Oecologia (2008) 156:765–772
DOI 10.1007/s00442-008-1040-z

PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data

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Received: 29 October 2007 / Accepted: 26 March 2008 / Published online: 30 April 2008
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Abstract Ecologists conduct isotopic incorporation experiments to determine the residence time of various stable isotopes in animal tissues. These experiments permit determining the time window through which isotopic ecologists perceive the course of diet changes, and therefore the scale of the inferences that we can make from isotopic data. Until recently, the results of these experiments were analyzed using first-order, one-compartment models. Cerling et al. (Oecologia 151:175–189, 2007) proposed an approach they named the reaction progress variable to: (1) determine how many compartments are needed to describe a pattern of istopic incorporation, and (2) to estimate the size and rate constant of each pool. We elaborate on the approach described by Cerling et al. (Oecologia 151:175–189, 2007) by providing a way to estimate average retention times for an isotope in a tissue (and its associate error) for multi-compartment models. We also qualify the interpretation of the parameters in multi-compartment models by showing that many possible mechanisms yield models with the same functional form. Multi-compartment models are phenomenological, rather than mechanistic descriptions, of incorporation data. Finally, we propose the use of information theoretic criteria to assess the number of compartments that must be included in models of isotopic incorporation.

Keywords Isotopic incorporation · Reaction progress variable · Stable isotopes

Communicated by Mark Chappell.

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Introduction

The measurement of naturally occurring stable isotopes (carbon, hydrogen, nitrogen, oxygen, and sulphur) in animal tissues is a powerful tool in the study of trophic ecology. The isotopic composition of an animal reflects the isotopic composition of its diet in a relatively predictable manner (DeNiro and Epstein 1978, 1981). However, after a diet change, the isotopic composition of an animal's tissues does not change instantaneously (Cerling et al. 2007a and references there). Different tissues and different animals incorporate the diet's isotopic composition at different rates (Tieszen et al. 1983; Carleton and Martínez del Rio 2005). Understanding the dynamics of isotopic incorporation is central to the interpretation of the data that isotopic ecologists gather in the laboratory and the field, and not surprisingly isotopic ecologists have devoted significant attention to the topic (reviewed by Cerling et al. 2007a).

Until recently, most isotopic incorporation studies used first-order, one-compartment models to describe isotopic incorporation data (Martínez del Rio and Wolf 2005, Fig. 1a). Recently, Cerling et al. (2007a) questioned the general use of these simple models and proposed the use of an alternative graphical approach to diagnose whether a data set might reveal the need for more than one compartment or pool to describe an isotopic incorporation data set. This method is potentially important because using the wrong model can lead to erroneous answers for the

