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Understanding the dynamics of $\delta^{13}C$ and $\delta^{15}N$ in soft tissues of the bivalve *Crassostrea gigas* facing environmental fluctuations in the context of Dynamic Energy Budgets (DEB)

A. Emmery[a, b, c, *], S. Lefebvre[c], M. Alunno-Bruscia[a] and S.A.L.M. Kooijman[d]

[a] Ifremer Dept. PFOM-PI, 11 Presqu'île du Vivier, 29840 Argenton, France
[b] Université de Caen Basse Normandie, UMR 100 Ifremer-UCBN PE2M, IBFA-Université de Caen Esplanade de la paix 14032 Caen cedex
[c] Université de Lille 1 sciences et technologies, UMR CNRS 8187 LOG, Station Marine de Wimereux 28 avenue Foch, 62930 Wimereux, France
[d] Vrije Universiteit, Dep. of Theoretical Biology, de Boelelaan 1085 1081 HV Amsterdam, The Netherlands

*: Corresponding author : Antoine Emmery, email address : antoine.emmery@ifremer.fr

Abstract :

We studied the dynamics of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ of an opportunistic suspension feeder the Pacific oyster (*Crassostrea gigas*) to better understand the factors that influence the trophic enrichment (trophic-shift, Δ) between primary producers and consumers. Most of the previous studies on this topic do not quantify mass fluxes or isotopic discrimination phenomena in the organism, which are two pillars in isotope ecology. We used a dynamic energy budget (DEB) approach (Kooijman, 2010) to quantify *i)* the fluxes of elements and isotopes in *C. gigas* soft tissues and *ii)* the impact of the scaled feeding level, the organism mass and the isotopic ratio of food on the "trophic-shift" Δ, and isotope turnover in tissues. Calibration and parametrization modelling were based on data from the literature. We showed that a five-fold increase in scaled feeding level leads to a decrease of the trophic-shift value of 35% for carbon and 43% for nitrogen. This can be explained by the molecule selection for the anabolic and/or catabolic way. When f increases due to the reserve dynamic formulation in the standard DEB model, the half-life of the isotopic ratio $t_{\delta}^{1/2}$ in tissues also decreases from 13.1 to 7.9 d for $\delta^{13}C$ and from 22.1 to 10.3 d for $\delta^{15}N$. Organism mass also affects the trophic-shift value: an increase of the individual initial mass from 0.025 g to 0.6 g leads to an enrichment of 22% for $\delta^{13}C$ and 21% for $\delta^{15}N$. For a large individual, these patterns show that a high structural volume has to be maintained. Another consequence of the mass effect is an increase of the half-life for $\delta^{13}C$ from 6.6 to 12.0 d, and an increase of the half life for $\delta^{15}N$ from 8.3 to 19.4 d. In a dynamic environment, the difference in the isotopic ratios between the individual tissues and the food ($\delta^{13}C_W - \delta^{13}C_X$) exhibits a range of variation of 2.02‰ for carbon and 3.03‰ for nitrogen. These results highlight the potential errors in estimating the contributions of the food sources without considering the selective incorporation of isotopes. We conclude that the dynamic energy budget model is a powerful tool to investigate the fate of isotopes in organisms.

Highlights

► We study the dynamics of $\delta^{13}C$ and $\delta^{15}N$ in soft tissues of a bivalve. ► When the food density increases, the trophic-shifts (Δ) for $\delta^{13}C$ and $\delta^{15}N$ decrease. ► An increase of the body mass results in an increase of the $\Delta^{13}C$ and $\Delta^{15}N$. ► Half-life of $\delta^{13}C$ and $\delta^{15}N$ are also affected by food density and body mass. ► DEB model is a powerful tool to investigate fate of isotopes in organisms.

Keywords : oyster; isotopic ratio; discrimination; trophic-shift; diet; DEB theory

1. Introduction

In recent years, understanding the ecological role of natural and cultivated suspension - feeding bivalves has gained increasing interest among marine ecologists (*e.g.* Dame, 1996; Newell, 2004). Bivalve populations exclusively inhabit the benthicpelagic interface and are a key link in the matter fluxes of coastal ecosystems. This is because they transfer organic and mineral suspended matter from the water column to sediments *e.g.* Considering their ability to filter a huge amount of pelagic matter (Doering and Oviatt, 1986), benthic suspension-feeders can exert a top-down control on phytoplankton communities in coastal ecosystems (Guarini et al., 2004; Cloern, 1982; Officer et al., 1982). Bivalves are mostly opportunistic and occupy an intermediate trophic niche between primary and secondary consumers. Consequently, they act as ecological indicators of the trophic state of the environment since they are sensitive to both the quality and quantity of the suspended organic matter that serves as their food source (Jennings and Warr, 2003; Lefebvre et al., 2009). Many bivalves can feed on a mixture of microalgae (phytoplankton and microphytobenthos) and detritus of marine (macroalgae) and terrestrial origin (Decottignies et al., 2007; Marin Leal et al., 2008).

Knowledge of the trophic role of bivalves in marine ecosystems has been improved by the use of stable isotope analysis (SIA) for tracing pathways of organic matter in food webs and for determining the contributions of different food sources to the organisms' diets (Marin Leal et al., 2008; Riera and Richard, 1996; Riera et al., 2002). Several laboratory studies have shown that the isotopic ratio of an organism, _13C and _15N, closely resembles that of the diet at steady state, though with a slight enrichment of heavier isotopes, *i.e.* 13C, 15N (DeNiro and Epstein, 1978, 1981). This enrichment, which is classically named the trophic-shift $\Delta = \delta_{consumer} - \delta_{diet}$ was

often considered to be constant across species and trophic levels with an average value of 1 ‰ for δ^{13}C and 3.5 ‰ for δ^{15}N (DeNiro and Epstein, 1978, 1981). This assumption has been widely applied in the literature to better understand the contribution of the different food sources to the diet of bivalves in coastal ecosystems (*e.g.* Riera et al., 1999; Dubois et al., 2007b).

Based on experimental and field data, studies by Vander Zanden and Rasmussen (2001) and McCutchan Jr et al. (2003), have shown that the Δ value has significant variation due to different factors. For instance, Tieszen et al. (1983), Suzuki et al. (2005), and Deudero et al. (2009) pointed out different Δ values for carbon and nitrogen amongst organs whereas studies by Gaye-Siessegger et al. (2004a), Mirón et al. (2006), and Adams and Sterner (2000) focused on the effects of the quality and nitrogen content of the diet on the trophic-shift. Focken (2001) and Barnes et al. (2007) concluded that the difference in the isotopic ratio between diet and consumer increased when feeding level increased (see Martínez del Rio et al., 2009, for a complete review). In the case of the bivalve *Crassostrea gigas*, the published Δ values are 0.9 ‰ for carbon and 5.4 ‰ for nitrogen (Yokoyama et al., 2008). However, those calculated by Dubois et al. (2007a) are 1.85 ‰ for δ^{13}C and 3.79 ‰ for δ^{15}N (Table 1). Determining and quantifying the factors that influence the trophic-shift is essential for trophic network studies. The Δ value makes it possible to correct isotopic signatures of consumers prior to incorporating them into mixing models (*i.e.* linear systems of mass balance equations that calculate contributions of different sources to a mixture). Therefore, the weak point in applying these models for food reconstruction is related to the estimation of appropriate Δ values (Phillips and Koch, 2002; Phillips, 2001; Phillips and Gregg, 2003). Another critical assumption is the steady-state equilibrium between the consumer and its diet which possibly does not occur under natural conditions. Several authors have used bio-energetic modelling approaches to circumvent this problem and to estimate the incorporation rate over time (Marin Leal et al., 2008; Olive et al., 2003).

