Temporal variation of $\delta^{13}C$ in particulate organic matter and oyster *Crassostrea gigas* in Marennes-Oléron Bay (France): effect of freshwater inflow

P. Riera*, P. Richard

Centre de Recherche en Ecologie Marine et Aquaculture de L'Houmeau, UMR 10, CNRS-IFREMER, BP5, Place du Séminaire, F-17137 L'Houmeau, France

ABSTRACT· The temporal variability of $\delta^{13}C$ in suspended particulate organic matter (POM) and oyster *Crassostrea gigas* along a salinity gradient was investigated from May 1992 to September 1993 within the estuarine bay of Marennes-Oléron (France). During this period the mean daily discharge of the Charente River exhibited large seasonal variation, with a high discharge from November 1992 to January 1993. Contrary to that at the river mouth and the marine littoral, $\delta^{13}C$ in POM and in oysters at mid-estuary was affected by the high flood period. The $\delta^{13}C$ values of POM decreased in mid-estuary and remained at low levels during the high discharge period, indicating an increasing contribution of terrestrial inputs to the estuarine POM pool. At the same site, a remarkable decrease of $\delta^{13}C$ in oysters occurred between December 1992 and March 1993 (after a time lag compared to the ambient POM), indicating incorporation of terrestrial organic matter in oyster tissues during the high flood discharge. The lag between the $\delta^{13}C$ decrease in POM and oysters is attributed to the time needed for oyster tissues to incorporate enough newly terrestrial light carbon to be recognized by the $\delta^{13}C$ measure (about 1 to 2 mo). This time interval depends on tissue turnover time. The $\delta^{13}C$ POM decrease (i.e. 1.3‰) cannot explain entirely the decrease observed in oysters (i.e. 2.3‰). In fact, the pattern exhibited by mid-estuarine oysters can be explained by the increasing contribution of terrestrial organic matter to their feeding, and the inability to preferentially utilize specific components of the estuarine POM that are ^{13}C-enriched.

KEY WORDS: Temporal variation · Carbon isotope ratio · *Crassostrea gigas* · Particulate organic matter · Freshwater inflow

INTRODUCTION

In estuarine environments, the seasonal variability of freshwater inflow may modify the transfer of organic matter within benthic and pelagic food webs. Because they are sedentary, estuarine bivalves must tolerate large fluctuations in the quality and the quantity of their food particles. Food sources available for bivalves are represented by a large pool of organic matter of different origins, sizes, nutritive values and biomass (Jørgensen 1990) and are also characterised by temporal variation resulting from hydrodynamic conditions in estuaries (Knox 1986). Particularly, the seasonality of

freshwater inflow can affect sediment transport and turbidity in estuaries (Uncles et al. 1988) but also can influence chemical and biological processes (Sharp et al. 1986, Cifuentes et al. 1988). In fact, nutrients carried down the river can stimulate phytoplankton production (Boynton et al. 1982) and increase food available for benthic invertebrates (Montagna & Yoon 1991). However, the response of primary production to freshwater inflow is not simple because it depends on the interactions between these different physical and chemical effects (Boynton et al. 1982). For example, high turbidity levels can limit primary production within estuarine waters by light limitation (Uncles et al. 1988, Monbet 1992). Thus, in very turbid estuaries, the effect of freshwater inflow on trophic links may be different from a simple transfer of organic matter up

*E-mail: priera@ifremer.fr

the food web resulting from river effect on primary production through nutrient availability.

In addition to inorganic nutrients, the quantitative importance of organic detritus that is carried by a river to its estuary has been pointed out by many authors (Meade 1969, Simenstad & Wissmar 1985, Cai et al. 1988, Mann 1988, Bird et al. 1992). The biomass of continental inputs can dominate over local primary production in salt marshes (Haines 1977) or in estuaries during low phytoplankton production (Cifuentes et al. 1988) because these inputs can contribute up to 75 % of total organic carbon available to estuarine consumers (Naiman & Sibert 1978). In coastal areas, the potential role of detritus as a food source for benthic invertebrates is now well recognized (Tenore 1982, Kemp 1986). The use of detritus derived from marine phanerogames as a food source was reported for the polychete Capitella capitata (Tenore 1981) and for the oyster Crassostrea virginica (Crosby et al. 1989). However, little is known about the potential utilization of detritus derived from terrestrial organic matter carried by rivers within estuarine food webs. Understanding the effect of freshwater inflow on trophic links in estuaries and coastal areas requires (1) pointing out the transfer of organic matter from terrestrial origin to higher trophic levels (2) documenting the temporal variations of these transfers that may be induced by seasonal variations in freshwater inflow.

