Functional Ecology

British Ecological Society

Functional Ecology 2010, **24**, 805–812 doi: 10.1111/j.1365-2435.2010.01700.x

Growth and catabolism in isotopic incorporation: a new formulation and experimental data

Scott A. Carleton* and Carlos Martinez del Rio

Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming 82071, USA

Summary

1. We propose a new model that uses an organism's change in mass to estimate isotope incorporation rates. Our model is a re-parameterization of a widely used one-compartment-first-order kinetic model that estimates the rate of isotopic incorporation as a function of time. If animals are growing exponentially and fractional growth rates are measured, the time model allows partitioning the contribution of growth and catabolic turnover to isotopic incorporation. The model can be extended to include more than one-compartment.

2. Our model makes the same assumptions but uses mass change as an independent variable. It estimates the ratio of the contribution of catabolic turnover to growth.

3. To test the model, we fed juvenile Nile tilapia (*Oreochromis niloticus*) at three different rations and measured their change in mass and tissue δ^{13}C isotopic composition for 128 days.

4. Fish grew exponentially and fractional growth rates differed significantly between rations. The contribution of catabolism (tissue replacement) to isotopic incorporation was 2·6 to 5 times higher than growth (tissue addition) in liver. In muscle tissue, the contribution of catabolism and growth were roughly equivalent for all rations.

5. When we used the parameters of the mass model to re-parameterize the time model we found a relatively good fit ($r^2 = 0{\cdot}8 \pm 0{\cdot}1$, mean $\pm$ SD) to the isotopic incorporation data.

6. The mass model estimated a smaller, and more biologically realistic range of δ_∞ values than did the time model which suggests that it might yield better results when isotopic incorporation experiments are not carried on long enough to yield estimates of the asymptotic isotopic value of an animal's tissues after a diet shift.

7. Rather than being alternatives, the time and mass models are complementary in that they allow us to see the same phenomenon from different perspectives.

Key-words: catabolism, growth, isotope incorporation, stable isotopes, δ^{13}C

Introduction

Understanding the rates at which different organisms and different tissues within organisms incorporate isotopes is essential to both interpret field isotopic data and reconstruct temporal changes in the diet of free ranging animals (Gulenix *et al.* 2007). Many studies have demonstrated that different tissues within an organism have contrasting rates of isotope incorporation (reviewed by Martínez del Rio *et al.*2009). Previous studies, however, have demonstrated that the ranking in the rate of isotopic incorporation among tissues seems to be preserved across taxa (Dalerum & Angerbjörn 2005; Logan *et al.* 2006; Reich, Bjorndal & Martinezdel Rio 2008). Briefly, splanchnic tissues with high protein turnover such as liver and gastrointestinal tract appear to have higher isotopic incorporation rates than structural tissues such as muscle and bone collagen (reviewed by Wolf, Carleton & Martinez del Rio 2009). The differences among tissues in isotopic incorporation rate are useful as they allow (i) tracking the timing of diet changes (Gulenix *et al.* 2007; Carleton *et al.* 2008), (ii) interpreting whether animal tissues have reached a constant value after a diet change (Carleton & Martinez del Rio 2005; Carleton *et al.* 2008), (iii) detecting events that occurred before the animal was captured (Bearhop *et al.* 2004a,b; Norris *et al.* 2005) and (iv) assessing degree and time scale of dietary specialization (Martínez del Rio *et al.* 2009).

Norris *et al.* (2005), for example, used a 'slow' tissue (red blood cells) of migratory American redstarts (*Setophaga ruticilla*) caught after arriving to their breeding grounds to assess differences in habitat use on the wintering grounds.