The isotope approach has some weaknesses due to the lack of ecological tools to quantify mass fluxes (elements) and isotopic discrimination phenomena during assimilation, growth, and maintenance of organisms. The present study therefore aims *i)* to describe and quantify the fluxes of elements and isotopes of an opportunistic suspension feeder, *i.e.* the Pacific oyster *Crassostrea gigas*, by using a dynamic energy budget (DEB) approach (Kooijman, 2010) and *ii)* to quantify the impact of factors influencing the "trophic-shift" Δ and isotope tissue turnover which is useful for trophic network studies and diet reconstruction. We based our methods on the study by Pecquerie et al. (2010) which is, to our knowledge, the first theoretical investigation of the impact of metabolism on stable isotope in the context of DEB

theory. Here we describe the first study with an application to *C. gigas*.

Table 1: Trophic-shift values (Δ, ‰) and half-life of the isotopic ratio ($t_\delta^{1/2}$, d) estimated for bivalve species and derived from literature during diet switching experiments. The Δ values refer to the enrichment of the whole body mass (non-defatted tissues). All individuals were fed *ad libitum* ($f = 1$). Temperature during the experiments was 15.9°C in Dubois et al. (2007a), between 15 and 17°C in Yokoyama et al. (2008), and 22°C in Yokoyama et al. (2005).

		Carbon δ^{13}C		*Nitrogen* δ^{15}N	
Study	Species	Δ	$t_\delta^{1/2}$	Δ	$t_\delta^{1/2}$
Dubois et al. (2007a)	*Crassostrea gigas*	1.85 (±0.194)	7.7	3.79 (±0.194)	15.1
	Mytilus edulis	2.17 (±0.324)	8.9	3.78 (±0.292)	14.1
Yokoyama et al. (2008)	*Crassostrea gigas*	0.9	1.05	5.4	1.19
Yokoyama et al. (2005)	*Ruditapes philippinarum*	0.6	−−	3.4	−−
	Mactra veneriformis	0.9	−−	3.6	−−

Material and methods

Standard Dynamic Energy Budget (DEB) model

The standard DEB model describes the rate at which an organism assimilates and utilizes energy for maintenance, growth,, and reproduction as a function of its state and its environment (Nisbet et al., 2000; Kooijman, 2010). Each metabolic transformation defines a chemical transformation in which five organic generalized compounds (food X, reserve E, reproduction buffer E_R, structure V, and faeces P) and four mineral compounds (carbon dioxide O, water H, dioxygen O, and nitrogenous waste N) can be involved according to the transformation type (Table 2). Water and dioxygen substrates are assumed to be non-limiting. Each compound is composed of the four most abundant elements in organic matter, namely carbon C, hydrogen H, oxygen O, and nitrogen N. The mass of each compound is expressed in C-moles, *i.e.* the amount of each element relative to the amount of carbon per compound. The formula for generalized compounds can be written as $\mathrm{CH}_{n_{Hj}}\mathrm{O}_{n_{Oj}}\mathrm{N}_{n_{Nj}}$ where n_{ij} is the proportion of atoms in an element i (i = H, O, N) relative to carbon in a compound j ($j = X, E, V, P$). In the DEB model, the biochemical composition of reserve, structure, and the reproduction buffer of *C. gigas* is constant over time (Table 2).

The total biomass of the individual (in C-moles) has contributions from reserve, structure, and the reproduction buffer and can be written as: $M_W = M_E + M_V + M_{E_R}$

where M_E, M_V, and M_{E_R} are the mass of the reserve, structure and reproduction buffer respectively. The standard DEB model defines a set of three transformations in living organisms, *i.e.* assimilation (conversion of food to reserve and products), growth (conversion of reserve to structure and products) and dissipation (conversion of reserve to products) where generalized compounds are metabolized (Kooijman, 2010; Pecquerie et al., 2010). Changes in the mass of reserve, structure, maturity, and reproduction buffer can be written as:

$$\frac{d}{dt}M_E = \dot{J}_{EA} + \dot{J}_{EC} \quad (1)$$

$$\frac{d}{dt}M_V = (\kappa \dot{J}_{EC} - \dot{J}_{EM})y_{VE} = \dot{J}_{VG} \quad (2)$$

$$\frac{d}{dt}M_H = (1-\kappa)\dot{J}_{EC} - \dot{J}_{EJ} = \dot{J}_{ER} \quad \text{if} \quad M_H < M_H^p, \quad \text{else} \quad \frac{d}{dt}M_H = 0 \quad (3)$$

$$\frac{d}{dt}M_{E_R} = \kappa_R \dot{J}_{ER} \quad \text{if} \quad M_H = M_H^p, \quad \text{else} \quad \frac{d}{dt}M_{E_R} = 0 \quad (4)$$

where $\dot{J}_{EA} = f\{\dot{J}_{EAm}\}L^2$ the assimilation flux ($f = 0$ if $M_H < M_H^b$) and $\dot{J}_{EC} = \{\dot{J}_{EAm}\}L^2\frac{ge}{g+e}(1+\frac{L}{gL_m})$ the catabolic flux. $e = \frac{\dot{v}[M_E]}{\{\dot{J}_{EAm}\}}$ represents the scaled reserve density and $g = \frac{\dot{v}[M_V]}{\kappa\{\dot{J}_{EAm}\}y_{VE}}$ represents the energy investment ratio. Maintenance fluxes are described by $\dot{J}_{EM} = [\dot{J}_{EM}]L^3$ for the somatic compartment and $\dot{J}_{EJ} = \dot{k}_J M_H$ for the maturity and reproduction compartment. The allocation to maturity and reproduction flux $\dot{J}_{ER}$ is described by the following expression $\dot{J}_{ER} = (1-\kappa)\dot{J}_{EC} - \dot{J}_{EJ}$. Initiation of allocation to reproduction occurs when individual reaches the threshold of maturity at puberty, *i.e.* $M_H = M_H^p$. The energy allocated to M_{E_R} is then converted into gametes (ovocyte or spermatozoa) with some efficiency denoted κ_R, and the remainder $1-\kappa_R$ is dissipated as overhead. Once enough energy has been accumulated in the reproduction buffer, *i.e.* when a certain gonado-somatic index (GSI, %) has been reached, and if the external temperature is above 20 °C, the buffer is completely emptied and further accumulation is possible (Pouvreau et al., 2006).

To determine the total biomass of the individual in grams (W, g of dry weight), we first calculated the molar weight of the compounds E, V, and E_R (w_E, w_V, and w_{ER} respectively) as: $w_j = \sum n_{ij}w_i$, where w_i is the molar weight of an element (g.mol^{-1}, Table 3). Therefore, W can be obtained from the following formula: $W = M_E w_E + M_V w_V + M_{E_R} w_{ER}$.

Dynamic Isotope Budget model (DIB)

The assumptions and equations of the DIB models used for this study are extensively detailed in Kooijman (2010); Pecquerie et al. (2010). The DIB model describes the changes in the isotope frequency γ_{ij}^{0} of reserve, structure, and reproduction buffer where 0 the isotope of an element i in a compound j, *e.g.* γ_{CE}^{13} is the frequency of ^{13}C in reserve.

The chemical reactions of compounds can be synthesized by a set of three macrochemical equations with a constant stoichiometry. In the simplest form, the assimilation macrochemical equation can be written as $X + O \rightarrow E + P + H + N + C$, growth leads to the production of structure from reserve, $E + O \rightarrow V + C + H + N$, and dissipation encompasses the transformation of reserve into mineral products through the following reaction $E + O \rightarrow C + H + N$. Nevertheless these macrochemical reactions do not provide any information on the fate of atoms or on the discrimination of isotopes.

