

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

Pascal Riera\*

Centre de Recherche en Ecologie Marine et Aquaculture de L'Houmeau, UMR 10, CNRS-IFREMER, BP 5,  
Place du Séminaire, F-17137 L'Houmeau, France

**ABSTRACT:** This study examined the  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  as a tracer of the origin of organic matter and as a means of determining the benthic food web structure in estuarine environments.  $\delta^{15}\text{N}$  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.  $\delta^{15}\text{N}$  data showed that the trophic position of oysters may vary in the estuarine bay. The  $\delta^{15}\text{N}$  values for the other invertebrates investigated revealed that apparent discrepancies may occur concerning the correspondence between  $\delta^{15}\text{N}$ , trophic level and feeding mode of invertebrates in estuarine ecosystems. In fact, these results suggest that  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  analyses, because  $\delta^{13}\text{C}$  only shows an average enrichment of 1‰ per trophic level (Rau et al. 1983, Fry & Sherr 1984); the enrichment in  $^{13}\text{C}$  allows a direct correspondence between a food source and its consumer to be estab-

lished (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,  $\delta^{15}\text{N}$  has been used successfully to investigate the trophic structure within aquatic ecosystems, since an increase in  $\delta^{15}\text{N}$  by about 3.5‰ per trophic level occurs as nitrogen is transferred (Minagawa & Wada 1984, Wada et al. 1987). Due to this trophic level effect for nitrogen isotopes,  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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

\*Present address: NIOO-CEMO, Postbus 140, 4400 AC, Yerseke, The Netherlands. E-mail: riera@cemo.nioo.knaw.nl

source in estuarine environments (Mann 1988) make tracing the trophic links in these habitats more complicated. Along an estuarine gradient within Marennes-Oléron Bay, France, a  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $\delta^{15}\text{N}$  in primary sources of organic matter and benthic invertebrates along an estuarine gradient in Marennes-Oléron 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  $\delta^{15}\text{N}$ , trophic level and feeding mode for benthic estuarine invertebrates.

## MATERIAL AND METHODS

The estuarine bay of Marennes-Oléron 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 Oléron Island to the west. The bay has an area of approximately 196 km<sup>2</sup> including extensive intertidal mudflats (112 km<sup>2</sup>), and it is surrounded by wide areas (110 km<sup>2</sup>) 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 10<sup>4</sup> km<sup>2</sup> 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 (Dechambenoit et al. 1977). The salt marshes lining the bay of Marennes-Oléron 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 Oléron 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  $\pm 1$  h. POM for isotope analyses was

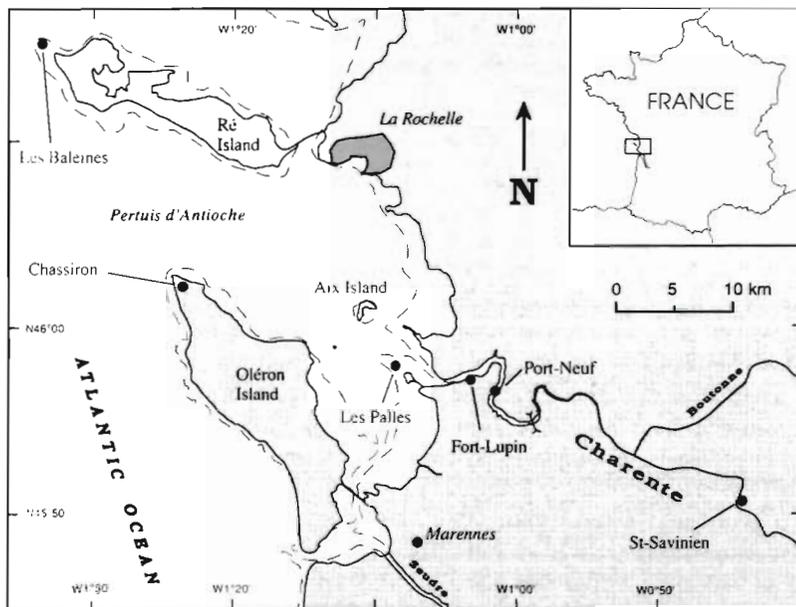


Fig. 1. Sampling sites in Marennes-Oléron Bay. Dashed line shows the limit of the intertidal mudflats and land areas are shaded

