

Donald L. Phillips · Peter M. Eldridge

Estimating the timing of diet shifts using stable isotopes

Received: 6 April 2005 / Accepted: 27 October 2005 / Published online: 3 December 2005
© Springer-Verlag 2005

Abstract Stable isotope analysis has become an important tool in studies of trophic food webs and animal feeding patterns. When animals undergo rapid dietary shifts due to migration, metamorphosis, or other reasons, the isotopic composition of their tissues begins changing to reflect that of their diet. This can occur both as a result of growth and metabolic turnover of existing tissue. Tissues vary in their rate of isotopic change, with high turnover tissues such as liver changing rapidly, while relatively low turnover tissues such as bone change more slowly. A model is outlined that uses the varying isotopic changes in multiple tissues as a chemical clock to estimate the time elapsed since a diet shift, and the magnitude of the isotopic shift in the tissues at the new equilibrium. This model was tested using published results from controlled feeding experiments on a bird and a mammal. For the model to be effective, the tissues utilized must be sufficiently different in their turnover rates. The model did a reasonable job of estimating elapsed time and equilibrium isotopic changes, except when the time since the diet shift was less than a small fraction of the half-life of the slowest turnover tissue or greater than 5–10 half-lives of the slowest turnover tissue. Sensitivity analyses independently corroborated that model estimates became unstable at extremely short and long sample times due to the effect of random measurement error. Subject to some limitations, the model may be useful for studying the movement and behavior of animals changing isotopic environments, such as anadromous fish, migratory birds, animals undergoing metamorphosis, or animals changing diets because of shifts in food abundance or competitive interactions.

Keywords Diet shift · Isotopic turnover · Tissues · Model

Introduction

Stable isotope analysis has been widely used to study animal feeding patterns in a variety of environments. Isotopic ratios in the various foods consumed are reflected in the animal's tissues, proportionate to the amount assimilated for each food source, after accounting for discrimination against heavier isotopes in the digestion and assimilation process (DeNiro and Epstein 1978, 1981). Thus, stable isotopes are often used to quantify contributions of different food sources to an animal's diet (Phillips and Gregg 2001), but they can also be used to indicate significant dietary changes. At some point in their life cycle, many animals experience a rapid change in the isotopic signatures of the foods they eat, either because of a change in diet and/or because of movement to a new environment with a different isotopic background. For example, anadromous Pacific salmon begin their life cycle in freshwater streams, then undergo moltification and migrate downstream to estuaries and eventually to the open ocean (Groot and Margolis 1991). Other fish, such as flounder and drum, undergo dramatic dietary changes as they settle and metamorphose from larval to adult forms (Bosley et al. 2002; Herzka et al. 2001); the same can be true for holometabolous insects (O'Brien et al. 2004). Migratory birds may cover large distances and have diets at their destination which are isotopically quite distinct from their point of origin (Hobson 1999).

Following a step change in the isotopic composition of their diets, animal tissues eventually come to isotopic equilibrium with their new diet as a result of both growth of new tissue and metabolic turnover of existing tissue (Fry and Arnold 1982). Isotopic composition turnover rates vary among tissues, with high rates in tissues such as blood plasma and liver, somewhat lower rates in muscle, and low rates in long-lived tissue such as

Communicated by David Post

D. L. Phillips (✉) · P. M. Eldridge
National Health and Environmental Effects Research Laboratory,
U.S. Environmental Protection Agency, 200 SW 35th St., Corvallis,
OR 97333, USA
E-mail: phillips.donald@epa.gov

bone (Tieszen et al. 1983). Increases in the mass of tissues through growth have an additional dilution effect which results in faster equilibration to the new diet than would occur by metabolic turnover alone. Since different tissues equilibrate to dietary isotopic changes at different rates, it should be possible to use isotopic signatures from multiple tissues to estimate the time at which a dietary step change occurred, given the tissue signatures sometime before the diet change and when sampled at an unknown length of time after the change. Hesslein et al. (1993) proposed using multiple tissue isotopic changes as a “clock”, but were prevented from doing so because the tissues they examined did not differ sufficiently in their turnover rates, and they did not elaborate on how this calculation would be done. The purpose of this paper is to formulate a model to estimate this time component, to validate it with experimental data from the literature, and to explore the limits of model reliability.

Methods

Model

A number of studies have demonstrated that tissue isotopic composition changes after a diet shift follow an exponential model (Ayliffe et al. 2004; Bosley et al. 2002; Hobson and Clark 1992; MacAvoy et al. 2001; Tieszen et al. 1983), in which half of the element of interest (usually C or N) is turned over during each constant half-life (HL) period. This can be attributed to both growth and metabolic turnover (Fry and Arnold 1982; Hesslein et al. 1993). As a starting point, Hesslein et al.’s (1993) formulation was chosen for an exponential model of isotopic change following a diet shift in broad whitefish:

$$C = C_n + (C_o - C_n)e^{-(k+m)t} \quad (1)$$

where C is the observed fish isotopic signature at some time t after the diet switch; C_o and C_n are the fish signatures in equilibrium with the old diet and new diet, respectively; and k and m are the instantaneous rate constants for growth and metabolic turnover, respectively. [Other exponential model formulations lump growth and metabolic turnover into a single rate constant (Hobson and Clark 1992; Tieszen et al. 1983), but this model allows separation of these two processes if data are available.] If k and m (or $k + m$) have been experimentally determined and the fish isotopic signature under the old diet is known, this equation has two unknowns, C_n and t . Estimation of these unknowns requires an additional constraint to allow a solution. If isotopic signatures have been measured in two different tissues with different turnover rates, this same equation can be written for each tissue:

$$\begin{aligned} C_1 &= C_{n,1} + (C_{o,1} - C_{n,1})e^{-(k_1+m_1)t} \\ C_2 &= C_{n,2} + (C_{o,2} - C_{n,2})e^{-(k_2+m_2)t} \end{aligned} \quad (2)$$

where the subscripts 1 and 2 refer to the two tissues. As an additional constraint to make the model solvable, it is assumed that the new equilibrium isotopic signatures ($C_{n,1}$ and $C_{n,2}$) will differ from those associated with the old diet ($C_{o,1}$ and $C_{o,2}$) by the same amount ΔC for each tissue. Thus:

$$\Delta C = C_{n,1} - C_{o,1} = C_{n,2} - C_{o,2} \quad (3)$$

Substituting this in Eq. 2 yields:

$$\begin{aligned} C_1 &= C_{o,1} - \Delta C \left(e^{-(k_1+m_1)t} - 1 \right) \\ C_2 &= C_{o,2} - \Delta C \left(e^{-(k_2+m_2)t} - 1 \right) \end{aligned} \quad (4)$$

This system of two non-linear equations in two unknowns can be solved numerically for ΔC (the change in equilibrium tissue isotopic signatures) and t (the time since the diet shift). Figure 1 illustrates the isotopic changes in two tissues following a step change in diet, and how ΔC and t relate to these changes.

Validation

To validate the model, data from two published studies on isotopic turnover rates in multiple tissues of a bird (Hobson and Clark 1992) and a mammal (Tieszen et al. 1983) were used. From each study pairwise data from three tissues representing fast, intermediate, and slow turnover rates were used. Hobson and Clark (1992) switched adult quail (*Coturnix japonica*) from a C_3

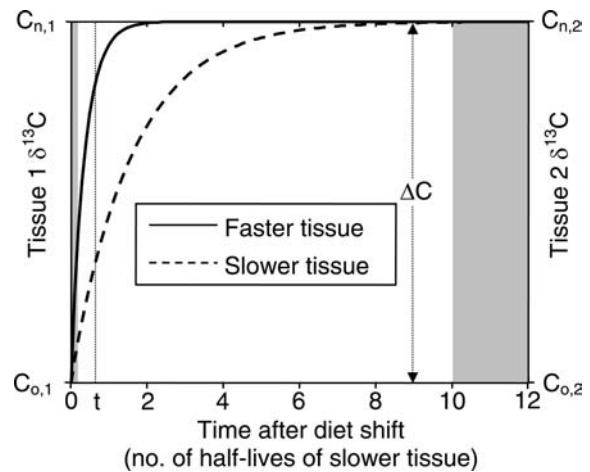


Fig. 1 Hypothetical isotopic changes in two tissues with faster and slower turnover after a diet shift at time 0. $C_{o,1}$ and $C_{o,2}$ are the isotopic composition ($\delta^{13}\text{C}$) under the old diet for tissues 1 and 2, $C_{n,1}$ and $C_{n,2}$ are the equilibrium isotopic composition under the new diet for tissues 1 and 2, and ΔC represents the isotopic shift in both tissues ($C_n - C_o$). The two tissues do not necessarily have the same $\delta^{13}\text{C}$ at time 0, but for illustrative purposes the left and right vertical scales have been adjusted to graph them with a common starting point. The vertical dotted line represents sampling at an unknown time t after the diet shift; the model is used to estimate both t and ΔC . The gray shaded regions represent very short and very long times after the diet shift (see discussion in text)

wheat-based diet to a C_4 corn-based diet, and then tracked changes in tissue $\delta^{13}C$ at up to nine sample times over 212 days. In a similar experiment, Tieszen et al. (1983) raised gerbils (*Meriones unguiculatus*) on a C_4 corn-based diet and then switched them to a C_3 wheat-based diet and tracked changes at six times over 155 days. Both studies modeled the time-varying $\delta^{13}C$ values in each tissue with an exponential equation equivalent to Eq. 1 above. Because adult quail and gerbils were used, the rate constant was attributed ($k + m$) all to metabolic turnover (m), assuming that growth (k) was zero. For fast, intermediate, and slow turnover tissues, we used data for liver (HL=2.6 days), blood (HL=11.4 days), and bone collagen (HL=173.3 days) from Hobson and Clark (1992), and liver (HL=6.4 days), muscle (HL=27.6 days), and hair (HL=47.5 days) from Tieszen et al. (1983), respectively, where $HL = \ln 2 / (k + m)$. For each sample date and pair of tissues, we used the “fsolve” routine in Matlab[®] to solve the system of equations for our model (Eq. 4) to estimate the time since the diet switch (t) and the equilibrium tissue changes in $\delta^{13}C$ (ΔC), given the initial and current $\delta^{13}C$ values for each of two tissues and the tissue rate constants. The model estimates (t_{est} and ΔC_{est}) were then compared to the actual values to assess the model’s performance.

Sensitivity analyses

Random variation in tissue isotopic signatures due to measurement and/or sampling error is to be expected and will affect the accuracy and precision of the model estimates. The effect of this stochastic variation is likely to depend on other details of the experimental situation. To determine the sensitivity of model estimates to

measurement and/or sampling error in different situations, a sensitivity analysis was conducted, using simulated data across realistic ranges of values of dietary isotopic shifts, ratios of slow HL/fast HL, and sample times. For four levels of dietary isotopic shift (ΔC : 2, 4, 8, and 16‰), four levels of the ratio between slow tissue and fast tissue HL (2, 4, 8, and 16), and six sample times after diet switching (t : 0.25, 0.5, 1, 2, 4, and 8 slow tissue HL), $\delta^{13}C$ values for two tissues were simulated at time 0 (time of diet switch) and time t . In order to introduce differences in the expected values of the faster and slower tissues relative to each other to reflect measurement error, the simulated isotopic signature of the faster tissue was then perturbed at time t by $+0.2\text{‰}$ and -0.2‰ , which is a typical reported precision for $\delta^{13}C$ measurements. For each of these 96 combinations of ΔC , slow HL/fast HL, and sample times, the deviations between the estimated elapsed time (t_{est}) and the actual elapsed time (t_{actual}) were examined as a measure of model sensitivity to measurement error across the gamut of realistic parameter values.