Isotopic discrimination in a macrochemical reaction is a three-step process. Compounds are first mobilized from a pool. Then, compounds are selected for the anabolic or catabolic fluxes according to their isotopic composition. Indeed, all three chemical transformations have an anabolic and catabolic aspect meaning that substrates have a dual function: they serve as a source for energy and building blocks. The catabolic route of any transformation uses substrates to produce energy. The anabolic route uses this energy and substrates as a source of building blocks to produce a given compound. Due to the difference in fate of substrate molecules, selection of molecules with particular isotopes can occur at the partitionning of anabolic and catabolic fluxes (Pecquerie et al., 2010; Kooijman, 2010). The number of molecules with one rare isotope in the anabolic route of a transformation is obtained from the mean of a Fisher's noncentral hypergeometric distribution. This selection depends on the odds ratio parameter value β which is defined as the ratio of probabilities of two isotopes (*i.e.* ^{13}C and ^{12}C) being selected for a particular route (Kooijman, 2010). This parameter allows the relative frequency of an isotope 0 of an element i in a compound j to be calculated for a given transformation k, n_{ij}^{0k}. $\beta = 1$ means that there is no selection between isotopes whereas $\beta > 1$ implies a discrimination against light isotopes. Finally, atom reshuffling occurs which describes the fraction of atoms in a chemical compound in a substrate which ends up in a product from a given transformation.

To fully describe the isotopic composition of an organism, the structure turnover is taken into account. This process is described by two coupled macrochemical reactions: the production of renewed structure from reserve, L_1: $E + O + V \rightarrow V + C + H + N$, and the degradation of structure, L_2: $V + O \rightarrow V + C + H + N$.

Structure turnover, which is part of the volume-specific somatic maintenance, states that the incoming flux of renewed structure is compensated by the outgoing flux of degraded structure and a part of the degraded structure is recycled to form renewed structure. Compound selections and atom reshuffling occur *i)* between reserve and structure *ii)* and between degraded structure and renewed structure. These three fluxes therefore have different isotopic compositions.

The isotopic ratios of reserve, structure and reproduction buffer are described by the following state equations:

$$\frac{d}{dt}\gamma^0_{iE} = \left(\frac{n^{0A}_{iE}}{n_{iE}} - \gamma^0_{iE}\right)\frac{\dot{J}_{EA}}{M_E}; \quad (5)$$

$$\frac{d}{dt}\gamma^0_{iV} = \left(\frac{n^{0G}_{iV}}{n_{iV}} - \gamma^0_{iV}\right)\frac{\dot{J}_{VG}}{M_V} - \left(\frac{n^{0L}_{iV}}{n_{iV}} - \gamma^0_{iV}\right)\frac{\dot{J}_{VL_1}}{M_V}; \quad (6)$$

$$\frac{d}{dt}\gamma^0_{iE_R} = \left(\frac{n^{0R}_{iE_R}}{n_{iE_R}} - \gamma^0_{iE_R}\right)\frac{\dot{J}_{ER}}{M_{E_R}}; \quad (7)$$

where, n_{iE}, n_{iV} and n_{iE_R} represents the frequency of an element i relative to that of carbon in compound of reserve, structure and/or reproduction buffer and n^{0A}_{iE}, n^{0G}_{iV}, and $n^{0R}_{iE_R}$ represent the relative frequency of an isotope 0 of element i (*i.e.* ^{13}C, ^{15}N) in a compound of reserve, structure and/or reproduction buffer during assimilation, growth and reproduction. $J_{VL_1} = -y^L_{VE}\dot{J}_{EL}$ represents the renewed structure flux with $\dot{J}_{EL} = \kappa_L \dot{J}_{EM}$. The γ notation is converted to δ notation (classically used in SIA) as $\delta_i = 1000((R_i - R_{ref})/R_{ref})$ where $R = \gamma^0_{ij}/(1-\gamma^0_{ij})$. The change of isotopic ratio of the whole body, γ^0_{iW}, is given by the weighted sum of each of the state variables:

$$\gamma^0_{iW} = \frac{\gamma^0_{iE}M_E + \gamma^0_{iV}M_V + \gamma^0_{iE_R}M_{E_R}}{M_E + M_V + M_{E_R}}$$

The framework, assumptions and equations of the standard DEB and DIB models used for this study have been extensively detailed in Kooijman et al. (2008); Kooijman (2010) and Pecquerie et al. (2010). Parameter estimation is performed following the procedure described by Lika et al. (subm). The zero-variate data used for the procedure are presented in the Table 4 and the set of DEB and DIB parameters obtained for *Crassostrea gigas* are presented in the Table 3. The model calibration was made on data from Dubois et al. (2007a) to obtain odds ratio values β for carbon and nitrogen isotopic discrimination (Fig. 1).

Trophic-shift and half-life of the isotopic ratio

The trophic-shift, *i.e.* Δ^{13}C and Δ^{15}N was calculated as the difference between the isotopic ratio of the consumer and the isotopic ratio of the food source, $\Delta = \delta_W - \delta_X$ in a constant environment. Considering that the structure turnover leads to the enrichment of structure, we estimated the derivative of the difference between δ_W and δ_X. We assumed that equilibrium between the individual and its food source is reached when derivative variations are lower than the threshold of 2 %, *i.e.* $\Delta_{threshold} = 2\,\%$. We also calculated the half-life of the isotopic ratio for δ^{13}C and δ^{15}N, from $t^{1/2}_{\delta^{13}\mathrm{C}}$ and $t^{1/2}_{\delta^{15}\mathrm{N}}$, respectively. The term $t^{1/2}_{\delta}$ corresponds to the time required to reach the half value of the isotopic ratio in the whole body δ_W at the equilibrium state.

Simulations

The dynamics of carbon and nitrogen stable isotopes, *i.e.* δ^{13}C and δ^{15}N, are simulated in soft tissues of an individual of *Crassostrea gigas* under four different scenarii to test for several effects:

Scenario 1 (S 1) Effect of scaled feeding level f: scaled feeding level is described by the scaled functional response f with $0 < f < 1$ (see Kooijman, 2010). Different scaled feeding levels are tested: $f = 0.2$, 0.4, 0.6, 0.8, 1 while temperature T and isotopic ratio of food source for carbon and nitrogen are constant.

Scenario 2 (S 2) Effect of the organism mass W: initial total dry mass of tissues (expressed in grams) at the start of simulations are $W_0 = 0.025$, 0.05, 0.1, and 0.6 g of dry weight. Temperature T and scaled feeding level are constant.

Scenario 3 (S 3) Effect of the isotopic ratio of food source: a varying signal of isotope food source for carbon and nitrogen, namely $\delta^{13}\mathrm{C}_X$ and $\delta^{15}\mathrm{N}_X$, are used. Temperature T and scaled feeding level are constant.

Scenario 4 (S 4) Effect of a varying environment: scaled feeding level, temperature T and food isotopic ratio are varying over time.

For all scenarios, only one type of food, *i.e.* a mono-specific culture of micro-algae is considered. Conditions for each scenario are summarised in Table 5.

Results

DIB model calibration

The calibration of the DIB model based on a fractionation experiment carried out on *C. gigas* by Dubois et al. (2007a) allowed us to estimate the odds ratio values

under controlled conditions of temperature ($T = 15.9°C$) and scaled feeding level ($f = 1$) over 90 days (Fig. 1). We assumed that the isotope selection, which depends on the odds ratio value, is equal in each metabolic function (assimilation, growth and dissipation, including structure turnover) for a given element. The estimated odds-ratio values are $\beta^{13}_{CW} = 1.008$ for the carbon and $\beta^{15}_{NW} = 1.0125$ for nitrogen. The carbon isotopic ratio of food, $\delta^{13}C_X$, shows an increase of $\approx 3‰$ during the experiment that leads to a slight increase of the $\delta^{13}C_W$ on the last sampling date whereas $\delta^{15}N_X$ shows higher variations of $\approx 20‰$ but shorter in time than those observed for $\delta^{13}C_X$. The model slightly underestimates nitrogen isotopic ratio at sampling times 8 and 15, but generally the simulations match the observations well.