*Correspondence author. E-mail: scott.carleton@uwyo.edu

Martínez del Rio *et al.* (2009) captured birds in the winter and used differences in the isotopic composition of feathers deposited in the summer with those of 'fast' tissues deposited in the winter to identify seasonal diet shifts in Chilean ovenbirds (*Cinclodes* sp.). These authors also compared the variation in the isotopic composition of fast and slow tissues to find out the time scale at which individuals shifted diets. Because of the amount of information that can be potentially gleaned by the variation in isotopic composition among the different tissues of a single individual (Bearhop *et al.* 2004a,b), ecologists have devoted a lot of attention to both constructing isotope incorporation models that describe the pattern of isotopic incorporation over time (Cerling *et al.* 2007; Martínez del Rio & Anderson-Sprecher 2008) and performing experiments that test these models (Carleton *et al.* 2008; reviewed by Wolf, Carleton & Martinez del Rio 2009).

In a seminal paper, Fry & Arnold (1982) recognized that the rate of isotopic incorporation after a diet shift is determined by both addition of new material to tissue ('growth') and by replacement of material exported from tissue as a result of catabolism ('catabolic turnover'). They proposed a simple model that relates change in body mass to the isotopic value of a growing animal's tissues. This model allows a qualitative assessment of the relative contribution of growth and catabolic turnover to isotopic incorporation. Hesslein, Hallard & Ramlal (1993) proposed a simple modification of the widely used, single-compartment, first-order kinetics model used to describe isotopic incorporation as a function of time. In this model

$$\delta_t = \delta_\infty - (\delta_\infty - \delta_0)\exp(-\lambda t), \qquad \text{eqn 1}$$

where δ_∞ and δ_0 are the asymptotic and initial isotopic values respectively, and λ is the fractional rate of isotopic incorporation (Wolf, Carleton & Martinez del Rio 2009). We will call equation 1 the 'time model'. Hesslein, Hallard & Ramlal (1993) demonstrated that in animals growing exponentially, the value of λ equals the sum of fractional net growth $k_g(k_g = w^{-1}[dw/dt])$ and the fraction ofturnover due to catabolic tissue replacement $k_c(\lambda = k_c + k_g)$. In this model w is an organism's mass. The purpose of this contribution is to describe and test a model that combines the mass-based approach of Fry & Arnold (1982) with the time based approach of Hesslein, Hallard & Ramlal (1993). This model is a re-parameterization of equation 1 as modified by Hesslein, Hallard & Ramlal (1993) and permits using mass data to find out the relative contribution of growth and catabolism to isotopic incorporation.

Following Hesslein, Hallard & Ramlal (1993) we assumed that animals were growing exponentially. If weight at time t (w_t) can be expressed as $w_t = w_0 \exp(k_g t)$, then $t = \ln(w_t/w_0)k_g^{-1}$. Substituting this time expression in equation 1 yields

$$\delta_t = \delta_\infty - (\delta_\infty - \delta_0)\exp(-(k_c - k_g)k_g \ln(\frac{w_t}{w_0})), \qquad \text{eqn 2}$$

which simplifies to

$$\delta_t = \delta_\infty - (\delta_\infty - \delta_0)(1 - \frac{\Delta w}{w_t})^{(1+\frac{k_c}{k_g})}, \qquad \text{eqn 3}$$

where $\Delta w = w_t - w_0$. We call the model represented by equation 3, the 'mass model'. When growth is the primary determinant of incorporation rate (i.e. when $k_g >> k_c$), as seems to be the case in the structural tissues of many growing ectotherms (Martínez del Rio *et al.* 2009), equation 3 reduces to a linear relationship between δ_t and $\Delta w/w_t$

$$\delta_t = \delta_0 + (\delta_\infty - \delta_0)\frac{\Delta w}{w_t}. \qquad \text{eqn 4}$$

Equation 4 represents a simple mixing model in which the isotopic composition of a tissue is a mixture of old and new tissue with fractional contributions equal to w_0/w_t and $\Delta w/w_t$, respectively (Kelly & Martínez del Rio 2010). Figure 1 illustrates the relationship between δ_t and $\Delta w/w_t$ for a range of k_c/k_g ratios. Note that the relationship is linear when $k_g >> k_c$ and curved as k_c/k_g increases.