In many coastal areas, stable isotope analysis has been used successfully to point out trophic links from primary producers to higher trophic levels, indicating different utilization of food sources by benthic and pelagic invertebrates (see Fry & Sherr 1984 for a review). Many of these studies focussed on the significance of spatial variations of isotopic composition in intertidal invertebrates (Stephenson & Lyon 1982, Hughes & Sherr 1983) while not considering temporal isotopic variation. Seasonal variability in $\delta^{13}C$ and $\delta^{15}N$ has been reported for benthic bivalves such as Macoma nosuta (Gearing et al. 1984) and Crassostrea sp. (Simenstad & Wissmar 1985, Conkright & Sackett 1986) but the sampling strategy involved in most of these studies did not allow for an easy interpretation of the temporal isotopic variability. The reasons for this are that generally not enough individuals were collected and sampling was infrequent and irregular. This may provide inadequate and incomplete information about the temporal variability of isotopic compositions in consumers and in their food sources. In Marennes-Oléron Bay, France, a preliminary study involving carbon stable isotope analysis was performed to assess the utilization of the main food sources within the particulate organic matter (POM) pool by the oyster Crassostrea gigas (Riera & Richard 1996). Along this estuarine gradient, oyster communities are subject to different hydrodynamic conditions over the annual cycle mainly induced by the seasonal variability of the Charente River discharge (Riera 1995, Riera & Richard 1996). These results indicated that oyster communities along a salinity gradient preferentially utilize different food sources; namely, terrestrial inputs, oceanic phytoplankton and benthic diatoms (Riera & Richard 1996). However, temporal $\delta^{13}C$ variability was not assessed adequately in that study because it included only 2 and 3 sampling dates for oysters and POM, respectively.

In the present study, we intended to investigate more precisely temporal variability of $\delta^{13}C$ in suspended POM and oysters Crassostrea gigas in the estuarine Marennes-Oléron Bay.

MATERIALS AND METHODS

Sampling area. The estuarine bay of Marennes-Oléron is a very shallow bay (average depth 4 m) located centrally 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. With an annual output of about 30×10^3 of Japanese oysters Crassostrea gigas, the Marennes-Oléron Bay is one of the most important sites of oyster production in Europe. It includes extensive bare intertidal mudflats and it is surrounded by wide areas of salt marshes that are entirely managed
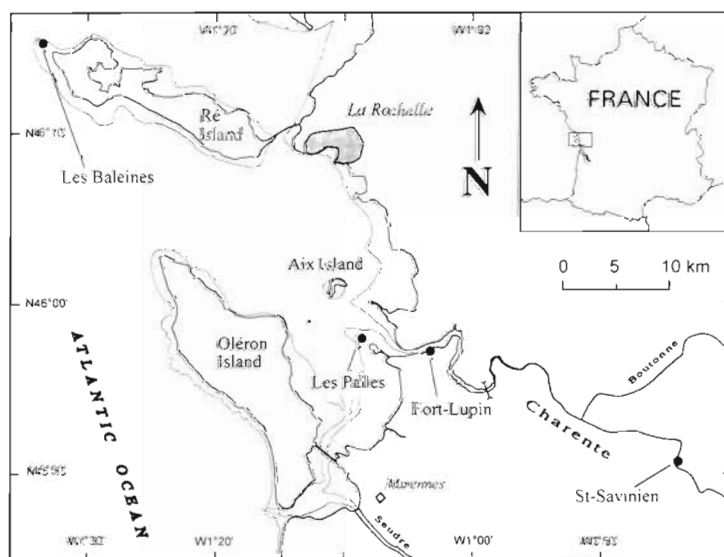

Fig. 1 Sampling sites, Marennes-Oléron Bay, France (--- indicates limit of intertidal mudflats)

and used for oyster cultivation. Thus, unlike the east coast of the United States, where many coastal areas are dominated by angiosperm marsh plants such as *Spartina alterniflora*, the salt marshes lining Marennes-Oléron Bay now consist of oyster ponds that are completely free of C4 plants. The absence of C4 plants is also true for the Charente Estuary because there are no marshes along the estuary itself.

Oceanic waters enter by the north entrance and move south with a residence time of 5 to 11 d (Bacher 1989). Freshwater flows into the bay mainly from the Charente River that drains 10^4 km^2 of agricultural and forested land, with only minor inputs of industrial or sewage effluents. During periods of high riverine discharge, some inputs from the Gironde Estuary, which flows into the ocean 40 km south of the Bay, can enter by the north entrance after having passed around Oléron Island and, to a lesser extent, with high tide through the south entrance within the bay (Dechambenoy et al. 1977).

Oyster sampling locations included 2 estuarine sites (Fort-Lupin, Les Palles) and a marine littoral site (Les Baleines) at the northwestern end of Ré Island (Fig. 1). The mid-estuarine site, Fort-Lupin, located 4 km from the mouth of the estuary, is dominated by muddy sediments. The site located right at the mouth of the Charente River, Les Palles, is a rocky reef with some patches of macroalgae and surrounded by extensive mudflats without any emergent marsh plants. The marine site, Les Baleines, is entirely rocky and is almost completely covered by macroalgae. Over the 200 km^2 of the bay's area, macroalgae are only present as small patches associated with rocky reefs and represent less than 0.05 km^2 (Callens 1994). In both rocky stations, *Fucus* sp. constitutes most of the macroalgae (Riera 1995). To characterize the terrestrial inputs carried into the bay, POM was sampled 50 km upstream in the Charente River, at St. Savinien where a dam prevents any tidal influence. For a normal flow of the Charente River, salinity typically ranges from 18 to 26‰ at Fort-Lupin, from 25 to 33‰ at Les Palles, depending on tide, and is always >35‰ at Les Baleines (Ravail-Legrand 1993).