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 m<sup>2</sup>. 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  $\mu\text{m}$  mesh) was laid upon the sediment surface and covered with a 5 mm thick layer of combusted silica powder (60 to 210  $\mu\text{m}$ ). The trays were held under light for 1 to 3 h, where the silica powder was kept moist by spraying filtered (0.2  $\mu\text{m}$ ) 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  $\mu\text{m}$  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. Macroalgae 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,  $\delta^{15}\text{N}$  data are presented for POM, SOM, organic matter sources and oysters and  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  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 CO<sub>2</sub>, N<sub>2</sub> was trapped on silica gel granules in a stopcock sample ampule and analyzed immediately after CO<sub>2</sub> 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  $\delta$  unit notation where  $\delta X = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 10^3$ , with  $R = {}^{13}\text{C}/{}^{12}\text{C}$  for carbon and  ${}^{15}\text{N}/{}^{14}\text{N}$  for nitrogen, and reported relative to the Pee Dee Belemnite standard (PDB) for carbon and to atmospheric N<sub>2</sub> for nitrogen. The typical precision of the complete analysis (i.e. combustion and mass spectrometric measurement) was  $\pm 0.1\%$  for carbon and  $\pm 0.2\%$  for nitrogen.

## RESULTS

$\delta^{15}\text{N}$  values of suspended POM, SOM and the main organic matter sources are presented in Table 1. POM  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  ranged from 6.2 to 7.7‰. The intertidal SOM  $\delta^{15}\text{N}$  values in the present study were similar to  $\delta^{15}\text{N}$  already reported for other coastal surficial muddy sediments (Couch 1989, Owens & Law 1989, Currin et al. 1995).  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values were comparable to the mean  $\delta^{15}\text{N}$  of 2.5‰ observed by Sweeney et al. (1978) for terrestrial organic matter. Schoeninger & de Niro (1984) reported  $\delta^{15}\text{N}$  for terrestrial plants between -5 and 18‰ but an isotopically lighter mean  $\delta^{15}\text{N}$  of 4‰ was reported recently by Fogel & Cifuentes (1993). The  $\delta^{15}\text{N}$  values

Table 1.  $\delta^{15}\text{N}$  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

Sample	St-Savinien	Les Palles	Chassiron	Les Baleines
Riverine POM	5.5 to 6.7 (n = 2)			
Terrestrial vegetation				
<i>Quercus robur</i>	2.3 to 4.2 (n = 2)			
<i>Ulmus carpinifolia</i>	5.8 (n = 1)			
<i>Carpinus betulus</i>	5.1 (n = 1)			
Gramineae	4.6 to 5.7 (n = 2)			
Estuarine POM		6.1 to 7.5 (n = 9)		
Intertidal SOM		6.2 to 7.7 (n = 10)		
Benthic diatoms		4.1 to 6.9 (n = 10)		
Oceanic POM				6.9 to 8.8 (n = 3)
Macroalgae				
<i>Fucus vesiculosus</i>		7.2 to 9.7 (n = 3)	6.5 to 6.6 (n = 4)	7.6 to 10.3 (n = 3)
<i>Fucus serratus</i>		7.1 to 8.8 (n = 6)	5.5 to 7.6 (n = 3)	5.8 to 7.3 (n = 3)
<i>Sargassum</i> sp.		8.6 (n = 1)	8.6 (n = 1)	5.9 to 9.0 (n = 2)
<i>Ulva</i> sp.		8.6 to 12.0 (n = 6)	7.5 to 9.6 (n = 6)	7.5 to 7.6 (n = 3)
<i>Enteromorpha</i> sp.		7.9 to 12.4 (n = 6)	7.2 to 9.4 (n = 4)	8.8 (n = 1)
<i>Laminaria</i> sp.			5.9 to 8.2 (n = 3)	6.2 to 8.3 (n = 3)
Calcareous algae			4.6 to 6.2 (n = 2)	8.2 (n = 1)

