The impact of metabolism on stable isotope dynamics: a theoretical framework

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Stable isotope analysis is a powerful tool used for reconstructing individual life histories, identifying food-web structures and tracking flow of elemental matter through ecosystems. The mechanisms determining isotopic incorporation rates and discrimination factors are, however, poorly understood which hinders a reliable interpretation of field data when no experimental data are available. Here, we extend dynamic energy budget (DEB) theory with a limited set of new assumptions and rules in order to study the impact of metabolism on stable isotope dynamics in a mechanistic way. We calculate fluxes of stable isotopes within an organism by following fluxes of molecules involved in a limited number of macrochemical reactions: assimilation, growth but also structure turnover that is here explicitly treated. Two mechanisms are involved in the discrimination of isotopes: (i) selection of *molecules* occurs at the partitioning of assimilation, growth and turnover into anabolic and catabolic sub-fluxes and (ii) reshuffling of *atoms* occurs during transformations. Such a framework allows for isotopic routing which is known as a key, but poorly studied, mechanism. As DEB theory specifies the impact of environmental conditions and individual state on molecule fluxes, we discuss how scenario analysis within this framework could help reveal common mechanisms across taxa.

Keywords: stable isotope ratios; discrimination mechanisms; dynamic energy budget theory; metabolism; reshuffling; molecule selection

1. INTRODUCTION

In ecological studies, stable isotope analysis (SIA) has been successfully applied to reconstruct diet and migration patterns of organisms, identify food-web structures and track flows of elemental matter within an ecosystem (Fry 2006). It relies on the existence of chemical elements with two or more stable isotopes that are unevenly distributed among compounds or compartments. Heavy isotopes of the commonest elements in the biosphere (¹³C, ²H, ¹⁸O, ¹⁵N) are rare, representing less than 1 per cent of the carbon, hydrogen, oxygen and nitrogen on earth. Yet there are well-documented, though small, variations in their proportions: e.g. leaves are typically depleted in ¹³C compared to atmospheric CO₂ (Farquhar *et al.* 1989).

The observation that animal tissues reflect the isotopic composition of their diet but are typically enriched

in ¹³C and ¹⁵N (DeNiro & Epstein 1978, 1981) is key to most SIA applications in ecology. However, the underlying mechanisms are still poorly understood (Martínez del Río *et al.* 2009). A large number of factors, all relating to metabolism, could explain the overall trophic increase in rare isotopes in animal tissues: nutritional status, body size, diet quality, assimilation efficiency, excretion form, protein turnover rates (Minagawa & Wada 1984; Adams & Sterner 2000; Vanderklift & Ponsard 2003; Barnes *et al.* 2007). Several models have been developed to investigate some of these factors (e.g. Ponsard & Averbuch 1999; Harvey *et al.* 2002; Olive *et al.* 2003; Marin-Leal *et al.* 2008). But the number of processes involved and the variability of observed patterns led Wolf *et al.* (2009) to renew their call both for more experiments and the development of theoretical models.

In the present study, we develop mechanistic theory that relates metabolism to isotope fluxes within an organism. Isotopes of atoms are embedded into molecules. To mechanistically understand isotope dynamics, we need to follow the fate of molecules within an organism and how atoms are redistributed among molecules during chemical transformations.

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Figure 1. Proposed mechanisms for stable isotope discrimination in a transformation: the transformation of one generalized compound into other compounds occurs in *several steps*. Mobilization does not modify the isotopic composition of the remaining compound. Discrimination occurs during (i) selection (circled cross symbol) of molecules according to their allocation to the catabolic (energy) and the anabolic routes (building blocks) and (ii) the reshuffling of atoms during the chemical reaction, if there is more than one substrate. If only one substrate is involved in the macrochemical reaction, then the products have the same isotopic composition as the substrate after selection: product 1 (P1) has the same isotopic composition as substrate 1 (S1) in the catabolic route. If two or more substrates and two or more products are involved in the reaction, then the isotopic ratio of e.g. P2 depends on the fraction of atoms that originate from S1 and S2. Here, most of S2 atoms went to P2. Colours describe the proportion of heavy isotopes.

Previous approaches made the implicit assumption that molecules during transformations were disassembled into their elemental components (Martínez del Río *et al.* 2009) and that fluxes of elements could be studied independently. We aim at developing a theoretical framework that relaxes this assumption.

We work within the framework of dynamic energy budget (DEB) theory (Kooijman 2010), which provides us with a framework that already specifies the impact of metabolism on molecule fluxes. DEB theory defines metabolism as a set of three chemical transformations—assimilation, growth and dissipation—where substrate molecules are transformed into product molecules. These transformations fully specify all mass fluxes within an organism, and between an organism and its environment. To follow the isotopic composition of these fluxes, new developments are nonetheless required. The turnover process, critical to understand isotopic incorporation rates (Martínez del Río *et al.* 2009), is implicit in the standard DEB framework and needs careful attention. Mechanisms for discrimination, i.e. change in isotopic composition, also need to be defined.

This paper first presents the extensions required to introduce stable isotopes within the DEB framework and the proposed mechanisms for isotopic discrimination in this framework. Second, we describe the resulting dynamic isotope budget (DIB) model. We then illustrate with numerical simulations the impact of body length and nutritional status on the isotopic composition of organisms experiencing constant and fluctuating isotopic composition of their food. Finally, we discuss how the framework can be used to explain

the observed variability in isotopic incorporation rates and discrimination factors among individuals and among species.

2. INTRODUCING STABLE ISOTOPE DYNAMICS WITHIN DEB THEORY

DEB theory provides a conceptual and quantitative framework for studying isotopic incorporation rates within an organism and discrimination mechanisms. To this end, the standard DEB model (Sousa *et al.* 2010) must be extended with a set of consistent assumptions at a more detailed level of organization. These assumptions should specify how isotopes of atoms in molecules travel through the organism ‘jumping’ from one molecule to another. In this section, we summarize the existing DEB framework (see table 1) and introduce the two DEB principles developed to study stable isotope dynamics: turnover of structure and recognition of anabolic and catabolic routes in transformations. We then detail the mechanisms for isotopic discrimination (figure 1).