Temporal variability of the Charente River discharge. The mean daily discharge of the Charente River (at St. Savinien), measured by the local water agency (DDE Charente-Maritime), exhibited large seasonal variability throughout the sampling period (Fig. 2). From May 1992 to November 1992, river discharge remained at low levels (5 to 40 m^3 s^{-1}). This low regime was followed by a high discharge period from

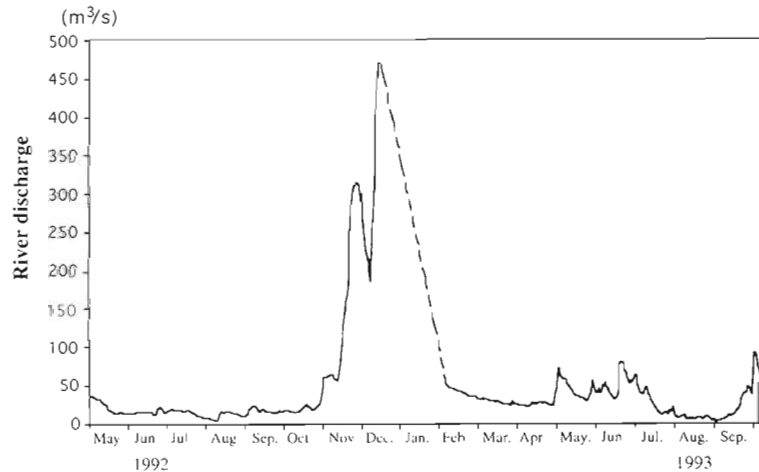

Fig. 2. Mean daily discharge (m^3 s^{-1}) of the Charente River at St. Savinien from May 1992 to September 1993 (data from DDE Charente-Maritime)

the end of November 1992 to the end of January 1993 with a winter peak flow at about 470 m^3 s^{-1}. The river flow then strongly decreased at the beginning of February 1993 and remained at low levels (mostly <50 m^3 s^{-1}) from spring 1993 to autumn 1993. No data are available for the period from the end of December 1992 to the end of January 1993 (dashed line) from the local water agency. However, the high discharge period remained as late as mid January 1993 when it began to decrease (Riera pers. obs.).

Sample collection and preparation. Oysters, suspended POM and sources were sampled at each sampling station within the period May 1992 to October 1993, except at the riverine site (St. Savinien) where there are no oysters. Oysters were collected by hand, taken to the laboratory, cleaned of epibionts and kept alive overnight in filtered water from the sampling site to allow evacuation of gut contents. They were then killed by freezing and dissected, and the tissues of the entire organism were treated with 10% HCl to remove any carbonate debris, rinsed with distilled water, and homogenized using a Polytron homogenizer. Tissues were then freeze-dried, ground to a powder using mortar and pestle and kept frozen until analysis.

Samples of POM were collected monthly from May 1992 to April 1993 at the different stations. On each sampling occasion, 20 l of water were pumped from about 50 cm under the water surface at high tide ±1 h. POM for isotope analyses was obtained by filtration of the water on precombusted Whatman GF/F glass fiber filters (0.7 µm) under moderate vacuum within 2 h after collection. Samples were acidified (10% HCl) to remove carbonates, freeze-dried and kept frozen until analysis.

The main sources that contribute to the suspended POM pool along the estuarine gradient were collected

on different occasions during the sampling period. Benthic diatoms were sampled on the large mudflat at Les Palles and separated using a procedure from Couch (1989) as modified by Riera & Richard (1996). Briefly, the surficial sediment with dense microalgal mats was scraped and then, in the laboratory, was spread on flat trays to a depth of about 1 cm. A nylon screen (63 µm mesh) was laid upon the sediment surface and it was covered with a 4 to 5 mm layer of combusted silica powder (60 to 210 µm). After 12 h, the top 2 mm of the silica powder was gently scraped and then filtered on previously combusted glass fiber filters, rinsed with distilled water, freeze-dried, and frozen. Macroalgae at rocky stations Les Palles and Les Baleines were taken 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 (*Quercus robur*, *Ulmus carpinifolia*, *Carpinus betulus*, Gramineae) in the catchment of the Charente River were collected in winter and spring at St. Savinien.

Stable isotope analysis. Samples for isotope analyses were combusted at 900°C using CuO as an oxydant in evacuated quartz tubes (Stump & Frazer 1973). The resulting CO_2 was purified using a cryogenic distillation method similar to that described by Boutton (1991), and its carbon isotope ratio measured with a Sigma 200 (CJS Sciences) double inlet, triple collector isotope ratio mass spectrometer. Data were reported in the standard $\delta^{13}C$ notation relative to the Pee Dee Belemnite standard (PDB) where $\delta^{13}C = [(R_{sample}/R_{PDB})-1] \times 10^3$, with $R = {}^{13}C/{}^{12}C$. Precision in the overall preparation and analysis was ±0.1‰.

RESULTS

$\delta^{13}C$ of food sources and suspended POM

Average values and variation in $\delta^{13}C$ of the main primary producers for the total sampling period are presented in Table 1. Terrestrial vegetation in the meadows and forests of the drainage basin of Charente River is dominated by C3 plants. Average $\delta^{13}C$ values for leaves of the most common plants, ranging from –30.9‰ (Gramineae) to –28.0‰ (*Quercus robur*), are similar to $\delta^{13}C$ values reported for terrestrial C3 vegetation of temperate areas (Degens 1969, Boutton 1991). These values are close to the POM $\delta^{13}C$ in the Charente River (Fig. 3). Benthic diatoms inhabiting intertidal mudflats near the river mouth had an average $\delta^{13}C$ value of –16.1‰, close to previously reported $\delta^{13}C$ for benthic diatoms in intertidal muddy sediments (Couch 1989, Currin et al. 1995). Diatoms were thus relatively enriched in ^{13}C compared with the suspended estuarine POM pool. The average $\delta^{13}C$ of macroalgae ranged from –18.8‰ (*Fucus vesiculosus*) to –10.7‰ (*Ulva* sp.) at Les Palles and from –30.5‰ (calcareous algae) to –15.9‰ (*Fucus vesiculosus*) at Les Baleines (Table 1).

