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Authors: Rio, Carlos Martínez del, and Carleton, Scott A.

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How fast and how faithful: the dynamics of isotopic incorporation into animal tissues

Carlos Martínez del Rio* and Scott A. Carleton

Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071-3166, USA (CMdR)
Cooperative Fish & Wildlife Research Unit, New Mexico State University, Las Cruces, NM 88003, USA (SAC)

* *Correspondent: cmdelrio@uwyo.edu*

The interpretation of isotopic data gathered in the field often demands knowing the rate at which isotopes are incorporated into different tissues and species, and the discrimination factor between tissues and diet. These 2 quantities are estimated in laboratory experiments on diet shifts in which results are interpreted using simple mathematical models, which we describe here. The simplest of these models assumes that each tissue can be represented as a well-mixed, single compartment that obeys 1st-order kinetics. Fitting this model to experimental data allows estimating discrimination factors and the instantaneous rate of isotopic incorporation, λ (the reciprocal of λ, $1/\lambda$, equals the average residence time, τ, of an atom in the tissue). In 1-compartment models the magnitude of λ equals the sum of catabolic turnover and mass-specific growth rate. Examination of available data suggests that the magnitude of λ scales with body mass to an exponent equal to approximately -0.25, differs between endotherms and ectotherms, and could be a useful feature in isotopic incorporation studies. We outline suggestions for the design and analysis of isotopic incorporation experiments and suggest that an increased data set of species and tissues can allow field researchers to estimate rates of incorporation from body size and growth rate data.

Key words: diet, incorporation, isotopic turnover, stable isotopes

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Field biologists interested in using stable isotopes to study animals face several decisions. Among them are what tissue to choose and how frequently to sample this tissue from the individuals in the study population. Independently of factors having to do with ease of collection and animal welfare (Pauli et al. 2009), tissue choice is important in isotopic studies for at least 2 reasons: Tissue to diet discrimination factors ($\Delta X_{tissue-diet} = \delta X_{tissue} - \delta X_{diet}$) can differ among tissues in predictable ways (i.e., bone carbonates tend to be enriched in ^{13}C—Crowley et al. 2010); and rates of isotopic incorporation differ among tissues and taxa (Dalerum and Angerbjörn 2005). The 2nd reason is the theme of this review. Animals do not incorporate the isotopic value of the resources that they consume instantaneously. Rather, the rate at which animals take in and lose elements determines how fast the isotopic value of a novel resource will be acquired in the tissues of an animal. Knowledge about how isotopic incorporation rates differ among individuals, tissues in these individuals, and different taxa is useful and sometimes necessary to interpret field isotopic data. These rates determine the temporal window of resource use captured in isotopic measurements (Martínez del Rio et al. 2009b). Tissues and taxa with high rates of isotopic incorporation reflect the values of resources consumed over a relatively short period of time, whereas those with low rates of incorporation reflect the values of resources over a longer time period. Knowledge about the magnitude of incorporation is not only important to interpret field isotopic data, it also can be used to infer the temporal variability of animal diets. By measuring several tissues with contrasting incorporation rates, we can determine whether individuals are specialists or generalists (Martínez del Rio et al. 2009a; Vander Zanden et al. 2010), and even determine the time-scale at which animals shift among diets with different isotope values (Opell and Powell 2009). A variety of stable isotope applications in animal ecology do not require detailed knowledge of isotopic incorporation, but many do. Isotopic incorporation rate data are essential whenever we are interesting in giving an isotopic application a temporal dimension.