Cerling *et al.* (2007) and Martínez del Rio & Anderson-Sprecher (2008) have suggested that multi-compartment models are often better descriptors of isotopic incorporation than the single compartment models represented by equations 1 and 3. The multi-compartment equivalent of equation 1 is:

$$\delta_t = \delta_\infty - (\delta_\infty - \delta_0)\left(\sum_{i=1} p_i e^{-\lambda_i t}\right), \qquad \text{eqn 5}$$

where $\sum_{i=1} p_i = 1$ (Cerling *et al.* 2007). If we assume that fractional growth rates are equal in all compartments (i.e. that for all i and j, all $k_{gi} = k_{gj} = k_g$), then equation 5 can be re-parameterized as:

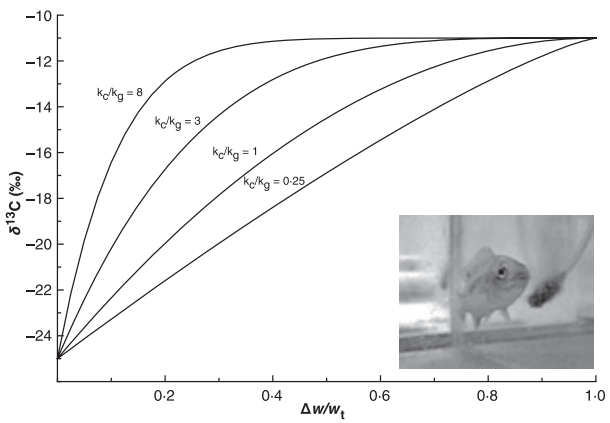

Fig. 1. The curvature of the relationship between the isotopic composition of a tissue ($\delta^{13}C$) and relative mass change ($\Delta w/w_t$) depends on the ration of catabolic turnover to growth rate (k_c/k_g). As the contribution of growth to isotopic incorporation increases, the curvature of the relationship decreases. If k_g greatly exceeds k_c, the relationship between $\delta^{13}C_t$ and $\Delta w/w_t$ is linear.

$$\delta_t = \delta_\infty - (\delta_\infty - \delta_0)\left(\sum_{i=1} p_i\left(1 - \frac{\Delta w}{w_t}\right)^{(1+k_i)}\right), \qquad \text{eqn 6}$$

where $k_i = k_{ci}/k_g$. Cerling *et al*. (2007) give a useful method to diagnose whether the pattern of isotopic incorporation described by the time model can be better described by a one- or a multi-compartment system. When data is transformed to a 'reaction progress variable',

$$(1 - F) = \frac{\delta_\infty - \delta_t}{\delta_\infty - \delta_0}, \qquad \text{eqn 7}$$

and $\ln(1 - F)$ is plotted against time, a decreasing straight line with a single slope diagnoses systems with a single compartment. A series of connected straight lines with different slopes diagnoses systems with multiple compartments (Cerling *et al*. 2007).

In a similar fashion, we can construct a reaction progress variable for the mass model and use it to diagnose whether the system is better described by a one or a multi-compartment model. By applying the same definition of $(1 - F)$ adopted by Cerling *et al*. (2007) to equation 3, we find that:

$$\ln(1 - F) = \ln\left(\frac{\delta_\infty - \delta_t}{\delta_\infty - \delta_0}\right) = \left(1 + \frac{k_c}{k_g}\right)\ln\left(1 - \frac{\Delta w}{w_t}\right). \qquad \text{eqn 8}$$

Thus, a double logarithmic plot of $\ln(1 - F)$ and $\ln(1 - \Delta w/w_t)$ should yield a straight line with slope equal to $(1 + k_c/k_g)$ for systems well-described by a one compartment model. Systems best described by multi-compartment models yield plots with connected straight lines with different slopes.