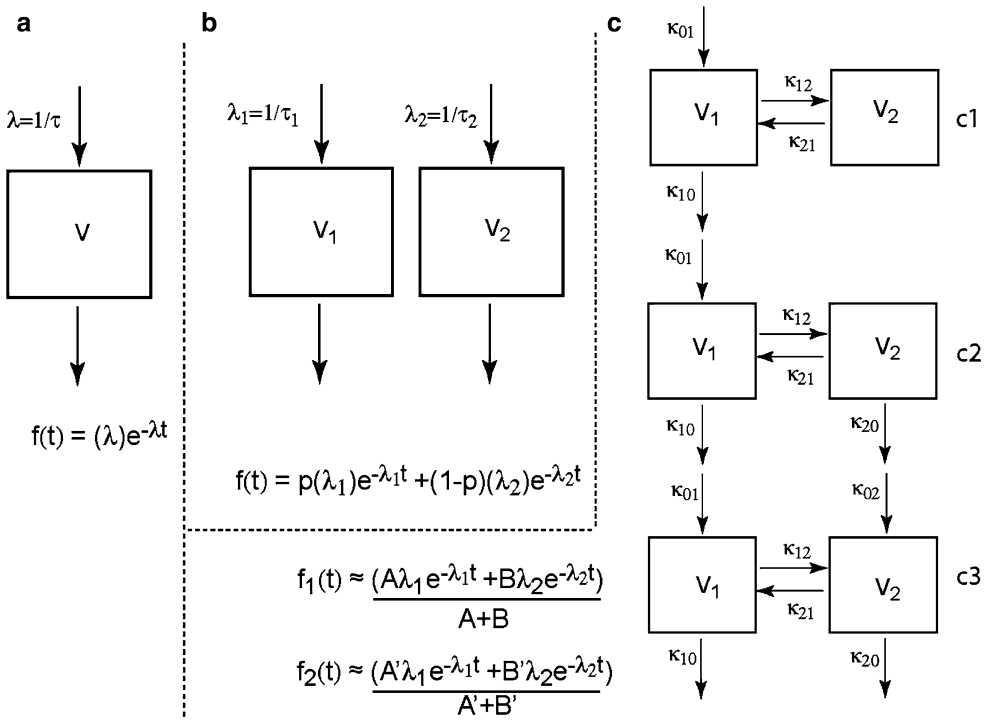

Fig. 1a–c One- and two-compartment models of isotopic incorporation. **a** shows the one-compartment model most widely used to describe isotopic incorporation data. The equations under the *box diagrams* represent the probability density function (PDF) for the residence time of element *X* in each model. **b** shows an interpretation of the structure of the model proposed by Cerling et al. (2007a, b). The *box* surrounding the two compartments indicates that the sample of tissue analyzed for its isotopic composition contains proportions p and $(1 - p)$ of each compartment ($p = V_1/(V_1 + V_2)$). **c** shows three possible mechanistic models all of which yield isotopic incorporation patterns that can be adequately described by bi-exponential PDFs. In these equations, λ_i and A and B (and A' and B' for compartment two) are complex functions of the micro-parameters (κ_{ij}) that govern the exchange of isotopes among compartments. Note that the compartments one and two have different PDFs

questions in which ecologists are interested. The approach championed by Cerling et al. (2007a) relies on "linearizing" the isotopic incorporation data and using least squares linear regression on the resulting linear segments to estimate the relative size of each pool/compartment and its "decay" (i.e., incorporation) constant (Ayliffe et al. 2004). Cerling et al. (2007a) base their method on what they call the reaction progress variable. The application of the reaction progress variable to animals is loosely based on the approach developed by Criss (1999, p. 142 and 205) to study closed multiphase geochemical systems.

Although the need to use the correct model to describe isotopic incorporation data is undeniable, and the method proposed by Cerling et al. (2007a) is appropriate for some applications, we believe that their model often has conceptual and statistical limitations. Here we describe what we believe are the limitations of the reaction progress variable approach and provide alternatives to its use. The alternatives that we outline recognize the need to sometimes use more complex models of isotopic incorporation, but overcome the reaction progress variable's limitations. Our reflections on the reaction progress variable approach are divided into a conceptual and a statistical section.

The conceptual limitations of the reaction progress variable

Quorsum isotopic incorporation studies?

Why are isotopic ecologists interested in the time course of the incorporation of the isotopic signature into an animal's tissues? The reason is that this information determines the time window through which isotopic ecologists perceive the course of diet changes in an animal (Newsome et al. 2007). The time scale of the inferences that we can make from isotopic data about an animal's use of resources is constrained by how fast its tissues incorporate the composition of its diet (Phillips and Gregg 2003; Hobson 1993; Norris et al. 2004). If the animal incorporates the isotopic signature of its diet very slowly, its tissues contain the elements of many meals in the past, and hence their composition integrates the isotopic signature of its diet over a long time interval. In contrast, if the animal incorporates the isotopic signature of its diet rapidly, the isotopic composition of its tissues is very responsive to changes in the isotopic composition of its diet. The rate at which animals incorporate the isotopic signal of their diets

differs among tissues (Tieszen et al. 1983; Hobson and Clark 1992). Some tissues, such as liver and plasma proteins have high turnover rates, and their isotopic composition reflects integration of recent dietary inputs. Others, such as bone collagen, exhibit low incorporation rates and their isotopic composition reflects integration of dietary inputs over longer time periods (Hobson and Clark 1992). This variation in the incorporation rate of different tissues is very useful (Newsome et al. 2007) and has led to the notion that we can use the difference in stable isotope measurements between tissues as a "clock" to date when animals shift diets (Phillips and Gregg 2003). Therefore, the main question that ecologists ask from isotopic incorporation data is what is the average "age" of an isotope in an organism and/or tissue? We believe that an accompanying question, and one that is seldom asked, is how much confidence can we place in this estimate. The reaction progress variable approach, at least in the form described by Cerling et al. (2007a), does not answer the latter question.

