

# Trophic typology of coastal ecosystems based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios in an opportunistic suspension feeder

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**ABSTRACT:** Our objective was to use stable carbon (C) and nitrogen (N) isotope ratios of the adductor muscles of cultured *Crassostrea gigas* to typify the trophic state of temperate coastal ecosystems. Young oysters were introduced on a regional geographical scale in 8 locations along the coast of Normandy (France) and sampled after 9 mo in cultivation. Food sources were also investigated using a mixing model based on food source isotopic composition data previously obtained. To strengthen the interpretation of trophic ecosystem functioning, values of stable isotope ratios were combined with environmental variables in a principal component analysis (PCA). Isotopic values of adductor muscles varied significantly between  $-19.94$  and  $-17.26\text{‰}$  for  $\delta^{13}\text{C}$  and between  $7.73$  and  $12.14\text{‰}$  for  $\delta^{15}\text{N}$ . PCA discriminated 2 groups of coastal ecosystems that differed in coastal hydrology, inputs of nutrients, and size of their respective watersheds. Our results suggest that isotopic signature differences between these 2 spatial groups appeared too important to be due to (1) variations in the isotopic ratios of food sources and (2) differing trophic step fractionation between locations. These differences are more probably linked with differences in oyster diets. Finally, we conclude that cultured *C. gigas* is a useful spatial bio-indicator of coastal ecosystem trophic functioning in temperate ecosystems and an interesting biological model for the determination of isotopic baselines.

**KEY WORDS:** *Crassostrea gigas* · Marine · Carbon and nitrogen stable isotopes · Trophic web · Isotopic baseline · Aquaculture · Oyster

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## INTRODUCTION

Coastal ecosystems located at the interface between continental and open-ocean zones exhibit complex ecological functions and trophic relationships that need to be better elucidated (Yokoyama et al. 2005). Particularly, it is necessary to better understand their dynamics, which vary at different temporal and spatial scales depending on the relative amounts of terrestrial or marine inputs, coastal hydrology, and the local food web structure (Cloern & Jassby 2008). Suspension-feeding invertebrates occupy a central place in the food webs of intertidal communities throughout the

world because they provide important links in the material and energy flows between primary producers and larger consumers (Jennings & Warr 2003). In temperate coastal ecosystems of western Europe, large areas are also devoted to the cultivation of bivalves, thus enhancing the latter's role in nutrient cycling processes (Riera 2007).

Many bivalves are considered to be generalists (as defined in Bearhop et al. 2004) and even opportunists since they can assimilate food from various sources according to their bioavailability (Lefebvre et al. 2009) in order to maximize rates of nutrient acquisition (Hawkins et al. 1996), and according to the occurrence

of competitors (Stuart & Klumpp 1984). Typically, intertidal bivalves feed on a mixture of microalgae (phytoplankton and microphytobenthos) and detritus of marine (macroalgae) and terrestrial origins (Hill et al. 2006, Decottignies et al. 2007, Marín Leal et al. 2008). As benthic sessile organisms, bivalves are good spatial indicators of the trophic state of their environment since they are subject to the quality and quantity of suspended organic matter that serves as their food source (Jennings & Warr 2003), albeit they are able to facultatively select food in their environment (Beninger et al. 2007). Within aquatic ecosystems, stable isotopes are widely used as time-integrating tracers of trophic interactions, environmental gradients, and carbon (C) and nitrogen (N) biogeochemical cycles within the system (Fry 2006).  $\delta^{13}\text{C}$  and/or  $\delta^{15}\text{N}$  values of bivalves (mussels, scallops, oysters) are also used to monitor watersheds and anthropogenic effects on freshwater and coastal ecosystems (Fry 1999, Jennings & Warr 2003).

In general, trophic studies based on stable isotopes of C and N need to be conducted such that the spatial and temporal scales are able to capture the variability in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values that is relevant to the research objectives (Boyce et al. 2001). Evidence of seasonal isotopic differences in consumer tissues has been reported mostly for invertebrates (Malet et al. 2007) and temporal variations have also been demonstrated to be site-specific (Fukumori et al. 2008, Marín Leal et al. 2008). Previously, spatial variability has only been studied on small scales, generally within bays (e.g. Dubois et al. 2007b) or along an estuarine gradient (e.g. Piola et al. 2006), within the same system with comparable functioning. Few studies involving invertebrates consider large spatial biogeographic scales (e.g. Jennings & Warr 2003, Hill et al. 2006). The capture of both temporal and spatial dynamics of coastal environments is a difficult task that necessitates integration of these 2 sources of variation. Changes in isotopic signatures following dietary change have generally been investigated in the laboratory (Dubois et al. 2007a) and results show that the time to reach a new isotopic equilibrium differs among species and even among tissues (Paulet et al. 2006). In bivalves, muscle tissues integrate long-term trophic information (i.e. months) because of their slow isotopic turnover rate (Lorrain et al. 2002, Paulet et al. 2006, Piola et al. 2006).

Our objective was to evaluate the cultivated bivalve *Crassostrea gigas* (Thunberg, 1793) as a bio-indicator that typifies the trophic functioning of temperate coastal ecosystems. We assumed that stable isotope values of *C. gigas* muscles reflect patterns in the availability and isotopic values of their food sources, and subsequent environmental conditions (e.g. nutrient sources and availabilities, salinity, temperature) in a given

coastal ecosystem, as supported by results on other species and other environments (Jennings & Warr 2003, Hill et al. 2006, Gustafson et al. 2007). The Pacific oyster *C. gigas*, an opportunistic suspension feeder, is a Japanese species that has been introduced in various temperate ecosystems worldwide because of its economic value for aquaculture and its capacity to thrive under different environmental conditions due to the breadth of its trophic niche. This sessile primary consumer may provide an interesting bio-indicator that can elucidate trophic ecosystem functioning worldwide. Stable isotopes of *C. gigas* muscle were investigated on a biogeographical scale along the coasts of Normandy (France) together with environmental conditions of the water column (nutrient concentrations, temperature, salinity, chl *a*, turbidity) and food availability to strengthen characterisation of ecosystems and to identify causes of the observed variability.

## MATERIALS AND METHODS

**Study area.** The study area is located on the northwest coastal domain of France in Basse-Normandie, which is one of the most important oyster farming areas of France and Europe ( $\sim 27\,000\text{ t yr}^{-1}$ ; Anonymous 2006). We selected 8 oyster farming areas in these macrotidal/megatidal and intertidal environments because of their distinctive characteristics (site hydrology, mean annual salinity of the water masses, nutrient inputs from rivers, growth of farmed species, especially oysters) around the Cotentin peninsula (J.-L. Blin et al. unpubl. data, Fig. 1).

