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Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and modelling as tools to estimate the trophic ecology of cultivated oysters in two contrasting environments

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Abstract:

Food sources for cultivated marine bivalves generally are not well identified, although they are essential for a better understanding of coastal ecosystems and for the sustainability of shellfish farming activities. In addition to phytoplankton, other organic matter sources (OMS), such as microphytobenthos and detritus (of terrestrial or marine origins), can contribute significantly to the growth of marine bivalves. The aim of this study was to identify the potential food sources and to estimate their contributions to the growth of the Pacific oyster (*Crassostrea gigas*) in two contrasting trophic environments of Normandy (France): the Baie des Veys (BDV) and the Lingreville area (LIN). Two sites were studied in the BDV area (BDV-S and BDV-N) and one in the LIN area. To estimate the contribution of each type of OMS, we used a combination of stable natural isotope composition ($\delta^{13}C$, $\delta^{15}N$) analysis of OMS and oyster tissue together with a modelling exercise. Field sampling was conducted every 2 months over 1 year. The sampled sources were suspended particulate organic matter from marine (PhyOM) and terrestrial (TOM) origins, microphytobenthos (MPB), detrital organic matter from the superficial sediment (SOM), and macroalgae (*Ulva* sp., ULV). A statistical mixing model coupled to a bioenergetic model was used to calculate the contributions of each different source at different seasons. Results showed that isotopic composition of the animal flesh varied with respect to the potential OMS over the year within each ecosystem. Significant differences were also observed among the three locations. For instance, the $\delta^{13}C$ and $\delta^{15}N$ values of the oysters ranged from −20.0 to −19.1‰ and from 6.9 to 10.8‰ at BDV-S, from −19.4 to −18.1‰ and from 6.4 to 10.0‰ at BDV-N, and from −21.8 to −19.4‰ and from 6.3 to 8.3‰ at LIN. The contributions of the different sources to oyster growth differed depending on the ecosystem and on the period of the year. Phytoplankton (PhyOM) predominated as the principal food source for oysters (particularly in the LIN location). MPB, TOM, and ULV detritus also possibly contributed to oysters' diet during summer

and autumn at the BDV-S and BDV-N sites. SOM was not considered an OMS since it was already a mix of the other four OMS, but rather a trophic reservoir that potentially mirrored the trophic functioning of marine ecosystems.

Introduction

Coastal aquatic ecosystems are complex mixing zones that exhibit diverse physical, chemical, and biological features, where the biogeochemical processes significantly affect the fate of allochthonous and autochthonous organic sources (Bianchi and Argyrou 1997; Herman et al. 2000; Maksymowska et al. 2000). Among the forcing factors that control food source assemblages in coastal and estuarine environments, the various hydrological characteristics (e.g. tidal range, strength and direction of currents, local geography, system morphology, and upland terrestrial inputs) differently affect both the production and availability of primary producers and thus their contributions to the diets of consumers (Doi et al. 2005; Vizzini and Mazzola 2006). In addition, the characteristics that vary seasonally (e.g. river flow, tidal cycle, temperature, radiation, and nutrients) cause temporal variations of organic matter sources (hereafter OMS) (Bianchi and Argyrou 1997; Richard et al. 1997) that increase complexity. Because food source assemblages of shallow and open shelf environments are commonly distinct from each other (Schröder-Adams 2006), the contribution of each food source in these complex environments is difficult to generalize and to assess, being highly specific to the studied ecosystems and even within the same ecosystem (Dubois et al. 2007a). Understanding trophic environments and elucidating trophic pathways in coastal ecosystems therefore are arduous tasks that need to be undertaken for optimal use and sustainable development of coastal shellfish ecosystems (Dame and Prins 1998; Vizzini and Mazzola 2006).

The available food supplies determine growth and development of macrobenthic suspension feeders, such as oysters (Kang et al. 2006). In coastal ecosystems and estuaries, a large variety of primary producers, such as benthic and/or pelagic microalgae, was shown to be accessible for bivalves' diets, with these primary sources being supplemented by detritus from several origins, such as vascular plants and macroalgae (McCallister et al. 2006). Phytoplankton was often described as the predominant food source, although depending on the studied ecosystems, terrestrial or microphytobenthos possibly contributed as well (Riera et al. 1999; Page and Lastra 2003). Elucidation of the contribution of OMS to the pool of sediment organic matter (SOM) deserves special attention in studies of intertidal food webs. SOM often represents an intermediate step between OMS and benthic primary consumers as a reservoir receiving and storing detritus of terrestrial or marine origins (Hsieh et al. 2000) before eventually becoming resuspended and available to suspension feeders (Herman et al. 2000).

The origin and fate of organic matter in the marine environment have been investigated by various approaches, such as lipid biomarkers (McCallister et al. 2006), chlorophyll pigments (Wysocki et al. 2006), C/N ratios, and stable isotope ratios (Andrews et al. 1998; Graham et al. 2001). Stable isotope ratio analyses, particularly of carbon and nitrogen, are powerful tools in deciphering the cycling and contribution of multiple organic sources in food webs (Fry 2006). Estimating the contributions of OMS to the diet of an organism requires knowing consumer-diet discrimination values (also known as fractionation values), i.e. the enrichment in heavy isotopes with respect to OMS. Many fractionation values are now available in the literature even if they are still subject to debate because these values are highly specific and linked to the origin of the food source and the feeding level (McCutchan et al. 2003; Gaye-Siessegger et al. 2004). Static

modelling approaches (e.g. mixing model IsoSource proposed by Phillips and Gregg 2003) allow estimates of the contribution of OMS to consumers' diet but unfortunately disregard the rate of incorporation of isotopic compounds, that is the time organisms take to acquire the signature of their food sources. Very few investigations have successfully showed, through bioenergetics models and organism metabolism, how assessing the time required for a consumer to acquire the signature of its food sources crucial to properly understand contributions of various food sources (Harvey et al. 2002; Herzka 2005)..

Oyster culture is one form of sustainable shellfish farming that can be conducted in the natural environment, which requires an adequate knowledge of the ecosystem trophic capacity (Dame and Prins 1998). The objective of this study was to determine the temporal dynamics of OMS contribution to the diets of cultivated oysters (*Crassostrea gigas*) in two contrasting trophic environments in Normandy (France), over an annual survey. We hypothesized that the diets of the cultured oysters change during the year, and that this change is ecosystem-specific. For this purpose we used carbon and nitrogen stable isotopes as tracers. Particular attention was paid to the dynamic aspect of the OMS contribution to oysters' diets by estimating their incorporation rate through time with bioenergetic modelling.

Methods

Study areas

Two different *C. gigas* culture areas were chosen in Normandy (Northwest coast of France, English Channel, Fig. 1) that had distinctive morphodynamical and hydrological variables, and very different biological performances of oysters in terms of growth, reproduction and survival. The first system was the Lingreville-sur-Mer culture area (hereafter LIN), which is located in the west coast of Cotentin Peninsula. This intertidal area, characterised by a macrotidal regime, is under direct exposure to the prevailing west wind. It is wide open to sea and expected to exhibit a high marine influence. Fine sand and coarse shell sand were the main sediments. The terrestrial inputs to this area come from a small river - La Sienne - with an average flow of 10.7 $m^3.s^{-1}$ (range 2.2 – 24.3 $m^3.s^{-1}$). The second area was the Baie des Veys (hereafter BDV), which is a macrotidal estuarine bay with an intertidal area of 37 km^2 located in the southwest part of the bay of Seine, English Channel. The maximum tidal amplitude averaged 8 m. This area is more protected from the prevailing wind than is the LIN area. BDV is influenced by four rivers, which are connected to the bay by the Carentan and Isigny channels in the western and eastern parts of the bay, respectively. Freshwater runoff is low in summer and high in winter, with an average flow of 13.1 $m^3.s^{-1}$ in the Carentan Channel (range 3.7 – 26.4 $m^3.s^{-1}$) and 20.1 $m^3.s^{-1}$ in the Isigny Channel (range 3.9 – 40.4 $m^3.s^{-1}$). The oyster culture area extends into the eastern part of the bay, on both soft-bottom and rocky environments. Since the BDV exhibits large spatial differences in biological performances (Costil et al. 2005) and in isotopic signatures (Dubois et al. 2007a) of *C. gigas*, two locations were chosen in this system. BDV-S in the south of the bay (Fig. 1) was characterized by estuarine influences and a muddy sand bottom. BDV-N, in the north of the bay, has a rocky bottom largely colonized by green and brown macroalgae (mainly *Ulva* sp. as well as *Fucus* sp.).

Sample collection and preparation

Oysters

As in traditional culture methods, oysters were reared 60 cm above the bottom in plastic culture bags put on iron tables. Juvenile oysters originating from the same batch were introduced to both systems (LIN and BDV-S and -N) two months before samplings started (i.e. in March 2004). Three samples, each corresponding to a pool of 10 oysters randomly extracted from oyster bags, were randomly collected in each site every two months, cleaned of epibionts and kept alive overnight in filtered sea water from their respective sampling areas to evacuate their gut contents. Oysters were individually measured, weighed, opened and carefully cleaned with distilled water to remove any debris from the shells. For each of the three samples (i.e. three pools of 10 randomly selected oysters), the whole individuals were freeze-dried, powdered, homogenised and conserved in safe light and humidity until isotopic analyses.