Results

Validation

From the Hobson and Clark (1992) feeding experiment on quail, after the diet switch paired $\delta^{13}C$ measurements were available at eight sample dates for liver/blood, four sample dates for liver/bone collagen, and five sample dates for blood/bone collagen. Model estimates of t_{est} and ΔC_{est} were made for each of the data points, except for the liver/blood pair 121 days after diet switching (Table 1) which had no valid solution (Eq. 4) because the observed slower tissue (blood) shift was slightly

Table 1 Model estimates (in bold) of time since diet shift (t_{est}) and equilibrium $\delta^{13}C$ change (ΔC_{est}) for pairs of tissues in quail [data from Hobson and Clark (1992)]

Tissue ₁	Tissue ₂	$k_1 + m_1$ (day ⁻¹)	$k_2 + m_2$ (day ⁻¹)	C_1 (‰)	$C_{o,1}$ (‰)	C_2 (‰)	$C_{o,2}$ (‰)	t_{actual} (day)	t_{est} (day)	equil. ΔC_1 (‰)	equil. ΔC_2 (‰)	ΔC_{est} (‰)
Liver	Blood	0.272	0.062	-22.17	-23.68	-22.89	-23.38	2	3.8	2.90	2.85	2.36
Liver	Blood	0.272	0.062	-21.68	-23.68	-22.48	-23.38	4	8.3	2.90	2.85	2.23
Liver	Blood	0.272	0.062	-21.28	-23.68	-22.19	-23.38	6	10.0	2.90	2.85	2.57
Liver	Blood	0.272	0.062	-21.17	-23.68	-21.88	-23.38	10	14.2	2.90	2.85	2.56
Liver	Blood	0.272	0.062	-20.78	-23.68	-21.59	-23.38	17	15.1	2.90	2.85	2.95
Liver	Blood	0.272	0.062	-20.58	-23.68	-20.78	-23.38	35	29.4	2.90	2.85	3.10
Liver	Blood	0.272	0.062	-20.74	-23.68	-20.38	-23.38	121	–	2.90	2.85	–
Liver	Blood	0.272	0.062	-20.50	-23.68	-20.39	-23.38	212	45.1	2.90	2.85	3.2
Liver	Bone	0.272	0.004	-20.78	-23.68	-20.98	-21.18	17	17.7	2.90	2.94	2.92
Liver	Bone	0.272	0.004	-20.58	-23.68	-20.79	-21.18	35	33.6	2.90	2.94	3.10
Liver	Bone	0.272	0.004	-20.74	-23.68	-20.00	-21.18	121	128.3	2.90	2.94	2.94
Liver	Bone	0.272	0.004	-20.50	-23.68	-19.80	-21.18	212	142.3	2.90	2.94	3.18
Blood	Bone	0.062	0.004	-21.59	-23.38	-20.98	-21.18	17	21.4	2.85	2.94	2.5
Blood	Bone	0.062	0.004	-20.78	-23.38	-20.79	-21.18	35	35.9	2.85	2.94	2.9
Blood	Bone	0.062	0.004	-20.38	-23.38	-20.38	-21.18	84	76.8	2.85	2.94	3.0
Blood	Bone	0.062	0.004	-20.38	-23.38	-20.00	-21.18	121	124.9	2.85	2.94	3.0
Blood	Bone	0.062	0.004	-20.39	-23.38	-19.80	-21.18	212	154.7	2.85	2.94	3.0

Sample times (t_{actual}) not shown did not have $\delta^{13}C$ for one of the two tissues. If the model was unable to calculate values for t_{est} or ΔC_{est} , this is indicated by –