The purpose of this review is to give readers a brief, and we hope, user-friendly compendium of the study and applications of isotopic turnover in animals so that readers can make an educated judgment about whether they need to consider data on incorporation rates in their research, and so that they can design and interpret informative studies that aim to elucidate the time course of isotopic incorporation in their study animals. We 1st describe the experiments that isotopic ecologists conduct to determine isotopic turnover and the mathematical and statistical tools that they use to summarize their outcomes. Then we summarize factors that determine, and hence potentially predict, the magnitude of isotopic incorporation among organisms and tissues. Finally, we provide guidelines for conducting isotopic incorporation experiments. Throughout this piece we attempt to identify what we believe are necessary and fertile areas for further investigation. This paper is not meant to be a comprehensive review of the now-vast literature on isotopic turnover and incorporation, but is instead a primer.

The Basic Kinetic Model

Simple experiments and simple models.—The metrics used to describe isotopic incorporation are estimated from the results of diet-shift experiments. Briefly, a group of animals that have been fed a diet with a certain isotopic composition are diet-shifted to a diet with a contrasting isotopic composition (Fig. 1). In a subsequent section we give details about and recommendations for experimental design and execution. A myriad of these diet-shift experiments have been conducted (Martínez del Rio et al. 2009b and references therein). Following Tieszen et al. (1983) and Hobson and Clark (1992), the majority of the authors of these studies used functions of the form:

$$\delta X(t) = a + be^{-\lambda t}, \quad (1)$$

to describe their data (Bearhop et al. 2002 and references therein). In this equation X is an element (H, C, N, or S), $\delta X_{tissue}(t)$ is the isotopic value of the tissue at time t (we use parentheses to indicate that δX_{tissue} is a function of time), a is the asymptotic isotopic value of the tissue after a diet switch, and b is the difference between the asymptotic isotopic value of the tissue and the isotopic value of the tissue prior to a diet switch; b represents the magnitude of the isotopic change in the tissue. The parameters a, b, and λ are estimated empirically.

Carleton and Martínez del Rio (2010) and Martínez del Rio and Anderson-Sprecher (2008) advocate 2 alternative forms of equation 1:

$$\delta X(t) = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{-\lambda t} \quad (2)$$

and

$$\delta X(t) = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{\frac{-1}{\tau}}. \quad (3)$$

Although equations 1, 2, and 3 are representations of the same model, in our opinion equations 2 and 3 have the virtue of making the biological meaning of the empirical parameters of equation 1 explicit: δX_∞ is the asymptotic value that the tissue reaches after the animal has reached isotopic steady state with the new diet and δX_0 is the initial isotopic value of the tissue (Fig. 1). The values of δX_0 and δX_∞ equal the isotopic value of the new and old diet plus their corresponding tissue to diet discrimination factors, respectively (i.e., $\delta X_0 = \delta X_0 + \Delta X_{tissue-old\ diet}$ and $\delta X_\infty = \delta X_\infty + \Delta X_{tissue-new\ diet}$).