of the benthic diatoms which inhabited the intertidal mudflats ranged from 4.1 to 6.9‰, encompassing the  $\delta^{15}\text{N}$  of  $5.9 \pm 1.9$ ‰ observed by Créach et al. (1997) for marsh benthic diatoms of the French Channel coast and were slightly heavier than the 3.9‰ reported by Couch (1989) for the salt marshes of the southeast coast of the United States. Currin et al. (1995) obtained much lighter  $\delta^{15}\text{N}$  (−1.1 to −0.1‰) in a North Carolina marsh, but these values were attributed to the abundance of  $\text{N}_2$ -fixing organisms such as cyanobacteria.  $\delta^{15}\text{N}$  of macroalgae varied between 7.1‰ (*Fucus serratus*) and 12.4‰ (*Enteromorpha* sp.) at the river mouth, compared with values from 4.6 (calcareous algae) to 10.3‰ (*Fucus vesiculosus*) at the marine littoral sites (Chassiron, Les Baleines). Mayer et al. (1988) reported a  $\delta^{15}\text{N}$  for *F. vesiculosus* of 8.1‰. Similar narrow ranges in  $\delta^{15}\text{N}$  values for macroalgae compared with  $\delta^{13}\text{C}$  values are found in the literature (see Currin et al. 1995). In Marennes-Oléron Bay,  $\delta^{13}\text{C}$  values for these macroalgae varied between −30.5 and −10.7‰ (Riera & Richard 1997).

$\delta^{15}\text{N}$  (mean  $\pm$  SD) for *Crassostrea gigas* and other invertebrates are presented in Fig. 2. Along the Charante Estuary, oyster  $\delta^{15}\text{N}$  values were  $11.5 \pm 0.8$ ‰ (n = 7) at Port-Neuf,  $10.0 \pm 1.4$ ‰ (n = 44) at Fort Lupin and  $9.0 \pm 1.0$ ‰ (n = 29) at Les Palles. At marine littoral sites, oyster  $\delta^{15}\text{N}$  were  $8.3 \pm 1.2$ ‰ (n = 27) at Chassiron and  $8.8 \pm 1.0$ ‰ (n = 27) at Les Baleines.  $\delta^{15}\text{N}$  for *C. gigas* were significantly different among the sampling sites (Kruskal-Wallis test,  $H = 42.1$ ,  $df = 4$ ,  $p < 0.001$ ). In the estuarine environment, *Hydrobia ulvae* had the lowest  $\delta^{15}\text{N}$  ( $7.7 \pm 0.3$ ‰, n = 5), *Littorina* sp. and *Patella* sp. had similar  $\delta^{15}\text{N}$  values ( $10.8 \pm 0.6$ ‰, n = 2, and  $10.8 \pm$

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

Sample	Les Palles	Les Baleines
<i>Littorina</i> sp.	−15.3 $\pm$ 1.3 (n = 2)	−15.3 $\pm$ 1.0 (n = 2)
<i>Patella</i> sp.	−16.2 $\pm$ 0.9 (n = 2)	−13.0 $\pm$ 0.7 (n = 2)
<i>Nereis</i> sp.	−19.7 $\pm$ 2.3 (n = 3)	−17.3 $\pm$ 2.0 (n = 2)
<i>Hydrobia ulvae</i>	−15.1 $\pm$ 0.2 (n = 5)	