Three sampling sites were on the west coast of Cotentin: the isles of Chausey ( $48^{\circ}53'31''\text{N}$ ,  $1^{\circ}47'96''\text{W}$ ), Lingreville-sur-mer ( $48^{\circ}56'61''\text{N}$ ,  $1^{\circ}35'33''\text{W}$ ), and Saint-Germain ( $49^{\circ}12'95''\text{N}$ ,  $1^{\circ}38'89''\text{W}$ ) from the south to the north. This coast is exposed to general currents of the North Atlantic drift, which are propagated eastward and deflected northward along the coast from the Baie du Mont Saint-Michel. The maximum spring tidal range is as much as 15 m, and is among the highest in the world. Thus, ecosystems of the west coast are megatidal (Levoy et al. 2000). The substrate is generally medium to coarse sand with some rock inclusions; however, near the coast and especially around the islands where currents form gyres, rocky platforms and tidal deltas locally modify the wave propagation patterns.

Five sampling sites were on the east coast of Cotentin and were more protected from the prevailing winds and marine currents than ecosystems on the west coast because of their location in the Baie de Seine. The maximum tidal range is 8 m, describing a macrotidal environment. Oyster farming sites in

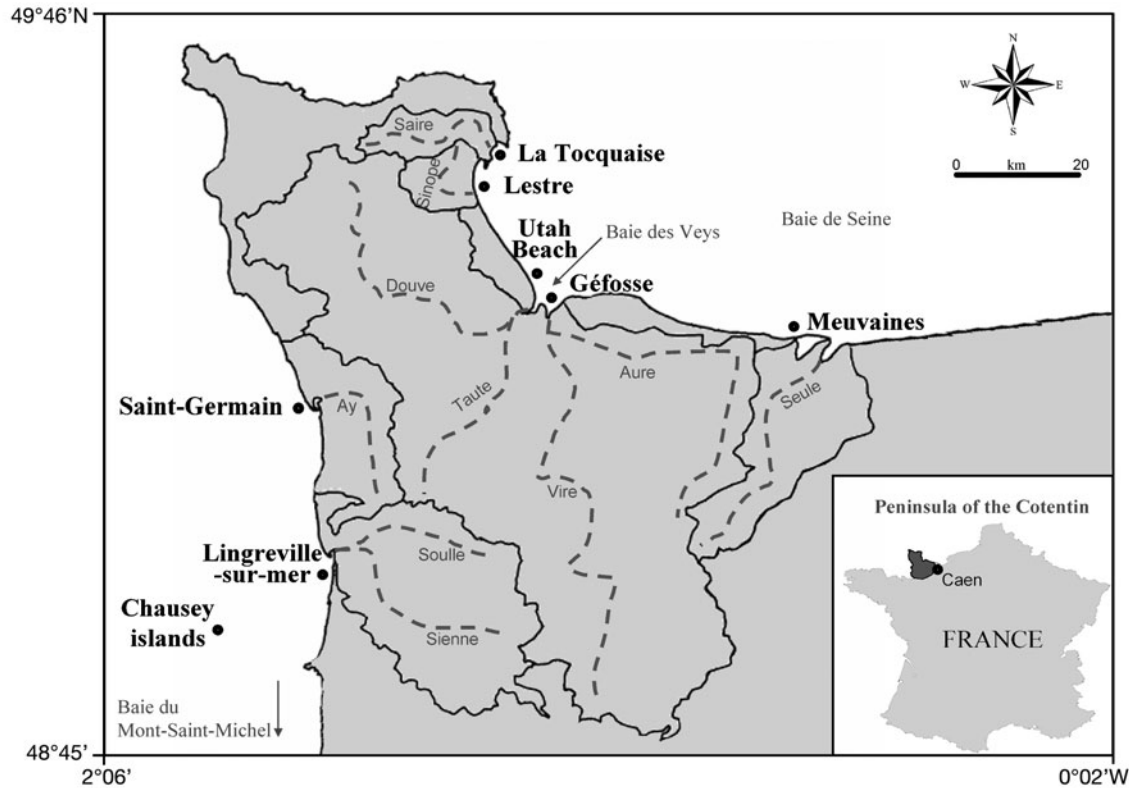


Fig.1. Cotentin peninsula (Normandy, France). Map showing the 8 sampling locations and their associated watersheds (solid lines) and main rivers (dashed grey lines) with their respective names

Géfosse (49° 23' 35" N, 1° 05' 98" W, in the Baie des Veys), Utah Beach (49° 25' 50" N, 1° 10' 62" W), Lestre (49° 32' 49" N, 1° 17' 39" W), and La Tocquaise (49° 35' 49" N, 1° 15' 18" W) are influenced by nutrients from the Baie des Veys (from south to north), an estuary in the western part of the Baie de Seine. Meuvaines (49° 21' 02" N, 0° 33' 75" W) represents one of the most recent oyster farming sites in Normandie. On average, the sediment grain size on the east coast is smaller than on the west coast and spatially more heterogeneous, with fine sand in the middle part of the intertidal zone and also with muddy sand especially in Lestre and Géfosse (F. Levoy pers. comm.).

**Sampling design.** Young Pacific oysters *Crassostrea gigas* (initial total live weight of 1.3 g) from the same batch (i.e. same history: age, method of culture and nutrition status) were introduced in the 8 systems in March 2005 and removed 9 mo later in December 2005. Their weight (taken before introduction and after removal) indicated high growth while reared in the study areas, providing consistently high footprint of each ecosystem in oyster tissues. Oysters were placed in each system (in the middle part of the intertidal area) such that daily immersion duration was equal to avoid error due to different feeding durations. Groups of 10 oysters were taken from each study area. They were individually weighed to estimate total live

weight, then killed by freezing, and dissected to separate the major parts, which were freeze dried. The dry flesh weight was measured on the adductor muscle alone and on the whole individual. Finally, the samples were frozen (−18°C) until further processing.

Simultaneously, surveys of water column parameters (temperature, chl *a* concentration, salinity, nitrate, ammonium, and phosphate) were conducted fortnightly in each study site using standard techniques (Aminot & Kérouel 2004). Watershed surface areas were provided by DIREN (Direction Régionale de l'Environnement Basse-Normandie).