Finding average retention times from isotopic incorporation data

For a one-compartment model with first-order kinetics, the average retention time and approximations of its error are easy to estimate. Martínez del Rio and Wolf (2005) have demonstrated that in such a system, the residence time of an element is distributed as an exponential density function of time (t):

$$f(t) = \frac{1}{\tau} e^{\frac{-t}{\tau}} \tag{1}$$

where τ is the average residence time (Fig. 1). Thus, in one-compartment systems with first-order kinetics, the incorporation of the isotopic composition of a new diet can be approximated by:

$$\delta X(t) = \delta X(\infty) - (\delta X(\infty) - \delta X(0)) e^{\frac{-t}{\tau}}, \tag{2}$$

where $\delta X(0)$ is the isotopic composition of the tissues before the diet shift and $\delta X(\infty)$ is the isotopic composition of the new diet assuming that the discrimination factor between tissues and diets, Δ, is 0. If $\Delta \neq 0$, then $\delta X(\infty) = \delta X(\text{diet}) + \Delta$. Martínez del Rio and Wolf (2005) provide a full derivation for Eq. 2.

All the parameters of Eq. 2, including τ, can be easily estimated by non-linear fitting procedures from isotopic incorporation data (Bates and Watts 1988). Equation 2 differs slightly from that used in most isotopic incorporation studies in its use of the reciprocal of the fractional incorporation rate ($\tau = 1/\lambda$, days; O'Brien et al. 2000) as a parameter to describe incorporation rate. We recommend the use of our alternative parameterization for two reasons: τ has a clear intuitive interpretation as the average retention (or residence) time of an element, and the non-linear routines used to fit Eq. 2 gives SE estimates for all its parameters, including $\hat{\tau}$. To estimate the time required for a tissue to replace 90% of an element, one only needs to multiply τ by $\text{Ln}(10) = 2.3$ [i.e., $t_{90} = \text{Ln}(10)\tau = 2.3\tau$]. A more intuitive interpretation is that after two average retention times, the tissues will have replaced $\approx$86.5% of an element. In previous studies, researchers estimated the fractional rate of incorporation ($\lambda = 1/\tau$) and used it to estimate half-lives of an element in a tissue [$t_{1/2} = -\tau \times \text{Ln}(2) = -\text{Ln}(2)/\lambda$]. The half-life is the median of the residence time distribution.

Cerling et al. (2007a) appropriately question the adequacy of one-compartment models for all situations and propose using more complex, multi-compartment models when these are needed. They suggest using the equation:

$$\delta X(t) = \delta X(\infty) - (\delta X(\infty) - \delta X(0)) \left(\sum_i p_i \mathrm{e}^{-\lambda_i t} \right). \tag{3}$$

Cerling et al. (2007a) interpret p_i as the fractional size of each "pool" (hence, $\sum_i p_i = 1$), and λ_i as the rate constants for each pool (Cerling et al. 2007a, p. 178). For reasons that will become clear in subsequent sentences, we prefer the following alternative parameterization:

$$\delta X(t) = \delta X(\infty) - (\delta X(\infty) - \delta X(0)) \left(\sum_i p_i e^{-\frac{t}{\tau_i}} \right) \tag{4}$$

again, with restriction $\sum_i p_i = 1$. Thus there are $k - 1 + k = 2k - 1$ free parameters in the k-compartment model (see Appendix 1). Equation 4 implies that the retention time for an element is distributed as a sum of exponential distributions,

$$f(t) = \sum_i \frac{p_i}{\tau_i} e^{-\frac{t}{\tau_i}}, \tag{5}$$

with average retention time equal to

$$\bar{\tau} = \sum_i p_i \tau_i. \tag{6}$$

Given sufficient data, and assuming that Eq. 4 is an appropriate description of an isotopic incorporation data set, the parameters of Eq. 4 can be estimated using a non-linear regression routine. Such routines typically also estimate SEs for the parameters and a variance covariance matrix (V) for the vector θ of the parameters involved in the estimation of $\hat{\bar{\tau}}$:

$$\theta = (p_1 \cdots p_{k-1}\ \tau_1\ \tau_2 \cdots \tau_k)^{\mathrm{T}}$$

Using the delta method (Stuart and Ord 1994), a variance (s^2) for $\hat{\bar{\tau}}$ can be estimated as:

$$s^2 = \left(\hat{\tau}_1 - \hat{\tau}_2\ \hat{\tau}_2 - \hat{\tau}_3 \cdot \cdot \hat{p}_1\ \hat{p}_1 \cdot \left(1 - \sum_{i=1}^{k-1} p_i\right)\right) \mathrm{V} \begin{pmatrix} \hat{\tau}_1 - \hat{\tau}_2 \\ \hat{\tau}_2 - \hat{\tau}_3 \\ . \\ . \\ \hat{p}_1 \\ \hat{p}_2 \\ . \\ (1 - \sum_{i=1}^{k-1} p_i) \end{pmatrix} \quad (7)$$

We provide a derivation for Eq. 7 in Appendix 1. Equations 6 and 7 complement Cerling's (2007a) reaction progress variable but leave the following questions unanswered:

1. How should we interpret the components of Eq. 5? That is, do the $p_i - s$ and $\tau_i - s$ in Eq. 4, respectively, represent the relative sizes of pools and the residence times in an organism or tissue?
2. How many compartments should we include in our attempts to describe isotopic incorporation patterns?

