under light for 1 to 3 h, where the silica powder was kept moist by spraying filtered (0.2 µm) seawater from the sampling site. The top 2 mm of the silica powder, into which the motile microalgae had migrated, were then gently scraped, and sieved through a 63 µm mesh to separate the diatoms from the remaining silica powder and from any nematodes or copepods which might have also migrated into the silica powder. Diatoms were finally collected on previously combusted Whatman GF/F glass fiber filters, washed with 1 M HCl, rinsed with Milli-Q water, freeze-dried and kept frozen (−80°C) until analysis. Macroalgal were present at rocky stations (Les Palles, Chassiron and Les Baleines). They were collected by hand, cleaned of epibionts, washed with 10% HCl to remove carbonates, rinsed with Milli-Q water and homogenized using a Polytron homogenizer. They were then freeze-dried, ground to a powder using a mortar and pestle and kept frozen (−80°C) until analysis. As samples of terrestrial organic matter, leaves of the dominant plants in the catchment of the Charente River were collected at St-Savinien.

Oyster sampling was carried out at each sampling station, except at St-Savinien where there are no oysters. Oysters were collected by hand, cleaned of epibionts and kept alive overnight at the laboratory in filtered water from the sampling site to allow evacuation of gut contents. Then they were killed by freezing, dissected and the flesh treated with 10% HCl to remove any carbonate debris from the shell, rinsed with distilled water and homogenized using a Polytron homogenizer. They were then freeze-dried, ground to a powder using mortar and pestle and kept frozen until analysis. Specimens of the other invertebrates were sampled during the period May to July 1992. The gastropod *Hydrobia ulvae* was taken from surficial sediment samples at low tides on the intertidal mudflat located south of the mouth of the Charente Estuary (Les Palles). Specimens of *Littorina* sp., *Patella* sp. and *Nereis* sp. were collected by hand on rocky reefs at Les Palles and Les Baleines. All individuals were cleaned of epibionts and the *Nereis* sp. and the flesh of molluscs (after dissection from the shell) were treated in the same way as oyster tissues. In this study, δ¹⁵N data are presented for POM, SOM, organic matter sources and oysters and δ¹³C and δ¹⁵N data are presented for *Nereis* sp., *Littorina* sp., *Patella* sp. and *H. ulvae*.

Samples for isotope analyses were prepared as described in Boutton (1991). Samples were combusted at 900°C using CuO as oxidant in evacuated quartz tubes (Stump & Frazer 1973). Before the purification of CO₂, N₂ was trapped on silica gel granules in a stopcock sample ampule and analyzed immediately after CO₂ collection (Mariotti 1982). The carbon and nitrogen isotope ratios were measured using a Sigma 200 (CJS Sciences) double inlet, triple collector isotope ratio mass spectrometer. Data are expressed in the standard δ unit notation where δX = [(Rsample/Rreference) - 1] x 10³, with R = ¹³C/¹²C for carbon and ¹⁵N/¹⁴N for nitrogen, and reported relative to the Pee Dee Belemnite standard (PDB) for carbon and to atmospheric N₂ for nitrogen. The typical precision of the complete analysis (i.e. combustion and mass spectrometric measurement) was ±0.1‰ for carbon and ±0.2‰ for nitrogen.

**RESULTS**

δ¹⁵N values of suspended POM, SOM and the main organic matter sources are presented in Table 1. POM δ¹⁵N values increased from 5.5 to 8.8‰ from the freshwater to the oceanic part of the bay, with intermediate values in the estuary (from 6.1 to 7.5‰). Intertidal SOM δ¹⁵N ranged from 6.2 to 7.7‰. The intertidal SOM δ¹⁵N values in the present study were similar to δ¹⁵N already reported for other coastal surficial muddy sediments (Couch 1989, Owens & Law 1989, Currin et al. 1993). δ¹⁵N values for leaves of the most common terrestrial plants in the meadows and forests of the drainage basin of the Charente River ranged from 2.3‰ (Quercus robur) to 5.8‰ (Ulmus carpinifolia). The δ¹⁵N values were comparable to the mean δ¹⁵N of 2.5‰ observed by Sweeney et al. (1978) for terrestrial organic matter. Schoneniger & de Niro (1984) reported δ¹⁵N for terrestrial plants between −5 and 18‰ but an isotopically lighter mean δ¹⁵N of 4‰ was reported recently by Fogel & Cifuentes (1993). The δ¹⁵N values
Table 1. $\delta^{15}$N values (‰) of POM, SOM and the main organic matter sources along the estuarine gradient in Marennes-Oléron Bay for the period May 1992 to October 1993