0.2‰, n = 2, respectively) and *Nereis* sp. had the highest mean  $\delta^{15}\text{N}$  ( $13.7 \pm 0.3$ ‰, n = 3). In the marine littoral environment, *Littorina* sp. and *Patella* sp. had  $\delta^{15}\text{N}$  of  $9.5 \pm 0.3$ ‰ (n = 2) and  $7.9 \pm 0.4$ ‰ (n = 2), respectively, and here as well *Nereis* sp. were also the most  $^{15}\text{N}$ -enriched, with a mean  $\delta^{15}\text{N}$  of  $12.7 \pm 2.7$ ‰ (n = 2). Corresponding  $\delta^{13}\text{C}$  values for these invertebrates are presented in Table 2. At both marine (Les Baleines) and estuarine (Les Palles) environments, *Littorina* sp. and *Patella* sp. were relatively  $^{13}\text{C}$ -enriched while *Nereis* sp. was more  $\delta^{13}\text{C}$ -depleted.

## DISCUSSION

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

In the present study, mean values for POM  $\delta^{15}\text{N}$  tended to increase slightly from riverine to oceanic environments (Fig. 2). Mariotti et al. (1984) reported a mean  $\delta^{15}\text{N}$  of 1.5‰ for suspended matter of predomi-

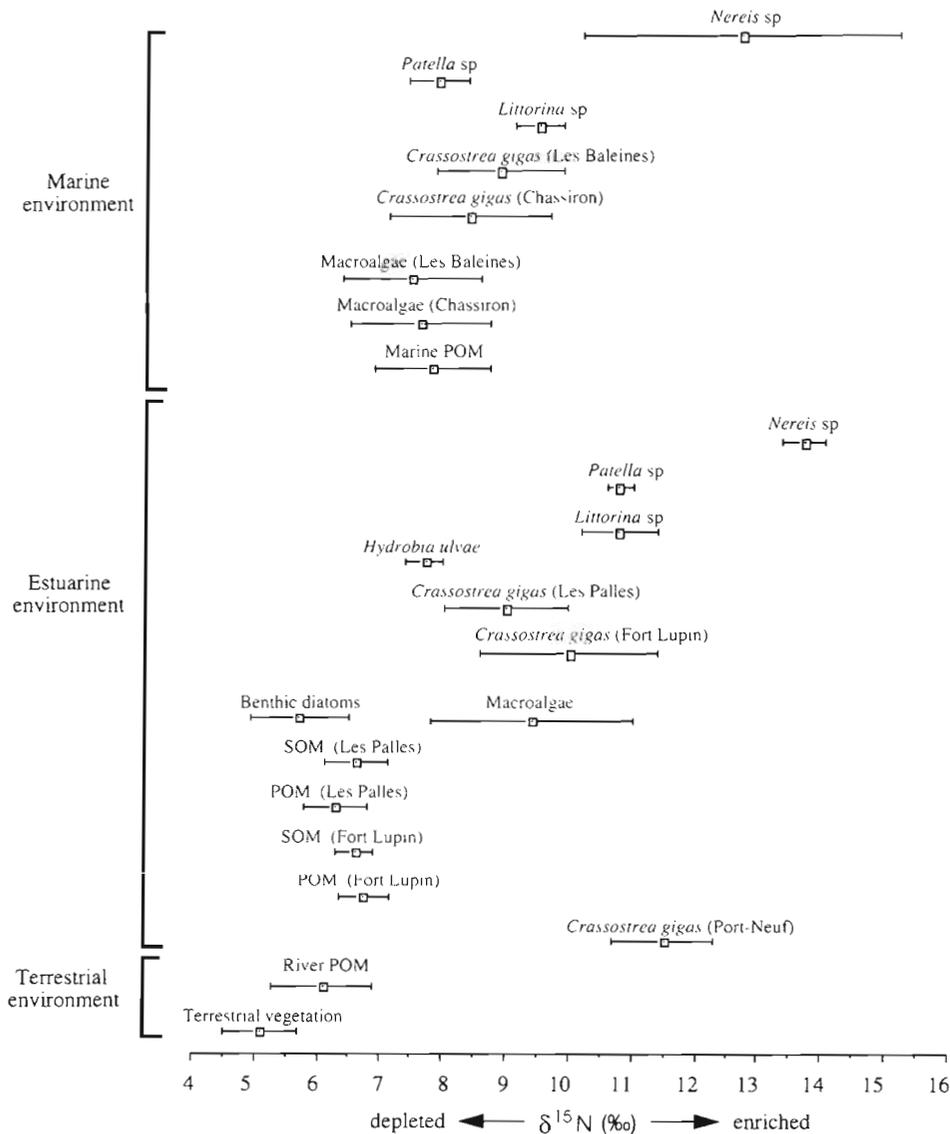


Fig. 2. Average  $\delta^{15}\text{N}$  (‰,  $\pm$  SD) of POM, SOM, organic matter sources, oyster *Crassostrea gigas* and other invertebrates (this study) in the terrestrial, estuarine and marine environments in Marennes-Oléron Bay during the period May 1992 to October 1993

nantly continental origin in the Scheldt Estuary (SW Netherlands) whereas a mean  $\delta^{15}\text{N}$  of 8.0‰ was observed in the North Sea. Similarly, a mean  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  of suspended particulate matter were reported throughout the sampling year (Cifuentes et al. 1988). Likewise,  $\delta^{15}\text{N}$  ranging from 2.6 to 10.6‰ was reported by Thornton & McManus (1994) for estuarine SOM, but they found no progressive  $^{15}\text{N}$ -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  $\delta^{15}\text{N}$  values.  $\delta^{15}\text{N}$  val-