(a) Standard DEB framework

DEB theory describes the rates at which an organism assimilates and uses energy for maintenance, growth and reproduction as a function of its state and its environment (i.e. food density and temperature; Kooijman 2010; Sousa *et al.* 2010). Each metabolic function defines a chemical transformation, which is key for a mechanistic understanding of stable isotope dynamics. These transformations involve four organic generalized compounds with constant elemental

Figure 2. Representation of the processes that impact the isotopic composition of structure in a time step (dt), (i) growth (standard DEB model), (ii) turnover and (iii) recycling, with dV_G , dV_L and dV_{Lr} the amounts of structure produced during these processes, respectively.

composition: food, reserve, structure and faeces, and four mineral compounds: carbon dioxide, water, dioxygen and N-waste (NH_3 , urea, etc.; Kooijman 2010, pp. 83–89). Three transformations are defined in the standard DEB framework:

- *assimilation*: the conversion of food to reserve and products (including faeces),
- *growth*: the conversion of reserve to structure and products,
- *dissipation*: the conversion of reserve to products.

The products leave the organism, and all three transformations require (environmental) dioxygen as substrate, which is here supposed to be non-limiting. The three transformations can be presented in the form of macrochemical reaction equations (Kooijman 2010, pp. 94–95), where we follow four elements (C, H, O and N). These macrochemical reaction equations specify the appearance and disappearance of all compounds. However, they do not provide the destination of each atom and new developments are required to follow atoms and isotopes of these atoms in transformations.

(b) New requirements within the DEB framework

Although DEB theory provides us with a detailed framework to study mass fluxes, new developments are required to follow isotope fluxes. In particular, we need to carefully specify one transformation that is key to understand isotopic incorporation rates: the turnover of structure. In order to study isotopic discrimination, we also need to specify the anabolic and the catabolic routes of each transformation for which molecules can be selected.

(i) Turnover of structure

In order to study the changes in the isotopic composition of *structure*, we need to define the *turnover* of structure. Unlike reserve, structure requires maintenance. This process is a significant part of the volume-specific somatic maintenance processes. It is, however, not explicitly described in the standard

Table 1. DEB assumptions and definitions on compounds, transformations and fluxes within an organism, as relevant for stable isotope dynamics.

DEB theory delineates four organic generalized compounds: food X , reserve E , structure V and faeces P . The biomass of an individual is composed of reserve E and structure V .
The elemental composition of the organic compounds is species-specific, but does not change throughout the life of the organism (strong homeostasis assumption).
The number of elements per compound is the same for all organic compounds. The standard DEB model deals with the four most abundant elements in living organisms: carbon C, hydrogen H, oxygen O and nitrogen N.
Mass of compounds is given in C-moles, i.e. the amount of each element is expressed relative to the amount of carbon in this compound. Compound formula is thus given by $\text{CH}_{nH}\text{O}_{nO}\text{N}_{nN}$ with n_{xi} the proportion of atoms of element x ($= \text{H, O, N}$) relative to carbon in compound i ($= X, E, V, P$).
The number of elements per compound fixes (and is equal to) the number of mineral compounds in each transformation. Hence, the mineral compounds that are associated with each transformation are: carbon dioxide C (CO_2), water H (H_2O), dioxygen O (O_2) and N-waste N (NH_3 , urea, etc.).
A transformation describes an irreversible conversion of <i>substrates</i> into <i>products</i> and is represented by a <i>macrochemical reaction equation</i> for which stoichiometry principles apply.
Fluxes of compounds can participate in three types of metabolic transformations only: assimilation, growth and dissipation.
Assimilation is the transformation of two substrates, food X and dioxygen O , into five products, reserve E , carbon dioxide C , water H , N-waste N and faeces P .
Growth is the transformation of two substrates, reserve E and dioxygen O , into four products, structure V , carbon dioxide C , water H and N-waste N .
Dissipation encompasses the transformations of two substrates, reserve E and dioxygen O , into three products, carbon dioxide C , water H and N-waste N .

DEB model as there is no *net* production of structure: the incoming flux of renewed structure is compensated by the outgoing flux of degraded structure. However,

these two fluxes may have different isotopic composition (figure 2). Thus, we need to quantify these fluxes and their isotopic composition. We further assume that part of the degraded structure can be recycled to form renewed structure (figure 2). For example, non-functional proteins could be broken down into amino acids that are reused to form new functional proteins.

(ii) *Catabolic/anabolic routes of a transformation*

We detail the *catabolic* and *anabolic* routes of each chemical transformation to allow selection of substrate molecules depending on their isotopic composition (figure 1). The catabolic route of a transformation uses *substrates to produce energy* for the anabolic route of the transformation. The anabolic route of a transformation uses this energy and *substrates as a source of building blocks* to produce a given compound. In the growth transformation for instance, reserve is used as a source of energy as well as building blocks to produce structure.

(c) *Discrimination mechanisms during transformations*

The standard DEB framework and the new developments presented in the previous section define a framework in which mass fluxes within an organism are carefully specified. This section presents our assumptions for the change in isotopic composition of these fluxes, i.e. *discrimination*. We define three steps in a transformation: mobilization, selection of molecules for anabolic and catabolic routes and reshuffling of atoms (figure 1). We assume that only the two last steps of a transformation could result in a change in isotopic composition.

H1. *Mobilization*. Mobilization of a compound from a pool does not modify the isotopic composition of this pool.

This assumption follows from the strong homeostasis assumption. For example, if we think of two large starch molecules, one that contains one ^{13}C and one with only ^{12}C , we can reasonably assume that their mass difference is not sufficiently different to allow selection.

H2. *Molecule selection*. Mobilized molecules have different probabilities to be selected for the anabolic or the catabolic route of a transformation depending on their isotopic composition (figure 1). The number of molecules with one rare isotope in the anabolic route of a transformation is given by the mean of a Fisher's noncentral hypergeometric distribution.

At this step, we assume that molecules can be selected according to their isotopic composition and that the probability of selecting one type of molecule for a given metabolic route depends on its fate. For example, once mobilized and monomerized, starch becomes available as small molecules of glucose; a molecule of glucose that contains a ^{13}C has a significant different weight compared with a molecule of glucose with only ^{12}C . A possible mechanism for selecting molecules with only light isotopes for the catabolic route for instance could be that light isotopes

have weaker binding and could be more easily broken down.