<table>
<thead>
<tr>
<th>Sample</th>
<th>St-Savinien</th>
<th>Les Palles</th>
<th>Chassiron</th>
<th>Les Baleines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riverine POM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terrestrial vegetation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercus robur</td>
<td>2.3 to 4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulmus carpinifolia</td>
<td>5.8 (n = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carpinus betulus</td>
<td>5.1 (n = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gramineae</td>
<td>4.6 to 5.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuarine POM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intertidal SOM</td>
<td>6.2 to 7.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benthic diatoms</td>
<td></td>
<td>6.1 to 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oceanic POM</td>
<td></td>
<td></td>
<td></td>
<td>6.9 to 8.8</td>
</tr>
<tr>
<td>Macroalgae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fucus vesiculosus</td>
<td>7.2 to 9.7</td>
<td>6.5 to 6.6</td>
<td>7.6 to 10.3</td>
<td></td>
</tr>
<tr>
<td>Fucus serratus</td>
<td>7.1 to 8.8</td>
<td>5.5 to 7.6</td>
<td>5.8 to 7.3</td>
<td></td>
</tr>
<tr>
<td>Sargassum sp.</td>
<td>8.6 (n = 1)</td>
<td>8.6 (n = 1)</td>
<td>5.9 to 9.0</td>
<td></td>
</tr>
<tr>
<td>Ulva sp.</td>
<td>8.6 to 12.0</td>
<td>7.5 to 9.6</td>
<td>7.5 to 7.6</td>
<td></td>
</tr>
<tr>
<td>Enteromorpha sp.</td>
<td>7.9 to 12.4</td>
<td>7.2 to 9.4</td>
<td>8.8 (n = 1)</td>
<td></td>
</tr>
<tr>
<td>Laminaria sp.</td>
<td></td>
<td>5.9 to 8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcareous algae</td>
<td>4.6 to 6.2</td>
<td>8.2 (n = 1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Average $\delta^{13}$C values (‰, ±SD) of benthic invertebrates analyzed in this study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Les Palles</th>
<th>Les Baleines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Littorina sp.</td>
<td>-15.3 ± 1.3</td>
<td>-15.3 ± 1.0</td>
</tr>
<tr>
<td>Patella sp.</td>
<td>-16.2 ± 0.9</td>
<td>-13.0 ± 0.7</td>
</tr>
<tr>
<td>Nereis sp.</td>
<td>-19.7 ± 2.3</td>
<td>-17.3 ± 2.0</td>
</tr>
<tr>
<td>Hydrobia ulvae</td>
<td>-15.1 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

$\delta^{15}$N of estuarine POM, SOM and organic matter sources

In the present study, mean values for POM $\delta^{15}$N tended to increase slightly from riverine to oceanic environments (Fig. 2). Mariotti et al. (1984) reported a mean $\delta^{15}$N of 1.5‰ for suspended matter of predomi-
nantly continental origin in the Scheldt Estuary (SW Netherlands) whereas a mean $\delta^{15}N$ of 8.8‰ was observed in the North Sea. Similarly, a mean $\delta^{15}N$ value of 1.9‰ for POM in the terrestrial part and 5.9‰ for estuarine POM were reported in the Tay Estuary, Scotland, UK (Thornton & McManus 1994). However, along the Delaware Estuary (USA), different patterns for $\delta^{15}N$ of suspended particulate matter were reported throughout the sampling year (Cifuentes et al. 1988). Likewise, $\delta^{15}N$ ranging from 2.6 to 10.6‰ was reported by Thornton & McManus (1994) for estuarine SOM, but they found no progressive $^{14}N$-enrichment along the estuarine gradient.

In estuarine environments, the POM pool is mainly detrital (Haines 1977, Mann 1988), which makes it difficult to follow the fluxes of organic matter and to determine the food sources from $\delta^{15}N$ values. $\delta^{15}N$ values of suspended and sedimented POM in estuaries are affected by processes of diagenesis (microbial mineralization). These processes reduce the amount of nitrogen and enrich the $^{15}N$ content of organic substrate due to a preferential utilization of $^{14}N$ (Thornton & McManus 1994). This isotope fractionation makes it difficult to interpret $\delta^{14}N$ values in the determination of food sources, as $\delta^{15}N$ may reflect biogenic processes associated with the decomposition of organic matter rather than its origin. $\delta^{15}N$ of the POM pool is all the more affected by diagenetic processes if it is rich in detritus; this is a characteristic of Marennes-Oléron Bay and, particularly, the lower Charente Estuary where the POM pool is largely dominated by detrital organic matter (Feuillet-Girard et al. 1994, Riera 1995). Further, the detritus of different origins included in the composition of the estuarine organic matter may have
been subject to different diagenetic processes (Billen & Lancelot 1988).