ues 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  $^{15}\text{N}$  content of organic substrate due to a preferential utilization of  $^{14}\text{N}$  (Thornton & McManus 1994). This isotope fractionation makes it difficult to interpret  $\delta^{15}\text{N}$  values in the determination of food sources, as  $\delta^{15}\text{N}$  may reflect biogenic processes associated with the decomposition of organic matter rather than its origin.  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values that are discordant with one another. Indeed, along the Sheepscot Estuary (ME, USA), Mayer et al. (1998) found that  $\delta^{13}\text{C}$  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  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  from detrital and living sources is not clear because nitrogen external to the original source can interfere with the determination of the source origin based on  $\delta^{15}\text{N}$  measurement of degrading organic matter.  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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.

### $\delta^{15}\text{N}$ of oysters

The differences in  $\delta^{15}\text{N}$  observed for oysters along the estuarine gradient show the diversity of their food sources (de Niro & Epstein 1981) and confirmed results obtained from  $\delta^{13}\text{C}$  values (Riera & Richard 1996). When the total estuarine gradient was considered it was more difficult to determine the food sources of oysters from  $\delta^{15}\text{N}$  than from stable carbon isotope data, as many sources have overlapping  $\delta^{15}\text{N}$  values (Table 1,

Fig. 2). In addition, the  $\delta^{15}\text{N}$  of these oysters showed a narrower range of values compared to the  $\delta^{13}\text{C}$  values, which varied between  $-25.2$  and  $-15.9\text{‰}$  (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  $\delta^{15}\text{N}$  is not as discriminating as  $\delta^{13}\text{C}$  for the characterisation of sources in estuarine environments.