Formally, we cannot select molecules in a flux to separate the flux into two sub-fluxes (the anabolic and catabolic). We can only select from a pool, and convert the flux to an infinitesimally small pool by multiplication with an infinitesimally small time increment dt . On the assumption that selection is at random, and that molecules with a heavy isotope have a deviating probability to be selected, the number of molecules with heavy isotopes follows Fisher's noncentral hypergeometric distribution (McCullagh & Nelder 1989). We choose to refrain from a full stochastic formulation of the selection process and use the mean value of this distribution to follow the fate of isotopes. In particular, we suppose that selection occurs at the separation of the catabolic and the anabolic fluxes. Further details can be found in Kooijman (2010, pp. 97–100).

H3. *Atom reshuffling*. Allocation of atoms of a substrate molecule to products is not random.

The concept of atom reshuffling recognizes that molecules are not completely disassembled into elements during chemical reactions. To illustrate why atom reshuffling might impact stable isotope ratios, we use the photosynthesis example: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + \text{O}_2$. The atoms of O_2 produced during photosynthesis for instance all originate from water, implying that the isotopic composition of O_2 depends only on the isotopic composition of the water, with the oxygen isotopic composition of CO_2 being irrelevant. To further illustrate this effect of atom reshuffling of substrates on the isotopic composition of a product, we detail the fate of the atoms from substrates to products in a transformation involving two substrates and two products: $S_1 + S_2 \rightarrow P_1 + P_2$ (figure 1). We present the theory for the general case of a transformation with multiple substrates and products in appendix C and further details can be found in Kooijman (2010, pp. 96–97). The reshuffling concept is particularly relevant to discrimination in assimilation in cases where the reserve and structure of food are distinguished or where two types of food are considered.

3. DIB MODEL

The DIB model describes the changes in reserve and structure of an organism, the amount of organic and mineral compounds exchanged with the environment, and the isotopic compositions of these different compounds. These changes depend on the state of the organism and the environmental conditions it experienced: food density, temperature and isotopic composition of its food. The standard DEB model determines the different mass fluxes involved in these changes. Our contribution specifies the proportions of isotopes in these fluxes.

(a) *Standard DEB model*

The full description of the standard DEB model, its notation, and the scheme of the different fluxes can be found in Sousa *et al.* (2010). An individual is described by two state variables: the reserve mass M_E

Table 2. State and forcing variables, parameter values and standard DEB equations for the dynamics of reserve and structure. Parameters are taken from Kooijman (2010) for a generalized animal with organic compounds $\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$. Rates are given at the reference temperature $T_1 = 293 \text{ K}$ ($=20^\circ\text{C}$).

variables		interpretation
M_E		reserve mass (mol)
M_V		structural mass (mol)
$e = M_E \dot{v} / (L^3 \{ \dot{J}_{EAm} \})$		scaled reserve density
$L = (M_V / [M_V])^{1/3}$		volumetric length (cm)
$L_f = L / \delta_M$		length (cm)
X		food density (mmol l^{-3})
T		temperature (K)
$f(X) = X / (X + K)$		scaled functional response
$c(T) = \exp(T_A / T_1 - T_A / T)$		temperature correction
parameters	value	interpretation
T_A	8000	Arrhenius temperature (K)
K	0.0159	saturation coefficient (mmol l^{-3})
$\{ \dot{J}_{EAm} \}$	0.0826	maximum surface-area-specific assimilation rate ($\text{mmol cm}^{-2} \text{ d}^{-1}$)
$[\dot{J}_{EM}]$	0.033	volume-specific somatic maintenance rate ($\text{mmol cm}^{-3} \text{ d}^{-1}$)
$[M_V]$	4	volume-specific structural mass (mmol cm^{-3})
$\dot{v}$	0.02	energy conductance (cm d^{-1})
κ	0.8	fraction of used reserve to growth + maintenance
y_{VE}	0.8	yield of structure from reserve in growth
$L_m = \kappa \{ \dot{J}_{EAm} \} / [\dot{J}_{EM}]$	2	maximum volumetric length (cm)
δ_M	0.2	shape coefficient
$g = \dot{v} [M_V] / (\kappa \{ \dot{J}_{EAm} \} y_{VE})$		energy investment ratio
equations		
	$\frac{d}{dt} M_E = \dot{J}_{EA} + \dot{J}_{EC},$	(3.1)
	$\frac{d}{dt} M_V = \dot{J}_{VG} = -(\kappa \dot{J}_{EC} - \dot{J}_{EM}) y_{VE}$	(3.2)
	with $\dot{J}_{EA} = c(T) f(X) \{ \dot{J}_{EAm} \} L^2$	(3.3)
	$\dot{J}_{EC} = -c(T) \{ \dot{J}_{EAm} \} L^2 \frac{ge}{g + e} \left(1 + \frac{L}{gL_m} \right)$	(3.4)
	and $\dot{J}_{EM} = -c(T) [\dot{J}_{EM}] L^3.$	(3.5)

(mol) and the structural mass M_V (mol). For simplicity, we do not discuss development and reproduction but the reasoning for stable isotope dynamics also applies. The equations for the dynamics of the mass of reserve and structure are given in table 2.

Assimilation (figure 3a) is defined by the following macrochemical reaction equation:

The stoichiometry of each transformation is specified by the different yield coefficients Y_{ij}^k . They define the number of C-moles of compound i produced per C-mole of compound j in transformation k and are ratios of two fluxes: $Y_{ij}^k = \dot{J}_{ik} / \dot{J}_{jk}$, with $\dot{J}_{ik}$ the flux of compound i and $\dot{J}_{jk}$ the flux of compound j taken as the reference rate of the transformation. Using the flux of food $\dot{J}_{XA}$ as the reference rate in assimilation, Y_{EX}^A defines the number of moles of E produced per mole of X . As some compounds (e.g. H_2O) can serve both as products and substrates, we need a sign convention for the calculation of the yield

coefficients. Substrate and product fluxes should have opposite signs and a substrate flux is taken to be negative (see appendix C). For example, the flux of reserve (product) in assimilation $\dot{J}_{EA}$ is positive while $\dot{J}_{XA}$, the flux of food (substrate), is negative. The yield coefficient Y_{XX}^A equals 1 by definition and we have $Y_{EX}^A = \dot{J}_{EA} / \dot{J}_{XA} = -y_{EX}$ and $Y_{PX}^A = -y_{PX}$, with y_{EX} and y_{PX} two model parameters. The elemental compositions of compounds are required for the calculation of the mineral yield coefficients and are model parameters (tables 1 and 2).