The proportions of C and N contents of organic material of different natures can also result in a plot of δ¹³C and δ¹⁵N values that are discordant with one another. Indeed, along the Sheepscot Estuary (ME, USA), Mayer et al. (1998) found that δ¹³C showed a gradient from primarily terrigenous inputs at the head of the estuary to a mixture of 2/3 to 1/3 terrigenous algal marine sources at the mouth, whereas δ¹⁵N varied from primarily terrigenous values at the head to primarily marine values at the mouth of the estuary. For these authors, the absence of a direct correspondence between δ¹³C and δ¹⁵N was due to the mixing of terrigenous inputs having a high C/N ratio with marine sources—consisting of plankton and macroalgal organic matter—having a low C/N ratio.

Finally, the determination of N sources for consumers on the basis of the δ¹⁵N of detrital sources is based on the hypothesis that isotopic ratios of detritus are close to those of the corresponding living plant. However, the correspondence between δ¹⁵N from detrital and living sources is not clear because nitrogen external to the original source can interfere with the measurement of the source origin based on δ¹⁵N measurement of degrading organic matter. δ¹⁵N for detritus derived from the angiosperm marsh plant Spartina alterniflora results largely from nitrogen sequestered by microbes from the environment and from adsorbed nitrogenous compounds rather than from the macrophyte substrate itself (Couch 1989, Currin et al. 1995). In fact, external N sources may account for up to 65% of the total N pool in detrital S. alterniflora (White & Howes 1994). For macroalgae, there have been no experimental studies assessing changes in δ¹⁵N of degrading macroalgal detritus. However, the relationships between microbes and detrital substrates are highly dependent on the chemical nature of the substrate considered (Rice 1982, Benner et al. 1984). Thus, the results obtained with detrital S. alterniflora may largely be due to some of its specific characteristics and are probably not applicable to more readily consumable macroalgal detritus.

**δ¹⁵N of oysters**

The differences in δ¹⁵N observed for oysters along the estuarine gradient show the diversity of their food sources (de Niro & Epstein 1981) and confirmed results obtained from δ¹³C values (Riera & Richard 1996). When the total estuarine gradient was considered it was more difficult to determine the food sources of oysters from δ¹⁵N than from stable carbon isotope data, as many sources have overlapping δ¹⁵N values (Table 1. Fig. 2). In addition, the δ¹⁵N of these oysters showed a narrower range of values compared to the δ¹³C values, which varied between -25.2 and -15.9‰ (Riera 1995). These results are consistent with previous data reported for a riverine mangrove (Primavera 1996) and with the suggestion made by Fry & Sherr (1984) and Couch (1989) that δ¹⁵N is not as discriminating as δ¹³C for the characterisation of sources in estuarine environments.

However, δ¹⁵N values of oysters can complement carbon isotopic ratios in analysing the utilisation of specific sources. At Les Palles, δ¹⁵N values are consistent with oysters (mean 9.0 ± 0.9‰) feeding predominantly on benthic diatoms (mean 5.7 ± 0.8‰) when considering the trophic δ¹⁵N enrichment of 3.5‰, which confirms previous results (Riera & Richard 1996). This δ¹⁵N change suggests that oysters fed directly on benthic diatoms because their difference in δ¹⁵N corresponds to 1 trophic level. In contrast, further up the estuary (Port-Neuf), the mean δ¹⁵N difference between oysters and terrestrial inputs (6.4‰) suggests oysters were 2 trophic levels higher than detritus of terrestrial origin. Since at Port-Neuf oysters incorporate carbon mainly from terrestrially derived organic matter (Riera & Richard 1996), these results suggest a trophic mediation between detritus of terrestrial origin and oysters. This mediation may occur through associated bacteria, as has been suggested for carbon transfer (Crosby et al. 1990, Langdon & Newell 1990). Further, it is likely that protozoa such as ciliates ingest detrital particles (Posch & Arndt 1996) and are themselves ingested by oysters (Le Gall et al. 1997). However, these enriched δ¹⁵N values for oysters may also be explained by a direct utilisation of nitrogen from a substrate of terrestrial origin which has been enriched in ¹⁵N through diagenesis and/or adsorbed nitrogenous compounds (see previous discussion). Then, considering this hypothesis, the nitrogen source for oysters in the upper Charente Estuary, where terrestrial detritus (high C/N) dominates, may have an origin partially different from that of the carbon source. At mid-estuary (Fort Lupin), intermediate δ¹⁵N values for oysters indicated that the oysters had a diet of mixed terrestrial and marine sources. At the marine littoral (Chassiron, Les Baleines), δ¹⁵N values could not confirm the dominant contribution of phytoplankton to the feeding of oysters (Riera & Richard 1996) because, unfortunately, δ¹⁵N was not measured on this source. Miyake & Wada (1967) reported a δ¹⁵N of 6‰ for marine phytoplankton. Considering this value, the N incorporated by these oysters could originate mainly from marine phytoplankton. However, as reported from δ¹³C analyses (Riera & Richard 1997), a contribution of macroalgae to oyster feeding cannot be excluded on the basis of δ¹⁵N.
Use of $\delta^{15}N$ to determine the structure of the benthic food web