Organic matter sources (OMS)

In these intertidal systems, suspension-feeders feed on a mixture of organic matter originating from three main local sources mixed twice daily by tides over the study areas (Doi et al. 2005; Vizzini and Mazzola 2006). First, marine water from the open sea provides marine suspended particulate organic matter that is mainly composed of phytoplankton (PhyOM). Second, river inputs supply suspended terrestrial organic matter (TOM) composed of vascular plant detritus and freshwater microalgae. Third, organic matter composed of sedimented macroalgal detritus (*Ulva* sp., ULV) and of microphytobenthos (MPB) is resuspended from the sediment to the water column by waves and tidal action (Lundsgaard and Olesen 1997). These local OMS were sampled in the two oyster culture areas, LIN and BDV. OMS samples were not collected at both BDV-S and BDV-N because potential variations in isotopic signatures of consumers within the BDV area are more likely due to variations in the food source composition of the mixture than to spatial variation in the isotopic signature of one food source (Dubois et al. 2007a).

Potential food sources in BDV and LIN were sampled every two months from May 2004 to May 2005 (a total of 7 samplings). Suspended particulate organic matter from marine and terrestrial origins was sampled where these sources originated and were undiluted. TOM was collected from freshwater ca. 2 km upstream from the river mouth during low tide in Isigny Channel for BDV, and in the Sienne River for LIN; PhyOM was collected at high tide in the open sea at a site ca. 1 km from each oyster culture area. Each time, two independent samples of water between 5 and 15 L from 0-50 cm depth were prefiltered onto a 200-µm mesh to remove the largest particles, and filtered onto pre-weighed, precombusted (450°C, 4 h) Whatmann GF/C glass-fibre filters, immediately after sampling. The total suspended particulate matter and suspended particulate organic matter were determined according to a standard weight measurement (Aminot and Kérouel 2004). Then, filters were treated with concentrated HCl fumes in order to remove carbonates before isotopic analyses (Lorrain et al. 2003).

Microphytobenthos (MPB) samples from BDV were collected by scraping the visible microalgal mats off the sediment surface adjacent to the culture area during low tide; in LIN, MPB samples were collected from arbitrary sites because microalgal mats were not visible. In each site, two independent samples were done. Benthic microalgae was extracted from the sediments using a sediment:Ludox HS-40% proportion of 1:2 and recovering the overlying MPB with a Pasteur pipette, a method slightly modified from Blanchard et al. (1988). The recovered microalgae were rinsed with distilled water, centrifuged, freeze-dried, and ground using mortar and pestle.

Two independent samples of the organic matter of the sediment compartment (SOM) were done at low tide using an aluminum corer with 15 cm diameter and 1 cm depth. Freeze-dried samples were sieved with a 250-µm screen to remove the largest sand particles, which supposedly are not ingested by oysters. Sediment subsamples for isotopic carbon analyses were treated with 1 N HCl (~12 h) to remove inorganic carbon. These subsamples then were rinsed with distilled water to remove the acid, freeze-dried, and ground. Subsamples for isotopic nitrogen analyses were not decarbonated because this has been reported to affect the $\delta^{15}N$ and total organic N values (Ryba and Burgess 2002).

Two independent samples of macroalgae ULV, which was the dominant species in the two shellfish culture areas, were collected by hand at low tide and cleaned carefully to remove epibionts before being freeze-dried and ground.

In order to estimate chlorophyll *a* (Chl *a*) contents of sediment and suspended particulate matter, fresh samples were extracted overnight with 90% acetone in the dark at 4°C. The chlorophyll extracts were measured on a Turner Designs TD 700 fluorometer (USA) following the method of Welschmeyer (1994).

Elemental and stable isotope analyses

Oysters and OMS samples were analysed using a CHN elemental analyser (EuroVector, Milan, Italy) for the particulate organic carbon (POC) and particulate nitrogen (PN) in order to calculate their C/N atomic ratio (C_{at}/N_{at}). Analytical precision for the experimental procedure was estimated to be less than 2% dry weight for POC and 6% dry weight for PN. The resultant gas of elemental analyses was introduced online into an isotope ratio mass spectrometer (IRMS) (GV IsoPrime, UK) to determine carbon and nitrogen isotopes. Stable isotopic data are expressed as the relative per mil (‰) differences between the samples and the conventional standard Pee Dee Belemnite (PDB) for carbon and air N_2 for nitrogen, according to the following equation:

$$\delta(X) = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] * 1000$$

where X(‰) is ^{13}C or ^{15}N abundance and R is the ^{13}C:^{12}C or ^{15}N:^{14}N ratios. The internal standard was the USGS 40 of the International Atomic Energy Agency ($\delta^{13}C$=-26.2; $\delta^{15}N$=-4.5). The typical precision in analyses was ±0.05‰ for carbon and ±0.19‰ for nitrogen. For each of the animal samples and OMS samples, respectively 2 and 4 tin caps were analysed to provide an accurate measurement of the intra-sample variability, knowing from preliminary investigations that OMS samples exhibited larger variability than consumers' samples. Mean and standard deviation of the independent samples were reported in the results section.

Statistical analyses

Because the two components of the isotopic signature respond together to some temporal and spatial change in the environment, multivariate analyses of variance (MANOVA) were used to assess the variability in isotopic composition of food sources and oyster tissues. For the response in food sources, the independent variables consisted of the location of the site (2 modalities), the date of the sampling (7 modalities), and the type of the food source (5 modalities). All interactions between factors were tested in this balanced factorial design. For oysters, only the spatial and temporal variables (and interactions between both factors) were considered. The assumptions of ANOVA also hold for MANOVA (Scheiner 1993): 1) subjects were independent; 2) the standardized

residuals (within separate groups of the MANOVA) were normally distributed (Kolmogorov test), and 3) homoscedasticity was guaranteed (Bartlett and Levene tests) but square-root transformation was required for food sources data to meet this condition. All other constraints for MANOVA were fully respected according to Von Ende (1993).

Modelling

As proposed by Phillips and Gregg (2003), a mixing model was used to calculate the contributions of the potential OMS (PhyOM, TOM, MPB, ULV, and SOM) to the oysters' diets. Among fractionation values available in the literature, two extreme sets of values (minima-maxima) were used to fully encompass possible solutions in the contribution of OMS. Hence, two scenarios were calculated using either 1.85‰ for $\delta^{13}C$ and 3.79‰ for $\delta^{15}N$ (scenario called hereafter Fract-A) as the only values for trophic fractionation available for this species by now (as obtained empirically using the diatom *Skeletonema costatum*; Dubois et al. 2007b) or 0.4‰ for $\delta^{13}C$ and 2.2‰ for $\delta^{15}N$ (scenario called hereafter Fract-B) (values for aquatic primary consumers; McCutchan et al. 2003).

This static approach was enhanced with an estimation of the turnover rate of the whole oyster tissues to take into account the incorporation rate of the OMS through a dynamic energy budget (DEB) model developed for this species (Pouvreau et al. 2006), as recently experienced with bioenergetics model on fishes (Harvey et al. 2002). In this model, the dynamics of growth and reproduction are described by three differential equations: the first one for the growth of the structural body volume, the second one for the dynamics of the energy reserves, and the third one for the storage and use of the energy allocated to development and reproduction. In such model, it is assumed that the assimilated energy is first stored in reserves, and these reserves are then used to fuel other metabolic processes (maintenance, growth, development and reproduction, see Pouvreau et al. 2006 for further details). Metabolic processes also depend on temperature. The turnover rate of energy and biomass of the whole oyster tissues can then be estimated by assessing the flux of energy entering the oyster through feeding and assimilation processes divided by the stock of energy in the animals. Both entering flux and stock of energy were estimated with an inverse method, using oyster weights and water temperatures collected in the two studied ecosystems (BDV and LIN).

Accordingly, the isotopic signature, $\delta^{13}C_t$ or $\delta^{15}N_t$ at time (t) depends on $\delta^{13}C_{t-1}$ and $\delta^{15}N_{t-1}$ at time (t_{-1}) and on a combination of isotopic signatures of OMS ($Mix\delta^{13}C$ or $Mix\delta^{15}N$) between t and t_{-1} and on its incorporation rate dependent on the turnover rate of the whole oyster tissues (Tr) during the same period:

$$\delta^{13}C_t = Mix\delta^{13}C + (Mix\delta^{13}C - \delta^{13}C_{t-1}) * \exp(-Tr * \Delta t) \text{ and}$$

$$\delta^{15}N_t = Mix\delta^{15}N + (Mix\delta^{15}N - \delta^{15}N_{t-1}) * \exp(-Tr * \Delta t)$$

Each combination of the potential sources was tested between t and t_{-1} with the sum of the different fraction being equal to 1.

$$Mix\delta^{13}C = f_{PhyOM} * \delta^{13}C_{PhyOM} + f_{TOM} * \delta^{13}C_{TOM} + f_{MPB} * \delta^{13}C_{MPB} + f_{ULV} * \delta^{13}C_{ULV} + f_{SOM} * \delta^{13}C_{SOM}$$

and

$$Mix\delta^{15}N = f_{PhyOM} * \delta^{15}N_{PhyOM} + f_{TOM} * \delta^{15}N_{TOM} + f_{MPB} * \delta^{15}N_{MPB} + f_{ULV} * \delta^{15}N_{ULV} + f_{SOM} * \delta^{15}N_{SOM}$$

with

$$f_{PhyOM} + f_{TOM} + f_{MPB} + f_{ULV} + f_{SOM} = 1$$

Interpolations of the OMS signature were calculated between t and t_{-1} and Tr was estimated daily. From all possible solutions to these equations, the 50 best solutions, corresponding to the 50 smallest isotopic distances (hereafter ID) were kept. ID is the difference between observed and calculated δ values as follow:

$$ID = \sqrt{\left(\delta^{13}C_{obs} - \delta^{13}C_{cal}\right)^2 + \left(\delta^{15}N_{obs} - \delta^{15}N_{cal}\right)^2}$$

Mean, minima and maxima of the contribution of the OMS were then calculated. The maximum ID (ID_{max}) corresponded to the needed tolerance in ID to produce the last 50th result. This modelling approach was done under Fortran 77 with Press et al. (2003) source codes.