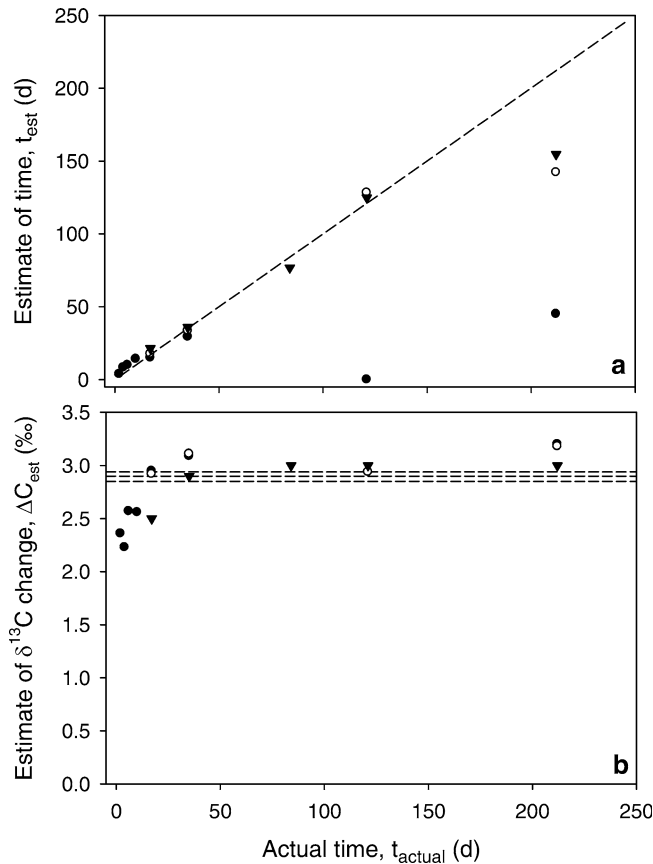


Fig. 2 Model results for quail (*Coturnix japonica*) data from Hobson and Clark (1992) for different tissue pairs [liver/blood (filled circle), liver/bone (open circle), blood/bone (filled triangle)]. **a** Model estimates of time since diet switch (t_{est}) versus actual time (t_{actual}) for the three tissue pairs. The line of equality is shown for reference. **b** Model estimates of equilibrated $\Delta^{13}C$ change (ΔC_{est}) versus actual time (t_{actual}) for the three tissue pairs. The observed equilibrated ΔC s for blood (2.85‰), liver (2.90‰), and bone collagen (2.94‰) from the feeding experiment are shown for reference (horizontal dashed lines). Both t_{est} and ΔC_{est} are shown as zero when the model was unable to estimate values for them

greater than the faster tissue (liver) shift. Other than this single data point, the estimated time since diet switching closely approximated the actual time through 121 days for all three pairs of tissues (Table 1, Fig. 2a). At 212 days, the model substantially underestimated the elapsed time for all three tissue pairs (Table 1, Fig. 2a). The estimates that Hobson and Clark (1992) made for equilibrated change in $\delta^{13}C$ after diet switching were very similar for liver, blood, and bone collagen, ranging from 2.85 to 2.94‰ (Table 1). For the first few sample dates, model estimates of ΔC_{est} were lower than this (~ 2.2 to 2.6‰; Table 1, Fig. 2b), but then more closely corresponded to the expected range. At 212 days, ΔC_{est} values were slightly higher, ~ 3.2 ‰ for liver/blood and liver/bone collagen tissue pairs.

The Tieszen et al. (1983) gerbil data set provided paired $\delta^{13}C$ measurements at six sample dates after diet switching for liver/muscle, liver/hair, and muscle/hair tissue pairs. No solutions could be found for Eq. 4 for the

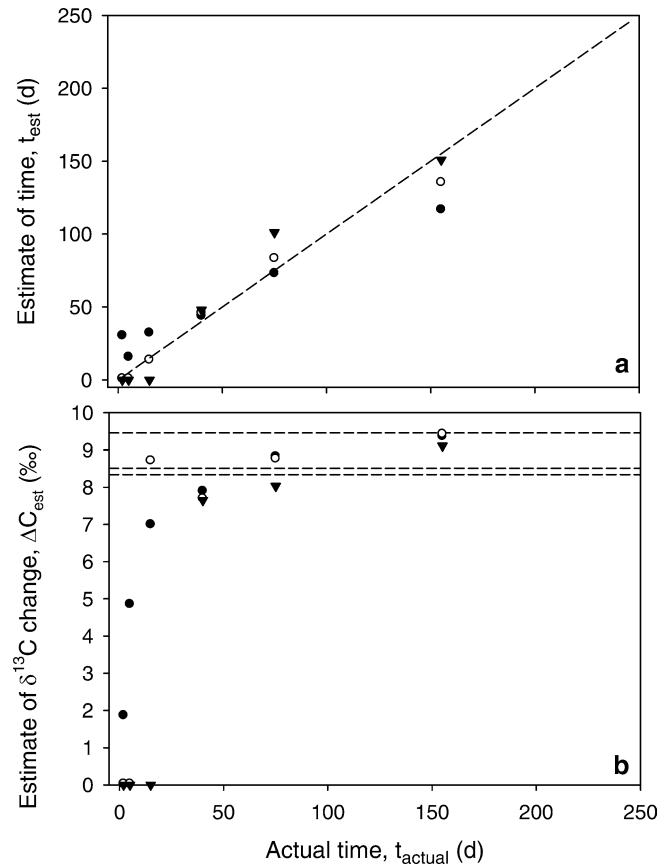


Fig. 3 Model results for gerbil (*Meriones unguiculatus*) data from Tieszen et al. (1983) for different tissue pairs [liver/muscle (filled circle), liver/hair (open circle), muscle/hair (filled triangle)]. **a** Model estimates of time since diet switch (t_{est}) versus actual time (t_{actual}) for the three tissue pairs. The line of equality is shown for reference. **b** Model estimates of equilibrated $\Delta^{13}C$ change (ΔC_{est}) versus actual time (t_{actual}) for the three tissue pairs. The observed equilibrated ΔC s for muscle (8.34‰), liver (8.51‰), and hair (9.46‰) from the feeding experiment are shown for reference (horizontal dashed lines). Both t_{est} and ΔC_{est} are shown as zero when the model was unable to estimate values for them

first two sample dates for liver/hair or the first three sample dates for muscle/hair, so there were no model estimates of t_{est} or ΔC_{est} (Table 2). Model estimates for t_{est} roughly tracked t_{actual} , but overestimated time since diet switching at the shortest times and underestimated it at the longest time (Table 2, Fig. 3a). Compared to the Hobson and Clark (1992) data set, there was more scatter around the line of equality of t_{est} and t_{actual} (Fig. 3a). Tieszen et al.'s (1983) estimated equilibrated changes in tissue $\delta^{13}C$ after diet switching also varied more than in the quail data set, ranging from 8.34‰ for muscle to 9.46‰ for hair (Table 2). For the earliest sample dates, model estimates of ΔC_{est} were substantially lower than this, but then rose to comparable values.