$\delta^{13}C$ values for riverine and estuarine phytoplankton were estimated from $\delta^{13}C$ of dissolved inorganic car-

Table 1 Average $\delta^{13}C$ (‰ ± SD) of the main food sources of oyster *Crassostrea gigas* along the estuarine gradient in the Marennes-Oléron Bay for the period May 1992 to October 1993. n = number of samples

| Food source	Station		
	St. Savinien	Les Palles	Les Baleines
Riverine phytoplankton	–36.7 ± 2.3 (n = 8)		
Terrestrial vegetation			
Quercus robur	–28.0 ± 0.2 (n = 4)		
Ulmus carpinifolia	–28.5 ± 1.7 (n = 3)		
Carpinus betulus	–28.2		
Gramineae	–30.9 ± 1.6 (n = 2)		
Estuarine phytoplankton		–23.5 ± 1.5 (n = 4)	
Benthic diatoms		–16.1 ± 0.7 (n = 7)	
Oceanic phytoplankton			–20.6 ± 0.8 (n = 6)
Macroalgae			
Fucus vesiculosus		–18.8 ± 0.4 (n = 5)	–15.9 ± 1.1 (n = 4)
Fucus serratus		–16.1 ± 1.9 (n = 8)	–17.5 ± 0.1 (n = 3)
Sargassum sp.			–19.4 ± 0.5 (n = 3)
Ulva sp.		–10.7 ± 1.3 (n = 10)	–18.6 ± 1.2 (n = 5)
Enteromorpha sp.		–16.7 ± 0.7 (n = 9)	–18.7
Laminaria sp.			–20.6 ± 1.4 (n = 4)
Calcareous algae			–30.5 ± 0.5 (n = 2)
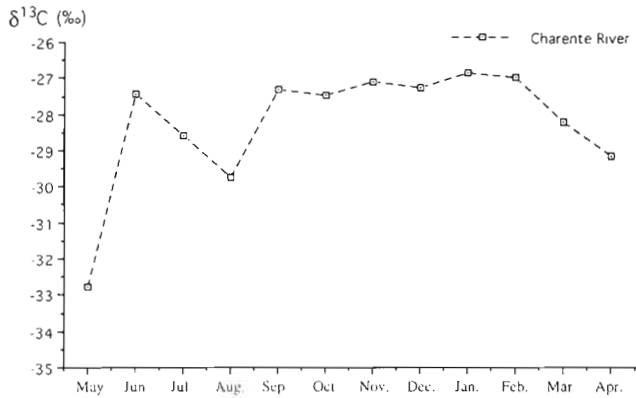
Fig. 3. Monthly $\delta^{13}C$ for suspended POM in the Charente River at the freshwater sampling site (St. Savinien) from May 1992 to April 1993

bon (DIC) by Riera & Richard (1996). $\delta^{13}C$ values for freshwater phytoplankton at St. Savinien ranged from -41.8 to $-32.4‰$ (average $-36.7‰$), and $\delta^{13}C$ for estuarine phytoplankton ranged from -25.4 to $-21.5‰$ (average $-23.5‰$). In fact, within the bay, the phytoplanktonic production is supported by nutrients from the Charente River and is mainly located in a well-defined area between the mouth of the estuary and Aix Island (Ravail et al. 1988). Off Marennes-Oléron Bay, oceanic POM $\delta^{13}C$ values of $-21.0‰$ were found by Fontugne & Jouanneau (1987) and values for POM <40 μm, which was taken as representative of true oceanic phytoplankton, ranging from -19.1 to $-21.5‰$ (average $-20.6‰$) were measured by Richard et al. (1997) from spring 1990 to spring 1993.

Monthly POM $\delta^{13}C$ from May 1992 to April 1993 are presented in Fig. 3 for the riverine station (St. Savinien) and in Fig. 4 for each oyster sampling station. At St. Savinien, POM $\delta^{13}C$ ranged from -32.8 to $-26.5‰$, while at the marine littoral site (Les Baleines) much higher $\delta^{13}C$ values were measured (-22.9 to $-20.1‰$). The 2 estuarine stations showed intermediate POM $\delta^{13}C$ (-24.4 to $-22.7‰$ at Fort-Lupin and -24 to $-21.7‰$ at Les Palles). $\delta^{13}C$ values of POM were significantly different among the sampling stations but were not significantly affected by the sampling dates (2-way ANOVA, $p < 0.0001$ and $p > 0.1$, respectively, for Station and Date).