Results

Isotopic signatures of organic matter sources (OMS)

Five OMS, i.e. PhyOM, TOM, MPB, ULV and SOM, were sampled every two months over two years in two contrasting environments, BDV and LIN. Carbon and nitrogen isotopic signatures of food sources did not co-vary ($P < 0.001$, Table 1) and there were no significant differences between BDV and LIN or among dates. For the interaction term Site x Date x Food Source, however, $\delta^{13}C$ and $\delta^{15}N$ values of OMS revealed some degree of temporal and spatial variability within each sampling area, BDV or LIN ($P < 0.01$). The isotopic signatures varied with the type of food source ($P < 0.001$, Table 1), suggesting that some of the food sources had distinct signatures from the others on the basis of dual $\delta^{13}C$ and $\delta^{15}N$ values during the survey (Figs. 2a, 2b).

Values of $\delta^{13}C$ were relatively stable over the survey (Figs. 2a, 2b). MPB (annual means = -18.2±2.4‰ in BDV and -18.0±1.4‰ in LIN) and ULV (annual means = -17.1±1.7‰ in BDV and -18.2±1.3‰ in LIN) were the most enriched OMS in ^{13}C, whereas TOM (annual means = -27.9±3.0‰ in BDV and -28.2±1.9‰ in LIN) was the most depleted, being very similar to an average C3 plant's isotopic composition. The carbon signatures of SOM and PhyOM were not different (Figs. 2, 3). Values of $\delta^{15}N$ of OMS were highly time-dependant both in LIN and BDV sampling areas (Fig. 2a, 2b). PhyOM was the most depleted OMS in ^{15}N, both for the BDV area (annual mean = 4.7±1.2‰) and for LIN area (annual mean = 4.9±1.2‰) while the nitrogen isotopic compositions of MPB, ULV, SOM, and TOM had higher values in both systems.

The $\delta^{13}C$ and $\delta^{15}N$ values of OMS required analysis together with other indicators of biomass and quality. The suspended material as PhyOM and TOM varied quantitatively and qualitatively both spatially and temporally. In terms of biomass, higher concentrations of Chl *a* (indicative of phytoplankton) were observed during spring and summer than at other times of year (Table 2). Sediment Chl *a* content (indicative of MPB biomass) also exhibited seasonal variations (Table 2), with a maximum in summer. The BDV area exhibited higher phytoplankton biomass (annual mean = 7.8±6.2 µg Chl *a*.L^{-1}) than the LIN area (annual mean = 2.7±1.3 µg Chl *a*.L^{-1}), although the Chl *a* content in sediment (indicative of MPB biomass) was higher in the LIN area (annual mean = 11.7±6.8 µg.g^{-1} dry sediment). Qualitatively, the POC/Chl *a* ratios (Table 2) of PhyOM and TOM were less than 100 during most of the year, indicating a dominance of live phytoplankton in the suspended organic matter pool, with the exception of the winter season during which detritus probably dominated. C/N ratios (Table 2) were often between 6 and 8, except for PhyOM and TOM during winter, which confirmed a significant presence of non-phytoplanktonic material in the suspended organic matter. Noticeably, ULV was the OMS that exhibited greatest variation in C/N atomic ratios among samplings, with summer maxima of 19.7±0.6 and 18.1±0.3 for BDV and LIN, respectively, indicating a weak physiological state.

The annual means of $\delta^{13}C$ and $\delta^{15}N$ for the SOM in the BDV area and the LIN area were -22.6±1.1 and 7.3±2.1‰, and -20.2±1.3 and 6.6±2.2‰, respectively (Fig. 3), which vary mostly between the values of the other four OMS (PhyOM, MPB, TOM, and ULV) over the year.

Isotopic signatures of *Crassostrea gigas*

The $\delta^{13}C$ and $\delta^{15}N$ of oysters did not co-vary ($P < 0.001$, Table 1). The isotopic signatures showed significant temporal differences ($P < 0.001$, Table 1) and spatial differences ($P < 0.001$, Table 1). The interaction between temporal and spatial factors was not significant, hence stressing that the seasonality of the isotopic signature of oysters followed parallel kinetics between sites for both elements. The $\delta^{13}C$ of oysters was more stable over time than $\delta^{15}N$ (Fig. 4a), particularly in BDV-N and BDV-S. The LIN oysters had more depleted ^{13}C signatures (-23.5±0.2 to -21.1±0.1‰) than did those in BDV. In general, the $\delta^{15}N$ of oysters increased during summer and autumn and then decreased (Fig. 4b). Based on annual means, the signatures of LIN oysters were more depleted in ^{15}N compared with those in BDV-N and BDV-S. The C/N ratios of oysters (Fig. 4c) varied with respect to seasons and sampling sites, increasing during spring and then decreasing to minima in winter.

Oysters reared in the three study sites had different growth and reproduction trends (Fig. 5); oysters gained weight in BDV-S until November-04, lost weight before March-05, and resumed growth between March and May-05. Oysters in BDV-N showed a smaller weight increase during the study. Comparatively, oysters in LIN showed a similar pattern to those in BDV-S, but resumed growth earlier and had a higher final weight. Notably, oysters had some differences in their turnover rates, with higher values during spring for BDV and during summer for LIN. The consequence may be that the reproduction effort (shown by weight loss in early summer) was greater in BDV-N and BDV-S than in LIN. Finally, turnover rates were low during winter and were equal to zero in certain cases (Fig. 5).

Contributions of OMS to oysters' diets

Among different possible scenarios, a four source scenario with PhyOM, TOM, MPB, and ULV (SOM excluded) provided the most relevant results because of the intermediate position of SOM isotopic signatures as compared with the others. In general, the contributions of OMS to oysters' feeding showed large spatial and temporal differences whichever scenario for fractionation was used (Tables 3, 4). In almost all cases, PhyOM was a major contributor to the oysters' diets, with a significant gradient from the estuarine location to the marine location on a yearly basis. By contrast, the contributions of TOM, MPB, and ULV to OMS increased while PhyOM decreased, resulting in an inverse relationship.

The modelling exercise provided exact solutions in most cases in BDV-S and in BDV-N since ID_{max} approached zero, in contrast to LIN when using scenario Fract-A (Table 3); however, this was improved by use of scenario Fract-B (Table 4). These discrepancies were due to the PhyOM $\delta^{15}N$ values, which were slightly higher than the fractionation-corrected values of oysters. In those cases, this means that PhyOM was probably the only food source used, with a possibility of some TOM contribution particularly in LIN and BDV-N in 2005.

When using the scenario Fract-A (Table 3), there were clear shifts in the diet through the seasons, with a larger contribution of PhyOM in spring; however, other OMS,

TOM, and MPB, contributed significantly in other seasons, especially in autumn in BDV locations. The contribution of ULV seemed insubstantial except in BDV-S in autumn. Solutions from scenario Fract-B (Table 4) gave similar shifts in diets, but with larger contributions of MPB and ULV. In general, the estimated contributions of microphytobenthos and ULV showed high intervals between their minima and maxima. This may be due to slight differences in $\delta^{13}C$ and $\delta^{15}N$ of these two OMS leading to an inability to state for the contribution of one or the other of these food sources in a statistical sense.

Discussion

New insights in ecological studies approaches

A prerequisite for understanding flows and trophic pathways in estuarine and marine ecosystems is the identification of food sources consumed by benthic consumers. Classically, in isotopic approaches to trophic studies, potential food sources are sampled at the same time as the consumers (Kang et al. 1999; Doi et al. 2005). This approach disregards both the incorporation rate of carbon and nitrogen into consumers' tissues and variation of the OMS $\delta^{13}C$ and $\delta^{15}N$ values over time. Our approach provides a more in-depth understanding of seasonal variability, as was recently shown for the temporal dynamics of stable isotopic signatures of fishes (Harvey et al. 2002; Gaye-Siessegger et al. 2004; Herzka 2005). The only two studies done on tissue turnover rates of *C. gigas* show that our model estimations are within the ranges of published turnover rates (ca 60 days during spring and autumn; Paulet et al. 2006; Dubois et al. 2007b). This approach is biologically more relevant because the tissue turnover rate of the consumers changes with season, reaching very low values during winter in our study. This may make it impossible to estimate the contributions of the various food sources during winter periods. The lack of modelling tools hampered interpretations of seasonal variations of $\delta^{13}C$ and $\delta^{15}N$ values of consumers and compelled scientists to roughly estimate incorporation rates of C and N (Page and Lastra 2003).