**Isotopic analyses.** Adductor muscles ( $n = 10 \text{ site}^{-1}$ ) were pulverized, and stored in a desiccator until isotopic analyses. Then, 1 mg ( $\pm 0.1 \text{ mg}$ ) of each sample (total  $n = 80$ ) was weighed with a microanalytical balance (Mettler Toledo MX5) and put in tin capsules ( $3.5 \times 5 \text{ mm}$  Sercon). Analyses were performed using an elemental analyser (Eurovector) for particulate C (% C) and N (% N) and an isotope ratio mass spectrometer (IRMS GV Isoprime instrument) for C and N isotopes. Analytical precision was estimated as 0.12 for  $\delta^{15}\text{N}$ , 0.05 for % N, 0.05 for  $\delta^{13}\text{C}$ , and 0.005 for % C. Results were expressed in the delta notation:

$$\delta^*X = [(^*X/X)_{\text{sample}} / (^*X/X)_{\text{standard}} - 1] \times 1000$$

where  $^*X$  and  $X$  are respectively the heavier and the

lighter stable isotopes of the elements analysed. Stable isotope data are expressed as the relative per mille (‰) differences between the samples and the conventional international standard Pee Dee Belemnite (PDB) for C and atmospheric N<sub>2</sub> for N.

**Statistical analyses.** Differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $n = 80$ ) and in hydrobiological variables among growing sites (Lingreville-sur-mer, Saint-Germain, Chausey, La Tocquaise, Lestre, Utah Beach, G efosse, and Meuvaines) were investigated using 1-way ANOVA in Minitab. Normality conditions (Kolmogorov-Smirnov test,  $p > 0.05$ ), homoscedasticity of data (Bartlett's test), and normality of residuals permitted the use of parametric tests. Finally, Tukey's pairwise comparisons were performed as post-hoc tests. Linear regressions were also performed within each ecosystem ( $n = 10$ ) and between ecosystems ( $n = 80$ ) to test for significant linear relationships between variables such as between oyster weights (total live weight, dry weight or adductor muscle dry weight) and  $\delta^{13}\text{C}$  as well as  $\delta^{15}\text{N}$  values. Principal component analysis (PCA, Canoco software), a multivariate analysis, was used to characterise the various studied ecosystems using means of environmental parameters ( $n = 19$  site<sup>-1</sup>), isotopic results, C/N ratios and oyster weights ( $n = 10$  site<sup>-1</sup>).

**Investigation of food sources.** A mixing model (IsoSource; Phillips & Gregg 2003) was performed to calculate the relative contributions of multiple potential food sources in the oysters' diets. For the model, mean C and N signatures of potential food sources were taken from a previous study in Lingreville-sur-mer and the Baie des Veys (see Fig. 2; Lefebvre et al. 2009). The isotopic values of organic matter in the Baie des Veys were used for the eastern locations with estuarine influence, while those in Lingreville-sur-mer were used for the western locations according to the results of PCA (see results section and Table 1). Results of food source contribution were aggregated *a posteriori* according to the method of Phillips et al. (2005) when appropriate.

To correct for the trophic shift existing between oyster adductor muscles, as primary consumers, and their potential food sources, we used a particular trophic step fractionation. First, the values ( $\Delta$ ) estimated from experimental oysters (whole body) reared on *Skeletonema costatum* (1.85‰ for  $\Delta\delta^{13}\text{C}$  and 3.79‰ for  $\Delta\delta^{15}\text{N}$ ; Dubois et al. 2007a) were identified as being the more reliable values for the species. Then, the specific values of McCutchan et al. (2003) for muscle enrichment (1‰ for  $\Delta\delta^{13}\text{C}$  and 0.8‰ for  $\Delta\delta^{15}\text{N}$ ) were added to strengthen the estimate done on the special tissue analysed here.  $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$  are therefore 2.9 and 4.7‰ respectively. Moreover, 3 sensitivity analyses were performed to test the response of the mixing

model of food source contribution to (1) the modification of isotopic values of food sources (values for the east and west coasts were permuted), (2) the change in trophic step fractionation ( $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$  were decreased by 1.85 and 3.79‰ instead of 2.9 and 4.7‰ respectively), and (3) a spatial difference in trophic step fractionation as supported by the results of Gaye-Siessegger et al. (2004) demonstrating a negative relationship between feeding level/growth and trophic step fractionation. Both  $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$  were decreased in proportion to the final oyster total live weight (TW), with a maximum decrease of 1‰ for the highest TW (for oysters located in Chausey and Utah Beach) and no change for the lowest TW (Saint-Germain). This rule can be synthesised in the following regression:

$$\Delta\delta^{13}\text{Cs (and } \Delta\delta^{15}\text{Ns)} = \Delta\delta^{13}\text{C} - 0.0379 \times \text{TW} + 0.4545$$

( $\Delta\delta^{13}\text{Cs}$  are spatialised). The new values for  $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$  were thus respectively 3.7 and 1.9‰ for the Chausey ecosystem, 3.8 and 2.0‰ for Lingreville-sur-mer, 4.7 and 2.9‰ for Saint-Germain (unchanged), 3.8 and 2.0‰ for G efosse, 3.7 and 1.9‰ for Utah Beach, 4.0 and 2.2‰ for Lestre, 4.2 and 2.4‰ for La Tocquaise, and 4.6 and 2.8‰ for Meuvaines.

## RESULTS

Mean individual dry weights (DW) and total live weights (TW) of oysters differed significantly among ecosystems (ANOVA, Table 1). C/N ratios of oyster adductor muscles differed significantly among sites, but differences were small (ANOVA, Table 1); indeed, values were all under 3.5  $\mu\text{g } \mu\text{g}^{-1}$ . Muscle DWs were linearly correlated with TWs ( $r^2 = 0.926$ ,  $n = 80$ ,  $p < 0.01$ ). Linear relationships between adductor muscle DW and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , or C/N ratios were not significant whether within ( $p > 0.05$ ,  $n = 10$ ) or between ( $p > 0.05$ ,  $n = 80$ ) the 8 ecosystems.

Isotopic values of oyster adductor muscles in the 8 sites were between  $-19.94$  and  $-17.26\%$  for  $\delta^{13}\text{C}$ , and between 7.73 and 12.14‰ for  $\delta^{15}\text{N}$  (Table 1). Isotopic ratios differed significantly among locations, both for C and N. Oysters on the west coast were less enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  than those on the east coast, as highlighted by a post-hoc Tukey's test (Table 1, Fig. 2). On the east coast, the  $\delta^{15}\text{N}$  of adductor muscles decreased concomitantly with distance from the mouth of the Baie des Veys estuary.