We answer these two questions in turn.

Pools or phases? The mechanistic interpretation of isotopic incorporation equations

Cerling et al. (2007a) interpret the $p_i - s$ and $\tau_i - s$ in Eq. 4 as relative pool sizes and reciprocals of first-order reaction constants. This interpretation assumes that the pools are independent, and that each sample is a composite to which each pool contributes in proportion to its relative size (Fig. 1b). This is most certainly not the case in physiological pools, which interact, in sometimes complex ways (e.g., Waterlow 2006, ch. 1). Multi-compartment models are widespread, and relatively well studied, in many fields of biology (Harte 1985). They are especially well developed in pharmacokinetics, where their properties and limitations have been recognized for decades (Gibaldi and Peerier 1982). The purpose of pharmacokinetic studies is to develop models to describe the fate of a drug in an organism and in an organism's tissues (Shargel and Yu 1999). This purpose is not that different from that of studies in isotopic incorporation, which aim to determine the residence time of different elements using stable isotopes as markers. Isotopic ecology can be informed by the long history of modeling in pharmacokinetics (Ette et al. 2003).

Pharmacokinetic models can be divided into two broad classes: mechanistic models and phenomenological models (Fleishaker and Smith 1987). The former rely on knowledge of the patterns of absorption, distribution, and excretion to create a mathematical representation of a drug's fate among the tissues of an organism. The latter aim to provide a quantitative description of the changes in the concentration of a drug in an organism and/or its tissues (Fleishaker and Smith 1987). Ideally, we should be able to establish a clear correspondence between a mechanistic and a phenomenological model, but this is not always possible, and in practice it is often not even needed. The models used in isotopic incorporation studies fall in the phenomenological realm. Consider the claim by Cerling et al. (2007a) about the meaning of the parameters p_i and $\lambda_i = 1/\tau_i$ in Eq. 4 as relative pool sizes and first-order rate constants for these pools. Figure 1b depicts graphically the only mechanistic structure consistent with this interpretation for a two-compartment system. Equation 4, however, is also consistent with the mechanistic structures illustrated in Fig. 1c1–c3. In these cases, however, its parameters have very different interpretations.

The case depicted in Fig. 1c1 has been well studied by pharmacologists. In this figure, $V1$ and $V2$ represent pool sizes and κ_{ij} represent first-order transfer constants between compartments. Compartment one is called the "central compartment" and is often used to represent plasma. Compartment two is often assumed to represent peripheral tissues, such as muscle and red blood cells (Shargel and Yu 1999). The κ_{ij} are also called "microconstants" and represent the fractional rate at which the material in pool i is transferred to pool j (Fleishaker and Smith 1987). In the case represented by Fig. 1c1 and at steady state (i.e., the pools are not growing), $\kappa_{01} = \kappa_{10} = \kappa$, and the values of λ_1 and λ_2 are given by solutions to the following system of simultaneous equations:

$$\lambda_1 + \lambda_2 = \kappa_{12} + \kappa_{21} + \kappa \quad (8)$$

$$\lambda_1 \lambda_2 = \kappa_{21} \kappa \quad (9)$$

(Gibaldi and Peerier 1982). The values of p_i are relatively complex combinations of V_i and κ_{ij} and are presented in detail by Gibaldi and Peerier (1982) and Fleishaker and Smith (1987). In all cases, the values of λ_i as combinations of κ_{ij} values depend on the structure of the mechanistic model. For a two-compartment model adequately described by Eq. 4, there are six possible mechanistic alternatives (for simplicity we only present three mechanistic alternatives in Fig. 1). For a three-compartment system, Eq. 4 is an adequate phenomenological description for 13 mechanistic alternatives (Fleishaker and Smith 1987)!