$\delta^{13}C$ of *Crassostrea gigas*

$\delta^{13}C$ values of oysters at the different sites during the period May 1992 to October 1993 are presented in Table 2. Oyster $\delta^{13}C$ values varied between -24.7 and $-19.7‰$ at mid-estuary (Fort-Lupin), between -20.3 and $-15.9‰$ at the mouth of the estuary (Les Palles) and between -21.5 and $-17.0‰$ at the marine littoral site (Les Baleines). $\delta^{13}C$ of oysters was significantly different among stations and among dates (2-way ANOVA, $p < 0.001$ for Station and Date), and the interaction between Station and Date was not significant ($p > 0.3$). For each oyster site, the temporal variability, as estimated by the coefficient of variation of monthly mean $\delta^{13}C$ values around the average $\delta^{13}C$ value over the total sampling period, ranged from 2.3% at Les Baleines to 3.8% at Fort-Lupin and Les Palles.

Table 2. $\delta^{13}C$ values (‰) of oyster *Crassostrea gigas* at the different sampling locations in the Marennes-Oléron Bay during May 1992 to October 1993 (values shown are ranges) n = number of individuals; ns: not sampled

| Date	Sampling location		
	Fort-Lupin	Les Palles	Les Baleines
1992			
May	-22.1 to -21.4 (n = 5)	-19.7 to -16.1 (n = 6)	-19.5 to -19.1 (n = 3)
June	-22.0 to -19.7 (n = 4)	-19.1 to -15.9 (n = 4)	-20.1 to -19.0 (n = 4)
July	-22.2 to -21.4 (n = 3)	-19.7 to -16.1 (n = 11)	-20.2 to -19.2 (n = 8)
August	-21.9 to -20.0 (n = 3)	-17.3 to -17.2 (n = 3)	-19.5 to -19.1 (n = 4)
September	-21.9 to -21.5 (n = 3)	-18.6 to -17.1 (n = 4)	-19.8 to -17.0 (n = 6)
October	-22.3 to -20.8 (n = 5)	-19.4 to -17.8 (n = 3)	-19.9 to -19.4 (n = 4)
November	-22.0 to -20.9 (n = 3)	-18.5 to -17.6 (n = 3)	-19.3 to -17.7 (n = 3)
December	-21.9 to -20.8 (n = 3)	-19.4 to -18.4 (n = 3)	-20.4 to -19.0 (n = 3)
1993			
January	-22.3 to -21.4 (n = 3)	-19.0 to -18.9 (n = 3)	-20.3 to -19.4 (n = 3)
February	-23.5 to -21.8 (n = 3)	-18.9 to -18.5 (n = 3)	-20.0 to -19.7 (n = 3)
March	-24.7 to -23.4 (n = 4)	-20.1 to -19.5 (n = 2)	-21.5 to -20.1 (n = 5)
April	-23.7 to -22.9 (n = 4)	-20.3 to -18.6 (n = 5)	-20.3 to -19.8 (n = 5)
August	-22.6 to -20.9 (n = 16)	-19.3 to -17.1 (n = 17)	-20.3 to -19.1 (n = 17)
October	-22.6 to -22.5 (n = 3)	-19.2 to -19.0 (n = 3)	ns
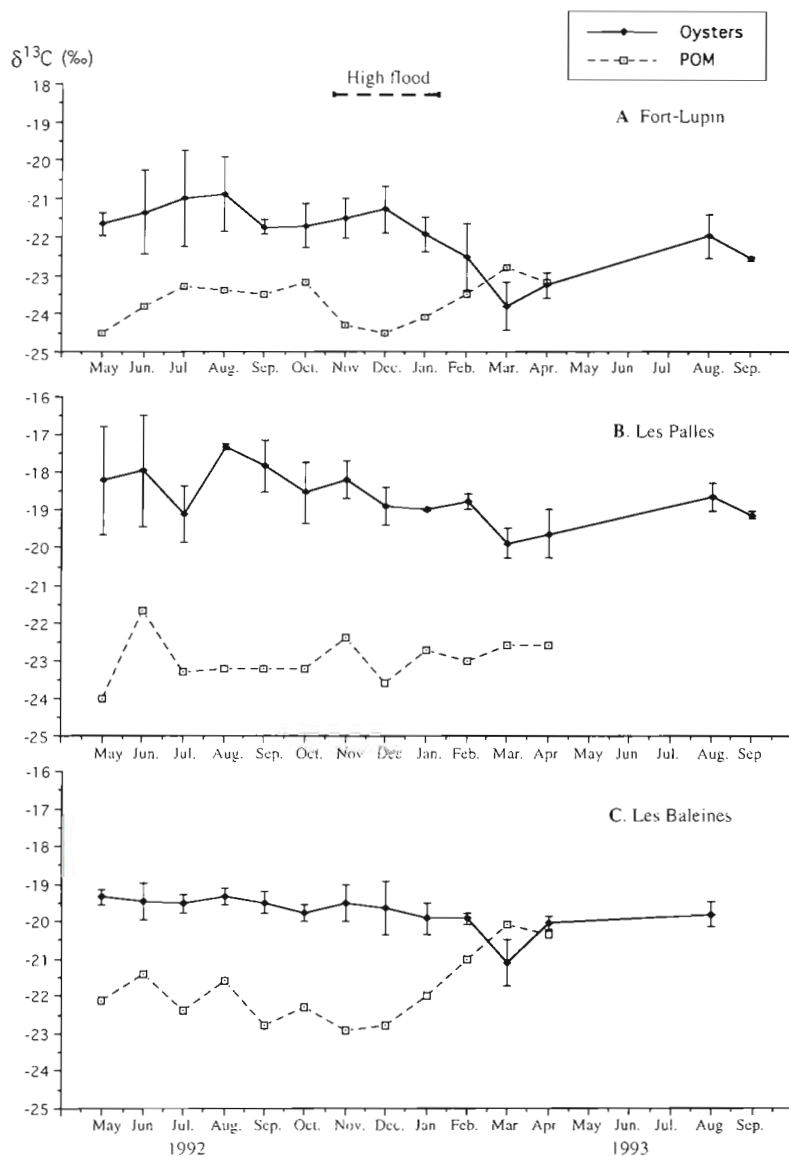
Fig. 4. Temporal variation in $\delta^{13}C$ of oysters (mean ± SD) from May 1992 to October 1993 and suspended POM from May 1992 to April 1993 in the estuarine bay of Marennes-Oléron at the 3 oyster sampling sites, Fort-Lupin (A), Les Palles (B) and Les Baleines (C). The 'high flood' line marks the period of high discharge from the Charente River