As for environmental variables, there were significant differences among locations except in temperature (ANOVA, Table 2). Based on post-hoc Tukey's test, there were clear differences in salinity between east and west coasts, and also in  $\text{NO}_3$ ,  $\text{PO}_4$  and  $\text{Si(OH)}_4$  (except for G efosse, Table 2). Trends were less pro-

Table 1. *Crassostrea gigas*. Weights (n = 10), isotopic carbon and nitrogen ratios, and elemental C/N ratios of adductor muscles (mean  $\pm$  SD) at sites along the west and east coasts of the Cotentin peninsula after 9 mo in cultivation (mean initial total live weight was 1.30 g). Differences among sites were tested for significance using 1-way ANOVA at  $p < 0.001$ . Means within each column that do not share a common superscript are significantly different at  $p < 0.05$  (post-hoc Tukey's test)

Location	Total weight (g)	Dry flesh weight (g)	Dry adductor muscle weight (g)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C/N ( $\mu\text{g } \mu\text{g}^{-1}$ )
<b>West coast</b>						
Chausey	38.4 (11.3) <sup>b</sup>	0.55 (0.23) <sup>b,c</sup>	0.09 (0.04) <sup>b,c,d</sup>	-19.70 (0.12) <sup>a</sup>	8.28 (0.32) <sup>a</sup>	3.26 (0.04) <sup>a</sup>
Lingreville-sur-mer	36.5 (11.3) <sup>b</sup>	0.69 (0.26) <sup>b,c</sup>	0.11 (0.05) <sup>c,d</sup>	-19.52 (0.25) <sup>b</sup>	9.36 (0.12) <sup>b</sup>	3.42 (0.06) <sup>c</sup>
St-Germain	12.0 (3.9) <sup>a</sup>	0.24 (0.08) <sup>a</sup>	0.02 (0.01) <sup>a</sup>	-19.07 (0.15) <sup>c</sup>	9.24 (0.35) <sup>b</sup>	3.35 (0.04) <sup>b</sup>
<b>East coast</b>						
Géfosse	35.0 (9.3) <sup>b</sup>	1.01 (0.35) <sup>c</sup>	0.16 (0.07) <sup>e</sup>	-18.04 (0.10) <sup>d</sup>	11.79 (0.23) <sup>f</sup>	3.31 (0.05) <sup>a,b</sup>
Utah Beach	38.3 (19.7) <sup>b</sup>	1.14 (0.57) <sup>c</sup>	0.15 (0.10) <sup>d,e</sup>	-17.53 (0.18) <sup>f</sup>	11.09 (0.16) <sup>e</sup>	3.33 (0.05) <sup>b</sup>
La Tocquaise	30.7 (10.2) <sup>a,b</sup>	0.57 (0.21) <sup>a,b,c</sup>	0.10 (0.04) <sup>b,c,d</sup>	-17.65 (0.18) <sup>e,f</sup>	10.47 (0.18) <sup>d</sup>	3.29 (0.03) <sup>a,b</sup>
Lestre	26.2 (8.1) <sup>a,b</sup>	0.41 (0.14) <sup>a,b</sup>	0.06 (0.03) <sup>a,b,c</sup>	-17.74 (0.14) <sup>e</sup>	10.65 (0.33) <sup>d</sup>	3.32 (0.08) <sup>a,b</sup>
Meuvaines	15.9 (3.4) <sup>a</sup>	0.30 (0.09) <sup>a</sup>	0.04 (0.02) <sup>a,b</sup>	-17.53 (0.17) <sup>f</sup>	9.77 (0.23) <sup>c</sup>	3.36 (0.04) <sup>b,c</sup>

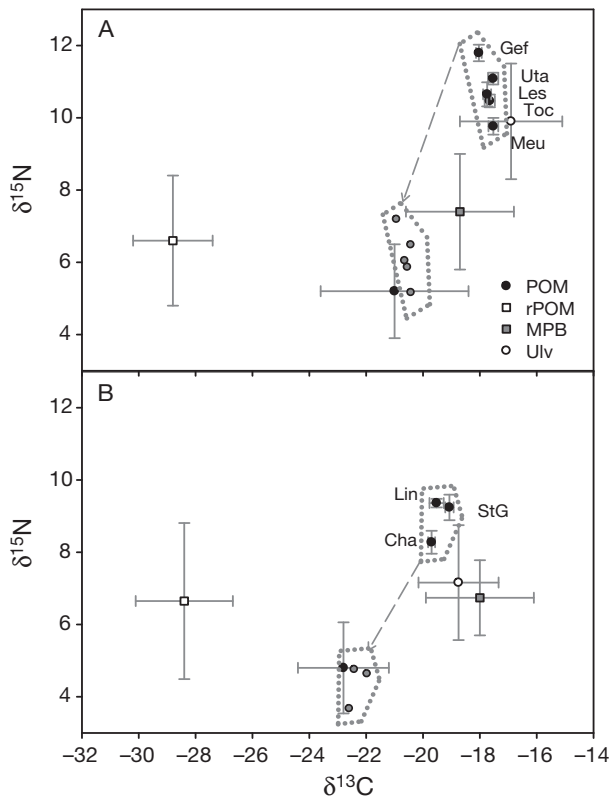


Fig. 2. *Crassostrea gigas*.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  SD) of adductor muscle (n = 10) and of the 4 potential food sources (n = 13) in the east coast of the Cotentin peninsula (A; reference site for food sources is the Baie des Veys), and in the west coast (B; reference site for food sources is Lingreville-sur-mer) from Lefebvre et al. (2009). POM: marine phytoplankton and suspended organic matter; MPB: microphytobenthos; Ulv: *Ulva* sp.; rPOM: riverine organic matter. Gef: Géfosse; Uta: Utah beach; Les: Lestre; Toc: La Tocquaise; Meu: Meuvaines; Lin: Lingreville-sur-mer; StG: Saint-Germain; Cha: Chausey. The corrected values after correction based on trophic step fractionation are indicated by the arrows and the envelopes (dotted grey lines)

nounced for chl *a* and  $\text{NH}_4$ , although values were generally lower on the west coast (Table 2). No clear trends were found for turbidity (Table 2). Related watershed surface areas of the 8 locations were given since coastal areas along the east or west coasts are interconnected by upward currents. However, it is worth noting that the total surface area of watersheds impacting the east coast is  $\sim 4\times$  larger than that of watersheds impacting the west coast (Table 2).