Sensitivity analyses

The effect of simulated 0.2‰ measurement errors on the precision of model estimates of t_{est} varied depending on

Table 2 Model estimates (in bold) of time since diet shift (t_{est}) and equilibrated ^{13}C change (ΔC_{est}) for pairs of tissues in gerbils (data from Tieszen et al. 1983)

Tissue ₁	Tissue ₂	$k_1 + m_1$ (day ⁻¹)	$K_2 + m_2$ (day ⁻¹)	C_1 (‰)	$C_{o,1}$ (‰)	C_2 (‰)	$C_{o,2}$ (‰)	t_{actual} (day)	t_{est} (day)	equil. ΔC_1 (‰)	equil. ΔC_2 (‰)	ΔC_{est} (‰)
Liver	Muscle	0.109	0.0251	-14.7	-12.9	-13.4	-12.4	2	30.6	-8.51	-8.34	-1.87
Liver	Muscle	0.109	0.0251	-16.9	-12.9	-14.0	-12.4	5	15.9	-8.51	-8.34	-4.86
Liver	Muscle	0.109	0.0251	-19.7	-12.9	-16.3	-12.4	15	32.4	-8.51	-8.34	-7.00
Liver	Muscle	0.109	0.0251	-20.6	-12.9	-17.7	-12.4	40	45.8	-8.51	-8.34	-7.75
Liver	Muscle	0.109	0.0251	-21.7	-12.9	-19.8	-12.4	75	73.2	-8.51	-8.34	-8.80
Liver	Muscle	0.109	0.0251	-22.3	-12.9	-21.3	-12.4	155	116.9	-8.51	-8.34	-9.40
Liver	Hair	0.109	0.0146	-14.7	-12.9	-11.0	-11.1	2	–	-8.51	-9.46	–
Liver	Hair	0.109	0.0146	-16.9	-12.9	-11.3	-11.1	5	–	-8.51	-9.46	–
Liver	Hair	0.109	0.0146	-19.7	-12.9	-12.7	-11.1	15	13.9	-8.51	-9.46	-8.72
Liver	Hair	0.109	0.0146	-20.6	-12.9	-14.9	-11.1	40	46.2	-8.51	-9.46	-7.75
Liver	Hair	0.109	0.0146	-21.7	-12.9	-17.3	-11.1	75	83.5	-8.51	-9.46	-8.80
Liver	Hair	0.109	0.0146	-22.3	-12.9	-19.2	-11.1	155	135.5	-8.51	-9.46	-9.40
Muscle	Hair	0.0251	0.0146	-14.7	-12.9	-11.0	-11.1	2	–	-8.34	-9.46	–
Muscle	Hair	0.0251	0.0146	-16.9	-12.9	-11.3	-11.1	5	–	-8.34	-9.46	–
Muscle	Hair	0.0251	0.0146	-19.7	-12.9	-12.7	-11.1	15	–	-8.34	-9.46	–
Muscle	Hair	0.0251	0.0146	-20.6	-12.9	-14.9	-11.1	40	47.0	-8.34	-9.46	-7.65
Muscle	Hair	0.0251	0.0146	-21.7	-12.9	-17.3	-11.1	75	101.2	-8.34	-9.46	-8.03
Muscle	Hair	0.0251	0.0146	-22.3	-12.9	-19.2	-11.1	155	150.9	-8.34	-9.46	-9.11

If the model was unable to calculate values for t_{est} or ΔC_{est} , this is indicated by –

the parameter values for isotopic diet shift (ΔC), ratio of slow HL/fast HL, and sample times (Fig. 4). Model imprecision in the face of measurement error, as indicated by the vertical separation between the paired curves for a given set of parameter values, was minimized at medium sampling times (1–2 slow tissue HLs) and increased at both lower and higher sampling times (Fig. 4). Imprecision increased as slow tissue HL and fast tissue HL became more similar; the upper and lower t_{est} bounds were narrowly focused around the 1:1 line for slow HL/fast HL = 16 but got progressively wider as this ratio decreased down to two. The model was unable to reach a solution for measurement errors in one direction (curves above the 1:1 line) after eight slow tissue HLs had elapsed since the diet shift. When the diet shift was only 2‰, this limit was reached after four slow tissue HLs. The model imprecision also generally increased as the isotopic diet shifts became progressively smaller from 16‰ down to 2‰ (Fig. 4).

Discussion

Validation

These results indicate that, given initial and current $\delta^{13}\text{C}$ values and rate constants for two tissues, the model was able to reasonably estimate the time since the diet shift and the subsequent equilibrated $\delta^{13}\text{C}$ change for both tissues, except at very short or very long times after the diet shift. These thresholds depended on the HL of the tissues utilized. In the quail data set, the two most problematic data points were the one for which no solution was found (121 days, liver/blood) and the extreme low outlier for t_{est} (212 days, liver/blood). The commonalities are that the two tissues used had the

fastest turnover times (HL of 2.6 days and 11.4 days for liver and blood, respectively), and the sampling time was at least 10 HL (for both tissues) after the diet shift. After this much elapsed time, both tissues have effectively come very close to a new isotopic equilibrium (Fig. 1; see also Fig. 1 in Hobson and Clark (1992)). The model depends on differential rates of isotopic change between multiple tissues after a diet shift, but there is little differentiation if the changes have already approached the new equilibrium for both tissues. After such extended periods of time, the expected differences between isotopic changes for faster and slower turnover tissues would be very slight and may be overwhelmed by random measurement error, making model estimates more unstable. In the gerbil data set, the data point for the two fastest turnover tissues (liver, muscle) at the longest time (155 days) gave a t_{est} estimate of 117 days, about 25% too low. While model accuracy may be beginning to deteriorate after this length of time, about five HL for muscle tissue, a new isotopic equilibrium was not yet approached to the same extent as in the quail data set for data points at >10 HL elapsed time. Thus, from the validation data it appears that elapsed time approximately between five and ten HLs of the slower turnover tissue may represent an upper threshold for useful estimates of t and ΔC from the model.