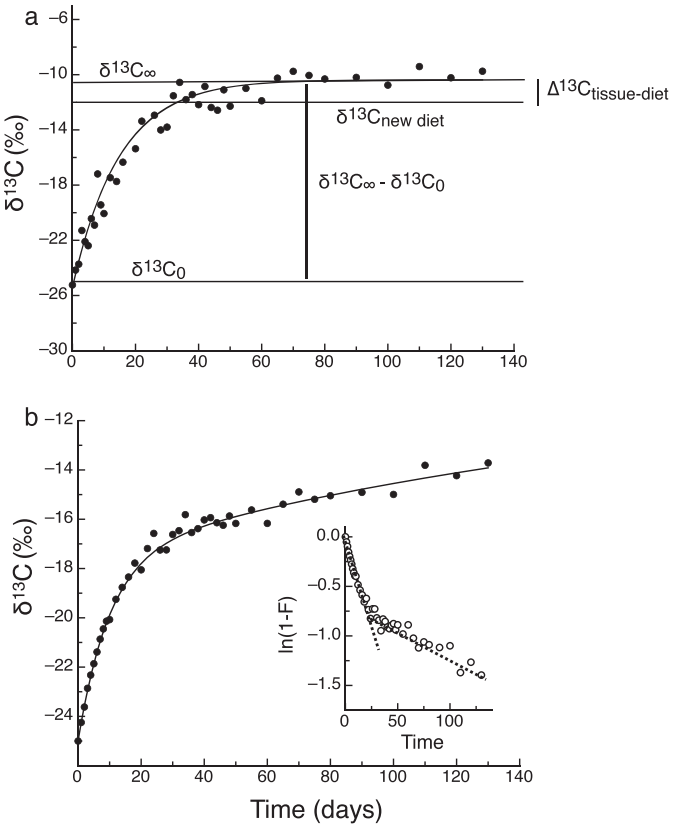

Fig. 1.—Graphic representation of a) parameters of 1-compartment isotopic incorporation models described in text, and b) a 2-compartment system. Inset represents the reaction-progress variable approach to diagnosing whether an isotopic incorporation system requires being described by a model with more than 1 compartment. Values in panels a and b were simulated assuming that $\delta^{13}C_\infty$ and $\delta^{13}C_0$ equaled −10‰ (± *SD* = 0.5‰) and −25‰ (± *SD* = 0.5‰), respectively. Average residence time in panel a was −20 days (± *SD* = 5 days). For panel b, we assumed the following values: λ_1 = 0.01 days^{-1}, λ_2 = 0.05 days^{-1} and P = 0.5. Values were simulated assuming that parameters had normally distributed errors.

Recognizing the biological meaning of the parameters in equations 1, 2, and 3 is informative because it forces us to recognize the assumptions that we make when we choose to describe isotopic incorporation data with an exponential model. Briefly, these equations represent the behavior of a well-mixed, 1-compartment system that obeys 1st-order kinetics (i.e., a constant fraction of the atoms in the pool or tissue enter and exit per unit time—Martínez del Rio et al. 2009a). The parameter λ is a fractional incorporation rate (with units equal to time^{-1}). At steady state, λ represents the instantaneous fractional rate at which materials enter and exit

the pool of elements in a tissue. Thus, if the animal is not growing, we can legitimately call λ an estimate of elemental turnover (Appendix I). Because this is not always the case, we refer to λ as an instantaneous incorporation rate.

In a system at steady state and obeying 1st-order kinetics, the residence times of the atoms of an element in a tissue are distributed as negative exponential density functions. Thus, the half-lives reported in many isotopic incorporation studies ($t_{1/2} = \ln(2)/\lambda$) represent the median of the distribution of residence times of an element in a tissue, and $1/\lambda = \tau$ defines the average age of an atom of element X in a tissue (Martínez del Rio and Anderson-Sprecher 2008). This average has variance equal to $1/\lambda^2$ (Stuart and Ord 1994). Chemical reactor theory provides another straightforward interpretation for λ. This parameter is the ratio of the net instantaneous rate of influx of materials into the pool ($\dot{v}$ in moles/time) divided by the size of the pool (A_T in moles of an element—Levenspiel 1999):

$$\lambda = \frac{\dot{v}}{A_T}. \quad (4)$$

Equation 4 allows estimating the total flux of an element through a tissue from isotopic incorporation data. The net flux of an element through a tissue equals the product of λ and A_T ($\dot{v} = \lambda A_T$ in moles/time). The isotopic incorporation data gathered by isotopic ecologists could be used to study patterns of elemental allocation into different tissues. To our knowledge, this potentially useful observation has not been applied yet.