Using the flux of reserve $\dot{J}_{EG}$ as the reference rate, growth (figure 3b) is defined by

with $Y_{EE}^G = 1$ and $Y_{VE}^G = y_{VE}$ the yield of structure over reserve in the growth transformation, a model parameter. Calculation of the yield coefficients of the growth transformation is detailed in the electronic supplementary material as an example.

Figure 3. Scheme of the metabolic transformations in a dynamic isotope budget (DIB) model: (a) assimilation, (b) growth and (c) volume-specific somatic maintenance: turnover of structure and other volume-specific somatic maintenance processes. Isotopic composition of a flux can change during: (circled cross symbol) selection of molecules for catabolic and anabolic routes and (filled star) reshuffling of atoms during transformation of substrates into products. *X* food, *E* reserve, *V* structure, *P* faeces, *O* dioxygen, *C* carbon dioxide, *H* water and *N* N-waste (e.g. NH_3 , urea).

Dissipation encompasses the transformation of reserve into mineral products in the following processes: somatic and maturity maintenance, development and the conversion of the reproduction buffer into offspring (overheads of reproduction). Using the flux of reserve j_{ED} as the reference rate, we obtain:

$$Y_{EE}^D E + Y_{OE}^D O \rightarrow Y_{CE}^D C + Y_{HE}^D H + Y_{NE}^D N \quad (3.8)$$

with $Y_{EE}^D = Y_{CE}^D = 1$ as reserve *E* is the only substrate and carbon dioxide *C* the only product containing carbon atoms. Among the dissipation processes, structure turnover does not modify structural mass but impacts its isotopic composition and should be detailed (figure 3c).

(b) Structure turnover

The turnover of structure can be described as two coupled macrochemical reactions: the production of

renewed structure *L1* and the degradation of structure *L2* (figure 3c). Using the flux of renewed and degraded structure j_{VL1} and j_{VL2} ($= -j_{VL1}$) as reference rates, we obtain:

$$L_1 : Y_{EV}^{L1} E + Y_{OV}^{L1} O + Y_{VrV}^{L1} V \rightarrow V + Y_{CV}^{L1} C + Y_{HV}^{L1} H + Y_{NV}^{L1} N \quad (3.9)$$

and

$$L_2 : V + Y_{OV}^{L2} O \rightarrow Y_{VrV}^{L2} V + Y_{CV}^{L2} C + Y_{HV}^{L2} H + Y_{NV}^{L2} N \quad (3.10)$$

with

$$j_{EL} = \kappa_L j_{EM}, \quad (3.11)$$

$$Y_{EV}^{L1} = \frac{j_{EL}}{j_{VL1}} = -\frac{1}{\gamma_{VE}^L} \quad (3.12)$$

and

$$Y_{V,V}^{L1} = \frac{\dot{J}_{V,L}}{\dot{J}_{VL1}} = \frac{\dot{J}_{V,L}}{\dot{J}_{VL2}} = Y_{V,V}^{L2} = -\kappa_{Lr} \quad (3.13)$$

where $\dot{J}_{EL}$ is the flux of reserve allocated to the turnover of structure, which is assumed to be a large fraction of $\dot{J}_{EM}$ the volume-specific somatic maintenance costs, $\dot{J}_{VL1}$ and $\dot{J}_{VL2}$ the renewed structure flux and the degraded structure flux, respectively, and $\dot{J}_{V,L}$ the (negative) recycled structure flux. The fraction of reserve allocated to the turnover of structure κ_L ($\in [0,1]$), the yield of structure from reserve in the turnover transformation y_{VE}^L and the fraction of degraded structure that is recycled κ_{Lr} are new model parameters.

(c) Isotope dynamics in reserve and structure

For clarity, we write the equations for ^{14}N and ^{15}N isotopes but the equations are general for any element. We define the ^{15}N isotope fraction in the reserve γ_{NE}^{15} , in the structure γ_{NV}^{15} and in the whole body (reserve + structure) γ_{NW}^{15} as follows:

$$\gamma_{NE}^{15} = \frac{n_{NE}^{15}}{n_{NE}} \quad (3.14)$$

$$\gamma_{NV}^{15} = \frac{n_{NV}^{15}}{n_{NV}} \quad (3.15)$$

and

$$\gamma_{NW}^{15} = \frac{n_{NW}^{15}}{n_{NW}} = \frac{n_{NE}^{15}M_E + n_{NV}^{15}M_V}{n_{NE}M_E + n_{NV}M_V} \quad (3.16)$$

with n_{NE}^{15} and n_{NV}^{15} the proportion of ^{15}N atoms in reserve and structure, respectively. Thus, $n_{NE}^{15}M_E$ and $n_{NV}^{15}M_V$ are the total number of ^{15}N atoms in reserve and structure, respectively.

In the literature, the variable δ quantifies the difference between the isotope ratio of a sample and a reference isotope ratio relative to that reference:

$$\delta^{15}\text{N} = 1000 \left(\frac{R_s - R_r}{R_r} \right) \quad (3.17)$$

with $R_s = ^{15}\text{N}/^{14}\text{N}$ the ratio of the frequencies of the heavy and light isotopes in the sample and R_r the international reference standard atmospheric N_2 for nitrogen, $R_r = 0.0036765$ (Fry 2006). As a ratio of ratios, the variable δ is appropriate to compare small differences but not to study dynamics. The ratio R_s relates nonetheless to the variable γ_{Nj}^{15} ($j = E, V, W$) as follows:

$$R_s = \frac{\gamma_{Nj}^{15}}{1 - \gamma_{Nj}^{15}}. \quad (3.18)$$

We use the δ notation in our results and we define the discrimination factor between compartments A and B as the following: $\Delta_{AB} = \delta^{15}\text{N}_A - \delta^{15}\text{N}_B$.