Principal component analysis using the environmental and biological variables (Tables 1 & 2) confirmed contrasts between the east and west coastal ecosystems (Fig. 3). Although the west coast appeared well correlated with salinity, with the isles of Chausey having the highest salinity ( $\sim 35$ ) because of their distance from the coast, ecosystems of the east coast were characterised by lower salinity ( $\sim 33.5$ ) because of their proximity to an estuary receiving freshwater from a large watershed (see Table 2) and higher nutrient concentrations leading to greater seston chl *a* concentrations (Fig. 3). Indeed, the east coast was more productive, with concentrations of chl *a*,  $\text{NO}_3$ , and  $\text{Si}(\text{OH})_4$  being approximately double those on the west coast of Cotentin. While  $\delta^{13}\text{C}$  variations among study sites appeared very well correlated with nutrients,  $\delta^{15}\text{N}$  values of adductor muscle tissue in each ecosystem reflected seston chl *a* concentrations (Fig. 3). Turbidity, C/N ratio and temperature showed lesser influence in the analysis (Fig. 3).

Results from the mixing model suggested that oysters on the west coast relied mainly on phytoplankton and marine organic matter sources (POM), while those on the east coast used benthic (microphytobenthos and algal detritus: MPB+Ulv) as well as planktonic sources (Fig. 4A). The contribution of rPOM was substantial only for Géfosse (the most estuarine location). On the east coast, the decrease in  $\delta^{15}\text{N}$  of oyster adductor mus-



Table 2. Environmental parameters (mean and SD) for each site along the west or east coast of the Cotentin peninsula between March and December 2005 (fortnight sampling; n = 19). Differences among sites were tested for significance using 1-way ANOVA at p < 0.05 (No significant differences were found for temperature, salinity or turbidity). Means within each column with different superscripts are significantly different at p < 0.05 (post-hoc Tukey's test)

Location	Temperature (°C)	Salinity	Turbidity (NTU)	Watershed area (km <sup>2</sup> )	Seston chl a (µg l <sup>-1</sup> )	NH <sub>4</sub> (µM)	NO <sub>3</sub> (µM)	PO <sub>4</sub> (µM)	Si(OH) <sub>4</sub> (µM)
<b>West coast</b>									
Chausey	14.61 (4.08)	35.09 (0.18) <sup>a</sup>	0.89 (0.38) <sup>a</sup>	~0	1.53 (1.21) <sup>a</sup>	0.52 (1.05) <sup>a</sup>	3.36 (4.26) <sup>a</sup>	0.27 (0.19) <sup>a</sup>	3.31 (1.59) <sup>a</sup>
Lingreville-sur-mer	15.05 (4.31)	34.79 (0.38) <sup>a</sup>	2.34 (4.06) <sup>a,b</sup>	759	1.93 (1.84) <sup>a</sup>	0.63 (0.92) <sup>a</sup>	3.81 (5.02) <sup>a</sup>	0.26 (0.22) <sup>a</sup>	3.08 (2.95) <sup>a</sup>
St-Germain	14.84 (4.21)	34.89 (0.38) <sup>a</sup>	2.77 (6.39) <sup>a,b</sup>	170	1.24 (0.70) <sup>a</sup>	0.89 (1.94) <sup>a,b</sup>	3.10 (4.63) <sup>a</sup>	0.32 (0.31) <sup>a,b</sup>	3.96 (3.29) <sup>a</sup>
<b>East coast</b>									
Géfosse <sup>1</sup>	14.57 (3.98)	33.50 (0.40) <sup>b</sup>	2.25 (1.57) <sup>a,b</sup>	3465	3.09 (2.85) <sup>a,b</sup>	0.66 (0.55) <sup>a</sup>	4.16 (7.65) <sup>a</sup>	0.24 (0.30) <sup>a</sup>	5.04 (4.90) <sup>a</sup>
Utah Beach	14.24 (4.55)	33.44 (0.56) <sup>b</sup>	2.40 (2.07) <sup>a,b</sup>	93	4.64 (5.75) <sup>b</sup>	1.09 (1.15) <sup>a,b</sup>	9.95 (14.18) <sup>b</sup>	0.54 (0.43) <sup>b</sup>	8.03 (7.32) <sup>b</sup>
La Tocquaise	14.06 (4.38)	33.68 (0.42) <sup>b</sup>	1.84 (1.25) <sup>a,b</sup>	197	2.41 (1.78) <sup>a,b</sup>	1.35 (1.28) <sup>a,b</sup>	9.13 (12.53) <sup>b</sup>	0.53 (0.38) <sup>b</sup>	8.41 (6.80) <sup>b</sup>
Lestre	14.19 (4.45)	33.65 (0.44) <sup>b</sup>	1.75 (0.85) <sup>a,b</sup>	197	3.32 (3.51) <sup>a,b</sup>	1.42 (1.27) <sup>a,b</sup>	8.73 (11.99) <sup>b</sup>	0.53 (0.38) <sup>b</sup>	8.04 (7.01) <sup>b</sup>
Meuvaines <sup>1</sup>	16.02 (3.57)	33.36 (0.23) <sup>b</sup>	3.22 (2.15) <sup>b</sup>	431	1.89 (1.13) <sup>a,b</sup>	2.28 (2.34) <sup>b</sup>	8.07 (10.12) <sup>b</sup>	0.41 (0.37) <sup>a,b</sup>	8.05 (6.67) <sup>b</sup>

<sup>1</sup>Environmental variables taken from Le Goff & Riou (2006)

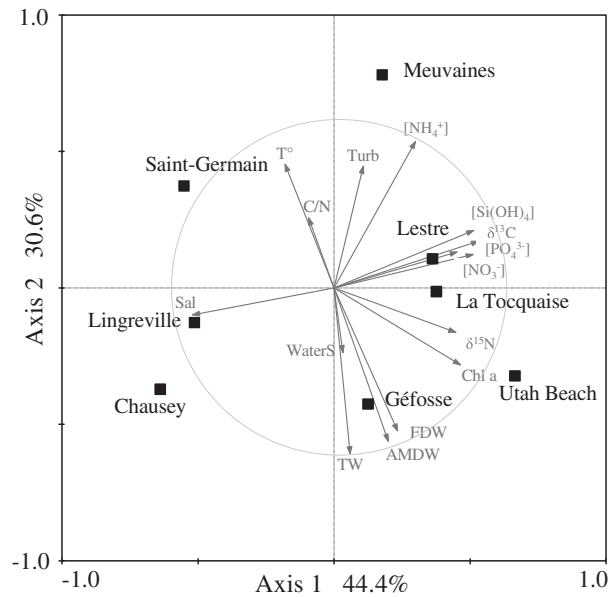


Fig. 3. Principal component analysis (PCA) to discriminate studied ecosystems using means (n = 10) of biological variables of *Crassostrea gigas* (total live weight, TW; dry flesh weight, FDW; dry adductor muscle weight, AMDW; carbon to nitrogen ratio, C/N; adductor muscle  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and means (n = 19) of environmental variables obtained *in situ* between March 2005 and December 2005 (temperature, T°; turbidity, Turb; nutrient concentrations; salinity, Sal; and seston chl a) as explanatory variables within each ecosystem. Watershed surface (WaterS) was a supplementary variable. The correlation circle stands for the variables

cles when moving away from the Baie des Veys mouth is demonstrated by the mixing model as a gradual decline in the use of benthic sources at that spatial scale (Fig. 4A). Whatever sensitivity analyses scenario is chosen, the difference between east and west coasts is evident (Fig. 4B–D). A decrease in trophic step fractionation for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  led to a general increase in MPB and Ulv in the oyster diets (Fig. 4B). Differences between the east and the west coasts were stronger when values for isotopic food sources were permuted from one coast to another (Fig. 4C), and when a decrease in trophic step fractionation was spatially correlated with oyster growth in the different ecosystems (Fig. 4D).