Effects of body size, temperature, and growth on isotopic incorporation.—Like most biological rates, the magnitude of λ should be influenced by body size and temperature (Brown et al. 2004). Inspection of equation 4 led us to predict that λ would be allometrically dependent on body mass for a given tissue (Carleton and Martínez del Rio 2005). More specifically, we predicted that the fractional rate of isotopic incorporation would scale with body mass raised to approximately the -0.25 power. This prediction stemmed from the observation that the flux of elements into a tissue ($\dot{v}$) should be approximately proportional to mass to the 0.75 power (Brown et al. 2004), whereas the size of most (albeit not all) tissues (and thus A_T) scales isometrically with body mass ($A_T \propto m_b$—Calder 1984). Thus, according to equation 4, the fractional rate of isotopic incorporation (λ) should be proportional to $m_b^{0.75}/m_b = m_b^{-0.25}$. Preliminary data on the rate of ^{13}C incorporation into the red blood cells of several bird species verify this prediction (Carleton and Martínez del Rio 2005), and several new data sets have established its generality for birds and fish (Bauchinger and McWilliams 2009; Weidel et al. 2011). A small data set on mammals suggests that the pattern holds for them as well (Bauchinger and McWilliams 2009), but the sample size for this group is small. The allometric dependence of λ on body mass is useful because it permits making educated guesses about the relative magnitude of isotopic turnover from an organism's body mass. For example, an increase in body mass of an order of magnitude ($10\times$) leads to a decrease by about one-half in the value of λ ($10^{-0.25} = 0.56$) or to an almost doubling in τ and $t_{1/2}$ ($10^{0.25} = 1.8$). According to these values, the tissues of a 10-kg coyote (*Canis latrans*) should retain carbon roughly 3 times longer than those of a 100-g least weasel (*Mustela nivalis*).

Gillooly et al. (2001) proposed that, to a 1st approximation, the magnitude of biological rates can be estimated as the product of an allometric function of body mass and an exponential function of body temperatures (see Martínez del Rio et al. 2009b). Thus, we should expect λ to depend on body temperature. Weidel et al. (2011) analyzed the dependence of λ on body size and temperature of 19 fish species. They found that the model best supported by data included only body mass. However, the data set included only experiments and measurements for a narrow temperature range. We believe that this is a hypothesis that demands attention. Although the temperature dependence of λ has not been established, available data clearly indicate that among vertebrates, ectotherms have much lower incorporation rates than do endotherms when body size has been accounted for (Warne et al. 2010).

Fry and Arnold (1982) were the 1st to recognize that rate at which tissues of an animal incorporate the isotopic value of resources is determined by both the addition of new material to the tissue (growth) and by the replacement of material exported from the tissue as a result of catabolism (turnover). Hesslein et al. (1993) proposed that the value of λ equals the sum of fractional (or mass-specific) net growth k_g ($k_g = m_b^{-1}(dm_b/dt)$) and catabolic turnover k_d ($\lambda = k_g + k_d$ [Appendix I]). If the animal is not growing (i.e., $k_g = 0$), then λ equals the value of catabolic turnover (k_d). If the animal is growing exponentially (i.e., if k_g is approximately constant), then we can measure growth and partition the contribution of net growth and catabolic turnover to λ. Carleton and Martínez del Rio (2010) describe an alternative formulation of equation 2 in terms of mass gain instead of time that allows estimating the relative contribution of k_g and k_d to λ.

Partitioning the contribution of growth and catabolism to λ is more complicated if the animal is not growing exponentially. If we measure the growth of the pool or tissue, we can find the function that describes the change of k_g in time and solve for k_d in the equation:

$$\delta X(t) = \delta X_\infty - (\delta X_\infty - \delta X_0) \exp\left\{ - \int_0^t \left[k_d + k_g(t)\right] dt \right\}. \quad (5)$$

Appendix I shows how this equation is derived. Note that equation 5 reduces to equation 2 for animals growing exponentially. Harvey et al. (2002) describe a very useful approach for the estimation of k_d from bioenergetic models and data on the isotopic value of resources that can be applied to animals growing in the field (Weidel et al. 2011). We emphasize that both equation 5 and the method proposed by Harvey et al. (2002) depend on assuming that a constant fraction of elements in a tissue are replaced per unit time and