Conversely, tissues with longer turnover times may not have yet exhibited much isotopic change when sampled a very short time after a diet shift (Fig. 1). Random measurement errors may be of a similar magnitude to the expected changes in slow turnover tissues over short time periods, making model estimates unstable under such conditions. This is demonstrated by the inability of the model to find solutions for three data points for gerbil muscle/hair and two data points for gerbil liver/hair for sample times less than 1/3 HL of hair

(HL = 47.5 days) after the diet shift (Table 2, Fig. 3a). While the model was able to estimate t_{est} and ΔC_{est} for two liver/hair data points less than ~ 0.1 HL of hair after the diet shift, it overestimated t and underestimated ΔC at these early sample times (Table 2, Fig. 3). These estimation problems were not observed at short sample times in the quail data set, probably because the slowest turnover tissue (bone collagen) was not sampled until 17 days after the diet shift. Thus there were no data points only a small fraction of a HL after the shift.

In the Hobson and Clark (1992) quail data set, the observed equilibrational shifts in $\delta^{13}\text{C}$ in the feeding experiment were very similar for all three tissues, ranging from 2.85 to 2.94‰, which met our assumption of equal ΔC s. This assumption was mildly violated in the Tieszen et al. (1983) gerbil data set, which had equilibrational ΔC s ranging from 8.34 to 9.46‰ for the three tissues. This is likely to be one reason for the poorer estimation precision of the model for this data set compared to the quail data, as verified by the greater scatter around the 1:1 line in Fig. 3a and the broader range of ΔC_{est} values (Fig. 3b). Differences in the degree of isotopic change for various

tissues following a diet shift may reflect preferential routing of different macromolecular forms (e.g., proteins, lipids) to different tissues in the consumer, rather than complete mixing (Phillips and Koch 2002). Similarly, the equilibrational shift in tissue isotopic signatures following a diet shift does not always equal the isotopic difference between the diets. Gerbil tissues had slightly smaller $\delta^{13}\text{C}$ shifts than the 9.6‰ difference in diets (Tieszen et al. 1983), and the same was true for quail tissues compared to a diet difference of 4.6‰ (Hobson and Clark 1992). This may also be due to preferential metabolic routing (K. Hobson, personal communication).

Few data are available in the literature on the relative magnitudes of isotopic shifts in different tissues following a diet shift. Ayliffe et al. (2004) modeled $\delta^{13}\text{C}$ changes in horse breath CO_2 and tail hair after a 13.0‰ diet shift from C_3 to C_4 grasses. After 21 weeks, breath CO_2 and tail hair had shifted 12.6 and 10‰, respectively. However, their analysis concluded that both breath CO_2 and hair incorporated several C pools with different turnover rates and the isotopic shifts had not yet come

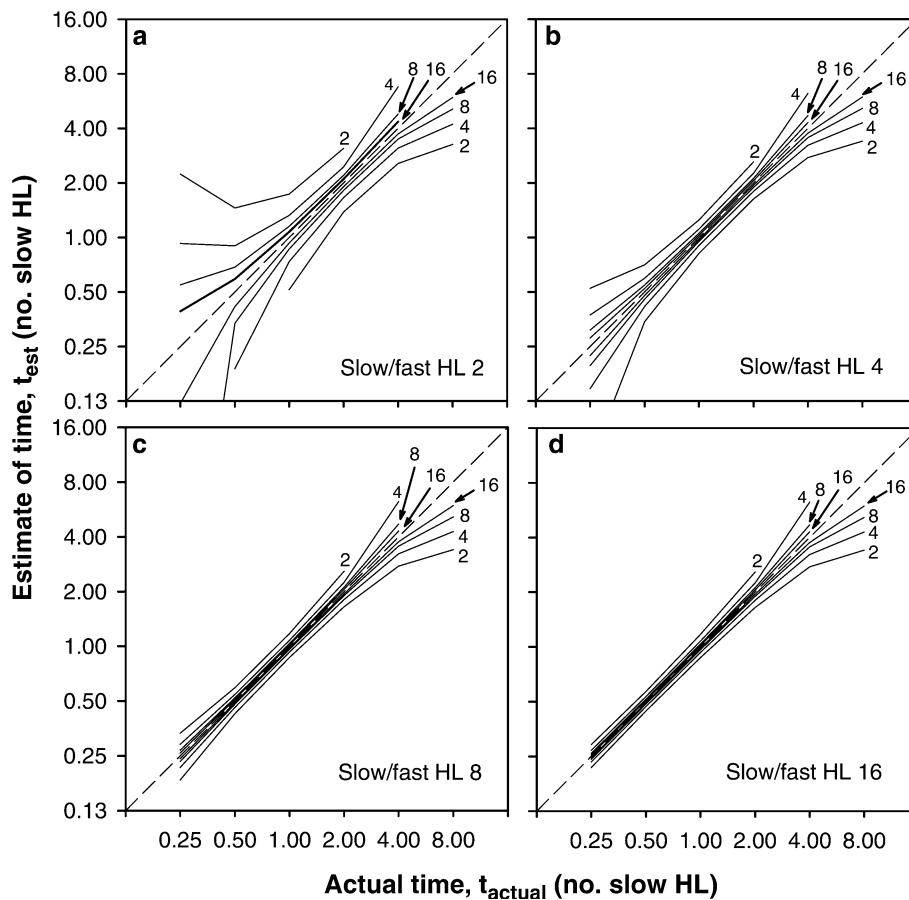


Fig. 4 Results of sensitivity analyses for effects of measurement error ($\pm 0.2\%$) on model estimates of time (t_{est}) for four ratios of slow tissue half-life to fast tissue half-life (a HL ratio 2, b HL ratio 4, c HL ratio 8, d HL ratio 16). For each ratio of slow tissue HL/fast tissue HL, model estimates of time (t_{est}) are plotted versus actual time values (t_{actual}) for a range of ΔC values (curve labels,