## DISCUSSION

The isotope composition of *Crassostrea gigas* adductor muscle clearly depended on geography, with differences between the west and east coasts of the Cotentin peninsula. Our assumption was that stable C and N isotope values of oyster muscles reflected the trophic

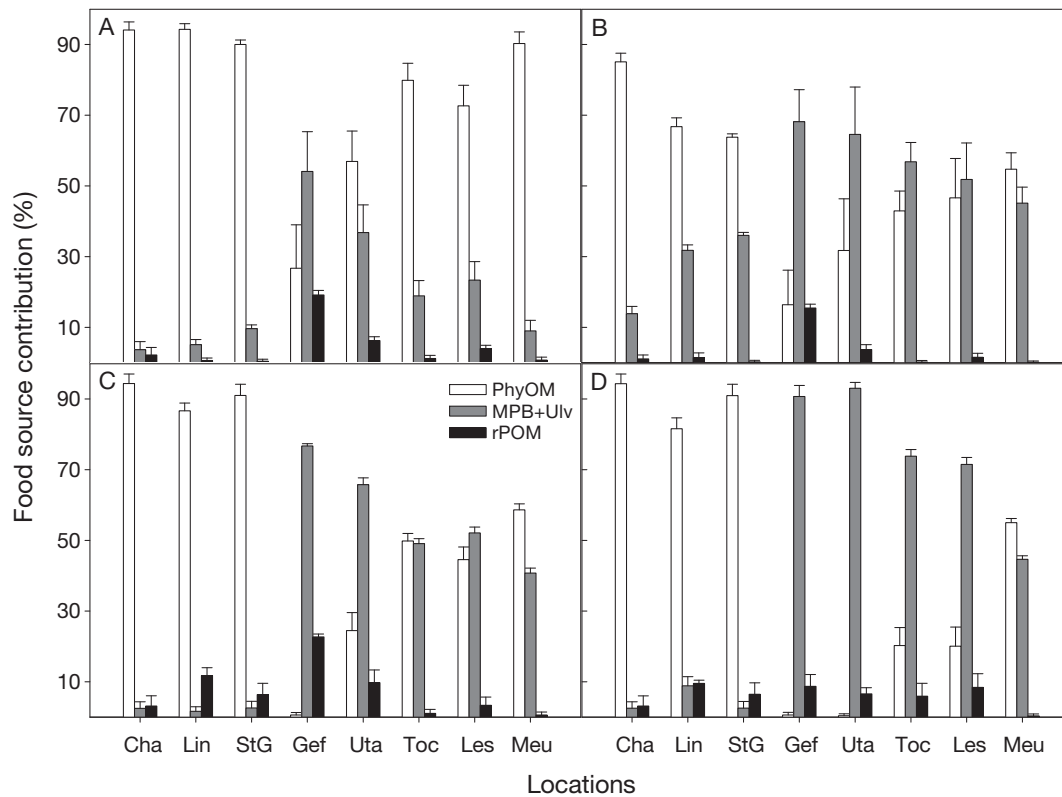


Fig. 4. Contributions (mean + SD) of the 4 main potential food sources (PhyOM: marine phytoplankton and organic matter; MPB: microphytobenthos; Ulv: *Ulv* sp.; rPOM: riverine organic matter) in the diets of *Crassostrea gigas* at each site along the west or east coast of the Cotentin peninsula, as obtained with a mixing model (Phillips & Gregg 2003) using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of oysters and of each of the 4 organic matter sources (Fig. 2). MPB and Ulv were aggregated *a posteriori* as suggested by Phillips et al. (2005). Cha: Chausey; Lin: Lingreville-sur-mer; StG: Saint-Germain; Gef: G efosse; Uta: Utah beach; Toc: La Tocquaise; Les: Lestre; Meu: Meuvaines. Tolerance values were 0.5‰ except for Chausey (1‰). (A) Trophic step fractionation ( $\Delta$ ) of 2.9‰ for  $\delta^{13}\text{C}$  and 4.6‰ for  $\delta^{15}\text{N}$ . (B) Sensitivity analysis 1: no additional  $\Delta$  for enrichment due to muscle;  $\Delta$  was 1.9‰ for  $\delta^{13}\text{C}$  and 3.8‰ for  $\delta^{15}\text{N}$ . (C) Sensitivity analysis 2: permutation of isotopic values of food sources between east and west coasts. (D) Sensitivity analysis 3:  $\Delta$  was spatialized and was dependent on oyster growth in the 8 locations. See 'Materials and methods' for details

functioning of coastal ecosystems, i.e. the availability of food sources and their isotopic values as a function of environmental gradients. Another source of variance for such spatial differences that merits discussion is trophic step fractionation.

#### Spatial variability of isotopic values of food sources

Due to their shallow depths and terrestrial inputs, coastal ecosystems are characterised by high spatial and temporal variability of environmental factors such as freshwater runoff, levels of irradiance, temperature, water movement, and different forms of C ( $\text{HCO}_3^-$  or dissolved  $\text{CO}_2$ ) and N ( $\text{NO}_3^-$  or  $\text{NH}_4^+$ ) sources. All of these factors may cause differences in the isotopic composition of the same organic matter source (Vizzini & Mazzola 2003). For example, typical  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for marine phytoplankton in temperate seas

range from  $-19.1$  to  $-22.0$ ‰ and from 3 to 12‰, respectively (Maksymowska et al. 2000). These authors showed that  $\delta^{15}\text{N}$  values of PhyOM varied according to season in the Gulf of Gdansk (differences of 1.6‰ during a year). This was a general pattern for other organic matter sources as well, with macroalgal signatures varying between 3 and 4.4‰ for  $\delta^{15}\text{N}$  and  $-17.4$  and  $-15.6$ ‰ for  $\delta^{13}\text{C}$ , and terrestrial organic matter signatures varying from 1.5 to 6.5‰ for  $\delta^{15}\text{N}$  and from  $-29.7$  to  $-27.3$ ‰ for  $\delta^{13}\text{C}$  (Maksymowska et al. 2000).