To test this re-parameterization, we switched growing Nile Tilapia between isotopically contrasting diets at three different rations, measured their rate of growth, and rate of isotopic incorporation into muscle and liver tissue. We predicted that 1) as ration levels increased tilapia would have increasing growth rates and the contribution of growth to isotopic incorporation would increase relative to catabolism, 2) the contribution of catabolism to isotopic incorporation would be higher in liver tissue when compared to muscle tissue across all rations, and 3) that the retention time for ^{13}C would decrease with increased ration for both liver and muscle tissue.

Materials and methods

We placed 150 Nile tilapia fingerlings (Americulture®; NM, USA, mass ≈1 g) in individual tanks (25 × 15 × 28 cm) and fed them *ad libitum* a commercial diet (Freedom Feeds Mash, $\delta^{13}C = -23{\cdot}95 \pm 0{\cdot}15$). Water flow in tanks was 79·2 L per day and temperature was maintained at 26 °C. After tilapia had tripled in mass (4·38 ± 0·44 g) they were randomly assigned to three different ration sizes (50 each @ 2%, 4% or 8% of body mass measured weekly) and fed a new corn-based diet (Isotapia, Table 1, $\delta^{13}C = -13{\cdot}45 \pm 0{\cdot}13$). Five fish from each treatment were collected, weighed, euthanized, and dorsal muscle and liver tissue were dissected for isotope analysis on days 0, 1, 2, 4, 8, 16, 32, 64 and 128.

ISOTOPIC ANALYSIS

Muscle and liver tissues were oven-dried at 55 °C to constant mass and ground to a homogenous powder. Lipids were extracted with petroleum ether. Briefly, tissues were soaked in petroleum ether three times for 72 h each. After lipid extraction, samples were dried, ground again, homogenized and placed in tin cups for isotopic analyses ($\delta^{13}C$ and $\delta^{15}N \approx 0{\cdot}09$ mg, %C and %N ≈ 5 mg). Unfortunately, $\delta^{15}N$ between diet 1 and diet 2 did not differ enough to make

Table 1. Composition, isotopic, and elemental analysis of our experimental corn-based diet (diet 2) including proportions added to diet

Diet	^{13}C (‰)	^{15}N (‰)	%C	%N	% Dry (kg^{-1})	mg kg^{-1}
Freedom feeds mash* (diet 1)	−23·95 ± 0·15	1·86 ± 0·07	46·3 ± 1·8	7·5 ± 0·21	–	–
Isotapia (diet 2)	−13·45 ± 0·13	3·22 ± 0·08	49·9 ± 0·2	5·4 ± 0·03	–	–
Individual ingredients					–	–
Corn gluten	−13·11 ± 0·04	2·90 ± 0·07	50·1 ± 0·9	10·2 ± 0·02	50·19	–
Corn starch	−9·90 ± 0·01	–	39·9 ± 0·5	–	15·68	–
Corn oil	−14·82 ± 0·04	–	65·1 ± 3·4	–	6·27	–
Corn syrup	−10·24 ± 0·05	–	28·6 ± 8·1	–	19·60	–
Methyl-cellulose	−29·50 ± 0·31	–	47·7 ± 0·8	–	1·57	–
α-cellulose	−24·80 ± 0·05	–	–	–	5·10	–
Sodium chloride	–	–	–	–	0·02	–
Vitamin mix	–	–	–	–	1·57	–
Mineral mix	–	–	–	–	–	–
Dicalcium phosphate	–	–	–	–	–	1500
Zinc	–	–	–	–	–	100
Iron	–	–	–	–	–	44
Manganese	–	–	–	–	–	25
Iodine	–	–	–	–	–	5
Copper	–	–	–	–	–	3
Selenium	–	–	–	–	–	0·3
Cobalt	–	–	–	–	–	0·05

This table also includes the elemental analysis and isotopic values of the diet fed to fish before the diet shift (diet 1). We do not list the ingredients of this diet because its composition is proprietary of Freedom Feeds®. Experimental diet was adapted from Gaye-Siessegger *et al*. (2003).

inferences about ^{15}N rates of isotopic incorporation and residence time. However, we have included the $\delta^{15}N$ and %N of each diet in Table 1.