However,  $\delta^{15}\text{N}$  values of oysters can complement carbon isotopic ratios in analysing the utilisation of specific sources. At Les Palles,  $\delta^{15}\text{N}$  values are consistent with oysters (mean  $9.0 \pm 0.9\text{‰}$ ) feeding predominantly on benthic diatoms (mean  $5.7 \pm 0.8\text{‰}$ ) when considering the trophic  $\delta^{15}\text{N}$  enrichment of  $3.5\text{‰}$ , which confirms previous results (Riera & Richard 1996). This  $\delta^{15}\text{N}$  change suggests that oysters fed directly on benthic diatoms because their difference in  $\delta^{15}\text{N}$  corresponds to 1 trophic level. In contrast, further up the estuary (Port-Neuf), the mean  $\delta^{15}\text{N}$  difference between oysters and terrestrial inputs ( $6.4\text{‰}$ ) 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  $\delta^{15}\text{N}$  values for oysters may also be explained by a direct utilisation of nitrogen from a substrate of terrestrial origin which has been enriched in  $^{15}\text{N}$  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  $\delta^{15}\text{N}$  values for oysters indicated that the oysters had a diet of mixed terrestrial and marine sources. At the marine littoral (Chassiron, Les Baleines),  $\delta^{15}\text{N}$  values could not confirm the dominant contribution of phytoplankton to the feeding of oysters (Riera & Richard 1996) because, unfortunately,  $\delta^{15}\text{N}$  was not measured on this source. Miyake & Wada (1967) reported a  $\delta^{15}\text{N}$  of  $6\text{‰}$  for marine phytoplankton. Considering this value, the N incorporated by these oysters could originate mainly from marine phytoplankton. However, as reported from  $\delta^{13}\text{C}$  analyses (Riera & Richard 1997), a contribution of macroalgae to oyster feeding cannot be excluded on the basis of  $\delta^{15}\text{N}$ .

### Use of $\delta^{15}\text{N}$ to determine the structure of the benthic food web

Due to the trophic effect for nitrogen,  $\delta^{15}\text{N}$  values can be used to classify animals by their trophic position and to establish an isotopic food web model. Thus,  $\delta^{15}\text{N}$  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  $^{15}\text{N}$  from plants (mean  $\delta^{15}\text{N}$ : 1.1‰) to predatory fishes (mean  $\delta^{15}\text{N}$ : 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  $\delta^{15}\text{N}$  of primary sources and consumers considered from the terrestrial to the marine littoral environments (Fig. 2). In the mouth of the Charente Estuary,  $\delta^{15}\text{N}$  values showed a direct trophic link between benthic diatoms and *Hydrobia ulvae* (Fig. 2). This result corroborates  $\delta^{13}\text{C}$  data showing a preferential utilisation of benthic diatoms (mean  $\delta^{13}\text{C}$ : -16.1‰; Riera 1995) as food source by *H. ulvae* (mean  $\delta^{13}\text{C}$ : -15.1‰; Table 2). Consequently, the present study provides further evidence of the trophic importance of microphytobenthos for consumers inhabiting intertidal mudflats in Marennes-Oléron Bay, as was pointed out for meiofauna by Riera et al. (1996).

Within the intertidal zone (Les Palles),  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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 microphytobenthos, 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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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 discrep-

ancy between  $\delta^{15}\text{N}$  and trophic level is given in this study by *Patella* sp. Individuals of *Patella* sp. had a mean  $\delta^{15}\text{N}$  value of 10.8‰ 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  $\delta^{15}\text{N}$  values (Fig. 2), and differed from *Patella* sp. and *Littorina* sp. by about 1 trophic level. These  $\delta^{15}\text{N}$  values could be consistent with a predatory feeding mode. However, the  $\delta^{13}\text{C}$  values for *Nereis* sp. showed relative  $^{13}\text{C}$ -depletion compared to other invertebrates, which may thus reflect a mixed diet that includes  $^{13}\text{C}$ -depleted and  $^{15}\text{N}$ -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 Riisgard (1991) for *N. diversicolor*.

In conclusion, this study strongly suggests that, along an estuarine gradient, the correspondence between  $\delta^{15}\text{N}$ , trophic level and feeding mode of invertebrates should be considered within each feeding habitat because  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values.

**Acknowledgements.** This work was supported by the Poitou-Charentes Region through the GIS Marennes-Oléron Program and by financial assistance to the author from IFREMER. Thanks are due to P. Richard and G. Blanchard for field support and useful comments on the manuscript. The comments of 4 anonymous reviewers also greatly contributed to improve the manuscript.