The recognition of a many-to-one relationship between a set of mechanistic models and a single mathematical description led pharmacologists to use the term "phases" rather than pools to refer to the sometimes distinct stages in the incorporation or disposal of a drug (Fleishaker and Smith 1987). Adopting this more neutral terminology in

isotopic incorporation studies may be appropriate, provided that authors are careful to distinguish this use of the term with the common usage of a physicochemical phase in isotope geochemistry.

Statistical considerations

One, two, three…how many compartments?

The reaction progress variable is a useful tool with which to make a preliminary diagnosis about the number of compartments in a system. Briefly, Eq. 3 can be rearranged into

$$(1-F)=\frac{\delta X(\infty)-\delta X(t)}{\delta X(\infty)-\delta X(0)}=\sum_i p_i e^{-\lambda_i t}. \quad (10)$$

We used λ instead of $(1/\tau)$ to maintain consistency with Cerling et al.'s (2007a, b) symbology. The reaction progress variable, $(1-F)$, is then log transformed and the transformed values are plotted against time. Note that Eq. 10 demands that we know the values of $\delta X(\infty)$ and $\delta X(0)$ without error. If the resulting plot is a straight line, the system can be well described by a single compartment model. If the resulting plot is a curve, one can use the graphical method of residuals (also known as "feathering" or "peeling") to identify each compartment. The method is described in detail by Cerling et al. (2007a) and by most pharmacokinetic textbooks (e.g., Shargel and Yu 1999). Feathering is a heuristically valuable method that can be carried out with graph paper and a hand-held calculator capable of fitting linear regressions using least squares.

The alternative to feathering is to make a preliminary visual assessment of the number of compartments from a plot of $\mathrm{Ln}(1-F)$ against time, and then find the parameters of Eq. 4 using a non-linear fitting procedure (Motulsky and Ransnas 1987). This method does not have the restrictive requirement of perfect knowledge about $\delta X(\infty)$ and $\delta X(0)$. These values and their errors are estimated by the method. There are a variety of non-linear fitting algorithms (linear descent, Gauss–Newton, and Levenberg–Marquardt; Motulsky and Christopoulos 2004), most of which find the least squares solutions to estimation equations that are non-linear in the parameters. The available non-linear fitting algorithms are iterative and because they are computationally intensive (at least relative to feathering) demand the use of a computer. Statisticians have repeatedly stressed the advantages of non-linear fitting procedures over their linearization equivalents (Motulsky and Ransnas 1987). One of the serious problems of linearization is that data transformations can distort experimental errors. The distorting effects of log-transforming data can be seen in Fig. 2 as well as in several figures in Cerling et al. (2007a). In these figures the value of the residuals along fitted lines increases with time in the reaction progress plots (Fig. 2a), but is relatively constant along the non-linear trend (Fig. 2b). Log transforms are commonly recommended when variation increases with the response level, but when variation is stable in the original scale, log transforms can inflate variation for small response values.

In many cases, such as the one illustrated in Fig. 2, it is hard to make a clear judgment on the number of compartments in the system. One possibility is to use non-linear regression and fit models with one, two, or three compartments and then judge the goodness of fit of these models. The conventional choice for goodness of fit, the coefficient of determination (r^2) will always improve as

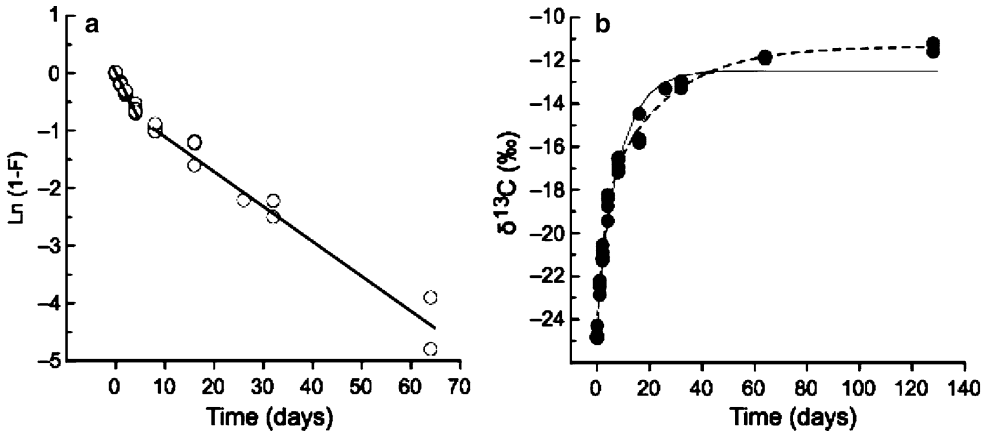

Fig. 2a, b Pattern of incorporation of ^{13}C into the liver of house sparrows (*Passer domesticus*). To construct **a**, we followed Cerling et al.'s (2007a, b) reaction progress variable approach. We used the average value of $\delta^{13}C$ of birds after 128 days on a cracked corn diet as an estimate of $\delta^{13}C(\infty)$ and the measurements on four individuals on day 0 of our experiment to estimate $\delta^{13}C(0)$. Note that this plot indicates that we need at most two compartments in a model of incorporation. The *straight lines* are for illustration only. In **b** we plotted equations fitted to a one-compartment (*solid curve*) or two-compartment (*dashed curve*) model