DISCUSSION

Effect of freshwater discharge on $\delta^{13}C$ of estuarine POM

On each sampling occasion, POM $\delta^{13}C$ exhibited a gradual isotopic ^{13}C-enrichment from freshwater to marine littoral waters that is likely caused by a progressive mixing of material originating from terrigenous and marine sources as shown by many other studies (Spiker & Schemel 1979, Tan & Strain 1983,

Fontugne & Jouanneau 1987, Fichez et al. 1993). The high discharge period of the Charente River (from the end of October 1992 to end of January 1993) coincided with decreased $\delta^{13}C$ values of suspended POM at mid-estuary (Fort-Lupin), which remained at relatively low levels through April 1993 (Fig. 4A). This pattern suggests an increasing contribution of more ^{13}C-depleted sources to the estuarine POM pool until February 1993 when the river returned to low discharge and $\delta^{13}C$ of mid-estuarine POM became increasingly more enriched and remained at higher levels through spring 1993. This $\delta^{13}C$ decrease of mid-estuarine POM is thus likely to result from the enhancement of terrestrial input poorly enriched in ^{13}C (Table 1) carried by the Charente River towards its estuary during the high flood period. Seasonal $\delta^{13}C$ variability of estuarine POM has been previously attributed to the dominance of terrestrial POM during high river flow regime or of the marine POM pool moving up the estuary during low discharge and high tidal inflow (Fontugne & Jouanneau 1981, Lettolle & Mariotti 1983). In the upper St. Lawrence River estuary (Canada), a seasonal decrease of $\delta^{13}C$ in POM was attributed to an increasing contribution of terrestrial organic matter resulting from the spring high discharge period (Pocklington & Tan 1987).

In the present study, the temporal variability of POM $\delta^{13}C$ in mid-estuarine waters confirms the important contribution of isotopically light terrestrial detritus within the Charente Estuary pointed out by Riera & Richard (1996). These $\delta^{13}C$ values for estuarine POM taken over 12 mo at Fort-Lupin also suggest that under more important high discharge periods there may be a larger range of $\delta^{13}C$ POM at one location than that observed along the spatial extent of the total estuary.

At the river mouth (Les Palles), $\delta^{13}C$ of POM is much less influenced by the high river influx of depleted terrestrial carbon (Fig. 4B). However, a small decrease in $\delta^{13}C$ was observed in December 1993 during the maximum river discharge measured. This lesser effect of the high flood period on POM $\delta^{13}C$ at the river mouth, compared to mid-estuary, can be explained by the

greater dilution of terrestrial inputs by marine waters (Cifuentes 1991).

Effect of freshwater discharge on $\delta^{13}C$ of marine POM

The temporal variability of POM $\delta^{13}C$ at the marine littoral site (Les Baleines) does not appear to be related to Charente River discharge (Fig. 4C), indicating minimal terrestrial input from the Charente Estuary at the NW end of Marennes-Oléron Bay, even during high discharge periods (Ravail-Legrand 1993). In fact, at the oceanic end of the bay, the turbidity is very low compared to riverine and estuarine waters, and the suspended POM includes mainly oceanic phytoplankton and detritus produced by macroalgae (Riera 1995). Off Marennes-Oléron Bay, values of phytoplankton $\delta^{13}C$ from −21.5 to −19.1‰ (Fontugne & Jouanneau 1987, Richard et al. 1997) are close to values given in the literature for oceanic plankton in temperate regions (see Gearing et al. 1984 for a data review). Macroalgae are abundant at this particular site (Les Baleines), and they show a large range of $\delta^{13}C$ values (from −30.5 to −15.9‰), as has already been reported (Simenstad & Wissmar 1985, Simenstad et al. 1993). However, the most common macroalga, *Fucus* sp., has $\delta^{13}C$ values from −18.8 to −15.9‰, more enriched than marine phytoplankton. Thus, the more positive $\delta^{13}C$ values for marine POM at diverse periods of the year (Fig. 4C) may be explained by a higher contribution of ^{13}C-enriched macroalgal debris within suspended POM.