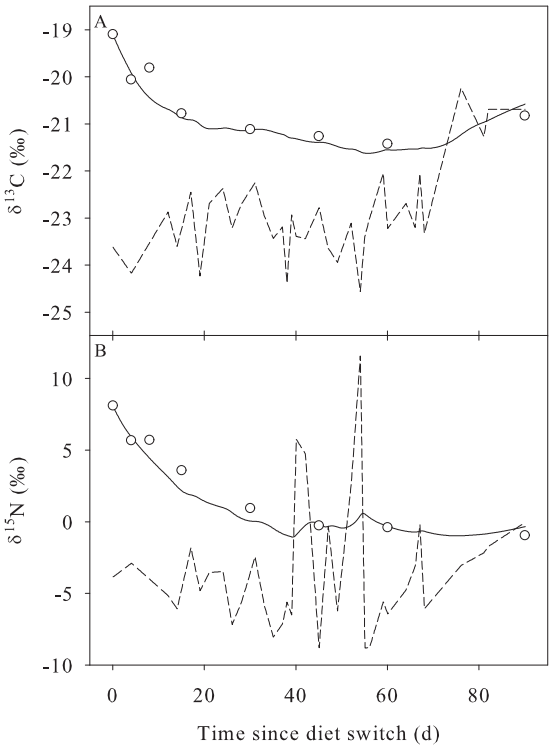

Figure 1: Simulated (solid lines) *versus* observed (dots) isotopic ratios of the oyster *Crassostrea gigas* tissues over time for carbon (upper panel) and nitrogen (lower panel) isotopes. The oyster diet switches from natural conditions (day 0) to a mono-specific algal diet of *Skeletonema costatum* with varying isotopic ratios (dashed lines). Data from Dubois et al. (2007a): scale functional response $f = 1$, temperature $T = 15.9°C$, initial mass $W_0 = 0.05\,g$, initial signature of oyster tissues $\delta^{13}C_{W_0} = -19.06‰$ for carbon and $\delta^{15}N_{W_0} = 8.11‰$ for nitrogen. For each sampling, oysters were kept alive overnight in filtered sea water to evacuate their gut contents.

S1: effect of scaled feeding level

A higher scaled feeding level results in a lower half-life of the isotopic ratio and a lower trophic-shift factors at the end of the experiment (Figs. 2 A and 2 B). As a corollary, an increase in f from 0.2 to 1 results in decreasing trophic-shift values from 3.01 ‰ to 1.93 ‰ for Δ^{13}C (Fig. 2 C) and from 5.46 ‰ to 3.06 ‰ for Δ^{15}N (Fig. 2 D). The estimated values for the half-life of the isotopic ratio exhibits the same pattern as that observed for the Δ values. For both δ^{13}C and δ^{15}N, there is a decrease of the half-life when f increases: $t^{1/2}_{\delta^{13}\mathrm{C}} = 13.1\,\mathrm{d}$, 12 d, 10.3 d, 8.9 d, 7.9 d, and $t^{1/2}_{\delta^{15}\mathrm{N}} = 22.1\,\mathrm{d}$, 17.4 d, 14.2 d, 12 d and 10.3 d, respectively for $f = 0.2$, 0.4, 0.6, 0.8 and 1.

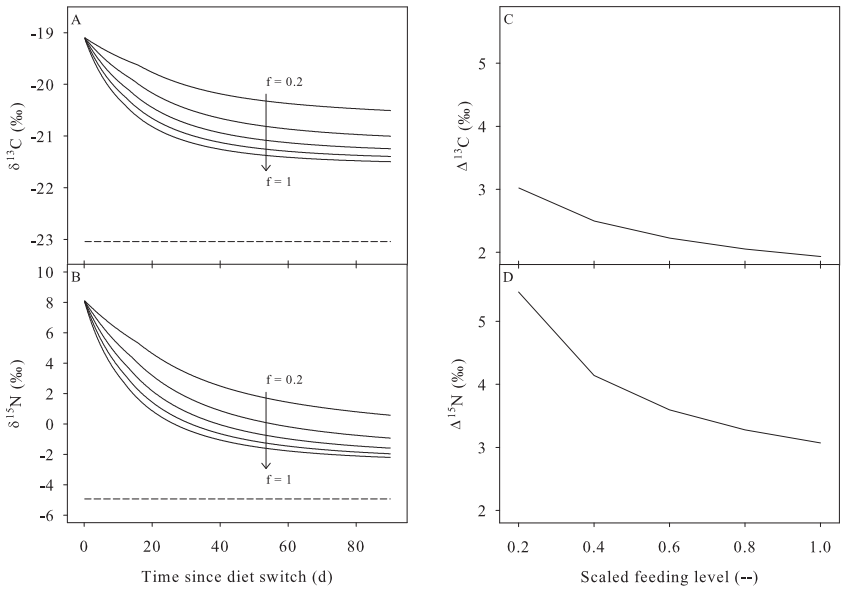

Figure 2: *Scenario S1*. *Left panels*: isotopic ratios of the oyster *Crassostrea gigas* tissues (solid lines) and of the food source (dashed lines) for different scaled feeding levels $f = 0.2$, 0.4, 0.6, 0.8, and 1 during a diet-switching simulation. *Right panels*: trophic-shift Δ values as a function of f. Graphs (A, C) and (B, D) relate to carbon and nitrogen stable isotopes respectively. Scenario conditions are described in Table 5. Final mass are $W_f = 0.08$, 0.18, 0.29, 0.43, and 0.59 g of dry weight for each scaled feeding level tested.

S2: effect of organism mass

A larger initial organism mass results in slower rate of change in δ_W during the experiment and higher trophic-shift factors at the end of the experiment (Figs. 3 A and 3 B). For $W_0 = 0.025\,\mathrm{g}$, 0.05 g, 0.1 g, and 0.6 g, the corresponding trophic-shift

values are respectively 1.85 ‰, 1.93 ‰, 2.03 ‰ and 2.37 ‰ for carbon, and 2.94 ‰, 3.06 ‰, 3.22 ‰ and 3.73 ‰ for nitrogen (Figs. 3 C and 3 D). For both δ^{13}C and δ^{15}N, the half-life values increase with increasing animal tissue mass: $t^{1/2}_{\delta^{13}\mathrm{C}} = 6.6$ d, 7.9 d 9.1 d and 12.0 d and $t^{1/2}_{\delta^{15}\mathrm{N}} = 8.3$ d, 10.3 d, 12.2 d and 19.4 d, respectively for $W_0 = 0.025$ g, 0.05 g, 0.1 g, and 0.6 g.