Isotope ratios were measured on a continuous flow isotope ratio mass spectrometer (FinniganDelta + XP, University of Wyoming's light Stable Isotope Facility) with samples combusted in a Costech elemental analyzer. The precision of these analyses was ±0·2‰ for both isotopes. Our standards were vacuum oil [$\delta^{13}C = -27{\cdot}5‰$, Vienna Pee Dee Belemnite (VPDB)] and ANU sucrose ($\delta^{13}C = -10{\cdot}5‰$, VPDB, NIST 8542). We included standards in every run to correct raw values obtained from the mass spectrometer. Stable isotope ratios were expressed using standard delta notation (δ) in parts per mil (‰) as:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000 \qquad \text{eqn 9}$$

where R_{sample} and $R_{standard}$ are the molar ratios of the heavy/light isotope of the sample and the reference, respectively. Samples were referenced against the international standard, the VPDB.

STATISTICAL ANALYSIS

We first verified that fish were growing exponentially by plotting $\text{Ln}(w_t)$ against time obtaining an r^2 value for each relationship and assessing the distribution of residuals. We then estimated growth rate as $k_g = \text{Ln}(w_t/w_0)/t$ (Ebert 1999) and tested for differences among rations in growth with a one-way ANOVA followed by Tukey's Honest Significant Difference. To diagnose whether one or more compartments were needed to describe the patterns of isotopic incorporation we plotted $\ln(1 - F)$ against $\ln(1 - \Delta w/w_t)$. This visual method does not allow making statistical inferences or to estimate the parameters of the incorporation model accurately (Martínez del Rio & Anderson-Sprecher 2008), therefore we used a Levenberg-Marquardt nonlinear fitting algorithm to estimate the parameters of equations 3 and 7 (JMP®). To avoid over-parameterizing the multi-compartment model we only attempted to fit a two-compartment model. The ratio of catabolic turnover to growth (k_{ci}/k_g) was estimated as a single parameter by this procedure. We used r^2 as a qualitative index of goodness of fit. We estimated the contribution of catabolism to the fractional rate of isotopic incorporation (k_c) as $(k_c/k_g)k_g$ and used standard propagation of error formulae to estimate this parameter's standard error. Following Martínez del Rio & Anderson-Sprecher (2008) we estimated carbon average retention times (τ) as $\tau = 1/\lambda = (1/[k_c + k_g])$.

Results

Fish grew exponentially (average $r^2 \pm \text{SD} = 0{\cdot}76 \pm 0{\cdot}16$, Table 2). Fractional growth rates differed significantly among treatments ($F_{2,123} = 62{\cdot}85$, $P = 0{\cdot}001$). Fish fed on the 2% ration grew more slowly than those fed on the 4% and 8% rations (Tukey's HSD $P < 0{\cdot}05$), but those fed on those two diets grew at similar growth rates (Tukey's HSD $> 0{\cdot}05$). The reaction-progress diagnostic procedure yielded straight lines with no evidence of more than one compartment (Fig. 2). Although the nonlinear algorithm always found locally optimal one-compartment models (Fig. 2), the two-compartment model was over-parameterized, resulting in singular Hessians and failure to converge. Incorporation of $\delta^{13}C$ was well described by the one-compartment model for both muscle and liver tissue (average $r^2 \pm \text{SD} = 0{\cdot}84 \pm 0{\cdot}13$ and $0{\cdot}78 \pm 0{\cdot}16$ respectively, Fig. 3). As predicted, the ratio of catabolic turnover to growth (k_c/k_g) was higher for liver than for muscle (Table 2, Fig. 4). In liver, the value of k_c was 2·6 to 5·4 times higher than the value of k_g. In contrast, the value of k_c in muscle was either of roughly the same magnitude as that of k_g (for fish fed on 2% and 4% rations) or a lot smaller (for fish fed on the 8% ration, Table 2). The relationship between the ratio of catabolism to growth (k_c/k_g) was more complex than expected. Although we expected this relationship to decrease with ration, for muscle, the value of k_c/k_g was roughly the same for fish fed on 2% and 4% rations, but dropped when fish were fed on the 8% ration. For liver, the value of k_c/k_g had a distinct maximum value when fish were fed the 4% ration (Fig. 4). We used the values for δ_∞ and $(\delta_\infty - \delta_0)$ estimated by equation 3 and $1 = k_c + k_g$ as the fractional incorporation rate in equation 1 to predict $\delta^{13}C_t$ as a function of time. The estimated parameters yielded a good fit to the data ($r^2 \pm \text{SD} = 0{\cdot}82 \pm 0{\cdot}13$ and $0{\cdot}79 \pm 0{\cdot}07$ for muscle and liver respectively). Estimated carbon residence times were consistently shorter for liver than for muscle (Table 3). However, we did not find the monotonic decrease in retention time as ration increased that we had expected (Table 3).