that this fraction does not vary during ontogeny. This assumption may or may not be correct. Note also that k_g refers to the mass-specific growth of the pool of an element in a tissue. Although it is tempting (and often justified) to use the change in total mass of an animal as a proxy to estimate k_g, this approximation will be poor if animals change in body composition and hence in elemental (i.e., C:N) ratios as they grow. Fry and Arnold (1982) provide an alternative to the analytical framework described here. Their approach does not require making any assumptions about the functional form of growth, and is very simple to use. Unfortunately, the interpretation of their method is qualitative and graphical and therefore only allows estimating whether growth contributes significantly to turnover or not, but it does not allow partitioning the relative contribution of growth and catabolic turnover to the rate of isotopic incorporation (Fry and Arnold 1982).

How do we incorporate information of tissue growth into studies of isotopic incorporation? It depends on the question that we are asking. If our goal is to estimate λ for comparative purposes, then our experiments must be done on animals at steady state—this might not be possible or physiologically realistic in animals with indeterminate growth. If our goal is to estimate the contribution of growth and catabolism to incorporation rates, then we must estimate the growth rate of the elemental pool of the tissue that we are investigating. In either case, we cannot ignore the influence of growth on the rate of isotopic incorporation. At the very least, researchers must report the changes in mass experienced by their subjects over the course of an isotopic incorporation experiment. Because growth can have an important effect on incorporation, we cannot afford to ignore it in field studies. Those that study ectotherms with seemingly determinate growth might be tempted to dismiss the effect of growth in the interpretation of isotopic field data. However, very large animals continue growing for a long time. Male African elephants (*Loxodonta africana*) for example, show seemingly indeterminate growth and female elephants do not reach asymptotic size until they are 15 years old (Shrader et al. 2006). The effect of growth on incorporation is particularly strong on structural tissues, such as collagen, which retain the isotopic composition of resources consumed during growth much longer than tissues with high protein turnover. The combined effect of a large body size and the slow tissues such as collagen, often studied in animals such as marine mammals and terrestrial ungulates, can complicate the interpretation of field data (Koch 2007). Elephant bones have a long isotopic memory.

Differences in isotopic incorporation among tissues.—Perga and Gerdeaux (2005) observed that the isotopic values of the muscle of whitefish (*Coregonus lavaretus*) reflected diet changes only in the summer, when the fish were growing. In the winter their isotopic value remained relatively constant. In contrast, the isotopic value of liver tracked that of diet throughout the year. This effect is a consequence of the much larger rate of catabolic turnover in liver than in muscle (Carleton and Martínez del Rio 2010). Even in nongrowing animals, there are large differences in isotopic incorporation among tissues (Hobson and Clark 1992; Tieszen et al. 1983).

Carleton and Martínez del Rio (2005) speculated that the primary determinant of the rate of isotopic incorporation in most tissues (whose isotopic composition is typically measured after lipids are extracted—Post et al. 2007) is protein turnover. This hypothesis implies that incorporation rates should be higher in the tissues of splanchnic (or visceral) organs than in muscle, epithelium, and nerve tissues. Available data are consistent with this hypothesis (Bauchinger and McWilliams 2009; Carleton et al. 2008). Bauchinger and McWilliams (2010) observed that tissues with high rates of isotopic incorporation are those that are more likely to change size during migration and fasting. Isotopic incorporation seems to be a predictor of the phenotypic flexibility of an organ. Perhaps the most widely used difference in isotopic incorporation among tissues is that observed between the various components of blood, a tissue that can be sampled easily and nonlethally. The noncellular components of blood have high incorporation rates, whereas the cellular components of blood have slow incorporation rates (Carleton et al. 2008).