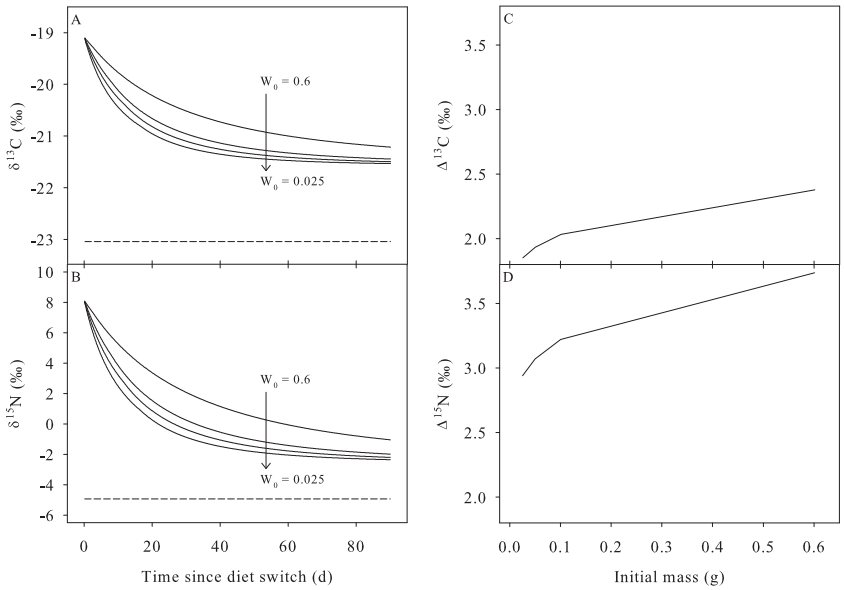

Figure 3: *Scenario S2*. *Left panels*: isotopic ratios of the oyster *Crassostrea gigas* tissues (solid lines) and of the food source (dashed lines) for different initial mass $W_0 = 0.025$, 0.05, 0.1, and 0.6 g of dry weight during a diet-switching simulation. *Right panels*: trophic-shift Δ values as a function of W_0. Graphs (A, C) and (B, D) relate to carbon and nitrogen stable isotopes, respectively. Scenario conditions are described in Table 5. Final masses are $W_f = 0.448$, 0.59, 0.81, and 2.18 g of dry weight for each initial mass tested.

S3: effect of the isotopic ratio of the food source

When the model is forced by varying signals of food isotopic ratio over time ($\delta^{13}\mathrm{C}_X$ and $\delta^{15}\mathrm{N}_X$) the amplitude of the $\delta^{13}\mathrm{C}_W$ and $\delta^{15}\mathrm{N}_W$ variations of *C. gigas* soft tissues are smoothed down compared with the food source signal (Fig. 4). The half-life of the isotopic ratios also varies as shown by the time-lag between both signals (Fig. 4). Finally, the difference in the isotopic ratio between the oyster tissues and the food source tends to increase over time (Figs. 4 C and 4 D).

Table 2: Elemental composition of the organic and mineral compounds used in this study for *Crassostrea gigas*. Values are estimated from the data of Whyte et al. (1990) and the procedures of Kooijman (2010).

	Organic comp.				*Minerals comp.*			
	X	V	E & E_R	P	C	H	O	N
Carbon C	1	1	1	1	1	0	0	0
Hydrogen H	1.8	1.78	1.79	1.8	0	2	0	3
Oxygen O	0.5	0.48	0.53	0.5	2	1	2	0
Nitrogen N	0.2	0.15	0.14	0.15	0	0	0	1

X: food
V: structure
E: reserve
E_R: reproduction buffer
P: faeces
C: carbon dioxide
H: water
O: dioxygen
N: nitrogenous waste

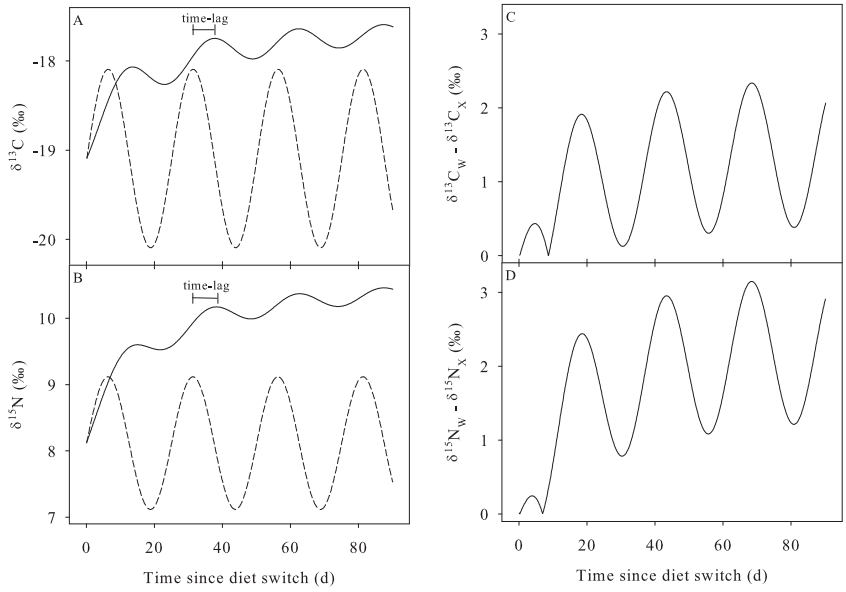

Figure 4: *Scenario S*3. *Left panels*: isotopic ratios of the oyster *Crassostrea gigas* tissues (solid lines) under varying conditions of food source signature (dashed lines). *Right panels*: difference in the isotopic ratio between the oyster tissues and the food source as a function of time. Graphs (A, C) and (B, D) relate to carbon and nitrogen stable isotopes respectively. Scenario conditions are described in Table 5. Final mass is $W_f = 0.59\,\mathrm{g}$ of dry weight.

Table 3: Estimated parameters for the *Crassostrea gigas* species. The parameter values come from the present study except for the parameters y^L_{VE}, κ_L and κ_{Lr} where values come from the study by (Pecquerie et al., 2010).

Symbols	Values	Units	Interpretations
T_1	293	K	Reference temperature
T_A	5722	K	Arrhenius temperature
T_L	277	K	Lower boundary tolerance range
T_H	318	K	Upper boundary tolerance range
T_{AL}	20000	K	Arrhenius temperature for lower boundary
T_{AH}	190000	K	Arrhenius temperature for upper boundary
$\{\dot{J}_{EAm}\}$	5.14^{-4}	$\mathrm{mol\,d^{-1}\,cm^{-2}}$	Maximum surface-area-specific assimilation rate
$\dot{v}$	0.04932	$\mathrm{cm\,d^{-1}}$	Energy conductance
$[M_V]$	4.25^{-3}	$\mathrm{mol\,cm^{-3}}$	Number of C-atoms per unit of structural body volume
$[J_{EM}]$	6.36^{-5}	$\mathrm{mol\,d^{-1}\,cm^{-3}}$	Volume-specific maintenance rate
κ	0.69	–	Fraction of reserve allocated to growth & maintenance
M^b_H	1.08^{-10}	mol	Maturation at birth
M^p_V	1.92^{-5}	mol	Mass of structure at puberty
M^p_H	5.46^{-5}	mol	Maturation at puberty
$\dot{k}_J$	0.002	$\mathrm{d^{-1}}$	Maturity maintenance rate coefficient
κ_R	0.95	–	Reproduction efficiency
y_{EX}	0.88	$\mathrm{mol\,mol^{-1}}$	Yield of reserve from food in assimilation
y_{VE}	0.776	$\mathrm{mol\,mol^{-1}}$	Yield of structure from reserve in growth
y^L_{VE}	0.63	$\mathrm{mol\,mol^{-1}}$	Yield of structure from reserve in turn-over of structure
κ_L	0.8	–	Fraction of volume-specific somatic maintenance
κ_{Lr}	0.47	–	Fraction of structure turnover that is recycled
β^{13}_{CW}	1.008	–	Odds ratio of the whole body for ^{13}C
β^{15}_{NW}	1.0125	–	Odds ratio of the whole body for ^{15}N
w_{C}	12	$\mathrm{g.mol^{-1}}$	Molar weight of C
w_{H}	1	$\mathrm{g.mol^{-1}}$	Molar weight of H
w_{O}	16	$\mathrm{g.mol^{-1}}$	Molar weight of O
w_{N}	14	$\mathrm{g.mol^{-1}}$	Molar weight of N

Table 4: Zero-variate data used in the parameter estimation procedure (Lika et al., subm) for the *Crassostrea gigas* species.