Jennings & Warr (2003) showed a negative correlation between salinity and  $\delta^{15}\text{N}$  of scallop tissue at large spatial scales and assumed it was due to higher  $\delta^{15}\text{N}$  values of the phytoplankton resulting from higher nutrient input from the watersheds. At our spatial scales, Mar n Leal et al. (2008) showed that the annual isotopic average of potential food sources for *Crassostrea gigas* expressed weak spatial differences within 2 of our studied systems that differ functionally

(i.e. G efosse in the Baie des Veys, east coast and Lingreville-sur-mer, west coast; Fig. 2), although they found more similar patterns in oyster isotopic values than in the present study. Generally, isotopic signatures of oyster muscles were more enriched in the Baie des Veys than at Lingreville-sur-mer (11.79‰ for  $\delta^{13}\text{C}$  in G efosse versus 9.36‰ in Lingreville-sur-mer, and  $-18.04$ ‰ for  $\delta^{15}\text{N}$  in G efosse against  $-9.52$ ‰ in Lingreville-sur-mer). This trend was also observed for the isotopic signatures of Ulv and PhyOM (only in C for PhyOM). Despite a more similar variation in C between PhyOM and oysters in G efosse compared with that in Lingreville-sur-mer, this resource cannot explain the huge variation in  $\delta^{15}\text{N}$  (2.4‰) in the tissue. Similarly, isotopic variations of Ulv appeared more consistent with those observed between oysters in Lingreville-sur-mer and the Baie des Veys, where Ulv was used less as a food source (Mar in Leal et al. 2008). Thus, the weak distinctions in food source isotopic values among ecosystems may account only a little of the significant isotopic differences found in oyster muscles as also supported by the sensitivity analysis (Fig. 4C). Other sources of variation (e.g. the spatial variability in trophic step fractionation or the spatial difference in the diet) must be considered to explain these differences.

#### Spatial variability in trophic step fractionation

Mean trophic step fractionation values (1‰ for  $\delta^{13}\text{C}$  and 3.4‰ for  $\delta^{15}\text{N}$ ) that have been estimated for many animals are generally used in the literature (McCutchan et al. 2003). Nevertheless, isotopic fractionation values may be influenced by species and biochemical forms of excretion (Vanderkluft & Ponsard 2003, Dubois et al. 2007a), age (Overman & Parrish 2001), tissue biochemical composition (Lorrain et al. 2002), diet and food quality (Hobson & Clark 1992, McCutchan et al. 2003), feeding level and nutritional status (Hobson et al. 1993, Gaye-Siessegger et al. 2004), environmental abiotic parameters (Vanderkluft & Ponsard 2003, Sweeting et al. 2007), and even by methods of sample preparation (McCutchan et al. 2003). Some of these sources of variability, specifically, species, age, and methods of sample preparation, could not have caused differences in oyster muscle isotopic values among our 8 systems. An advantage of the biological model used in our study (*Crassostrea gigas*) is that many environmental conditions are controlled because culture methods are standardised in Normandy; specifically, oysters are grown in plastic culture bags at similar densities, put on iron tables at 60 cm above the bottom, with the same immersion time at each study site. The other sources of variability, i.e.

environmental parameters, feeding level, and diet composition are discussed below. Few studies of trophic step fractionation exist for bivalves, so the following discussion is based on vertebrates.

Trends in  $\delta^{15}\text{N}$  fractionation values of sea bass muscles were weaker with temperature than with diet (Sweeting et al. 2007). Nevertheless, Barnes et al. (2007) found that N and C fractionation was affected by temperature. For instance, the difference in fractionation was  $-0.6$ ‰ for  $\delta^{15}\text{N}$  and 0.5‰ for  $\delta^{13}\text{C}$  when temperature changed by  $5^\circ\text{C}$  ( $0.1$ ‰  $^\circ\text{C}^{-1}$ ); however, in our study, between-site differences in average temperatures appeared to be too small ( $\sim 1.2^\circ\text{C}$ ) to explain the observed variability in isotopic fractionation of oyster muscles.

Several studies emphasize that both the amount of the diet consumed and the individual metabolic rate could influence  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of tissues (e.g. Gaye-Siessegger et al. 2004, Barnes et al. 2007). Indeed, starvation and low feeding levels of the Nile tilapia *Oreochromis niloticus* resulted in enrichment of  $^{13}\text{C}$  in lipids and  $^{15}\text{N}$  in protein (Gaye-Siessegger et al. 2007). Laboratory tests have shown that periods of reduced nutrient intake can cause  $^{15}\text{N}$  enrichment (from 0.5‰ to 1.5‰ depending on the tissue) in growing birds (Hobson et al. 1993). Both C and N isotope values differed significantly in carp fed at different rates; there was a negative relationship between trophic step fractionation and feeding level of up to 1‰ for both C and N (Gaye-Siessegger et al. 2004). Barnes et al. (2007) found similar trends in sea bass. Actually, oyster final weight differed by up to 3.2× among our 8 systems, providing evidence of different food availabilities. However,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were not significantly correlated with growth and possibly with feeding level, supporting the idea that spatial differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  may not be due to a spatialised trophic step fractionation. This assumption is also supported by the sensitivity analysis (Fig. 4D) which showed that spatial differentiation in trophic step fractionation according to the trends of Barnes et al. (2007) and Gaye-Siessegger et al. (2004) amplified the difference in oyster diet food source contribution between east and west coasts.

Diet-tissue fractionation and turnover rates were also influenced by diet quality (Hobson & Bairlein 2003, Miron et al. 2006). For example, nectarivorous bats fed with amaranth had higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (1.1‰ for N and 1.9‰ for C), albeit with lower nitrogen content, than those fed with soya (Miron et al. 2006). Hobson & Clark (1992) found lower values for N tissue fractionation (1.7‰ for  $\delta^{15}\text{N}$  and 1‰ for  $\delta^{13}\text{C}$ ) in crows *Corvus brachyrhynchos* raised on a diet of perch *Perca flavescens* than crows raised on a plant-based diet (3‰ for  $\delta^{15}\text{N}$  and 2.2‰ for  $\delta^{13}\text{C}$ ). In 2 of our study



sites (Géfosse and Lingreville-sur-mer), Marín Leal et al. (2008) showed that food sources for oysters were similar, but their proportions in the diets differed, in agreement with our results. Therefore, the oyster diets did not completely differ spatially and fractionation might not have been affected by diet quality in the range of values observed in our study.