The changes in isotope fraction in the reserve γ_{NE}^{15} is obtained by assuming that there is no isotope selection during mobilization of reserve (H1. Mobilization):

$$\frac{d}{dt} \gamma_{NE}^{15} = \left(\frac{n_{NE}^{15A}}{n_{NE}} - \gamma_{NE}^{15} \right) \frac{\dot{J}_{EA}}{M_E} \quad (3.19)$$

with n_{NE}^{15A} the proportion of ^{15}N in the reserve flux in assimilation $\dot{J}_{EA}$ (equation (3.3)). The expression for n_{NE}^{15A} is given in equation (A 1) and the full derivation for equation (3.19) is given in the electronic supplementary material.

Only the flux of renewed structure $\dot{J}_{VL1}$ impacts the isotopic composition of structure (H2. molecule selection), not the degraded flux of structure $\dot{J}_{VL2}$ (H1. mobilization):

$$\begin{aligned} \frac{d}{dt} \gamma_{NV}^{15} = & \left(\frac{n_{NV}^{15G}}{n_{NV}} - \gamma_{NV}^{15} \right) \frac{\dot{J}_{VG}}{M_V} \\ & + \left(\frac{n_{NV}^{15L1}}{n_{NV}} - \gamma_{NV}^{15} \right) \frac{\dot{J}_{VL1}}{M_V} \end{aligned} \quad (3.20)$$

with n_{NV}^{15G} the proportion of ^{15}N in the flux of structure in growth $\dot{J}_{VG}$ (equation (3.2)) and n_{NV}^{15L1} the proportion of ^{15}N in the flux of renewed structure $\dot{J}_{VL1}$ (equation (3.11)). The expression for n_{NE}^{15G} and n_{NV}^{15L1} are given in equations (A 2) and (A 3) and the full derivation for equation (3.20) is given in the electronic supplementary material.

4. SIMULATIONS

In this section, we illustrate the implementation of the theory by studying three scenarios, each involving two identical organisms experiencing changes in the isotopic value of their food. We assume in all simulations that assimilation discriminates against heavy isotopes while growth and structure turnover discriminate against light isotopes. We follow individual growth, changes in isotopic values in reserve and structure, and the resulting changes at the whole body level. Temperature is kept constant in all simulations, but food levels can be different. At ‘high’ food level, the scaled functional response f is set to 0.9; at ‘low’ food level, $f = 0.2$. Parameters are given in tables 2 and 3.

(a) Incorporation rate depends on individual length

Simulation 1 illustrates that large individuals have slower isotopic incorporation rates (Martínez del Río *et al.* 2009 and references therein). We study the effect of a switch in the $\delta^{15}\text{N}$ of the food for individuals of different lengths. Both individuals experience high food level but individual 2 is born earlier than individual 1. Hence, at the time of diet switch, individual 2 is larger than individual 1 (figure 4a). When the switch occurs, $\delta^{15}\text{N}$ in reserve is the same for both individuals (figure 4b). As recycling of structure during turnover discriminates against light isotopes and individual 2 is older than individual 1 at the switch, $\delta^{15}\text{N}$ values in structure are different between the two individuals (figure 4c). Although individual 1 has a lower isotopic value in structure than individual 2, individual 1 reaches subsequently a new ‘equilibrium’ value faster (figure 4d).

(b) Discrimination factor depends on the reserve density

Observations made at the individual level showed an increase of the diet–tissue discrimination factor in

Table 3. Specific state variables and parameter values for the DIB model. State variables and parameters are dimensionless.

state variables		interpretation
n_{NE}^{15}		proportion of ^{15}N atoms in reserve
n_{NV}^{15}		proportion of ^{15}N atoms in structure
$\gamma_{NE}^{15} = n_{NE}^{15}/n_{NE}$		fraction of ^{15}N in reserve
$\gamma_{NV}^{15} = n_{NV}^{15}/n_{NV}$		fraction of ^{15}N in structure
parameters	value	interpretation
κ_L	0.8	fraction of volume-specific somatic maintenance allocated to structure turnover
κ_{Lr}	0.4762	fraction of structure turnover that is recycled
y_{EX}	0.8	yield of reserve from food in assimilation
y_{PX}	0.1	yield of faeces from food in assimilation
y_{VE}^L	0.63	yield of structure from reserve in turnover of structure
$\beta_{NX}^{15A_a}$	0.999	odds ratio in assimilation
$\beta_{NE}^{15G_a}$	1.005	odds ratio in growth
$\beta_{NE}^{15L1_a}$	1.007	odds ratio in the production of renewed structure
$\beta_{NV}^{15L2_a}$	1.007	odds ratio in the degradation of structure
α_{EX}^{NA}	0.8	reshuffling coefficient for nitrogen from X to E in assimilation
α_{VE}^{NG}	0.8	reshuffling coefficient for nitrogen from E to V in growth
α_{VE}^{NL1}	0.4737	reshuffling coefficient for nitrogen from E to V in turnover
α_{VV}^{NL1}	0.4737	reshuffling coefficient for nitrogen from V to V in turnover

starvation conditions (Adams & Sterner 2000; Oelbermann & Scheu 2002; Gaye-Siessegger *et al.* 2007). In simulation 2, we illustrate this effect in low food conditions rather than starvation conditions as the model is not specified for strong starvation conditions, i.e. when the reserve is not sufficient to cover maintenance costs. We study the effect of nutritional status after a switch in the $\delta^{15}\text{N}$ of the food: after the switch, individual 2 experiences low food levels such that no growth occurs subsequently (figure 5a).

In low food conditions, isotopic incorporation rates are slower both in reserve and structure (figure 5b,c). As no growth occurs after the switch for individual 2, the recycling of structure also results in a larger discrimination factor in structure after day 475. Interestingly, the $\delta^{15}\text{N}$ value for the whole body in individual 2 becomes larger than individual 1 earlier than in structure (day 325, figure 5d). This pattern is explained by the differences in isotopic values in reserve and structure and the relative contributions of reserve and structure to the total biomass. In low food conditions, the reserve to structure ratio, i.e. the reserve density, is lower. As structure is heavier than reserve, it results in a higher isotopic value in the total biomass in individual 2.