Symbols	Values	Units	Interpretations	References
a_b	5.5	d	Age at birth at	Rico-Villa et al. (2010)
a_p	93	d	Age at puberty	pers. com.
L_b	0.008	cm	Length at birth	Rico-Villa et al. (2009)
L_p	2.4	cm	Length at puberty	pers. com.
L_i	45	cm	Maximum length observed	Van der Veer et al. (2006)
W_{DW}^b	5^{-9}	g	Dry weight at birth	Rico-Villa et al. (2010)
W_W^p	0.2	g	Wet weight at puberty	pers. com.
W_W^i	1430.6	g	Ultimate wet weight	pers. com.
R_i	2.7e6	eggs/d	maximum reproduction rate	pers. com.
a_m	4745	d	Life span	from Van der Veer et al. (2006)
r_B	0.002	d^{-1}	von Bertalanffy growth rate	Van der Veer et al. (2006)

Table 5: Conditions of simulations for each scenario. f relates to the scaled feeding level (–), T relates to the temperature (°C), W_0 relates to the initial mass of the organism (g of dry weight), and δ notation relates to isotopic ratio (‰).

Scenarii ↓; Conditions →	T	f	W_0	$\delta^{13}C_X$	$\delta^{15}N_X$	$\delta^{13}C_W$	$\delta^{15}N_W$
$S1$, "scaled feeding level" effect:	16	varying	0.05	−23.04	−4.93	−19.06	8.11
$S2$, "Organism mass" effect:	16	1	varying	−23.04	−4.93	−19.06	8.11
$S3$, "isotopic ratio of food" effect:	16	1	0.05	varying	varying	−19.06	8.11
$S4$, "Varying environment" effect:	varying	varying	0.05	varying	varying	−20.98	−1.30

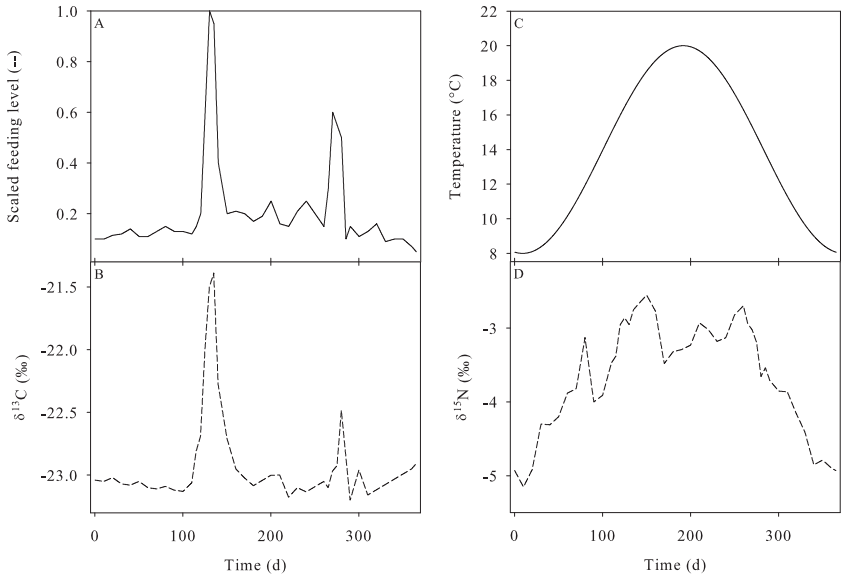

Figure 5: Forcing variables over time used for the scenario S 4. (A) f values, (B) isotopic ratio of food source for carbon (‰), (C) temperature (°C) and (D) isotopic ratio of food source for nitrogen (‰).

S4: effect of a varying environment

As in the previous experiment, the amplitude of the variations in the food isotopic ratio is smoothed down in the animal tissues: strong variations of $\delta^{13}C_X$ (Fig. 5 B) over a short time period result in small variations in $\delta^{13}C_W$ in animal tissues (Fig. 6 A). The difference in the isotopic ratios between the individual tissues and the food ($\delta_W - \delta_X$) clearly varies over time with a range of 2.02 ‰ and 3.03 ‰ for carbon and nitrogen, respectively (Figs. 6 C and 6 D). During a spawning event (day 175), both $\delta^{13}C_W$ and $\delta^{15}N_W$ of oyster abruptly change regardless of the variations in the isotopic composition of food.

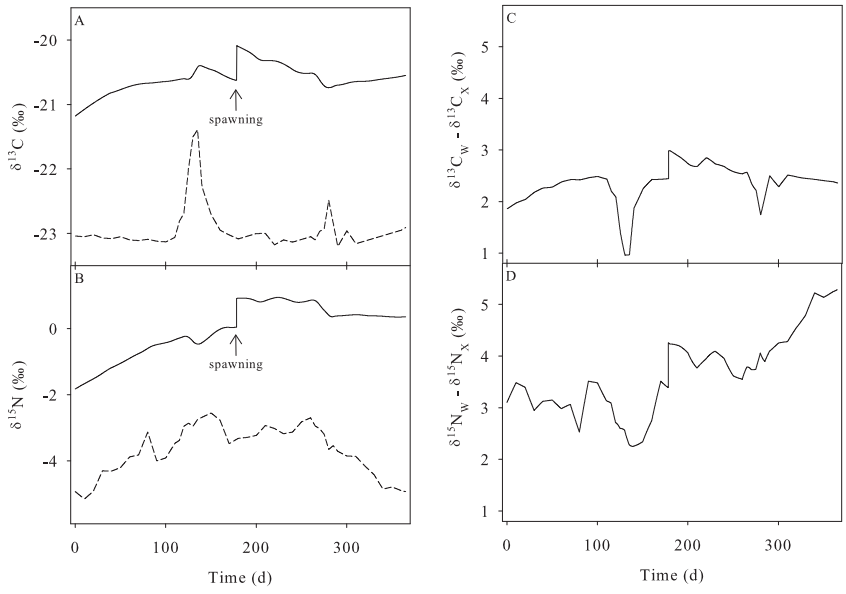

Figure 6: *Scenario S4*. *Left panels*: isotopic ratios of the oyster *Crassostrea gigas* tissues (solid lines) under varying conditions of scaled feeding level, temperature and food isotopic ratios (dashed lines). *Right panels*: difference in the isotopic ratio between the oyster tissue and the food source as a function of time. Graphs (A, C) and (B, D) relate to carbon and nitrogen stable isotopes respectively. Scenario conditions are described in Table 5. Final mass is $W_f = 0.18$ g of dry weight.

Discussion

Variable trophic-shift

DEB theory (Kooijman, 2010) can be used to quantify variations in the trophic shift for a marine bivalve *Crassostrea gigas* in response to varying scaled feeding lev-

Table 6: Trophic-shift values (Δ, ‰) and half-life of the isotopic ratio ($t_\delta^{1/2}$, d) derived from the literature during diet-switching experiments carried out with different feeding levels. All Δ values refer to the enrichment of the whole body mass (non-defatted tissues) except for the study by Barnes et al. (2007) which is only on muscle. In the studies by Gaye-Siessegger et al. (2003, 2004b) and Focken (2001), Δ values are recalculated on the basis of dry flesh mass at the equilibrium state and the feeding level is expressed in $\mathrm{g.kg^{-0.8}.d^{-1}}$. f relates to the scaled feeding level (–), T relates to the temperature (°C), and W_0 relates to the initial mass of the organism (g of dry weight).