parameters are added and thus can lead to over-parameterization. In the present application we favor the information theoretic approach advocated by Burnham and Anderson (2002) and widely adopted in ecological studies (Hobbs and Hilborn 2006; Stephens et al. 2007). This approach has a good theoretical foundation and is based on the idea that we should adopt parsimonious models, which avoid under- and over-fitting and give accurate approximations to the interpretable information in the data available (Anderson and Burnham 2001). Briefly, one can estimate the Akaike Information criterion (AIC) for each model and choose the model with the lowest AIC value. Following Burnham and Anderson (2002) we suggest using the small-sample AIC (AICc), if the ratio of number of data points (n) to the number of parameters in the model (K) is small, as is typically the case in biological studies of stable isotopes (Burnham and Anderson 2002):

$$\mathrm{AICc} = n\left[\mathrm{Ln}(2\pi) + 1 + \mathrm{Ln}\left(\frac{\mathrm{SSE}}{n}\right)\right] + 2K + \frac{2K(K+1)}{(n-K-1)}, \quad (11)$$

where SSE is the error sum of squares. AICc can be estimated with a calculator from the output of the non-linear fitting procedure. Models with low AICc values are better supported by data than models with high values. Equation 11 assumes a normal error model consistent with the least squares criterion [see Burnham and Anderson (2002) for details.]

Designing and analyzing isotopic incorporation experiments

Our comments on the reaction progress variable can be summarized in a series of recommendations about how to analyze isotopic incorporation data. The first step is to follow the suggestions of Cerling et al. (2007a) and plot $\mathrm{Ln}(1 - F)$ against time. We believe that this step in the reaction progress approach is an invaluable diagnostic tool for two reasons: it allows doing a preliminary diagnosis of the maximal number of compartments that must be included in the non-linear models, and it allows identification of those cases in which using a multi-compartment model is inappropriate. For example, Cerling et al. (2007b) describe systems that are best described by functions of the form:

$$\delta X(t) = \begin{cases} \delta X(0) & \text{if } 0 \le t \le d \\ \delta X(\infty) - (\delta X(\infty) - \delta X(0))e^{\frac{-(t-d)}{\tau}} & \text{if } t > d \end{cases} \quad (12)$$

where d is a delay (also called transit time). The average retention time in such a system equals $\tau + d$, and the plot of $\ln(1 - F)$ against time has a flat section followed by a descending straight line. Once it has been diagnosed that the system can be described by one, two, or three compartments, we recommend using a non-linear fitting routine to estimate the value of the parameters of Eqs. 2, 4. Given the wide availability of computer programs that perform non-linear fitting effectively, the "feathering" step in the reaction progress variable approach can be omitted.

After constructing a set of two or three alternative models, the value of AICc can be calculated to assess which of these models is best supported. Burnham and Anderson (2002) propose using the difference in AICc ($\Delta_i = \mathrm{AICc}_i - \mathrm{AICc}_{\mathrm{min}}$, where $\mathrm{AICc}_{\mathrm{min}}$ is the lowest value in a comparison) as a measure of the plausibility of an alternative model. They suggest that high values of Δ_i ($\Delta_i > 2$) indicate low support for the alternative model (Burnham and Anderson 2002, p. 70). Equations 6, 7 can be then used to estimate the average retention time of an element in a tissue and its variance in the best model. If Δ_i cannot distinguish between two competing models ($\Delta_i < 2$), it is important to ask whether the two models estimate divergent estimates of $\bar{\tau}$. To our knowledge, this is an exercise that has not yet been done for biological systems.