(c) *Fluctuations in the isotopic composition of the food*

Seasonal isotopic variation at the base of a food web is commonly propagated to higher levels of a food web, but with decreasing amplitude (e.g. Kiriluk *et al.* 1995; Harvey *et al.* 2002). In simulation 3, we study this pattern by simulating a seasonal variation in the $\delta^{15}\text{N}$ of the food. Individuals are grown in high and low food levels, respectively. Individual 2 is thus

smaller than individual 1 throughout the experiment (figure 6a) and its reserve density is lower. The signature of their food is fluctuating (sinusoid) but is the same for the two individuals (figure 6b).

We observe the same effects of body length as in simulation 1: the larger individual has a slower isotopic incorporation rate which results in a lag between the reserve and the food isotopic value and this lag increases as the individual is growing (figure 6b). However, the isotopic composition of reserve in individual 2 (low food conditions) follows closely the isotopic composition of the food. The individual is small enough, which compensates the effect of a slower incorporation rate when reserve density is small (simulation 2). The isotopic composition of reserve in turn impacts the isotopic composition of structure (figure 6c). The amplitude of the variations of the isotopic composition of both reserve and structure is decreasing as the individuals grow (figure 6b,c) which is consistent with observed patterns (Harvey *et al.* 2002, and references therein). The differences in $\delta^{15}\text{N}$ at the whole body level are also explained by the differences in reserve density between the two individuals as in simulation 2 (figure 6d).

This simulation shows that we can simulate small individuals with $\delta^{15}\text{N}$ levels equal or greater than larger conspecifics (Kiriluk *et al.* 1995). In reserve, this pattern is owing to faster incorporation rates in small individuals (figure 6b). At the whole body level, this pattern is explained by lower reserve density in individual 2 (figure 6d). This simulation emphasizes the value of a bioenergetic approach that integrates the dynamics of the environment with no *a priori* fixed discrimination factor values to understand observed patterns. We show that the model can be used to disentangle different factors.

Figure 4. Simulation 1. (a) Growth in length of individuals born on different dates but experiencing the same conditions (temperature, food density). Changes in $\delta^{15}\text{N}$ in (b) reserve, (c) structure and (d) the whole body (reserve and structure) after a switch in the food $\delta^{15}\text{N}$.

5. DISCUSSION

In this research, we introduced stable isotopes within DEB theory, in order to study in a mechanistic way the impact of metabolism on isotopic incorporation and discrimination within an organism. The new steps involve careful bookkeeping of atoms and isotopes embedded into molecules during transformations. We also required some new components beyond 'standard' DEB, notably recognition of anabolic and catabolic routes in transformations, so as to allow molecule selection and an explicit treatment of structure turnover. Our simulations illustrate that part of the variability in isotopic incorporation rates and discrimination factors among individuals can be explained by differences in body length and nutritional status. We also show the potential of the model to explain patterns in dynamic environments.

We make two important steps towards a mechanistic understanding of the impact of metabolism on stable isotope dynamics. First, we formulate discrimination mechanisms for each of three metabolic functions: assimilation, growth and turnover. The production of excreted products such as CO_2 or N-waste involves a linear combination of these three functions, and can therefore be derived. Here, our approach differs from Ponsard & Averbuch (1999) who treated discrimination during excretion as a single mechanism. In our representation, changes in the isotopic composition of the total N-waste flux could be

explained by changes in the relative contribution of each metabolic function, which may offer new interpretations of observed patterns. Our approach is also consistent with an observation by Carleton & Martínez del Río (2005) who observed that variations in respiration rate, taken as a proxy for metabolic rate, and incorporation rate of ^{15}N and ^{13}C were decoupled. These authors suggested that turnover processes should not be estimated by the total respiration flux. Our approach recognizes that respiration has contributions not only from turnover processes but also from other dissipation processes (development, maintenance of gradients, movement, etc), and from growth and assimilation in growing and feeding individuals. Thus, respiration is not an explanatory variable in our approach.

Second, the new approach allows us to recognize that molecules are not disassembled into their elemental components during transformations. This step was identified by Martínez del Río *et al.* (2009) as important for improving our understanding of stable isotope dynamics in an organism. Other approaches implicitly assume complete mixing of atoms during transformations, thereby preventing the study of isotopic routing. To our knowledge, only one previous study incorporated routing into a mixing model for $\delta^{13}\text{C}$ by assuming that carbon in food proteins was routed preferentially into tissue proteins (Martínez del Río & Wolf 2005). In our approach, the reshuffling principle

Figure 5. *Simulation 2.* (a) Growth in length of individuals grown in the same conditions until diet switch. Individual 2 experiences a decrease in food density after the switch. Changes in $\delta^{15}\text{N}$ in (b) reserve, (c) structure and (d) the whole body after a switch in the food $\delta^{15}\text{N}$ are represented.

allows specification of a non-random allocation of atoms of a particular substrate to a particular product. This mechanism impacts isotopic composition of a given product when two substrates are involved in the transformation. This mechanism is particularly relevant when several food items are considered or when reserve and structure of the food items are considered (e.g. Kuiper *et al.* 2004; Kooijman *et al.* 2007).

In this paper, we adhere rigorously to the assumptions of standard DEB theory, and require that any extensions are consistent with standard DEB principles. This philosophy determines our representation of recycling of structure. A reader could reasonably ask why we do not return the recycled part to reserve first; the answer is that it would require an additional parameter to describe the transformation of structure into reserve (overhead), but much more important it would change the reserve dynamics. For similar reasons, we assume that mobilization does not impact the isotopic composition of the pool from which molecules are mobilized because of the strong homeostasis assumption. The equation describing reserve dynamics (equation (3.1)) is derived from the weak homeostasis assumption Kooijman (2010) and departing from this assumption has far-reaching consequences, e.g. for determining the chemical composition of reserve and structure from data on body composition or to explain the

transition from multiple reserves to single reserve systems in evolutionary contexts (Kooijman 2010).

Although mobilization does not change the isotopic composition of substrate, our simulations lead to the same conclusions to those in Ponsard & Averbuch (1999). These authors found that a dynamic equilibrium between assimilation and excretion could explain trophic enrichment in nitrogen in a fully grown animal: if excretion favours the dissipation of light isotopes in the environment, assimilation should be less biased towards light isotopes. In our simulations, assimilation also discriminates against heavy isotopes and growth and structure turnover discriminate against light isotopes with a higher probability, i.e. odds ratio in assimilation is closer to 1 with the value 1 corresponding to no-selection situations (see table 3 and appendix B). The result is that total biomass, composed of reserve and structure, is heavier than food. Odds ratio values are chosen such that the biomass–food discrimination factors are in the range of observed values (Vanderklift & Ponsard 2003).