There were instances during this study when it was only possible to calculate contributions with a large tolerance especially for LIN or for the two last sampling periods in BDV-N, particularly for $\delta^{15}N$ (see ID_{max} Tables 3, 4). Several assumptions can be made. As debated below, fractionation is still a matter of discussion. Another possibility could be that a food source with a low $\delta^{15}N$ value could have been missed, which seems unlikely considering that microalgae often have the lowest $\delta^{15}N$ values in coastal systems (Fry 2006). More likely, because PhyOM is an assemblage of several species of microalgae and oysters can select food particles through pre-ingestive sorting, low $\delta^{15}N$ microalgae in the community could have been preferentially selected, for example, small flagellates over diatoms (Bougrier et al. 1997). While some previous studies have shown the predominance of diatoms in BDV (Jouenne et al. 2007), flagellates occurred significantly in LIN area (B. Véron pers comm).

The generalisation of modelling tools will greatly improve estimation of the dietary plasticity of consumers in general; however, whether fractionation estimates also are important factors, they are still debated among ecologists using stable isotopes to study food webs. In this study, we used fractionation values (Fract-A) estimated by experiments on oysters fed with the diatom *S. costatum* (Dubois et al. 2007b) in comparison with other fractionation values (Fract-B) for aquatic primary consumers (McCutchan et al. 2003). The use of these two extreme scenarios allowed us to perform a sensitivity analysis of our

results validating the main trends; notably, whichever scenario was used, there was a gradient in the utilisation of PhyOM between the two areas and the three sites, as well as plasticity in the oysters' diets through the seasons. Nevertheless, it is likely that each OMS has a different fractionation, reflecting the nature and quality of the assimilated food, and also the feeding level itself (Gaye-Siessegger et al. 2004). This complicates the problem, because fractionation values would potentially change over time. Consumer signatures could also change during starvation, as was seen in BDV-S and LIN during the winter, when these changes could not be attributed to incorporation of food sources. Finally, recent studies (e.g. Post et al. 2007) pointed out the importance of correcting the value of $\delta^{13}C$ depending on the percentage of lipid in tissue since lipids are depleted in ^{13}C. They proposed a correction using the C/N ratio. However, this correction is not applicable to oysters which store most of their reserves in the form of glycogen instead of lipids (Costil et al. 2005) and mainly during spring (Fig. 3c). Furthermore, no fractionation values take into account this previous lipid correction making it difficult to interpret at the moment. This calls again for further physiological and modelling study to describe mechanistically and dynamically fractionation instead of using empirical constant values as done in ecological studies up to now.

Time-dependent variations of OMS signatures are site-specific in coastal environments

Most of the OMS available for the nutrition of bivalves in such systems are primary producers or detritus originating from primary producers (Riera and Richard 1996). It is well known that $\delta^{13}C$ and $\delta^{15}N$ of primary producers depend on the seasonal availabilities of mineral nutrients and their origin, and the metabolic fractionation, which is species-specific (Kang et al. 1999; Savoye et al. 2003; Vizzini and Mazzola 2003). In our study, these characteristics allowed for good discrimination of the five food sources (PhyOM, TOM, SOM, MPB, and ULV) by use of a dual C and N isotope approach, although differences between MPB and ULV were not significant on a yearly basis. Overall, the isotopic ratios of OMS obtained in this study were in the range of values from other coastal ecosystems (Maksymowska et al. 2000 and references therein). Noticeably, carbon isotopic compositions of OMS were more discriminating as well as less time-dependent than nitrogen isotopic compositions. The small differences between $\delta^{15}N$ values of OMS are easily altered by the high variability in mineral nitrogen availability due to complex biogeochemical processes.

As supported by the statistical analysis, the differences in OMS signatures were mostly due their time variability which was system-specific. This underlined that it is important to survey OMS signatures over seasons for each studied ecosystem since it is difficult to explain and to predict the temporal and spatial variability of stable isotopic compositions of the OMS. For instance, $\delta^{13}C$ values of PhyOM were more depleted in LIN than BDV, potentially reflecting the different abiotic conditions for phytoplankton production supported by differences in Chl *a* biomass (Table 2). In the same way, MPB was more depleted in $\delta^{13}C$ or $\delta^{15}N$ in the two systems than in muddy sediment (Riera and Richard 1996). Apart from the question of the biomass produced, coarse sediment certainly permits greater circulation and availability of dissolved nutrients than in fine sediments, leading to signatures closer to those of phytoplankton. We also observed similar gradients in our two systems, with MPB in the BDV area (estuarine influence and fine sediment) being more enriched in ^{15}N than in the LIN area (marine influence and coarse sediment), although, conversely, the biomass of MPB was higher in LIN than in BDV. Finally, a low $\delta^{15}N$ generally characterizes TOM while the marine component has relatively higher values (Maksymowska et al. 2000). Higher values of $\delta^{15}N$ in our systems could have been related

to the influence of sewage inputs (Pruell et al. 2006) or inputs from a human- impacted drainage basin such as in the Schelde estuary (Middelburg and Nieuwenhuize 1998).

Trophic plasticity of the opportunistic suspension-feeder *Crassostrea gigas*

The trophic ecology of intertidal bivalves such as cockles, mussels, or oysters in European Atlantic estuaries has been investigated previously by use of natural stable isotopes (Riera and Richard 1997; Kang et al. 1999; Page and Lastra 2003; Piola et al. 2006); they all suggested that both PhyOM and MPB constituted most of the bivalves' diets in intertidal areas, with variation in their relative importance depending on the location. In our study, the spatial and seasonal variations of oysters' $\delta^{13}C$ and $\delta^{15}N$ signatures suggested that OMS contributed to their diets in different ways among the three locations, but that PhyOM was a major contributor in all trophic environments and with both fractionation scenarios we used. We found that, on a yearly basis, the PhyOM constituted as much as 50 to 80% of oysters' diets in the most marine environment (LIN) and 21 to 47% (BDV-S) and 33 to 60% (BDV-N) in estuarine environments (Tables 3, 4). The contribution of PhyOM was greater with increased exposure to the open sea and decreased with freshwater input.

Nevertheless, the PhyOM contribution varied according to season and location. Other food sources (TOM, MPB, and ULV) can contribute alternately during non-bloom periods. The contribution of the different OMS depends on their relative abundance in the ecosystems and also on the oysters' ability to select their food by preingestive sorting (Barillé et al. 1997; Ward et al. 1998). Actually, suspended organic matter in seawater often contains mainly phytoplankton, which is preferentially ingested by oysters (Dupuy et al. 2000). The contribution of PhyOM as the main OMS during spring and summer has been reported in several studies and relies on planktonic bloom events confirmed by high Chl *a* concentrations in the water (Table 2). Phytoplankton's predominant contribution to oysters' feeding in the LIN area probably was determined by the oceanic influence on this ecosystem in contrast to the estuarine influence in the BDV area. During the months where phytoplankton biomass decreased (November to March, Table 2, estimated from POC/Chl *a* content), the oysters used other available food sources to satisfy their energetic requirements (Tables 3, 4).

Oysters are opportunistic suspension feeders and use whatever materials comprise detritus in their habitat (Hsieh et al. 2000; Dubois et al. 2007a) even if this contribution usually remains low (Deegan and Garritt 1997). The abundance and availability of such alternative food sources depend on the nature of each ecosystem, particularly on its hydrodynamic features and freshwater inputs (Riera and Richard 1997). The contribution of TOM to oysters' diets was particularly obvious in the BDV-S location (Tables 3, 4) due to the influence of terrestrial contributions from Isigny Channel, supporting the incorporation of terrestrial detritus into coastal food webs as suggested by Riera and Richard (1997). Actually, the two BDV locations were impacted by freshwater twice daily due to partial saline stratification during flow tide; the BDV-S being more impacted than the BDV-N (Costil et al. 2005). The contribution of TOM also was especially strong during periods of high freshwater inputs (end of autumn, winter, and early spring). In general, it is assumed that detritus (TOM and macroalgal detritus) could be part of the diet of bivalves in the presence of a high bacterial biomass that guarantees the degradation of refractory materials and acts as an intermediary organic matter source (Crosby and Newell 1990; Langdon and Newell 1990).