in ‰). The paired labeled curves show the difference in t_{est} values when measurement errors of $+0.2$ and -0.2% are imposed. The 1:1 line where $t_{\text{est}} = t_{\text{actual}}$ is shown for comparison, which represents model results in the absence of measurement error. Missing data points on a ΔC curve for a given value of t_{actual} indicate that the model was unable to achieve a solution

to equilibrium, which were both eventually expected to achieve the 13‰ shift evident in the diet. In a diet shift experiment examining isotopic changes in mouse blood, muscle, and liver, MacAvoy et al. (2005) found that the equilibrium isotopic shifts in the three tissues were within 0.6‰ of each other for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$, except liver $\delta^{13}\text{C}$, which had not yet reached equilibrium by the end of the experiment. After fitting exponential curves to experimental diet shift data, Hobson and Clark (1993) for crows and Podlesak et al. (2005) for yellow-rumped warblers found 1.7–2‰ greater shifts in blood cells than plasma. However, in the data of Podlesak et al. (2005), this may be explained by model lack of fit as the fitted curves did not go through the day 0 values for plasma, and the difference between day 0 and day 75 (apparently at equilibrium with new diet) values was close to that for blood cells. Several other studies switched diets and examined multiple tissues in mammals and birds, but the results were not presented in a way that easily allows comparison of isotopic shifts for the different tissues after equilibration to the new diet (Hilderbrand et al. 1996; Pearson et al. 2003).

Trials with the model using unequal isotopic shifts for the two tissues showed that ΔC_{est} converged on the fast tissue shift, and that t_{est} tended to be underestimated if the slow tissue had the smaller shift, and overestimated if the slow tissue had the larger shift. Unequal tissue shifts of the magnitude seen in the Tieszen et al. (1983) data set do not appear to create a serious estimation problem, but differences of several ‰ may invalidate the assumptions of the model and hence its results.

Sensitivity analyses

The measurement error sensitivity analyses corroborated the patterns of model stability thresholds that were noted in the experimental validation data. Measurement errors caused divergence of model results from expected values for both short elapsed times (0.25 slow tissue HL) and long elapsed times (eight slow tissue HL). In the latter case, measurement errors in one direction caused the model to be unable to achieve a solution because the observed slow tissue shift was larger than the fast tissue shift, which was also seen in the experimental validations at long elapsed times. This similarity in results lends credence to the interpretation that model instability at high and low sample times is due to decreased signal to noise ratios in the differences in tissue isotopic shifts relative to background levels of measurement error. For 0.2‰ measurement errors, the upper threshold was reached at a shorter sample time (four HL) when the isotopic difference between diets was only 2‰. Thus a reasonable rule of thumb might be that isotopic shifts in diet should exceed ten times the measurement error for adequate model performance.

From a theoretical standpoint, the resolving power of the model should be highest when the difference in the isotopic shifts in the two tissues ($C_1 - C_{o,1}$ vs $C_2 - C_{o,2}$) is

maximized (the maximum vertical separation between the two curves shown in Fig. 1). This point can be determined by rearranging Eq. 4 to express the difference between the two tissue changes as a function of the other terms:

$$(C_1 - C_{o,1}) - (C_2 - C_{o,2}) = -\Delta C \left(e^{-(k_1+m_1)t} - e^{-(k_2+m_2)t} \right) \quad (5)$$

The maximum value of this function can be found by differentiating and setting the derivative to zero, which occurs where:

$$t = \frac{\ln(k_2 + m_2) - \ln(k_1 + m_1)}{(k_2 + m_2) - (k_1 + m_1)} \quad (6)$$

For example, for two tissues with slow HL/fast HL = 4, this translates to a sample time of 0.4 HL of the slower tissue after the diet shift. However, the presence of measurement error may alter this theoretical optimum sampling time, as seen by the fact that model precision was actually best at a sampling time of 1 slow tissue HL in the sensitivity analyses for slow HL/fast HL = 4 (Fig. 4). This is likely due to the fact that measurement errors can have a disproportionate effect on the apparent slow tissue isotopic changes after such relatively short times, during which the expected change is not yet very large.

Limitations

Besides the parameter limits discussed above, beyond which model results become unstable, several other factors may affect the degree to which this model may be fruitfully applied in field studies. First, this method requires prior knowledge of, or pilot studies to determine, estimates of rate constants for the tissues examined. It is not necessary to split out separate metabolic turnover (m) and growth rate (k) constants, as opposed to estimating a combined rate ($k + m$), but this may be done if desired. In the case of adult organisms, such as in the quail and gerbil studies described earlier, the growth component may be negligible. The most preferable situation is having first-hand experimental determinations of tissue rate constants for the study organism. Failing this, information from the literature about tissue turnover rates for the study organism or related species might be used. In the absence of such information, a rapid assessment of the turnover rates might be possible by sampling the study organisms at two times after the diet shift. While the time between the diet shift and the initial sample is unknown and is to be estimated by the model, the isotopic change over a fixed interval between the two samples can be used to estimate the tissue turnover rates. This procedure is apt to result in more estimation uncertainty than an extended controlled experiment with more measurements, but replicated paired measurements can provide adequate HL esti-

mates (Phillips 1989), which would allow the model to estimate t and ΔC .