		Carbon δ^{13}C		*Nitrogen* δ^{15}N		*Experimental conditions*		
Study	Species	Δ	$t_\delta^{1/2}$	Δ	$t_\delta^{1/2}$	f	T	W_0
This study	*Crassostrea gigas*	3.01	13.1	5.46	22.1	$f = 0.2$	16	––
	〃	2.49	12.0	4.14	17.4	$f = 0.4$	16	––
	〃	2.22	10.3	3.59	14.2	$f = 0.6$	16	––
	〃	2.05	8.90	3.27	12.0	$f = 0.8$	16	––
	〃	1.93	7.9	3.06	10.3	$f = 1$	16	––
	〃	1.85	6.6	2.94	8.3	$f = 1$	16	0.025
	〃	1.93	7.9	3.06	10.3	$f = 1$	16	0.05
	〃	2.03	9.1	3.22	12.2	$f = 1$	16	0.1
	〃	2.37	12.0	3.73	19.4	$f = 1$	16	0.6
Barnes et al. (2007)	*Dicentrarchus labrax*	1.38 (±0.49)	–	4.02 (±0.43)	–	Low	16 (±0.03)	––
	〃	1.38 (±0.48)	–	3.92 (±0.46)	–	Medium	16 (±0.03)	––
	〃	1.55 (±0.43)	–	3.79 (±0.36)	–	High	16 (±0.03)	––
	〃	1.23 (±0.37)	–	4.23 (±0.27)	–	Low	11.1 (±0.05)	––
	〃	1.00 (±0.48)	–	4.82 (±0.30)	–	Medium	11.1 (±0.05)	––
	〃	1.09 (±0.56)	–	4.27 (±0.37)	–	High	11.1 (±0.05)	––
Gaye-Siessegger et al. (2003)	*Oreochromis niloticus*	2.34	–	5	–	2.5	27 (±0.1)	––
	〃	1.86	–	4.7	–	6.0	27 (±0.1)	––
	〃	1.34	–	4.5	–	10.0	27 (±0.1)	––
	〃	1.37	–	4.6	–	15.0	27 (±0.1)	––
Gaye-Siessegger et al. (2004b)	*Cyprinus carpio*	3.51	–	1.69	–	3.2	27 (±0.3)	––
	〃	2.19	–	1.44	–	9.6	27 (±0.3)	––
	〃	1.82	–	1.27	–	16	27 (±0.3)	––
	〃	1.65	–	1.13	–	22.4	27 (±0.3)	––
Focken (2001)	*Oreochromis niloticus*	0.69	–	0.6	–	5	27 (±0.2)	––
	〃	0.8	–	0.9	–	10	27 (±0.2)	––
	〃	1.05	–	1	–	20	27 (±0.2)	––

els, initial mass of oyster, and isotopic ratio of the food. Stable isotope analysis has helped to understand the diet of natural and cultivated suspension-feeding bivalves in marine ecosystems (Marin Leal et al., 2008; Riera and Richard, 1996; Riera et al., 2002). These analyses assume that the organism is in equilibrium with its food source. To judge this, the trajectory of the isotopic signal of food and the enrichment factor must be known. Most ecological investigations have used the concept of a constant trophic-shift because of the absence of an ecological tool to quantify the impact of different factors on the metabolism of the organism. A few studies have used bioenergetics-based models to investigate the impact of factors on the isotope dynamic of an organism (*e.g.* Harvey et al., 2002) or to estimate food-source contributions (Marin Leal et al., 2008). However, none of these studies described a dynamic and variable isotopic discrimination.

Link between trophic-shift and scaled feeding level

In our study, Δ^{13}C and Δ^{15}N were both affected by scaled feeding level. A five times increase of the scaled feeding level ($f = 0.2$ to $f = 1$) leads to a decrease of the Δ value of 35 % for carbon and 43 % for nitrogen (Fig. 2). These patterns and the range of variation of the Δ value are consistent with previous findings concerning *Cyprinus carpio* (Gaye-Siessegger et al., 2004b). A decrease of 52 % in Δ^{13}C and 33 % in Δ^{15}N was found between the lowest and highest feeding levels for this species. Another fish species (*Oreochromis niloticus*) showed a decrease of 41 % of the carbon trophic-shift when feeding level increased by a factor of 6 but no clear pattern was found for Δ^{15}N (Gaye-Siessegger et al., 2003). For the European sea bass (*Dicentrarchus labrax*) Barnes et al. (2007) showed a slight decrease of 5 % of the Δ^{15}N at 16 °C and a decrease of 11 % for Δ^{13}C at 11 °C between low and high feeding levels, although no clear pattern was observed for Δ^{13}C at 16 °C and Δ^{15}N at 11 °C. However, for *Cyprinus carpio* Focken (2001) found an increase of the Δ for both carbon and nitrogen with increasing feeding levels (Table 6). For the Δ^{15}N pattern, Focken (2001) suggested that a nutritional stress due to a high protein concentration in food may have occurred during the experiment. The authors further assumed that during the liponeogenesis that occurs at the highest feeding level, the newly formed lipids had a higher ^{13}C content than the lipids absorbed directly from food.

The fate of compounds through anabolism and catabolism, as well as the description of isotopic discrimination during metabolic transformations (assimilation, growth and dissipation) are critical to understand the impact of scaled feeding level on the isotopic ratio of an organism. During compound transformation, the probability of a molecule to be selected for the catabolic or anabolic route depends on its

isotopic composition. In the present study, we assumed that the isotopic discrimination is equal for assimilation, growth, dissipation, and structure turn-over (see section *DIB model calibration*). The biochemical composition of reserve, structure, and reproduction buffer is constant over the life cycle (strong homeostasis assumption, Kooijman, 2010) implying that only the amount of the pools E, V, and E_R, and their respective isotopic ratios, can change over time according to the food characteristics (Kooijman, 2010). Therefore, for a given isotopic ratio of the food, the probability of a "light molecule" to be selected for the anabolic route is higher when $f = 1$ (high scaled feeding level) than when $f = 0.2$ (low scaled feeding level). This phenomenon is supported by the fact that food and energy reserve cannot be considered as infinite (large) pools, *e.g.* as illustrated by the low level of primary production frequently observed in coastal marine ecosystems during winter. Isotopic discrimination related with maintenance of the organism, *i.e.* the somatic maintenance including structure turn-over, can also have a significant effect on the isotope dynamics of the whole body. In the standard DEB model, maintenance processes have priority over growth and maturation or reproduction. The importance of somatic maintenance relative to assimilation increases for decreasing ingestion levels. This leads to a strong enrichment of the whole body at low scaled feeding levels.

Link between trophic-shift and individual mass

An increase from 0.025 g to 0.6 g of the initial mass results in an enrichment of 22 % for Δ^{13}C, and 21 % for Δ^{15}N respectively (Fig. 3). This enrichment can be explained by the somatic maintenance because growing individuals increase their structural volume. In our model, the somatic maintenance flux is proportional to the structural volume. Since the structure turn-over selects for heavy isotopes during an animal's life, big individuals have a larger amount of structure to maintain and are consequently heavier in terms of isotopic ratio than small individuals.

Our results cannot be compared easily with literature data as, to our knowledge, no controlled diet-switching experiment, *e.g.* under constant conditions of feeding level and temperature, has been carried out on individuals of the same species with different initial body mass. Sweeting et al. (2007a,b) found a weak negative correlation between the δ^{13}C values and the mass of muscle and liver in *D. labrax* reared over a 2 yr experiment under a constant isotopic ratio of food, though with seasonal variations of temperature and natural daylight cycle. The authors also found a correlation between the δ^{15}N values and liver mass, but this correlation was difficult to interpret. We think that this could have been due to confounding effects of mass, temperature, and experimental duration on sea bass metabolism. Trueman et al. (2005) showed that δ^{15}N varied inversely with growth rate in the Atlantic salmon

Salmo salar during a controlled feeding experiment which is consistent with the fact that salmon were fed on a depleted diet at the start of the experiment.