Our simulations show that the model can capture the known effects of body length and nutritional status on incorporation rates and discrimination factors among conspecifics in constant and variable food isotopic ratios. As our framework is fully consistent with standard DEB theory, we can fully exploit its properties to further study a variety of scenarios where confounding factors might be involved. At present, we

Figure 6. *Simulation 3.* (a) Growth in length of individuals grown in the same temperature conditions but different food levels. Both individuals experience fluctuations in the isotopic composition of their food. Changes in $\delta^{15}\text{N}$ in (b) reserve, (c) structure and (d) the whole body are represented.

can study the impact of variable temperature and variable food densities. Short-term starvation situations where maintenance costs can be paid from reserve can also be studied, an issue that was not taken into account in the bioenergetic approach developed by Harvey *et al.* (2002) for fish muscle for instance. Strong starvation situations where maintenance costs cannot be covered by reserve are, however, more complex. Responses to these situations can be typically species-specific. Our treatment of structure turnover opens, however, possibilities to treat structure shrinking as a mechanism to cope with strong starvation. The degraded structure might only be partially renewed during structure turnover which results in shrinking. This approach is consistent with the approach developed by Tolla *et al.* (2007) where somatic maintenance is covered by both reserve and structure.

In the present framework, we can relate model outputs to measurements at the whole body level, which integrate tissues with fast and slow turnover rates. To further link model variables to sub-individual data, new developments are nonetheless required. Tissues could be modelled as proportion of both reserve and structure with variable maximum reserve densities and structure turnover rates. Moreover, studies report non-destructive methods that follow stable isotope ratios in time for a given individual in particular tissues such as blood cells or protein plasma, and

also in hairs and feathers (Martínez del Río *et al.* 2009). Measurements in time are of particular importance for the application of DEB theory (Kooijman *et al.* 2008). Application of the theory to such datasets could be a fruitful strategy for better understanding the impact of dynamic environments. Measurements of the exchanges with the environment together with growth are, however, required, e.g. food, faeces, but also N-waste production and reproductive outputs for instance. Our framework could guide the design of such experiments.

Searching for common mechanisms across taxa is perhaps the most promising contribution of this new theory. DEB theory is built on the premise that the mechanisms for the organization of metabolism are not species-specific (Sousa *et al.* 2010). Body size scaling relationships in DEB theory provide a mechanism for the decrease in the specific respiration rate with species maximum size (Kooijman 2010; Sousa *et al.* 2010). These relationships could help reveal which physiological traits are key to explain common patterns across taxa and which traits could be responsible for variations across taxa. As emphasized by Ponsard & Averbuch (1999), the inter-species variability of the tissue–diet discrimination factor may not be random. Understanding the mechanisms responsible for this variability would increase the potential applications of SIA in field studies to species for which no experimental data are available. Following DEB principles

and body size scaling relationships in particular, we also suggest that the variability of incorporation rates of stable isotopes in comparable tissues may also not be random among species as they are determined by metabolic rates. Furthermore, our framework may also account for differences in C:N ratios in reserve and structure and differences in biochemical form of excretion as the chemical composition of molecules is specified. Scenarios could be designed to study why ammonotelic organisms show lower $\delta^{15}\text{N}$ enrichment than ureotelic or uricotelic organisms (Vanderklift & Ponsard 2003) or why herbivorous fish tend to have a more variable food-to-biomass discrimination factor (Mill *et al.* 2007).

This work presents new theory for stable isotope dynamics within an organism in the context of DEB theory. Important steps are made towards a mechanistic understanding of isotopic incorporations rates and discrimination factors among individuals and among species. The theory provides mechanisms for discrimination and deals with dynamic environments, thereby providing a sound basis for further scenario analysis to understand patterns in data and learn more about metabolic organization in general.

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APPENDIX A. ISOTOPIC COMPOSITION OF FLUXES

This section describes how we obtain n_{NE}^{15A} , n_{NV}^{15G} and n_{NV}^{15L} , the proportions of ^{15}N that enter the pools of reserve and structure during assimilation, growth and structure turnover (equations (3.19) and (3.20)). Reserve and structure are here products of these transformations.

$$n_{NE}^{15A} = \alpha_{EX}^{NA} n_{NX}^{15Aa} \frac{1}{y_{EX}}, \quad (\text{A } 1)$$

$$n_{NV}^{15G} = \alpha_{VE}^{NG} n_{NE}^{15Ga} \frac{1}{y_{VE}} \quad (\text{A } 2)$$

and

$$n_{NV}^{15L1} = \alpha_{VE}^{NL1} n_{NE}^{15L1a} \frac{1}{y_{VE}} + \alpha_{VV}^{NL1} n_{NV}^{15L2a} \frac{1}{\kappa_{Lr}}. \quad (\text{A } 3)$$

The dimensionless reshuffling coefficients α_{ps}^{Nk} ($0 \leq \alpha_{ps}^{Nk} \leq 1$) specify what fraction of N atoms in substrate s ends up in product p in transformation k ($k = A, G, L1, L2$). For instance, the number of N atoms that goes in E from X is fixed by the reshuffling coefficient α_{EX}^{NA} . This coefficient does not depend on the isotope fraction of X . Reshuffling coefficients can be defined as model parameters or given by stoichiometry requirements if we assume complete reshuffling (see appendix C and the electronic supplementary material). Note that n_{NV}^{15L1} the proportion of ^{15}N that enter structure during turnover depends on two substrates, reserve and structure, and n_{NV}^{15L2a} the proportion of ^{15}N in the recycled flux of structure $\dot{J}_{VrL}$.