Preferential utilization within the POM pool by oysters

Average $\delta^{13}C$ differences between POM in suspension and oysters during the total sampling period (Fig. 5) are too large to be explained only by the metabolic ^{13}C enrichment that occurs during the assimilation of food (about 1‰ according to DeNiro & Epstein 1978 and Rau et al. 1983). In fact, the $\delta^{13}C$ differences also result from biochemical and/or physiological processes that lead a consumer to preferentially utilize one or several components of the POM pool. Here, these differences may be considered as an index of preferential utilization by oysters of specific components that are relatively enriched in ^{13}C among diverse potential food sources. Although it is apparent for each oyster community, the magnitude of this preferential utilization differs among the sampling stations but also between sampling dates. It was most noteworthy at the mouth of the Charente River
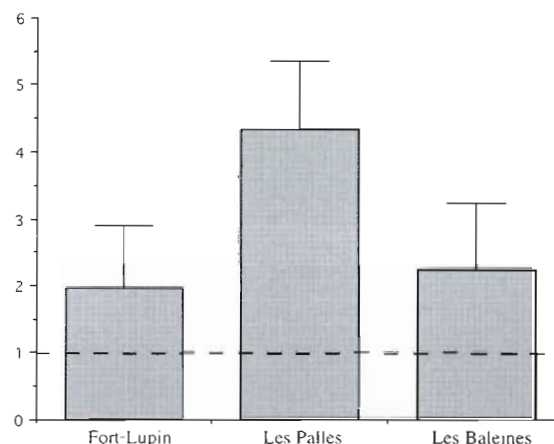
Differences in $\delta^{13}C$
between oysters and suspended POM (‰)

Fig. 5. Average differences (+SD) between $\delta^{13}C$ of oysters (*Crassostrea gigas*) and $\delta^{13}C$ of suspended POM for the period May 1992 to April 1993 at the different sampling sites. Horizontal dashed line shows average carbon trophic enrichment

(Les Palles), where it reached 5.7‰ in May 1992 (Fig. 4B).

Effect of freshwater inflow on $\delta^{13}C$ of oysters

Mid-estuary oysters

Between December 1992 and March 1993, a remarkable decrease in $\delta^{13}C$ values in oysters was observed at Fort-Lupin (Fig. 4A), indicating a gradual incorporation of light carbon in oyster tissues. We attribute this decrease to an increasing contribution to oyster consumption of terrestrial organic matter input resulting from the high discharge period because (1) $\delta^{13}C$ of oysters began to decrease during the high discharge period of the Charente River, indicating enhancement of the contribution of terrestrial organic matter within estuarine POM, (2) in the upper reaches of this estuary, most of the carbon requirement of *Crassostrea gigas* originates from detritic terrestrial organic matter (Riera & Richard 1996), and, (3) the freshwater phytoplankton carried by the Charente River is too depleted to contribute significantly to oyster feeding (Riera & Richard 1996), and the phytoplankton production within the Charente Estuary is strongly limited by high turbidity (Ravail-Legrand 1993).

This result is in accordance with the suggestion of Conkright & Sacket (1986) to explain the decrease in $\delta^{13}C$ (i.e. 2.2‰) observed for the oyster *Crassostrea virginica* between dry and rainy seasons in a marine bay of Florida. This temporal isotopic variability was mainly attributed by these authors to an increasing

contribution of terrestrial inputs carried by drainage waters to oyster feeding.

The decrease in $\delta^{13}C$ values of these oysters occurred after a time lag of between 1 and 2 mo compared to the suspended POM (Fig. 4A). This lag can be attributed to the time needed for oyster tissues to incorporate enough of the 'new' terrestrial light carbon for it to be recognizable by the $\delta^{13}C$ measurement. This time interval depends on tissue turnover time (Anderson et al. 1987).

However, the seasonal decrease in estuarine POM $\delta^{13}C$ (i.e. 1.3‰) cannot entirely explain the larger isotopic variation observed in these oysters (i.e. 2.3‰). In fact, the decrease in $\delta^{13}C$ of oysters is progressive, converging towards $\delta^{13}C$ values of the POM and leading to narrow differences between $\delta^{13}C$ of oysters and POM from February 1993 to April 1993 (Fig. 4A). One interpretation for this convergence is a reduction of the preferential utilization of specific components within POM by oysters (i.e. the more ^{13}C-enriched components). Such a progressive decrease of $\delta^{13}C$ of oysters towards $\delta^{13}C$ of POM was not observed at Les Palles and Les Baleines (Fig. 4B, C). However, the winter decrease of $\delta^{13}C$ in mid-estuarine oysters can have 2 other interpretations.

The first is that, during high river flow, ^{13}C depletion in freshwater DIC may explain a part of the $\delta^{13}C$ decrease in oysters through phytoplanktonic production. Simenstad & Wissmar (1985) attributed the seasonal variations of oyster $\delta^{13}C$ to an increasing contribution of freshwater input within the estuary which could influence $\delta^{13}C$ in autotrophs and thus, $\delta^{13}C$ of primary consumers. Indeed, these authors observed a seasonal decrease in $\delta^{13}C$ of primary consumers (i.e. Crassostrea sp.) of estuarine and nearshore environments of the Hood Canal (Washington, USA) and a seasonal variation of $\delta^{13}C$ of DIC in superficial waters of riverine, estuarine and nearshore environments, the lowest $\delta^{13}C$ values in estuarine DIC occurring during winter. In the Charente River, a decrease of $\delta^{13}C$ DIC was observed in winter 1993 (Riera & Richard 1996) that could have lowered phytoplankton $\delta^{13}C$. However, in the Charente Estuary, although freshwater inflow carries large amounts of nutrients, the phytoplanktonic activity is strongly limited by a high turbidity due to sediment resuspension (Ravail-Legrand 1993, Riera 1995). Moreover, the main phytoplankton production of the Charente Estuary, which takes place between the mouth of the estuary and Aix Island (Ravail et al. 1988), is not a dominant food source for oysters, even at Les Palles, because these oysters preferentially utilize benthic diatoms as a food source (Riera & Richard 1996).