### Difference in ecosystem trophic functioning

C and N isotopic differences among sites are probably due to the discrimination existing between pelagic and benthic food resources used as already shown by 2 previous studies in Lingreville-sur-mer (west coast) and Géfosse (east coast) (Marín Leal et al. 2008, Lefebvre et al. 2009). Indeed, among primary producers, low  $^{15}\text{N}$  enrichment values are found in phytoplankton in Normandy (Marín Leal et al. 2008). Oyster diets on the west coast were more pelagic than on the east coast. Based on Hsieh et al. (2000), who found a gradient in  $^{13}\text{C}$ -depleted values in oyster tissues from the open sea to the Chiku River in a Taiwan lagoon, the oyster C values in our study, particularly those of the west coast, reflected the more seaward stations. This could explain why Chausey, which is an island that is far from terrestrial inputs and had the highest salinity, was the most oceanic area in the multivariate analysis and had oysters that were less enriched in the heavy C and N isotopes. Actually,  $^{13}\text{C}$  enrichment indicates that oysters on the east coast rely significantly on food items from benthic environments (France 1995). These oysters may rely on various potential food sources: phytoplankton blooms which are greater on the east coast (except in Meuvaines, see chl *a* concentrations in Table 2), microphytobenthos that live in mudflat areas like sites in the Baie des Veys, and terrestrial inputs from one of the most important estuaries in Normandy (Marín Leal et al. 2008). Although C isotope values were quite similar for locations on the east coast, except for Géfosse, N isotope values displayed a gradient from Géfosse (within Baie des Veys) to La Tocquaise (the northernmost station) and Meuvaines (the easternmost station), with the gradient being linked to distance from the Baie des Veys. Multivariate analysis showed that N isotope variations were very well correlated with chl *a* concentrations in the water column and with the final weight of oysters. Variability in  $\delta^{15}\text{N}$  values has been linked to the effects of the watershed and also to anthropogenic nutrient sources such as sewage, urban runoff, industrial wastes, and contaminated groundwater (Fry 1999), which is confirmed by the salinity gradient between east and west coasts in the multivariate analysis. Likewise, total watershed surface areas impacting coastal zones are 4× larger on

the east than on the west coast. The gradient in N was certainly due to terrestrial inputs delivered into estuaries and coastal systems by rivers draining agricultural and/or urban wastes that are enriched in N and other nutrients. Oysters inhabiting intertidal areas on the east coast study sites relied mostly on the benthic food web, albeit to a lesser extent than in the Westerschelde estuary, which is located in one of the highest population density and industrial areas in Europe (The Netherlands; Riera et al. 2000). This confirms the estuarine influence and ecosystem productivity correlation.

The general outcome of the mixing model applied to our data confirmed that oysters reared on the west coast of Cotentin relied mainly on POM (i.e. phytoplankton) in contrast to oysters from the east coast that relied on a mixture of benthic–pelagic sources. These results are in agreement with previous studies done at smaller spatial scales in the same area (Marín Leal et al. 2008, Lefebvre et al. 2009). Similarly, Decottignies et al. (2007) and Malet et al. (2007) found that both benthic and pelagic resources were exploited by oysters in 2 estuarine embayments of the French Atlantic coast. Thus, resuspension processes combined with the nature of the sediment (fine sand and muddy sand) may contribute to the mixing and greater availability of benthic organic matter for the oysters located on the east coast of Cotentin. In contrast, the nature of sediment (medium to coarse sand) along the west coast and the higher hydrodynamic processes may not favour the development or accumulation of benthic organic matter that could be used by oysters once resuspended (Marín Leal et al. 2008).

As concluded by Jennings & Warr (2003) and Barnes et al. (2009), there is a statistical link between bivalve isotopic composition and environmental variables, such as salinity (and consequently nutrient concentrations) and temperature. In coastal ecosystems, however, we conclude that biogeographic differences are probably due more to differences in distributions and availabilities of food source biomass than to differences in isotopic values of one dominant food source (phytoplankton), as assumed by these authors. We also conclude that trophic step fractionation could not explain spatial differences at the scale of our study.

Oysters are known to be selective suspension feeders that can facultatively select food in suspension in their environment (Beninger et al. 2007). Hence, there is a possibility that the isotopic values of oysters reflect more the microalgal component of seston rather than the detrital component (Ulv and rPOM). Another suspension feeder such as *Crepidula fornicata*, which is well known as an indiscriminate suspension feeder (Beninger et al. 2007), would be an interesting model for the investigation of trophic typology of coastal eco-

systems. However, Lefebvre et al. (2009) showed that the diet of *C. fornicata* in the Baie des Veys, where oysters incorporated both microalgae and detritus (rPOM and Ulv), relied almost exclusively on a mixture of microalgae (phytoplankton and microphytobenthos). To our best knowledge, there is no study concerning selective feeding in oysters using isotopes and more research is needed in this area. The use of cultivated oysters ensures low noise in the results due to standardized age, life cycle, densities, and immersion time. Thus, this study showed that oysters could provide an interesting bio-indicator to typify and monitor marine temperate ecosystem trophic functioning at large spatial and possibly interannual scales. In addition, differences in isotopic ratios of food sources and trophic step fractionation may be considered at larger spatial scales if strong environmental gradients (e.g. in temperature or nutrients) are expected (Jennings & Warr 2003, Barnes et al. 2009). These results also have implications for the determination of trophic baselines using bivalves in marine systems, when the trophic position of higher consumers is estimated in food webs (Post 2002, Jennings & Warr 2003, Fukumori et al. 2008). Utilization of different food sources on a regional scale resulted in changes in  $\delta^{15}\text{N}$  values of oysters of up to ~4‰, which is equivalent to one trophic level.

**Acknowledgements.** We thank SMEL and IFREMER for technical assistance through the HYDRONOR and REMONOR programs, Sea Pen Scientific Writing LLC (J. Purcell) for editorial assistance, P. Legoué for geographical information, S. Dubois for valuable comments on the study, and M.-P. Bataillé for help in isotope analysis. We also thank 4 anonymous reviewers for help in improving the manuscript. This work was supported by the Regional Council of Basse Normandie, the Agence de l'Eau Seine-Normandie, DIREN Basse-Normandie, and DRAM/IFOP through the POMOYSTER program.

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Editorial responsibility: Matthias Seaman,  
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Submitted: November 25, 2008; Accepted: June 29, 2009  
Proofs received from author(s): September 4, 2009