Discussion

We used an alternative model that depends on relative mass change rather than time to estimate the incorporation of a diet's isotopic value into an animal's tissues. Our experimental test of this new model was driven by three predictions. First, we predicted that as ration levels increased, tilapia growth rates would increase and that the contribution of growth to isotopic incorporation would increase relative to the contribution of catabolism. As predicted, growth increased with increasing ration (Table 2). However, the

Table 2. Parameters of equation 3 estimated by nonlinear regression (δ_∞, $\delta_\infty - \delta_0$ and k_c/k_g), experimentally (k_g) or by multiplying k_c/k_g by k_g

Ration (%)	Tissue	δ_∞ (‰)	$\delta_\infty - \delta_0$ (‰)	k_c/k_g	k_c (day^{-1})	k_g (day^{-1})
2	Muscle	−13·2 ± 0·8	7·0 ± 0·7	1·1 ± 1·1	0·003 ± 0·003	0·002 ± 0·001
	Liver	−10·7 ± 2·5	9·0 ± 2·5	2·6 ± 1·6	0·006 ± 0·004	0·002 ± 0·001
4	Muscle	−12·7 ± 1·3	7·9 ± 1·2	1·3 ± 0·7	0·010 ± 0·006	0·008 ± 0·001
	Liver	−13·1 ± 0·6	7·8 ± 0·6	5·4 ± 1·1	0·044 ± 0·011	0·008 ± 0·001
8	Muscle	−9·5 ± 0·3	11·1 ± 0·4	0 ± 0·2	0 ± 0·002	0·012 ± 0·002
	Liver	−11·9 ± 0·4	8·1 ± 0·4	3·0 ± 0·6	0·032 ± 0·009	0·012 ± 0·002

Values are estimates ± SE.