The second interpretation is that the increase in the lipid content associated with gamete production in Crassostrea gigas (Héral & Deslous-Paoli 1983) could explain the $\delta^{13}C$ decrease in oysters because lipids are much more ^{13}C-depleted than proteins and carbohydrates (De Niro & Epstein 1977, Tieszen et al. 1983). However, in Marennes-Oléron Bay this seasonal increase in lipid content is observed from June to mid-August (Deslous-Paoli et al. 1982); whereas, in the present study, the $\delta^{13}C$ decrease in oysters at Fort-Lupin occurred in winter.

Thus, the decrease in $\delta^{13}C$ exhibited by mid-estuarine oysters (Fort-Lupin) can be explained by (1) the increasing contribution of terrestrial organic matter to their feeding and (2) the inability of oysters to preferentially utilize specific components among the ambient POM pool that are more enriched in ^{13}C. In fact, the preponderance and the persistence of freshwater input within the estuary during high flood can physically limit the tidal inflow of marine water towards the upper estuary and, thus, the transfer of marine food sources from the river mouth towards the upper estuary. Indeed, during high runoff conditions, continental water forces coastal waters further offshore and can cover the shallow sills (Drinkwater 1986). As these hydrodynamic conditions occur, the high river discharge may reduce the diversity of potential food sources available for oysters inhabiting mid-estuary, limiting their possibility to preferentially utilize specific sources. Particularly, high nutritive food sources like benthic diatoms that are enriched in ^{13}C (Table 1) may become poorly available to these oysters. In fact, near the mouth of the Charente River large intertidal mudflats support an important biomass of benthic diatoms (Cariou-LeGall & Blanchard 1995). These benthic diatoms can be resuspended in the water column along with surficial sediments and, during normal flow of the river, may be carried into the upper estuary during flood tide, thereby becoming available to oysters. During spring, as the river returned to low discharge (Fig. 2), oyster $\delta^{13}C$ increased towards less negative values (i.e. similar to $\delta^{13}C$ values before the high flood period), indicating a higher contribution of ^{13}C-enriched sources to their feeding. Moreover, the return of oyster $\delta^{13}C$ to less negative values (after a time lag compared to POM) confirms, a posteriori, the influence of the high discharge period on feeding requirements of Crassostrea gigas.

River mouth oysters

The high freshwater discharge period does not influence oyster $\delta^{13}C$ at the river mouth (Les Palles) as much as it does at mid-estuary. In fact, between May 1992 and January 1993, temporal $\delta^{13}C$ of these oysters showed little monthly variation (Fig. 4B). After this

time, oyster δ^{13}C followed a trend which was similar to that of oysters from Fort-Lupin but occurred later (between February 1993 and April 1993) and at a lesser magnitude. This lesser impact compared to mid-estuary can be attributed to a progressive dilution of riverine inputs by marine waters along the estuary and a longer time interval for the riverine POM in transit towards the river mouth (Cifuentes 1991). The absence of a strong δ^{13}C decrease in oysters at the river mouth confirms the previous results of Riera & Richard (1996) that these oysters preferentially utilize benthic diatoms made available by the resuspension of surficial sediment. The results of the present study suggest that benthic diatoms are preferentially utilized by these oysters during the whole year, even during high river flood, when terrestrial food sources are more readily available.

Marine littoral oysters

Oysters from the marine site (Les Baleines) showed little δ^{13}C monthly variability (Fig. 4C) suggesting (1) an overall stability of their food sources during the sampling period and (2) an absence of influence of the Charente River discharge on their feeding. These data confirm previous results of Riera & Richard (1996), indicating a predominance of oceanic phytoplankton (Table 1) in the feeding of these oysters, and are in accordance with carbon isotopic results for bivalves in similar environments (Incze et al. 1982). However, although macroalgae are unlikely to be considered as a main food source for oysters, their contribution cannot be excluded from δ^{13}C measured in this study (Table 1). Indeed, in coastal areas where macroalgae are abundant, Duggins et al. (1989) reported that the carbon derived from Laminaria sp. and Alaria fistulosa detritus can be incorporated in significant levels by many deposit-feeders and also by the bivalve Mytilus edulis.

In summary, the results of this study point out the effects of seasonal freshwater inflow on the sources of food particles consumed by estuarine oysters Crassostrea gigas. However, the response of oyster feeding to the temporal variation of freshwater inputs is clearly dependent on their position along the estuarine gradient. The δ^{13}C of mid-estuarine oysters can be influenced by the seasonal variability of the river flow through the increasing contribution of terrestrial inputs within the estuarine POM and the reduction of the diversity of food sources available to bivalves. Thus, this study also suggests the importance of interactions between hydrodynamic and biological processes in the transfer of organic matter within intertidal food webs.

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