SOM as a trophic reservoir and as an ecological indicator

In our study, the temporal variations of SOM isotopic compositions demonstrated the differential contributions of PhyOM, TOM, MPB, and ULV as primary sources to the sedimented organic matter pool during different seasons (Fig. 3). Overall, carbon isotopic compositions of sediments are a function of the ecosystems' primary productivity (Gu et al. 1996). Isotopic values of SOM from the two sites noticeably differed, supporting the idea that the OMS contributions to this reservoir in the two ecosystems also differed. It could be another piece of evidence (together with the results for oysters) that the two systems function differently in a trophic sense. For instance, depletion in ^{13}C was observed in the BDV area during the winter (January-05 = -23.9‰), due to the influence of TOM during the rainy season (terrestrial influence). Similar behaviours were shown by the nitrogen abundances, with greater values during spring and summer, both for the BDV area (July-04 = 11.8‰) and for the LIN area (March-05 = 9.9‰), possibly due to proliferation of benthic microalgae and accumulation of macroalgal detritus on the superficial sediments. In aquatic environments, the SOM acts as an important source of primary organic matter for intertidal macroconsumers because most of the detritus is in the superficial layers of sediment (up to 5 cm; Josefson et al. 2002); this is especially true for suspension feeders once this organic matter is resuspended by numerous environmental factors (tides, swale, etc) (Herman et al. 2000; Kang et al. 2006; Usui et al. 2006). Our results suggested that in the BDV area, the resuspension processes of sediment have a major influence on food availability and diversity (e.g. MPB, macroalgal detritus), influenced by the interaction between seasons and tidal cycles. We assume here that sediment acts as a reservoir and as a reactor governing the availability of detrital organic matter, such as TOM and ULV (Dubois et al. 2007a), but also living material such as MPB . For instance, the contribution of macroalgal detritus (ULV) was surprisingly not higher in BDV-N, although this area is characterized by hard substrata and pebbles largely colonized by macroalgae. A recent investigation of trophic relationships in hard-bottom communities revealed that the main potential food source was macroalgal detritus, but that it was mostly exported and not consumed locally (Behringer and Butler 2006). Therefore, we assume that macroalgal detritus was exported and included in soft sediment in the southern bay (BDV-S) where it contributed to OMS in the autumn. However, it remains difficult to take into account signatures of SOM as a food source in our mixing models because each of the four other food sources may possibly contribute considerably to its signature. This lead to diffuse solutions in the mixing model (Phillips and Gregg 2003). However, it is not possible to estimate how the OMS signatures change during degradation in the sediment or how much the biomass of bacteria, meiofauna or even macrofauna contribute to $\delta^{13}C$ or $\delta^{15}N$ of SOM. Referring to the works of Currin et al. (1995), labile material kept their signatures during degradation while refractory material did not. In our study, the signature of *Ulva* material or dead benthic and planktonic microalgae could not have varied much during their degradation in contrast to TOM material which probably could have varied. On the other hand, the biomass of bacteria and meiofauna is generally supposed to be 10% of the total organic matter in the sediment (Herman et al. 1999). For these reasons (low isotopic transformation and low contribution of the sediment live biomass), it could be reasonably assumed that SOM did not differ considerably from the mixing of the primary sources. Finally, macrofauna (deposit and suspension feeders) and especially cultured oysters could also interact strongly with sediment via the production of biodeposit (i.e. pseudo-feces and feces). These biodeposits are potentially resuspended and re-consumed by the filter feeders themselves (Orvain et al. 2003). In addition to the biodeposits, mucus is produced by the oyster which is known to favour the growth of benthic algae (Cognie and Barillé 1999). Nothing is known about the

possible re-consumption of mucus although this labile and soluble substance could not be stable in the environment for an extended period. Also, the $\delta^{13}C$ or $\delta^{15}N$ of the organic matter of the feces could potentially differ from the original material due to differential digestion and absorption (as a kind of fractionation) occurring between different types of organic matter while in the digestive tract. Similar results were reported for terrestrial mammals (Sponheimer et al. 2003), fish (Franco-Nava et al. 2004) or crustaceans (Gorokhova and Hansson 1999). The $\delta^{13}C$ or $\delta^{15}N$ of the organic matter of the pseudo-feces could also potentially be different from PhyOM since oysters are capable of sorting before ingestion (Barillé et al. 1997). To conclude, filter-feeders such as oysters could interact with and change $\delta^{13}C$ or $\delta^{15}N$ of SOM via biodeposits which could then be resuspended and re-consumed by themselves.

In addition, sediment type undoubtledly plays an important role in the availability and biomass of MPB. Normally, muddy sediments (as in the BDV area) exhibit greater MPB biomass than sandy sediments (Kang et al. 2006), which is contrary to our results for Chl *a* content (Table 2). Similar trends were reported for three European coastal areas characterised by different tidal regimes, and were attributed to differences in the thickness of the photic zone, which is greater in sandy sediments than in muddy ones (Miles and Sundbäck 2000). However, the contribution of MPB was low in the diet of LIN oysters (Tables 3, 4) possibly because of the predominance of epipsammic communities that can not be easily detached from sand grains (de Jonge and Colijn 1994).

Conclusion

This study confirms that the oyster *C. gigas* is an opportunistic filter-feeder showing a large trophic plasticity depending on ecosystem functioning. The dynamic survey of potential OMS in two ecosystems showed that the time variability in $\delta^{13}C$ or $\delta^{15}N$ was site-specific. The physical, chemical, and biological phenomena specific to each ecosystem influenced the variability and availability of OMS, which could contribute differentially to the trophic chain. This was taken into account together with a variable turnover rate of oyster tissues through an original modelling approach combining mixing and bioenergetic models, in order to calculate the contribution of potential OMS to the oyster's diet over time. Whatever the site studied, phytoplankton is a major contributor to oyster's diet, but other OMS as microphytobenthos, macroalgae detritus or terrestrial organic matter, contributed significantly outside the period of phytoplankton blooms (spring) particularly in the more estuarine site. We argued that organic matter from the sediment (SOM) could not be included as an independent OMS since it was already a "reservoir" of the potential OMS for oysters. Hence, $\delta^{13}C$ or $\delta^{15}N$ of SOM mirrored the trophic functioning of the ecosystem. Although OMS contributions were very sensitive to the fractionation value used in the model, this did not change the main conclusions about time variability and site-specific OMS contributions to oyster's diet.

Knowledge of the contributions of OMS to benthic macroinvertebrate feeding, particularly to diets of bivalves in culture, would improve understanding of the trophic relationships among the biological compartments of marine food webs as well as helping to estimate the trophic capacity of marine ecosystems. Ecological processes of these environments are influenced by the biomass and species of the cultivated bivalves, which affect the biodeposition and resuspension processes, as well as the cycles of important elements like nitrogen and carbon. Additionally, other biotic interactions occur because feeding by cultivated oysters in an ecosystem certainly is in relation to the abundance of other suspension-feeders, which may or may not compete for the same food (Dubois et al.

2007a). In this perspective, the development of dynamic models focused on the ecosystems' typology, and where physical variables, energy flows, and biological compartments are considered, would ideally take into account the variety and the availability of organic matter sources for bivalves as well as their trophic niche.

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Table 1 Summary of multivariate MANOVA results for $\delta^{13}C$ and $\delta^{15}N$ for organic matter sources and for oysters from two bays in northern France during 2004-5 (*df*, degrees of freedom; MS, mean squares; *F*, Fischer's *F*; *P*, probability).

Sample	Source of variation	Statistic of Wilks' lambda	*F*	*df*	*Denom. df*	*P*
Organic matter sources	Element (C+N)	0.090	2114.33	1	209	<0.001
	Site	0.992	1.59	1	209	0.208
	Type	0.739	18.46	4	209	<0.001
	Date	0.953	1.71	6	209	0.120
	Site*Type	0.979	1.12	4	209	0.350
	Site*Date	0.996	0.14	6	209	0.991
	Type*Date	0.888	1.09	24	209	0.355
	Site*Type*Date	0.825	1.84	24	209	<0.010
Oysters	Element (C+N)	0.002	43890.51	1	104	<0.001
	Site	0.426	70.10	2	104	<0.001
	Date	0.705	7.26	6	104	<0.001
	Site*Date	0.854	1.48	12	104	0.143

Table 2 Temporal variations of chlorophyll *a* (Chl *a*), POC/Chl *a* ratios and C/N ratios for studied sites in Normandy (BDV and LIN) during 2004-2005. PhyOM: marine organic matter (µgChl *a*.L^{-1}), TOM: terrestrial organic matter (µgChl *a*.L^{-1}) and SOM: sedimented organic matter (MPB biomass, µgChl *a*.g^{-1} dry sediment). mean ± SD (n=2).

Variable	Site	Source	May-04	Jul-04	Sep-04	Nov-04	Jan-05	Mar-05	May-05
Chl *a*									
	BDV								
		PhyOM	13.7±0.2	14.1±0.2	5.2±0.1	1.1±0.0	1.0±0.0	0.8±0.0	11.8±0.1
		TOM	80.7±2.3	71.9±11.4	68.4±9.9	5.9±0.0	4.7±0.0	5.4±0.1	25.8±0.3
		SOM	5.4±1.4	8.5±3.1	16.3±1.5	5.1±0.7	3.8±1.5	3.5±1.4	11.8±2.3
	LIN								
		PhyOM	3.1±0.0	3.2±0.2	3.9±0.2	1.3±0.0	0.8±0.0	4.3±0.2	2.7±0.1
		TOM	6.2±0.1	150.8±6.7	32.0±0.3	2.2±0.1	1.9±0.2	2.9±0.0	5.1±0.2
		SOM	2.4±0.0	21.8±2.8	19.3±0.4	12.2±0.2	8.2±0.4	8.2±0.3	10.1±0.2
POC/Chl *a*									
	BDV								
		PhyOM	16.6±0.4	20.0±0.2	12.5±0.0	64.9±0.1	78.5±0.2	91.8±0.1	30.2±0.0
		TOM	7.3±0.1	9.5±0.3	18.9±0.3	71.4±0.1	100.0±0.4	51.2±0.1	15.8±0.3
	LIN								
		PhyOM	49.7±0.6	72.0±0.1	20.7±0.1	41.2±0.0	121.9±0.1	18.8±0.1	79.8±0.1
		TOM	72.1±0.6	5.3±0.2	13.4±0.2	85.2±0.3	264.5±0.3	105.2±0.2	57.8±0.2
C/N									
	BDV								
		PhyOM	6.8±0.1	6.8±0.1	7.5±1.3	7.0±0.0	7.3±0.1	7.6±0.2	6.7±0.0
		TOM	7.3±0.2	7.0±0.1	9.7±0.1	9.1±0.4	11.5±0.7	10.0±0.3	7.4±0.0
		ULV	11.8±0.5	19.7±0.6	10.7±0.9	8.5±0.1	9.3±0.1	8.7±0.0	9.8±0.1
		MPB	7.1±0.6	7.5±0.0	7.5±0.0	8.2±0.1	8.3±0.3	8.7±0.0	9.2±0.0
	LIN								
		PhyOM	6.1±0.1	7.1±0.1	7.1±0.3	6.1±0.1	14.2±0.3	6.9±0.3	6.3±0.0
		TOM	9.5±0.0	6.6±0.2	8.9±0.1	9.2±0.6	10.7±0.1	10.1±0.1	9.3±0.2
		ULV	8.1±0.1	18.1±0.3	14.0±0.2	10.1±0.0	9.2±0.0	9.1±0.1	10.8±0.1
		MPB	7.8±0.3	7.4±0.0	4.4±0.0	7.8±0.0	8.4±0.1	7.0±0.0	7.7±0.2