In Fig. 2 and Table 1, we describe the results of following the steps that we propose here for a data set on the incorporation of ^{13}C into the liver of house sparrows (*Passer domesticus*). Δ_i indicated that a two-compartment model was superior to a one-compartment model [$\mathrm{AICc}(2) \ll \mathrm{AICc}(1)$, Table 1]. We estimated the average retention time for both

Table 1 House sparrows were fed on a C3 diet for 4 months (cracked wheat, $\delta^{13}C = -25.4 \pm 0.2$) and then shifted to a C4 diet (cracked corn, $\delta\ ^{13}C \pm SD = -11.3 \pm 0.2$). Birds were euthanized at intervals (see Fig. 2) and their livers dissected, dried at 45°C to constant weigh, defatted and ground to a fine powder. Their isotopic composition was determined with a continuous flow mass-rationing spectrometer on line with a CHN analyzer (Finnigan Delta+XP, University of Wyoming's light Stable Isotope Facility). The equations describing isotopic incorporation for a one and a two-compartment model were fitted using JMP. SEs and small-sample Akaike information criterion (*AICc*) values were calculated with the formulae in the text

		Average retention time ($\hat{\bar{\tau}} \pm$ SE)	AICc
One compartment	$\delta^{13}C(t) = -12.5 - (11.4)\exp\left(\frac{-t}{8.4}\right)$	8.4 ± 0.9	83.1
Two compartments	$\delta^{13}C(t) = -11.3 - (13.4)\left\{\left(0.4 * \exp\left(\frac{-t}{2.5}\right)\right) + \left(0.6 * \exp\left(\frac{-t}{23.3}\right)\right)\right\}$	14.2 ± 1.6	39.9
Δ_i[a]			43.2

[a] $\Delta_i = \mathrm{AICc}_i - \mathrm{AICc}_{\mathrm{min}}$, where $\mathrm{AICc}_{\mathrm{min}}$ is the lowest value in a comparison

models and found biologically significant differences in the two estimates (Table 1). Using alternative models to estimate average retention time can lead to different inferences. It is fundamentally important to recognize that Δ_i measures the relative plausibility of a model given the data. Thus, model selection depends strongly on the design of the isotopic incorporation experiment (Cerling et al. 2007a). We emphasize that AICc gives a *statistical* criterion to assess the superiority of one model over another given the data. If there are theoretical or empirical reasons for the number of compartments that the system should have, these reasons should be weighed in the choice of models. Given the paucity of mechanistic models of isotopic incorporation models (Martínez del Rio and Wolf 2005), we probably have to rely on the phenomenological approach that we have proposed.

In summary we propose adopting Cerling et al.'s (2007a, b) reaction progress variable approach as a diagnostic tool to assess the number of compartments needed to describe an incorporation data set. Non-linear regression approaches and information theoretic criteria can then be used to determine the best supported model given the data. The resulting parameter estimates can be used to inform ecological studies. We again emphasize that the resulting model is a statistical, rather than a mechanistic, description of the pattern of incorporation. Progress in the study of isotopic incorporation demands that we understand the process of incorporation mechanistically. We find the significance of isotopic incorporation information in field ecological studies. We will find its meaning in physiological research.

Acknowledgements Seth Newsome gave us useful comments. We are grateful to Thure Cerling for developing the reaction progress variable and for comments on drafts of this manuscript. Martínez del Rio was supported by NSF grant ISBN (0421738). The experiments reported here were approved by the University of Wyoming Institutional Animal Care Committee and were conducted by Scott Carleton.

Appendix 1 A variance estimate for $\hat{\bar{\tau}}$

We derive our results for the k-compartment model using the parameterization:

$$\theta = (p_1 \cdots p_{k-1}\ \tau_1\ \tau_2 \cdots \tau_k)^T$$

Estimation of this form with $2k-1$, instead of $2k$ parameters results in a non-singular estimate of its variance covariance, V, but estimates should incorporate the restriction that forces $0 \le p_i < 1$ for all i, and $\sum_i p_i = 1$. For the k compartment, the average retention time is:

$$\bar{\tau} = \sum_{i=1}^{k} p_i\tau_i = p_1\tau_1 + \cdots + p_{k-1}\tau_{k-1} + \left(1 - \sum_{i=1}^{k-1} p_i\right)\tau_k$$

Applying the multivariate delta method, we pre- and post-multiply V by the vector of first partial derivatives:

$$s^2 = \mathrm{Var}(\bar{\tau}) = \left\langle \frac{\delta\bar{\tau}}{\delta\theta} \right\rangle^T V \left\langle \frac{\delta\bar{\tau}}{\delta\theta} \right\rangle.$$