Positive correlations of increasing age, length, and mass with the δ^{15}N enrichment of organisms have been frequently reported for marine fish species in field studies (Badalamenti et al., 2002; Lindsay et al., 1998, and references therein). However, for the δ^{13}C, this pattern is difficult to observe since the classical enrichment between two different trophic levels ranges from 0 ‰ to 1 ‰. This relationship for δ^{15}N should nevertheless be interpreted carefully due to the complexity of interactions between species and their environment. Indeed, an old individual can be enriched in heavy isotopes due to an increase in mass and/or a change in the trophic position, *i.e.* a change in the diet and/or in the size of prey. None of the current ecological tools make it possible to discriminate and quantify the effects of these two factors on the δ^{15}N enrichment across trophic levels. Jennings et al. (2002a,b) studied trophic network structures by applying stable isotope analysis on size-structured production. Dynamic energy and isotope budget models can be valuable in this context since isotopic discrimination is modelled mechanistically.

Half-life of the isotopic ratio

The half-life of the isotopic ratio in the whole body decreases when the scaled feeding level increases as explained in the scenario S 1. Indeed, when the scaled functional response remains constant, the scaled reserve density is equal to the scaled functional response, namely $e = f$. Moreover, J_{EC} is a function of the amount of energetic reserve M_E and of structural volume M_V. Consequently, when the scaled feeding level increases, e and J_{EC} increase, which leads to a rapid reserve mobilisation and a decrease of the compound half-life of reserve.

For two individuals of the same species with different body mass, the larger one will have more reserve and structure than the smaller one under constant conditions. If the pools of reserve and structure are big, one compound of the pool will remain for longer before being mobilized and used for a particular metabolic function than in a small pool. This implies that the bigger the organism, the longer the residence time of a compound (see scenario S 2, Fig. 3).

The difference between $t^{1/2}_{\delta^{13}\mathrm{C}}$ and $t^{1/2}_{\delta^{15}\mathrm{N}}$ for both scenarios S 1 and S 2 can be partially explained by the difference in isotopic discrimination between carbon and nitrogen. There are different odds-ratio values for carbon and nitrogen with $\beta^{13}_{CW} < \beta^{15}_{NW}$, which implies that the $\delta^{13}\mathrm{C}_W$ reaches the equilibrium with the food source faster than the $\delta^{15}\mathrm{N}_W$ in a given pool. This effect is also increased because the reserve dynamic is faster than the structure dynamic. The biological composition is different (Table 2), though constant throughout the life span due to the strong

homeostasis assumption (see Kooijman, 2010). Only the amounts of reserve and of structure can vary relative to each other, leading to the property that the chemical composition, and thus the C:N ratio of the whole body can change. This difference leads to different dynamics of isotopes among compartments. The use of two or more compartments is clearly an advantage to describe isotope dynamics (see review by Martínez del Rio et al., 2009).

Dynamic equilibrium between the food source and the individual

The trophic-sift value Δ is estimated when the isotopic ratio of an individual is constant compared with the isotopic ratio of the food, which is assumed to be constant. The scenario S 3 shows the possible errors that may be introduced into the estimation of Δ when δ_X varies. In controlled feeding experiments, bivalves are frequently fed on phytoplankton species which have complex and variable isotope dynamics (Riera and Richard, 1997; Savoye et al., 2003; Malet et al., 2008; Bodineau et al., 1998). Even under controlled conditions, the complex life cycle of microalgae does not allow the attainment of a constant isotopic ratio during any experiment. This effect of the δ_X variations is well illustrated in Figure 1 where the $\delta^{13}C_X$ exhibits an increase of ≈ 3 ‰ from day 0 to day 75 of the experiment, resulting in an enrichment of the whole body on the last sampling date. The effect of mass on the isotopic ratio of *Crassostrea gigas* is also well illustrated by the Figure 4. Indeed, the organism increases its body mass throughout the simulation. This results in: *i)* an increase of the mean difference between δ_W and δ_X, *i.e.* the oyster is heavier in terms of isotopic ratio than its food, and *ii)* a decrease of the rate of change in δ_W value in larger organisms.

In simulations of the natural environment over one year (Fig. 6) the discrimination of isotopes in *C. gigas* soft tissues results from the combined effects of organism mass, varying scaled feeding level, temperature, and isotopic ratio of food (see results of S 1, S 2 and S 3). Temperature that influences metabolic rates (assimilation, dissipation, and growth) should only affect the rate of isotopic discrimination in oyster tissues, but not the Δ value itself. For this reason, we considered a varying temperature for our study. The difference in the isotopic ratios between the individual tissues and the food in a dynamic environment, *i.e.* $\delta_W - \delta_X$, exhibits a range of variation of 2.02 ‰ for carbon and 3.03 ‰ for nitrogen (Figs. 6 C and 6 D). This range of variation emphasizes the potential errors that can occur when static traditional approaches are used to access to the contribution of food sources for interdidal suspension-feeders (Dubois et al., 2007a). Indeed, the isotopic ratio of a consumer is corrected from the discrimination factor Δ and then compared with the isotopic ratio of food sources with a $\delta^{13}C$ - $\delta^{15}N$ plot. A mixing model (Phillips, 2001) can then

be used to quantify contribution of the different food sources to the consumer diet. The weakness of this method, which has been widely applied in coastal ecosystems to study the benthic invertebrate diets (*e.g.* Riera et al., 2004; Riera and Richard, 1996; Kang et al., 2003) is related to the estimation of the trophic-shift value. For example, Dubois et al. (2007a) report a difference of 0.85 ‰ and 0.79 ‰ for carbon and nitrogen Δ values (namely the difference between the commonly assumed: $\Delta^{13}C = 1.00$ ‰ and $\Delta^{15}N = 3.50$ ‰ and their estimations: $\Delta^{13}C = 1.85$ ‰ and $\Delta^{15}N = 3.79$ ‰) lead to a difference of 13 %, 11 %, and 9.4 % in the contribution of the microphytobenthos to the *C. gigas* diet for three data sets (see Dubois et al., 2007a). It is therefore understandable that a range of variation of 2.02 ‰ and 3.03 ‰ (Fig. 6) can introduce significant errors into the contribution of the food source. The equilibrium assumption of static mixing models does not consider food (isotope) assimilation flux as a dynamic process, which therefore introduces another bias into the estimation of the long-term effect of the diet. This is because the isotopic ratio of an organism reflects the isotopic ratio of past (recent) and present food.

The use of a standard DEB model is of increasing interest to capture the bioenergetics and physiology of molluscs, *e.g.* *Mytilus edulis* (Rosland et al., 2009; Van Haren and Kooijman, 1993) and *Crassostrea gigas* (Pouvreau et al., 2006; Bourlès et al., 2009; Bernard et al., in press.; Ren and Schiel, 2008) according to environmental fluctuations. Although most applications of DEB models deal with energy budgets, the DEB theory also specifies the elemental composition to access to a more detailed level of metabolic organisation. Our description of the biochemical composition of *C. gigas* in a standard DEB model and the recent development of the dynamic isotope budget (Kooijman, 2010) concepts allow us to investigate two critical points in isotopic ecology: the impact of scaled feeding level and organism mass on isotope incorporation and discrimination. To our knowledge, the Dynamic Energy Budget theory is the first to propose a mechanistic description of isotope fluxes and discrimination among assimilation, growth, and dissipation in living organisms. Furthermore, the use of a dynamic isotope budget required only three more parameters than in the standard DEB models. Although estimation methods for DEB parameters are still in development, rapid progress has been made (Lika et al., subm). Our study gives a first calibration of the DIB model based on the data by Dubois et al. (2007a), but some improvements are still required in relation to both modelling and experimental procedures. For instance, a fractionation experiment involving two or more feeding levels during a growth survey of oyster could provide useful uni-variate data set (*i.e.* mass, length, C:N ratio against time) to refine the parameter estimation in the covariation method of Lika et al. (subm).

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