Table 3 Contributions of OMS (%) to the diets of oysters from two bays (3 sampling sites, BDV-S, BDV-N, and LIN) in northern France during 2004-5, Data as mean (min-max), calculated from a mixing model by Phillips and Gregg (2003) coupled to a DEB model (Pouvreau et al. 2006) considering a trophic fractionation (Fract-A) of 1.85‰ for $\delta^{13}C$ and 3.79‰ for $\delta^{15}N$ (Dubois et al. *in press*), – : no turnover rate. ID_{max} is the needed tolerance for isotopic distance (ID) to calculate the 50^{th} best results.

Location/Source	May-Jul 2004	Jul-Sep 2004	Sep-Nov 2004	Nov 2004-Jan 2005	Jan-Mar 2005	Mar-May 2005	Year
BDV-S							
Marine organic matter (PhyOM)	76.3 (73.0-78.0)	54.1 (39.0-70.0)	7.2 (2.0-14.0)	–	–	40.2 (39.0-43.0)	47.2
Terrestrial organic matter (TOM)	8.6 (8.0-10.0)	13.3 (7.0-19.0)	38.4 (36.0-40.0)	–	–	56.7 (54.0-60.0)	27.6
Microphytobenthos (MPB)	7.5 (0.0-17.0)	21.4 (0.0-42.0)	4.4 (0.0-10.0)	–	–	2.1 (0.0- 7.0)	9.2
Ulva sp.(ULV)	7.6 (0.0-15.0)	11.2 (0.0-23.0)	50.0 (48.0-52.0)	–	–	1.0 (0.0- 4.0)	15.9
ID_{max}	0.0	0.0	0.0			0.3	
BDV-N							
Marine organic matter (PhyOM)	78.1 (75.0-80.0)	60.4 (46.0-75.0)	61.6 (34.0-88.0)	34.8 (23.0-45.0)	60.2 (44.0-77.0)	39.8 (38.0-43.0)	59.9
Terrestrial organic matter (TOM)	10.7 (10.0-12.0)	7.5 (2.0-13.0)	8.2 (0.0-17.0)	6.7 (1.0-12.0)	39.5 (23.0-56.0)	57.6 (55.0-61.0)	21.8
Microphytobenthos (MPB)	6.0 (0.0-13.0)	20.8 (0.0-41.0)	25.5 (20.0-49.0)	36.1 (1.0-76.0)	0.3 (0.0- 1.0)	1.9 (0.0- 6.0)	12.3
Ulva sp. (ULV)	5.3 (0.0-12.0)	11.3 (0.0-23.0)	4.7 (0.0-10.0)	22.3 (0.0-42.0)	0.0 (0.0- 0.0)	0.8 (0.0- 3.0)	6.1
ID_{max}	0.0	0.0	0.0	0.0	0.8	0.7	
LIN							
Marine organic matter (PhyOM)	76.6 (73.0-83.0)	82.0 (81.0-84.0)	92.8 (85.0-100.0)	–	65.1 (57.0-75.0)	47.7 (44.0-50.0)	80.4
Terrestrial organic matter (TOM)	21.9 (17.0-25.0)	0.0 (0.0- 0.0)	6.3 (0.0- 15.0)	–	34.2 (25.0-54.0)	0.0 (0.0- 0.0)	11.1
Microphytobenthos (MPB)	0.7 (0.0- 3.0)	11.1 (1.0-19.0)	0.6 (0.0- 2.0)	–	0.6 (0.0- 2.0)	48.7 (41.0-56.0)	8.2
Ulva sp. (ULV)	0.8 (0.0- 3.0)	6.8 (0.0-18.0)	0.3 (0.0- 1.0)	–	0.2 (0.0- 1.0)	3.6 (0.0- 9.0)	7.8
ID_{max}	0.4	0.5	1.0		2.2	1.5	

Table 4 Contributions of OMS (%) to the diets of oysters from two bays (3 sampling sites, BDV-S, BDV-N, and LIN) in northern France during 2004-5. Data as mean (min-max), calculated from mixing model by Phillips and Gregg (2003) coupled to a DEB model (Pouvreau et al. 2006) considering a trophic fractionation (Fract-B) of 0.4‰ for $\delta^{13}C$ and 2.2‰ for $\delta^{15}N$ (McCutchan et al. 2003), – : no turnover rate. ID_{max} is the needed tolerance for isotopic distance (ID) to calculate the 50^{th} best results.

Location/Source	May-Jul 2004	Jul-Sep 2004	Sep-Nov 2004	Nov 2004-Jan-2005	Jan-Mar 2005	Mar-May 2005	Year
BDV-S							
Marine organic matter (PhyOM)	36.3 (33.0-40.0)	18.8 (3.0-35.0)	3.1 (2.0- 6.0)	–	–	22.0 (17.0-27.0)	21.2
Terrestrial organic matter (TOM)	10.5 (9.0-12.0)	17.0 (11.0-23.0)	28.2 (26.0-32.0)	–	–	24.5 (14.0-34.0)	19.3
Microphytobenthos (MPB)	25.9 (0.0-52.0)	23.3 (0.0-46.0)	0.8 (0.0- 3.0)	–	–	34.1 (2.0-69.0)	21.9
Ulva sp. (ULV)	27.3 (3.0-51.0)	40.9 (28.0-54.0)	67.9 (66.0-72.0)	–	–	19.4 (0.0-37.0)	37.6
ID_{max}	0.0	0.0	0.4			0.0	
BDV-N							
Marine organic matter (PhyOM)	38.0 (34.0-42.0)	21.8 (2.0-41.0)	29.9 (2.0-57.0)	27.6 (24.0-31.0)	60.0 (45.0-75.0)	33.7 (31.0-36.0)	33.7
Terrestrial organic matter (TOM)	12.6 (11.0-14.0)	28.1 (0.0-57.0)	10.5 (2.0-19.0)	0.4 (0.0- 2.0)	39.6 (25.0-55.0)	40.5 (35.0-45.0)	19.7
Microphytobenthos (MPB)	26.1 (0.0-52.0)	37.8 (21.0-54.0)	24.6 (0.0-50.0)	5.1 (0.0-14.0)	0.4 (0.0- 1.0)	16.9 (0.0-34.0)	21.0
Ulva sp. (ULV)	23.2 (0.0-47.0)	2.8 (2.0-12.0)	35.1 (29.0-41.0)	66.9 (62.0-70.0)	0.0 (0.0- 0.0)	9.0 (0.0-19.0)	25.5
ID_{max}	0.0	0.0	0.0	0.1	0.5	0.0	
LIN							
Marine organic matter (PhyOM)	47.6 (44.0-51.0)	53.0 (51.0-55.0)	89.2 (86.0-92.0)	–	94.9 (88.0-100.0)	5.0 (2.0- 7.0)	50.7
Terrestrial organic matter (TOM)	20.6 (17.0-25.0)	0.0 (0.0- 0.0)	0.3 (0.0- 1.0)	–	3.9 (0.0- 12.0)	0.0 (0.0- 0.0)	10.9
Microphytobenthos (MPB)	14.7 (0.0-31.0)	32.0 (15.0-46.0)	8.2 (3.0-13.0)	–	0.8 (0.0- 3.0)	90.3 (81.0-98.0)	27.2
Ulva sp. (ULV)	17.1 (0.0-33.0)	15.0 (0.0-34.0)	2.3 (0.0- 7.0)	–	0.4 (0.0- 2.0)	4.7 (0.0-12.0)	21.9
ID_{max}	0.0	0.1	0.2		1.7	1.0	

Figure Captions

Fig. 1 Sampling locations (BDV-S, BDV-N and LIN) in Normandy (France): the Baie des Veys (a: BDV) and the Lingreville area (b: LIN). In the BDV area, two locations were established (BDV-S and BDV-N).