Should we use models with more than 1 compartment?—The existence of disparities in the incorporation rates of tissues that exchange materials is inconsistent with the 1-compartment assumption implicit in equation 1. Indeed, researchers investigating protein turnover have routinely considered models with more than 1 compartment to describe their data (Waterlow 2006). Cerling et al. (2007a) proposed a useful tool to assess whether isotopic incorporation models should include more than 1 compartment. Equation 3 can be rearranged to yield:

$$\frac{\delta X_\infty - \delta X_{tissue}(t)}{\delta X_\infty - \delta X_0} = e^{-\lambda t}. \quad (6)$$

Cerling et al. (2007a) call equation 1 the reaction progress variable and denote it by (1 − F), where F measures how much relative ''progress'' the tissue has done in reaching its asymptotic value after time t (Fig. 1). In a 1-compartment system, ln(1 − F) is a decreasing linear function of time with slope equal to $-\lambda$ (Fig. 1):

$$\ln(1-F) = \ln\left[\frac{\delta X_\infty - \delta X_{tissue}(t)}{\delta X_\infty - \delta X_0}\right] = -\lambda t. \quad (7)$$

Systems with many compartments can be adequately described by the following equation:

$$\delta X(t) = \delta X_\infty - (\delta X_\infty - \delta X_0)\left[\sum_{i=1}^{n-1} p_i e^{-\lambda_i t} + (1 - \sum_{i=1}^{n-1} p_i) e^{-\lambda_n t}\right]. \quad (8)$$

In those systems, a plot of ln(1 − F) against t results in a sequence of lines of increasingly shallow slope (Fig. 1). The number of lines represents the number of compartments.

Although the reaction progress variable is valuable in diagnosing whether a system might be better described by models that include more than 1 compartment, alternative methods that rely on nonlinear fitting and model comparisons

are needed to estimate the parameters of the best-supported model. Martínez del Rio and Anderson-Sprecher (2008) give details of how this is done. In summary, one can use the reaction progress variable of Cerling et al. (2007a) to diagnose whether fitting a model with more than 1 compartment is merited and to do a preliminary assessment on the number of compartments in the model. Then one can use a nonlinear fitting routine on each of these models and use model comparison metrics (such as Akaike's information theoretic criterion—Burnham and Anderson 2002) to determine which of these models is best supported by the data. Martínez del Rio and Anderson-Sprecher (2008) recommend summarizing the average retention time of the multi-compartment system defined by:

$$\delta X(t) = \delta X_\infty - (\delta X_\infty - \delta X_0)\left[\sum_{i=1}^{n-1} p_i e^{-\frac{t}{\tau_i}} + (1 - \sum_{i=1}^{n-1} p_i) e^{-\frac{t}{\tau_n}}\right] \quad (9)$$

as

$$\tau = \sum_i^n p_i \tau_i, \quad (10)$$

to characterize the dynamics of incorporation of a multi-compartment system with a single number. For multicompartment systems there is no simple way to characterize the system by a single rate constant, and the half-life ($t_{1/2}$) must be estimated by solving an awkward equation numerically:

$$\frac{1}{2} = \sum_{i=1}^{n-1} p_i e^{-\frac{t}{\tau_i}} + (1 - \sum_{i=1}^{n-1} p_i) e^{-\frac{t}{\tau_n}}. \quad (11)$$

The results of several studies have revealed that with few exceptions (Ayliffe et al. 2004), the model best supported by data includes at most 2 compartments (Bauchinger and McWilliams 2010; Carleton et al. 2008; Warne et al. 2010), and when 2-compartment models are better supported by data these estimate longer retention times than 1-compartment models (Bauchinger and McWilliams 2010; Carleton et al. 2008).

Considerations of Experimental Design

The type of tissue that a researcher plans to use in a field study dictates the choice of experimental design. Some tissues can be harvested relatively noninvasively, and thus allow experimental designs in which many measurements can be done on a single animal. Because blood cells and plasma proteins are convenient to sample and favored by field ecologists, we will use them to exemplify the design and analysis experiments in which animals can be sampled repeatedly. A typical experiment involves maintaining a group of animals on a diet of a given isotopic composition until one is reasonably sure that the tissues are in isotopic steady state, and then shifting the isotopic composition of the diet and measuring the isotopic composition of blood over time. In this design, one can fit equations 2 or 3 (or their multicompartment equivalents) to each individual using a nonlinear fitting routine (typically a Gauss–Newton iterative algorithm—Bates and Watts 1988). If the sample of animals in the experiment is reasonably large, the parameters of these equations should have near-normal distributions (Stuart and Ord 1994). Hence, we can estimate confidence intervals for these parameters in the standard way and then conduct ordinary parametric statistics (e.g., *t*-tests and analysis of variance) to make inferences about them.