### LITERATURE CITED

- Bacher C (1989) Etude de la capacité trophique du bassin de Marennes-Oléron: utilisation d'un modèle couplé de transport particulière et de croissance de l'huître *Crassostrea gigas*. Th. doct. Univ Brest
- Benner R, Maccubbin AE, Hodson RE (1984) Preparation, characterization and microbial degradation of specifically radiolabelled [ $^{14}\text{C}$ ] lignocelluloses from marine and freshwater macrophytes. *Appl Environ Microbiol* 47:381-389
- Billen G, Lancelot C (1988) Modelling benthic nitrogen cycling in temperate coastal ecosystems. In: Blackburn TH, Sorensen J (ed) Nitrogen cycling in coastal marine environments. Wiley, New York, p 341-378
- Boutton TW (1991) Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. In: Coleman DC, Fry B (eds) Carbon isotopes techniques. Academic Press, San Diego, p 155-171
- Cifuentes LA, Sharp JH, Fogel ML (1988) Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. *Limnol Oceanogr* 33:1102-1115
- Couch CA (1989) Carbon and nitrogen stable isotopes of meiobenthos and their food resources. *Estuar Coast Shelf Sci* 28:433-441

- Créach V, Schricke MT, Bertru G, Mariotti A (1997) Stable isotopes and gut analyses to determine feeding relationships in saltmarsh macroconsumers. *Estuar Coast Shelf Sci* 44:599–611
- Crosby MP, Newell RIE, Langdon CJ (1990) Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. *Limnol Oceanogr* 35: 625–639
- Curran CA, Newell SY, Paerl HW (1995) The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Mar Ecol Prog Ser* 121:99–116
- Day JW, Hall CAS, Kemp WM, Yanez-Arancibia A (1989) Estuarine ecology. John Wiley & Sons, New-York
- Dechambenoy C, Pontier L, Sirou F, Vouvé J (1977) Apport de la thermographie infrarouge aéroportée à la connaissance de la dynamique superficielle des estuaires (système Charente-Seudre-Anse de l'Aiguillon). *CR Acad Sci Paris Sér D* 284:1269–1272
- de Niro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 45:341–351
- Feuillet-Girard M, Héral M, Abrioux MF, Fontugne M (1994) Carbone organique dissous et particulaire de la colonne d'eau et de l'interface eau-sédiment du bassin de Marennes-Oléron: influence des huîtres. *Oceanol Acta* 17: 271–284
- Fogel ML, Cifuentes LA (1993) Isotope fractionation during primary production. In: Engel MH, Macko SA (eds) Organic geochemistry: principles and applications. Plenum Press, New York, p 73–98
- Fry B, Sherr EB (1984)  $\delta^{13}\text{C}$  measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib Mar Sci* 27:13–47
- Haines EB (1977) The origin of detritus in Georgia salt marsh estuaries. *Oikos* 29:254–260
- Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis. *Mar Ecol Prog Ser* 84:9–18
- Jennings S, Reñones O, Morales-Nin B, Polunin NVC, Moranta J, Coll J (1997) Spatial variation in the  $^{15}\text{N}$  and  $^{13}\text{C}$  stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. *Mar Ecol Prog Ser* 146:109–116
- Langdon CJ, Newell RIE (1990) Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. *Mar Ecol Prog Ser* 58:299–310
- Le Gall S, Bel Hassen M, Le Gall P (1997) Ingestion of a bacterivorous ciliate by the oyster *Crassostrea gigas*: protozoa as a trophic link between picoplankton and benthic suspension-feeders. *Mar Ecol Prog Ser* 152:301–306
- Mann KH (1988) Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. *Limnol Oceanogr* 33:910–930
- Mariotti A (1982) Apports de la géochimie isotopique à la connaissance du cycle de l'azote. *Th Etat Sci, Univ Paris 6*