Fig. 2 Temporal variations of $\delta^{13}C$ (a and b) and $\delta^{15}N$ (c and d) for organic matter sources of Baie des Veys (BDV: a and c) and Lingreville area (LIN: b and d) during 2004-2005. PhyOM: marine organic matter, MPB: microphytobenthos, TOM: terrestrial organic matter, ULV: *Ulva* sp. The vertical bars indicate ± SD of the mean for n=2.

Fig. 3 Temporal variations of $\delta^{13}C$ (a) and $\delta^{15}N$ (b) for sedimented organic matter (SOM) of two locations (BVD and LIN) in Normandy (France) during 2004-2005. □ BDV site and ● LIN site. The vertical bars indicate ± SD of the mean for n=2.

Fig. 4 *Crassostrea gigas*. Characteristics of oyster in three locations (BDV-S, BDV-N and LIN) in Normandy (France) during 2004-2005. a: $\delta^{13}C$ variations, b: $\delta^{15}N$ variations and c: C/N atomic ratios. The vertical bars indicate ± SD of the mean for n=3. The isotopic signatures of oysters are shown without correction due to fractionation.

Fig. 5 *Crassostrea gigas*. Turnover rate and oysters' growth curves (simulation and observation) of three locations (BDV-S, BDV-N and LIN) in Normandy (France) during 2004-2005. a: BDV-S, b: BDV-N and c: LIN. ○ Observations, ▬ Simulation, and --- Turnover rate. The vertical bars indicate ± SD of the mean for n=30.

Fig 1

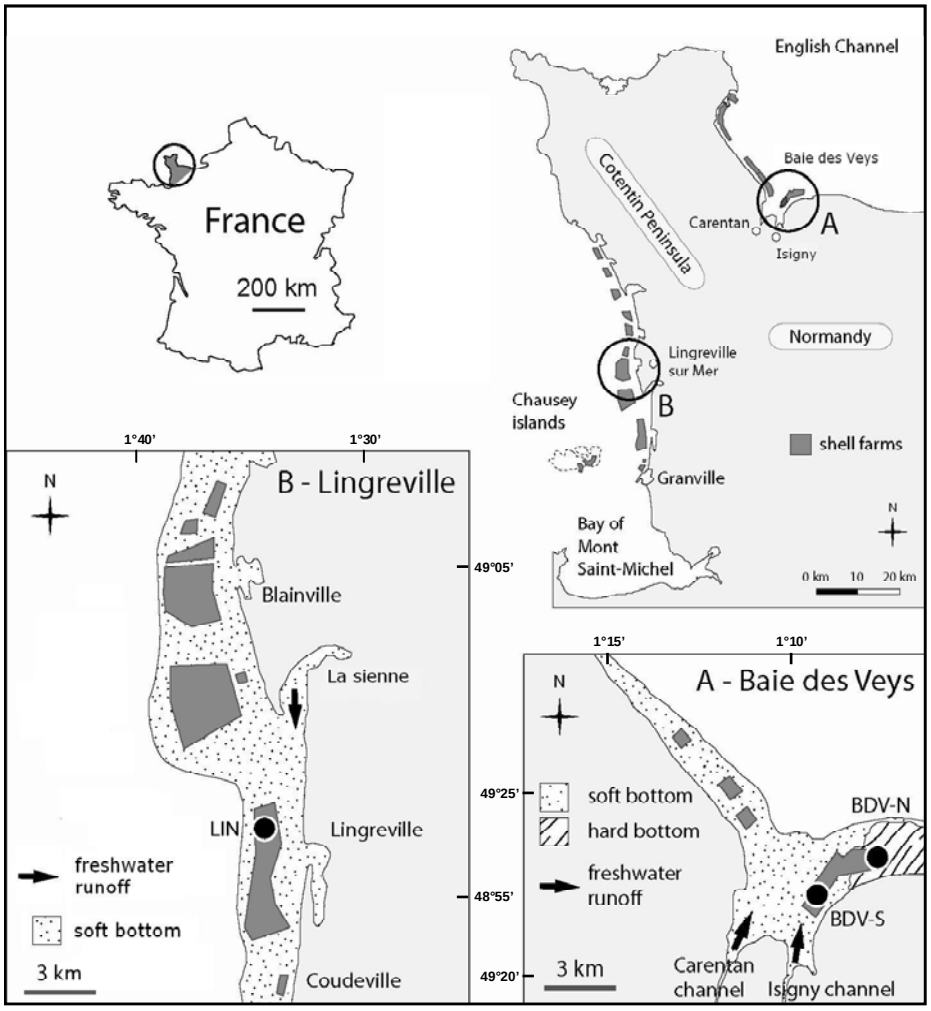

English Channel
France
200 km
Baie des Veys
Cotentin Peninsula
Carentan
A
Isigny
Normandy
Lingreville sur Mer
B
Chausey islands
shell farms
Granville
Bay of Mont Saint-Michel
0 km 10 20 km
N
1°40'
1°30'
B - Lingreville
49°05'
Blainville
La sienne
LIN
Lingreville
freshwater runoff
soft bottom
3 km
Coudeville
1°15'
1°10'
A - Baie des Veys
49°25'
soft bottom
hard bottom
freshwater runoff
BDV-N
BDV-S
48°55'
49°20'
3 km
Carentan channel
Isigny channel

Fig 2

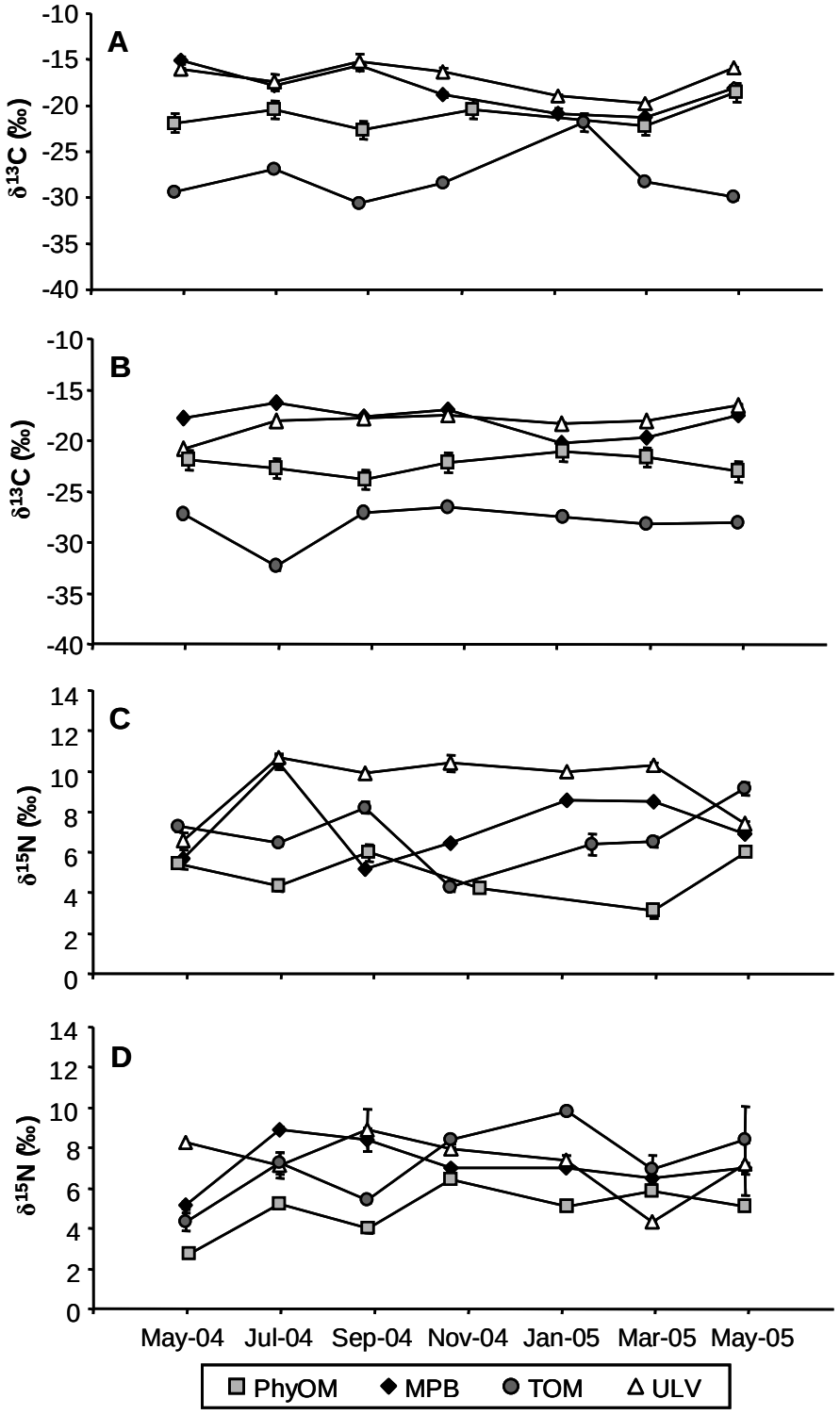

A
B
C
D
δ13C (‰)
δ15N (‰)
-10
-15
-20
-25
-30
-35
-40
14
12
10
8
6
4
2
0
May-04
Jul-04
Sep-04
Nov-04
Jan-05
Mar-05
May-05
PhyOM
MPB
TOM
ULV