How long and how frequently to sample?—Two questions should be considered before conducting these experiments: how often should tissues be measured and for how long should the experiments be conducted? The answer to the 1st question is that, given economic constraints and the need to minimize harm to animals, tissues should be harvested as frequently as possible. Many isotopic incorporation experiments have sampled tissues at intervals that follow a geometrical (2^n) sequence rule (e.g., sampling at 1, 2, 4, 16, …, days). Cerling et al. (2007b) suggest that this sampling schedule might prevent researchers from detecting fast compartments and recommend a sampling protocol that follows a $2^{n/2}$ rule (i.e., sampling at 1, 1.4, 2, 2.8, 4, 5, 6, …, days). This recommendation has to be qualified by the ecologically relevant time windows that researchers might be interested in and by cost. The schedule of Cerling et al. (2007b) includes twice as many sampling dates as the 2^n schedule.

The experiment should be conducted for as long as needed for the tissues to reach isotopic equilibrium with the new diet. Bates and Watts (1988) and Berges et al. (1994) give formal justification for the need to prolong the experiment to equilibrium. How can we determine a priori the duration of an experiment? We cannot, but we can use allometry to make educated guesses. One can assume a 1-compartment model and use allometric equations to approximate how long an experiment must be conducted for avian blood to reach 95% of the new ^{13}C steady state value after a diet change:

$$t_{95\%} = \frac{\ln(20)}{a(m_b)^b}, \quad (12)$$

where $a(m_b)^b$ is the allometric equation relating λ with body mass, and $b \approx -0.25$. Unfortunately, the allometric equations available are restricted to a few taxa (birds and fish—Bauchinger and McWilliams 2009; Weidel et al. 2011), are derived from relatively small species, and apply to only a few tissues.

Some of the same considerations that apply to experiments in which tissues of an animal can be sampled repeatedly also apply to experiments in which tissues must be harvested after an animal has died (liver, muscle, and bone collagen). Timing of measurements must be spaced sensibly and measurements must be done for long enough to achieve isotopic equilibrium. However, data must be analyzed differently. The nonlinear regression routine used to estimate the parameters of incorporation equations is the same; however, in this case we cannot estimate confidence intervals in the standard fashion. There are 3 ways to estimate the confidence intervals for these parameters when we cannot estimate them for a sample of individual subjects that were measured repeatedly.

First, we can obtain estimates of asymptotic confidence intervals. Most computer programs that include nonlinear fitting routines give these in their output. Second, one can use Monte Carlo simulations, and 3rd, use likelihood methods (Motulsky and Christopoulos 2003), or both the 2nd and 3rd methods. Motulsky and Christopoulos (2003) describe in detail how these methods work and the situations in which each is appropriate. Hilborn and Mangel (1997) provide an accessible discussion of the advantages and disadvantages of simulation and likelihood methods to estimate confidence intervals for the parameters of nonlinear models.

In summary, we have described a simple theoretical framework that allows designing and interpreting the results of isotopic incorporation experiments. The outcomes of these experiments (discrimination factors, average retention times, and rates of isotopic incorporation) are needed to interpret field data. The allometric dependence of isotopic incorporation parameters is a particularly useful feature of the framework described here. Its wide applicability in field studies is only hindered by the paucity of data sets on a wider variety of taxa, tissues, and animals with larger M_b. Conducting 1 more isotopic incorporation experiment on a new species, a functional group of species, and on a variety of tissues is not only important to understand the isotopic data for that particular species in the field, it also adds to a body of data that, one hopes, will make performing those experiments unnecessary in the future.