Second, the model requires that the consumer's tissues be in equilibrium with the original diet before the diet shift occurs. Prior to the step change in diet being studied, it is important that both the short-term and long-term diet have been uniform enough for both fast and slow turnover tissues to be equilibrated to the same dietary isotopic composition. This would primarily be a consideration where tissue turnover time is very long or temporal variation in diet is high. Very long turnover tissues, such as bone, integrate the isotopic composition of assimilated diet over extended periods. During this time, variations in dietary isotopic composition would be reflected in shorter turnover tissues, which could result in a slightly different tissue baseline for a diet shift. Similarly, isotopic equilibration with diet may be a problem in areas with high temporal variation in isotopic ratios as Post (2002) and others have found in some aquatic food webs.

A third factor that might complicate the use of the model is utilization of endogenous reserves that are isotopically reflective of the earlier diet. Migratory birds, for example, utilize fat and sometimes protein stores during long-distance flights (Battley et al. 2000), but some birds may continue to draw on endogenous reserves after arrival in the new isotopic environment (Morrison and Hobson 2004). For tissues that lose mass, it may be possible to still use the model if the growth rate constant k is specified as a negative number that reflects the rate of mass loss, although we have not evaluated its use in these situations. However, the model is predicated on essentially a step change in diet and the subsequent mixing of old and new dietary isotopic signatures in multiple tissues. If there is a substantial preferential metabolism of tissues reflecting the old diet, rather than complete mixing, the model may not be applicable.

Summary

In summary, the model did a good job of estimating the time since a diet shift (t) and the equilibrated isotopic shift (ΔC) except when the elapsed time was a small fraction of the longer HL or more than 5–10 HL for both tissues, or when isotopic diet shifts were small (≤ 10 times measurement error). Model performance is best when the assumption of equal isotopic shifts among tissues is closely met. Subject to data availability and meeting the assumptions of the model, it may be useful for studying the movement and behavior of animals moving between different isotopic environments, such as anadromous fish (Groot and Margolis 1991), migratory birds (Hobson 1999), animals undergoing metamorphosis (Bosley et al. 2002; Herzka et al. 2001), or animals changing diets because of changes in food abundance (Ben-David et al. 1997) or social interactions (Ben-David et al. 2004). For the model to be effective, the tissues utilized must be sufficiently different in their turnover rates. Separating

blood samples into cellular and plasma components, which have markedly different turnover rates, offers the possibility of non-destructive sampling which could be advantageous for wildlife studies (Hobson and Clark 1993).

Acknowledgements The information in this document has been funded by the U.S. Environmental Protection Agency. It has been subjected to the Agency's peer and administrative review, and approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. We thank Keith Bosley, Claudio Gratton, Diane O'Brien, Bob Ozretich, David Post, and two anonymous reviewers for constructive reviews.

References

- Ayliffe LK et al. (2004) Turnover of carbon isotopes in tail hair and breath CO₂ of horses fed an isotopically varied diet. *Oecologia* 139:11–22
- Battley PF, Piersma T, Dietz MW, Tang S, Dekinga A, Hulsman K (2000) Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proc R Soc Lond Ser B Biol Sci* 267:191–195
- Ben-David M, Hanley TA, Klein DR, Schell DM (1997) Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. *Can J Zool* 75:803–811
- Ben-David M, Titus K, Beier LR (2004) Consumption of salmon by Alaskan brown bears: a trade-off between nutritional requirements and the risk of infanticide? *Oecologia* 138:465–474
- Bosley KL, Witting DA, Chambers RC, Wainwright SC (2002) Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Mar Ecol Prog Ser* 236:233–240
- 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
- Fry B, Arnold C (1982) Rapid ¹³C/¹²C turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* 54:200–204
- Groot C, Margolis L (eds) (1991) Pacific Salmon life histories. UBC Press, Vancouver, BC
- Herzka SZ, Holt SA, Holt J (2001) Documenting the settlement history of individual fish larvae using stable isotope ratios: model development and validation. *J Exp Mar Biol Ecol* 265:49–74
- Hesslein RH, Hallard KA, Ramlal P (1993) Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}\text{S}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. *Can J Fish Aquat Sci* 50:2071–2076
- Hilderbrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C (1996) Use of stable isotopes to determine diets of living and extinct bears. *Can J Zool* 74:2080–2088
- Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314–326
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: turnover of ¹³C in tissues. *Condor* 94:181–188
- Hobson KA, Clark RG (1993) Turnover of ¹³C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. *Auk* 110:638–641
- MacAvoy SE, Macko SA, Garman GC (2001) Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. *Can J Fish Aquat Sci* 58:923–932
- MacAvoy SE, Macko SA, Arneson LS (2005) Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. *Can J Zool* 83:631–641

- Morrison RIG, Hobson KA (2004) Use of body stores in shorebirds after arrival on high-Arctic breeding grounds. *Auk* 121:333–344
- O'Brien DM, Boggs CL, Fogel ML (2004) Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. *Oikos* 105:279–291
- Pearson SF, Levey DJ, Greenberg CH, Martinez del Rio C (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon signatures in an omnivorous songbird. *Oecologia* 135:516–523
- Phillips DL (1989) Propagation of error and bias in half-life estimates based on two measurements. *Arch Environ Contam Toxicol* 18:508–514
- Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171–179
- Phillips DL, Koch PL (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia* 130:114–125
- Podlesak DW, McWilliams SR, Hatch KA (2005) Stable isotopes in breath, blood, feces, and feathers can indicate intra-individual changes in the diet of migratory songbirds. *Oecologia* 142:501–510
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718
- Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57:32–37