Due to the trophic effect for nitrogen, $\delta^{15}N$ values can be used to classify animals by their trophic position and to establish an isotopic food web model. Thus, $\delta^{15}N$ can reflect some characteristics of the feeding regimes of animals (Schoeninger & de Niro 1984). On Mediterranean reefs, Jennings et al. (1997) observed a general trend corresponding to an enrichment in $^{15}N$ from plants (mean $\delta^{15}N$: 1.1‰) to predatory fishes (mean $\delta^{15}N$: 13.8‰) with intermediate values for benthic invertebrates. In the present study, a food web representation can be established for the first trophic levels on the basis of the $\delta^{15}N$ of primary sources and consumers considered from the terrestrial to the marine littoral environments (Fig. 2). In the mouth of the Charente Estuary, $\delta^{15}N$ values showed a direct trophic link between benthic diatoms and Hydrobia ulvae (Fig. 2). This result corroborates $\delta^{13}C$ data showing a preferential utilisation of benthic diatoms (mean $\delta^{13}C$: -16.1‰; Riera 1995) as food source by H. ulvae (mean $\delta^{13}C$: -15.1‰; Table 2). Consequently, the present study provides further evidence of the trophic importance of macrophytobenthos for consumers inhabiting intertidal mudflats in Marennes-Öleron Bay, as was pointed out for meiofauna by Riera et al. (1996).

Within the intertidal zone (Les Palles), $\delta^{15}N$ values of Hydrobia ulvae and both Patella sp. and Littorina sp. differed by about 3‰ (Fig. 2), whereas these taxa have been reported to occupy the same trophic position, corresponding to primary consumers (Sauriau et al. 1989). As their $\delta^{15}N$ were separated by about 1 trophic level, these grazers could be considered to occupy different trophic levels, potentially leading to an incorrect interpretation of their feeding mode. In fact, though, within the intertidal zone of the Charente Estuary mouth (Les Palles), 2 habitat types (as defined by Day et al. 1989) can be distinguished, which correspond to 2 feeding habitats for these grazers: (1) the intertidal mudflat surrounding the reef, dominated by macrophytobenthos, which is occupied by Hydrobia ulvae; and (2) the rocky reef, covered by macroalgae, which is occupied by both Patella sp. and Littorina sp. If the mean $\delta^{15}N$ value of macroalgae in the estuary (Fig. 2) is considered to reflect the base of a food chain, then the trophic position of Patella sp. and Littorina sp. is consistent with the herbivore feeding mode reported by Sauriau et al. (1989). However, although their $\delta^{15}N$ was not determined, epiphytic microalgae and/or epilithic microalgae that cover the reef could also contribute significantly to the feeding of these grazers. That macroalgal epiphytes contribute to the diet of benthic marine invertebrates was hypothesized by Jennings et al. (1997). A further illustration of the apparent discrepancy between $\delta^{15}N$ and trophic level is given in this study by Patella sp. Individuals of Patella sp. had a mean $\delta^{15}N$ value of 10.6‰ in the Charente Estuary and 7.9‰ in the marine littoral of Ré Island, while they occupy the same trophic level at both rocky reefs. In estuarine and oceanic environments, Nereis sp. showed the highest mean $\delta^{15}N$ values (Fig. 2), and differed from Patella sp. and Littorina sp. by about 1 trophic level. These $\delta^{15}N$ values could be consistent with a predatory feeding mode. However, the $\delta^{13}C$ values for Nereis sp. showed relative $^{13}C$-depletion compared to other invertebrates, which may thus reflect a mixed diet that includes $^{13}C$-depleted and $^{15}N$-enriched sources. This is consistent with the ability of Nereis to behave as a carnivore and/or a scavenger but also as a suspension feeder, as reported by Rübsämad (1991) for N. diversicolor.

In conclusion, this study strongly suggests that, along an estuarine gradient, the correspondence between $\delta^{15}N$, trophic level and feeding mode of invertebrates should be considered within each feeding habitat because $\delta^{15}N$ values at the base of food chains depend on the diversity of habitats and primary sources. Also, this study recognizes the importance of an adequate sampling strategy, taking the different habitats into account, for establishing an isotopic food web model from $\delta^{15}N$ values.

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