- Mariotti A, Lancelot C, Billen G (1984) Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Scheldt estuary. *Geochim Cosmochim Acta* 48:549–555
- Mayer LM, Macko SA, Cammen L (1988) Provenance, concentrations and nature of sedimentary organic nitrogen in the Gulf of Maine. *Mar Chem* 25:291–304
- Minagawa M, Wada E (1984) Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochim Cosmochim Acta* 48:1135–1140
- Miyake Y, Wada E (1967) The abundance ratios of  $^{15}\text{N}/^{14}\text{N}$  in marine environments. *Res Oceanogr Works Jpn* 9:37–53
- Owens NJP, Law CS (1989) Natural variations in  $^{15}\text{N}$  content of riverine and estuarine sediments. *Estuar Coast Shelf Sci* 28:407–416
- Posch T, Arndt H (1996) Uptake of sub-micrometre- and micrometre-sized detrital particles by bacterivorous and omnivorous ciliates. *Aquat Microb Ecol* 10:45–53
- Primavera JH (1996) Stable carbon and nitrogen isotope ratios of penaeid juveniles and primary producers in a riverine mangrove in Guimaras, Philippines. *Bull Mar Sci* 58: 675–683
- Rau GH, Mearns AJ, Young DR, Olson RJ, Schafer HA, Kaplan IR (1983) Animal  $^{13}\text{C}/^{12}\text{C}$  correlates with trophic level in pelagic food webs. *Ecology* 64:1314–1318
- Rice DL (1982) The detritus nitrogen problem: New observations and perspectives from organic geochemistry. *Mar Ecol Prog Ser* 9:153–162
- Riera P (1995) Origine et devenir de la matière organique dans un écosystème estuarien à vocation conchylicole. Utilisation du traçage isotopique naturel. *Th doct, Océanogr, Univ Bordeaux I*
- Riera P, Richard P (1996) Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. *Estuar Coast Shelf Sci* 42:347–360
- Riera P, Richard P (1997) Temporal variation of  $\delta^{13}\text{C}$  in particulate organic matter and oyster *Crassostrea gigas* in Marennes-Oléron Bay (France): effect of freshwater inflow. *Mar Ecol Prog Ser* 147:105–115
- Riera P, Richard P, Grémare A, Blanchard G (1996) Food source of intertidal nematodes in the Bay of Marennes-Oléron (France), as determined by dual isotope analysis. *Mar Ecol Prog Ser* 142:303–309
- Risgård HU (1991) Suspension feeding in the polychaete *Nereis diversicolor*. *Mar Ecol Prog Ser* 70:29–37
- Sauriau PG, Mouret V, Rincé JP (1989) Organisation trophique de la malacofaune benthique non cultivée du bassin ostréicole de Marennes-Oléron. *Oceanol Acta* 12: 193–204
- Schoeninger MJ, de Niro MJ (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim Cosmochim Acta* 48:625–639
- Stump RK, Frazer JW (1973) Simultaneous determination of carbon, hydrogen and nitrogen in organic compounds. Lawrence Livermore Lab, Rep UCID 16198, University of California, Davis
- Sweeney RE, Liu KK, Kaplan IR (1978) Oceanic nitrogen isotopes and their uses in determining the source of sedimentary nitrogen. In: Robinson BW (ed) Stable isotopes in the earth sciences. Science Information Division, Department of Scientific and Industrial Research, Wellington, p 9–26
- Thornton SF, McManus J (1994) Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems—evidence from the Tay Estuary, Scotland. *Estuar Coast Shelf Sci* 38:219–233
- Wada E, Terazaki M, Kabaya Y, Nemoto T (1987)  $^{15}\text{N}$  and  $^{13}\text{C}$  abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. *Deep Sea Res* 34:829–841
- White DS, Howes BL (1994) Nitrogen incorporation into decomposing litter of *Spartina alterniflora*. *Limnol Oceanogr* 39:133–140