The dimensions of V force this result to be a scalar. The components of the partial derivative vectors are:

$$\frac{\delta\bar{\tau}}{\delta p_i} = \hat{\tau}_i - \hat{\tau}_k \quad i = 1, \ldots, k-1$$

$$\frac{\delta\bar{\tau}}{\delta p_i} = p_i \quad i = 1, \ldots, k-1$$

$$\frac{\delta\bar{\tau}}{\delta p_i} = \left(1 - \sum_{i=1}^{k-1} \hat{p}_i\right) = \hat{p}_k$$

In the case of $k = 2$, the variance reduces to:

$$(\hat{\tau}_1 - \hat{\tau}_2 \quad \hat{p} \quad 1-\hat{p}) V \begin{pmatrix} \hat{\tau}_1 - \hat{\tau}_2 \\ \hat{p} \\ 1-\hat{p} \end{pmatrix}.$$

Most non-linear regression programs include V in their output.

References

Anderson DR, Burnham KP (2001) Commentary on models in ecology. Bull Ecol Soc Am 82:160–161

Ayliffe LK, Cerling TE, Robinson T, West AG, Sponheimer M, Passey BH, Hammer J, Roeder B, Dearing MD, Ehleringer JR (2004) Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. Oecologia 139:11–22

Bates DM, Watts DG (1988) Nonlinear regression and applications. Wiley, New York

Burnham KP, Anderson DR (2002) Model selection and multimodel inference. Springer, New York

Carleton SA, Martínez del Rio C (2005) The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). Oecologia 144:226–232

Cerling TE, Ayliffe LK, Dearing MD, Ehleringer JR, B. Passey BH, Podlesack DW, A. Torregrossa A, West AG (2007a) Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151:175–189

Cerling TE, Bowen GJ, Ehleringer JR, Sponheimer M (2007b) The rection progress variable and isotope turnover in biological systems. In: Dawson TE, Siegwolf RTW (eds) Stable isotopes as indicators of ecological change. Academic Press, New York

Criss RE (1999) Principles of stable isotope distribution. Oxford University Press, New York

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Ette EI, Williams PJ, Kim YH, Lane JR, Liu M, Cappareli EV (2003) Model appropriateness and population pharmacokinetic modeling. J Clin Pharmacol 43:610–623

Fleishaker JC, Smith RB (1987) Compartment model analysis in pharmacokinetics. J Clin Pharmacol 27:922–926

Gibaldi M, An D. Peerier (1982) Pharmacokinetics. Dekker, New York

Harte J (1985) Consider a spherical cow: a course in environmental problem solving. Kaufmann, Los Altos

Hobbs TN, Hilborn R (2006) Alternatives to statistical hypothesis testing in ecology: a guide to self-teaching. Ecol Appl 16:5–19

Hobson KA (1993) Trophic relationships among high Arctic sabirds: insights from tissue-dependent stable-isotope models. Mar Ecol Prog Ser 95:7–18

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181–188

Martínez del Rio C, Wolf BO (2005) Mass-balance models for animal-isotopic ecology. In: Starck M, Wang T (eds) Physiological and ecological adaptations to feeding in vertebrates. Science Publishers, Enfield

Motulsky HJ, Christopoulos A (2004) Fitting models to biological data using linear and non-linear regression: a practical guide to curve fitting. Oxford University Press, Oxford

Motulsky HJ, Ransnas LA (1987) Fitting curves to data using non-linear regression: a practical and non-mathematical review. FASEB J 1:365–374

Newsome SD, Martínez del Rio C, Phillips DL, Bearhop S (2007) A niche for isotopic ecology. Front Ecol Environ 5:429–436

Norris DR, Marra PP, Kyser TK, Sherry TW, Ratcliffe TM (2004) Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. Proc R Soc Lond B Biol Sci 271:59–64

O'Brien DM, Schrag DP, Martínez del Rio C (2000) Allocation to reproduction in a hawkmoth: a quantitative analysis using stable isotopes. Ecology 81:2822–2831

Phillips DL, Eldrige PM (2006) Estimating the timing of diet shifts using stable isotopes. Oecologia 147:195–203

Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oeologia 136: 261-269

Shargel L, Yu BCA (1999) Applied biopharmaceutics and pharmacokinetics. McGraw Hill, New York

Stephens PA, Buskirk SW, Martinez del Rio C (2007) Inference in ecology and evolution. Trends Ecol Evol 22:192–197

Stuart A, Ord JK (1994) Kendall's advance theory of statistics. Distribution theory, vol 1. Arnold, London

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37

Waterlow JC (2006) Protein turnover. CABI International, Cambridge