The coefficients n_{Ns}^{15ka} specify the proportion of ^{15}N in substrate s ($s = X, E, V$) in the *anabolic* component of transformation k , i.e. after selection (A2. molecule selection). To study the properties of the model in a simple and deterministic way, we evaluate n_{Ns}^{15ka} with an approximation of the mean of a Fisher's noncentral hypergeometric distribution using the odds ratios β_{Ns}^{15ka} (see equation (B 1)) and κ_{ka} the different fractions of the substrate fluxes that go into the anabolic routes:

$$\kappa_{Aa} = \frac{\dot{J}_{XAa}}{\dot{J}_{XA}} = \frac{y_{EX}}{Y_{EX}^a} \quad (\text{A } 4)$$

$$\kappa_{Ga} = \frac{\dot{J}_{EGa}}{\dot{J}_{EG}} = \frac{y_{VE}}{Y_{VE}^a} \quad (\text{A } 5)$$

$$\kappa_{L1a} = \frac{\dot{J}_{ELa}}{\dot{J}_{EL}} = \frac{y_{VE}^L}{Y_{VE}^{L1a}} (1 - \kappa_{Lr}) \quad (\text{A } 6)$$

and

$$\kappa_{L2a} = -\kappa_{Lr} \quad (\text{A } 7)$$

with for instance $\dot{J}_{XAa} = -\dot{J}_{EA}/Y_{EX}^a$ and $Y_{EX}^a = 1$ by definition of a building block, i.e. all carbon atoms of the food compound in the anabolic component of assimilation are allocated to reserve. Similarly $Y_{VE}^a = Y_{VE}^{L1a} = 1$ by definition of the anabolic component. Values for parameters y_{EX} , y_{VE} and y_{VE}^L are provided in tables 2 and 3.

APPENDIX B. SELECTION FROM FLUXES

Transformations k_a and k_c correspond to the anabolic and catabolic routes of transformation k ($k = A, G, L$), respectively.

We must have $n_{Ns}^{15k} \dot{J}_{sk} = n_{Ns}^{15ka} \dot{J}_{ska} + n_{Ns}^{15kc} \dot{J}_{skc}$ or $n_{Ns}^{15k} = n_{Ns}^{15ka} \kappa_{ka} + n_{Ns}^{15kc} (1 - \kappa_{ka})$. We have $n_{Ns}^{15k} = \gamma_{Ns}^{15k}$ (as mobilisation does not change the isotope ratio of a given compound) and introduce an odds ratio β_{Ns}^{15ka} for the ^{15}N in compound s (E or V for instance) in transformation k_a . The number of isotopes in the anabolic flux times a time increment follows Fisher's noncentral hypergeometric distribution (H2. molecule selection) with approximate mean:

$$n_{Ns}^{15ka} \simeq \frac{2n_{Ns}^{15k} \beta_{Ns}^{15ka}}{\sqrt{B^2 + 4(1 - \beta_{Ns}^{15ka}) \beta_{Ns}^{15ka} n_{Ns}^{15k} \kappa_{ka} - B}}; \quad n_{Ns}^{15kc} = \frac{n_{Ns}^{15k} - n_{Ns}^{15ka} \kappa_{ka}}{1 - \kappa_{ka}} \quad (\text{B } 1)$$

for $B = n_{Ns}^{15k} - (1 - \kappa_{ka}) - (n_{Ns}^{15k} + \kappa_{ka}) \beta_{Ns}^{15ka}$, with parameters β_{Ns}^{15ka} and κ_{ka} , the two new parameters for transformation k . We must have

$$n_{Ns}^{15k} \geq n_{Ns}^{15ka} \kappa_{ka} \quad \text{and} \quad B^2 + 4(1 - \beta_{Ns}^{15ka}) \beta_{Ns}^{15ka} n_{Ns}^{15k} \kappa_{ka} \geq 0. \quad (\text{B } 2)$$

If $\beta_{Ns}^{15ka} = 1$, we have $n_{Ns}^{15ka} = n_{Ns}^{15k}$ and the process is unselective.

For further details, the DEBtool routine `fnchd.m` provides the expected value of Fisher's non-central hypergeometric distribution, its approximation, and the corresponding mean of the binomial distribution

for small samples sizes for comparison purposes (<http://www.bio.vu.nl/thb/deb/deblab/debtool/>).

APPENDIX C. RESHUFFLING

The dimensionless reshuffling coefficient α_{ps}^{Nk} ($0 \leq \alpha_{ps}^{Nk} \leq 1$) specifies what fraction of N atoms in substrate s ends up in product p in transformation k (H3. Atom reshuffling).

Given n_{Ns}^{15k} the relative frequency of ^{15}N in all substrates $s \in S$ in transformation k , the proportions n_{Np}^{15k} are given for $p \in P$ by

$$n_{Np}^{15k} = - \sum_{s \in S} \alpha_{ps}^{Nk} n_{Ns}^{15k} \frac{\dot{J}_{sk}}{\dot{J}_{pk}} = - \sum_{s \in S} \alpha_{ps}^{Nk} n_{Ns}^{15k} \frac{1}{Y_{ps}^k} \quad (\text{C } 1)$$

with S and P the substrates and products in transformation k , respectively, and $\sum_{p \in P} \alpha_{ps}^{Nk} = 1$. If n_s substrates and n_p products exist, the number of reshuffling parameters is $(n_p - 1)n_s$.

The isotope fraction in the flux of structure during growth for instance is given by

$$n_{NV}^{15G} = -\alpha_{VE}^{NG} n_{NE}^{15G} \frac{\dot{J}_{EG}}{\dot{J}_{VG}} = \alpha_{VE}^{NG} n_{NE}^{15G} \frac{1}{Y_{VE}}. \quad (\text{C } 2)$$

If we suppose complete reshuffling, i.e. mixing, of all substrate atoms of nitrogen in a given transformation, the isotope ratios of that element are equal in all products, so for product $p \in P$ we have

$$n_{Np}^{15k} = n_{Np} \sum_{s \in S} \frac{\dot{J}_{sk} n_{Ns}^{15k}}{\dot{J}_{sk} n_{Ns}} = n_{Np} \sum_{s \in S} \frac{n_{Ns}^{15k}}{n_{Ns}} \quad (\text{C } 3)$$

$$\text{and } \alpha_{ps}^{Nk} = \frac{n_{Np} \dot{J}_{pk}}{\sum_{p \in P} n_{Np} \dot{J}_{pk}} = \frac{n_{Np} Y_{ps}^k}{\sum_{p \in P} n_{Np} Y_{ps}^k} \quad (\text{C } 4)$$

with $\dot{J}_{sk}$ taken as the reference rate for the yield coefficients Y_{ps}^k .

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