Fig 3

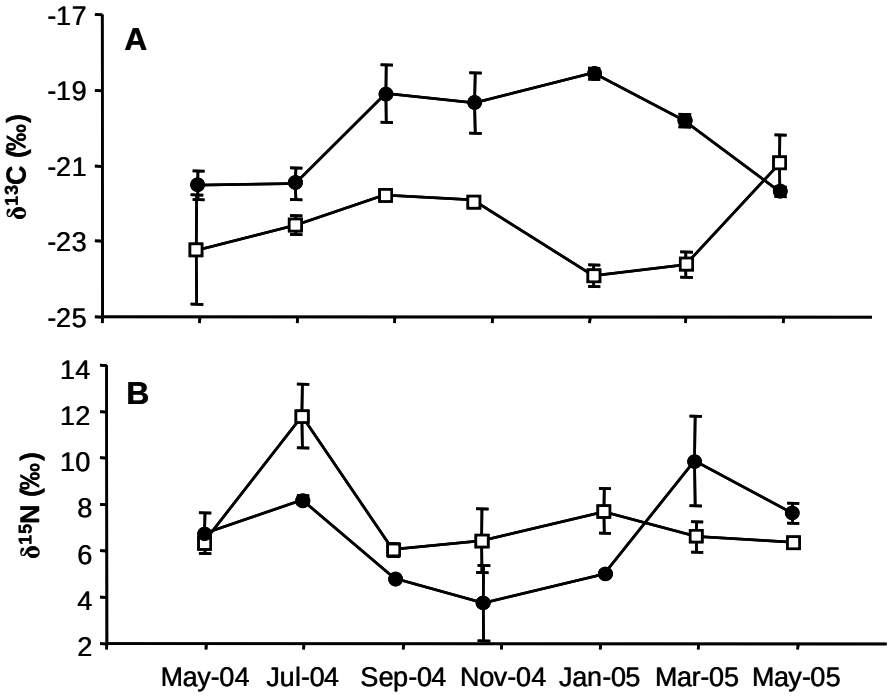

A
B
δ13C (‰)
δ15N (‰)
-17
-19
-21
-23
-25
14
12
10
8
6
4
2
May-04
Jul-04
Sep-04
Nov-04
Jan-05
Mar-05
May-05

Fig 4

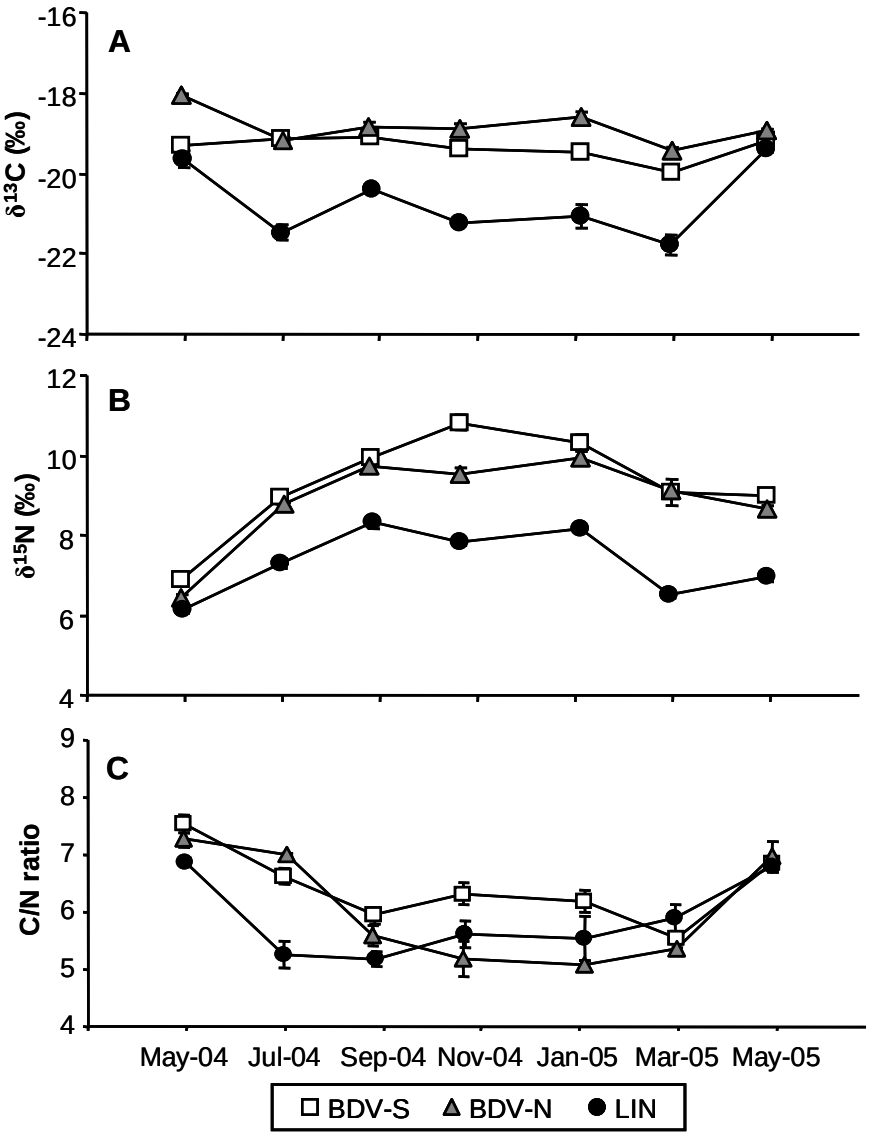
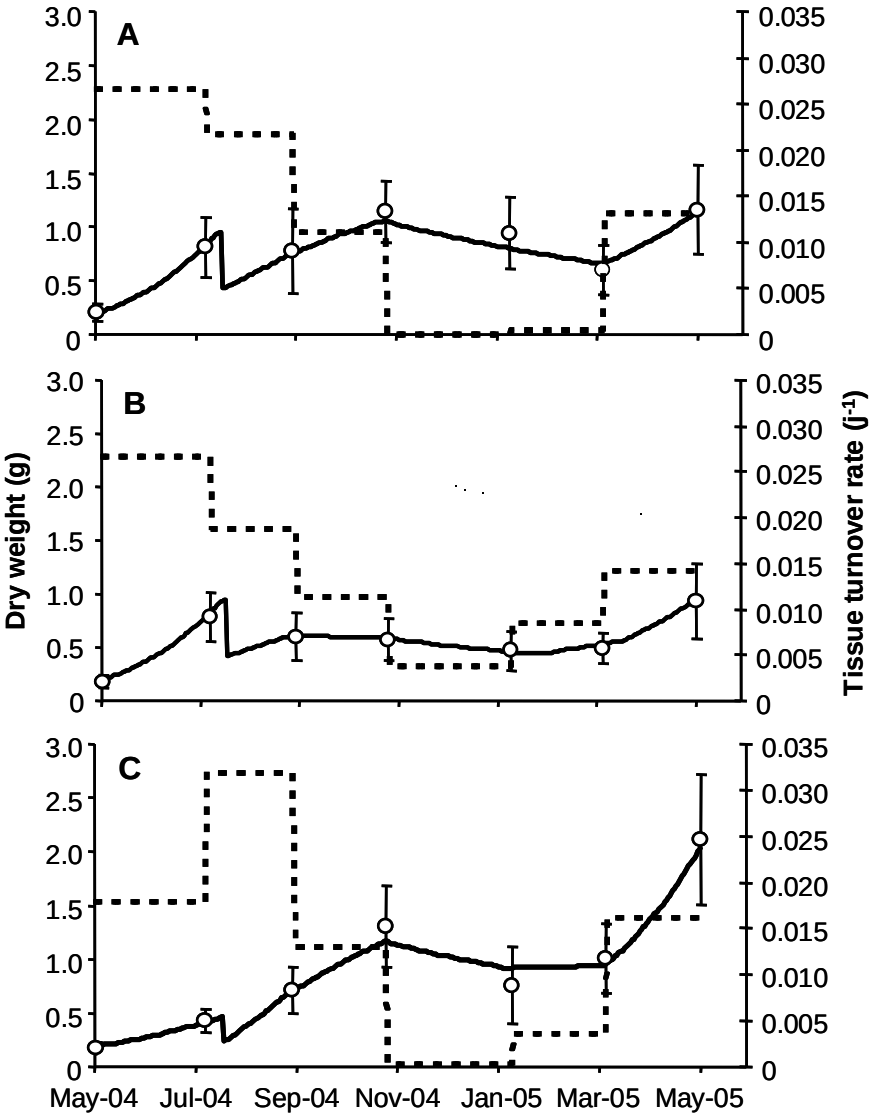

A
B
C
δ13C (‰)
δ15N (‰)
C/N ratio
-16
-18
-20
-22
-24
12
10
8
6
4
9
8
7
6
5
4
May-04
Jul-04
Sep-04
Nov-04
Jan-05
Mar-05
May-05
BDV-S
BDV-N
LIN

Fig. 5


A
B
C
Dry weight (g)
Tissue turnover rate (j-1)
3.0
2.5
2.0
1.5
1.0
0.5
0
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0
May-04
Jul-04
Sep-04
Nov-04
Jan-05
Mar-05
May-05