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APPENDIX I

Consider a tissue that contains A_T moles of element X (where X is C, N, H, or S). Then consider the amount (A_H) of the heavy isotope (^2H, ^{13}C, or ^{15}N) in this pool. This amount equals the product of A_T times the fraction of heavy isotopes (f_{Hb}) in the pool: $A_H = f_{Hb}A_T$. We call k_s and k_d (with units equal to time^{-1}) the fractional rates at which the element enters and leaves the pool, respectively, and f_{Hd} and f_{Hb} the fractions of the heavy isotope in the incoming materials (d stands for diet) and in the pool (b stands for body), respectively (Fig. 1). Then,

$$\frac{dA_H}{dt} = \frac{d(A_T f_{Hb})}{dt} = f_{Hb}\frac{dA_T}{dt} + A_T\frac{df_{Hb}}{dt} \quad (A1)$$

and

$$\frac{dA_H}{dt} = A_T(k_s f_{Hd} - k_d f_{Hb}). \quad (A2)$$

If we combine equations A1 and A2, we get:

$$\frac{df_{Hb}}{dt} = (k_s f_{Hd} - k_d f_{Hb}) - f_{Hb}\left(\frac{1}{A_T}\right)\frac{dA_T}{dt}. \quad (A3)$$

Equation A3 can be simplified by recognizing that $\left(\frac{1}{A_T}\right)\frac{dA_T}{dt} = (k_s - k_d)$:

$$\frac{df_{Hb}}{dt} = k_s(f_{Hd} - f_{Hb}), \quad (A4)$$

or

$$\begin{aligned}\frac{df_{Hb}}{dt} &= (k_s - k_d + k_d)(f_{Hd} - f_{Hb}) \\ &= \left[\left(\frac{1}{A_T}\right)\frac{dA_T}{dt} + k_d\right](f_{Hd} - f_{Hb}).\end{aligned} \quad (A5)$$

To simplify notation, we define $\left(\frac{1}{A_T}\right)\frac{dA_T}{dt}$ as $k_{g(t)}$. Assuming that at time 0, $f_{Hb} = f_{Hb}(0)$, and that $f_{Hd}(t)$ is constant ($f_{Hd}(t) = f_{Hd}$), then we can integrate equation A5 to yield:

$$f_{Hb}(t) = f_{Hd} - [f_{Hd} - f_{Hb}(0)]\exp - \int_0^t [k_g(t) + k_d]\,dt. \quad (A6)$$

If the animal is growing exponentially, and hence $k_g + k_d = \lambda$ is a constant, then we have the more familiar:

$$f_{Hb}(t) = f_{Hd} - [f_{Hd} - f_{Hb}(0)]e^{-\lambda t}. \quad (A7)$$

To be able to satisfy mass balance, we have couched this model in terms of the fractional abundance of the heavy isotope, f_H. To transform these equations into the more widely used delta notation, we need to recognize that when the heavy isotope is (usually) rare (i.e., $f_H << 0.1$), $f_H \approx R_{sample}$ and $f_H \approx R_{standard}$ $(1{,}000\delta X + 1)$. Substituting this expression for f_H into equation A7 leads to equation A8:

$$\delta X_{tissue}(t) = \delta X_{diet} - [\delta X_{diet} - \delta X_{tissue}(0)]e^{-\lambda t}, \quad (A8)$$

where $\delta X_{tissue}(0)$ is the value at the beginning of the experiment prior to the diet shift and δX_{diet} is the value of the new diet. This equation assumes that there is no tissue to diet discrimination. When we include a discrimination factor to account for the difference in isotopic value between tissues and diet (substitute δX_{diet} for $\delta X_\infty = \delta X_{diet} + \Delta X_{tissue-diet}$), we have equation 3.