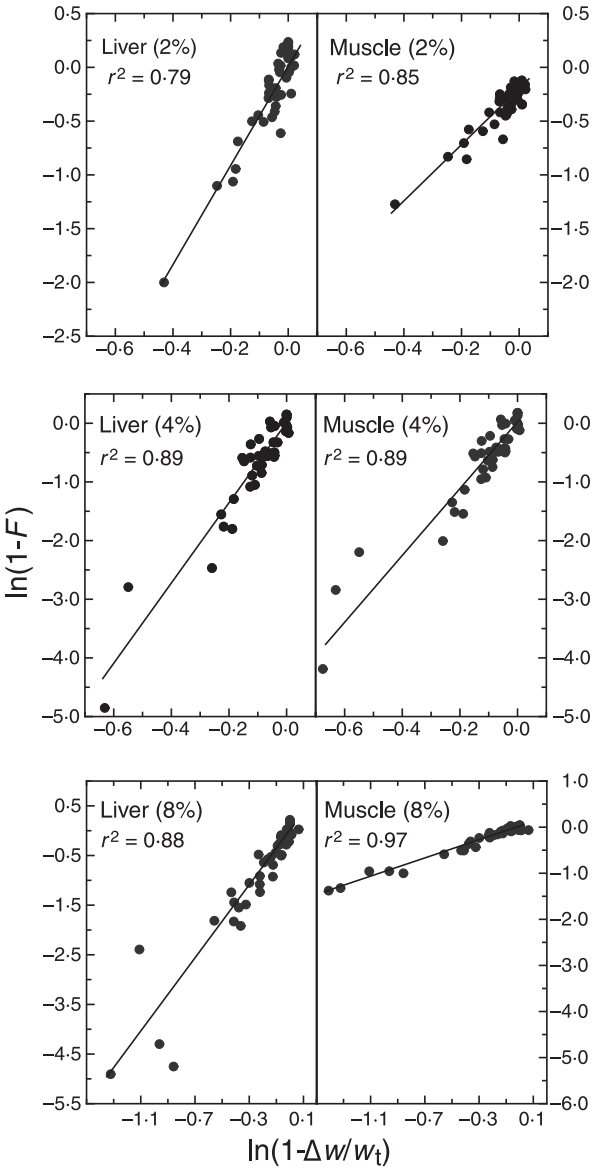

Fig. 2. Plots of the natural log of the reaction progress variable $(1 - F)$ against natural log $(1 - \Delta w/wf)$ revealed that a model with a single compartment describes the data adequately. In this case, there seems to be no need to invoke more than one-compartment to describe isotope incorporation. [Correction added after online publication 16 June 2010: position of the regression lines in the top two panels.]

contribution of growth relative to catabolism decreased (albeit only slightly for muscle) then increased for both tissues with increasing ration (Fig. 4). Second, we predicted that the contribution of catabolism to isotopic incorporation would be higher in liver than in muscle. Indeed, the contribution of catabolism to isotopic incorporation was higher in liver than in muscle across all three feeding levels (Fig. 4). Conversely, growth contributed more to isotopic incorporation in muscle than liver. Finally, we predicted that as ration and growth increased the retention time (τ) for $\delta^{13}C$ would decrease for both muscle and liver. Fish fed on 2% ration had the longest average carbon retention time (Table 3). However, the retention time of carbon was similar or slightly higher in fish fed on 8% than on the 4% ration.

Our discussion is divided into two broad sections. The first one evaluates the potential advantages and disadvantages of the two parameterizations of the isotopic incorporation model. We contend that the two expressions of the model (mass or time) should be deemed as complementary, rather than alternative. In the second section of this discussion, we consider explanations for the patterns revealed by our experimental study and their consequences for field studies. Specifically, we examine why splanchnic tissues such as liver usually have higher isotopic incorporation rates, we discuss the potentially complex interplay of growth and catabolism as determinants of isotopic incorporation, and finally we consider the potential reasons why we failed to find simple relationships between ration, growth rate, and isotopic incorporation.

MASS OR TIME: ADVANTAGES AND DISADVANTAGES OF TWO INDEPENDENT VARIABLES

Richard Feynman (1967), a prominent physicist, emphasized the importance of having different derivations of the mathematical expressions that describe the same physical process. Different formulations permit perceiving the same phenomenon from different angles. We believe that framing isotopic incorporation equations from the perspective of mass and time change plays this useful role. To assess the relative merits of the two parameterizations of the incorporation rate model, we first list their assumptions. Both models assume that we can interpret a tissue as being comprised of one compartment that incorporates materials with first-order kinetics (Martínez del Rio *et al.* 2009). Cerling *et al.* (2007) and others have criticized this assumption, but we emphasize that this assumption is shared by both forms of the model. Like the time model, the re-parameterization that we have proposed can be extended to include more than one compartment and the methods to compare the merit of these more complex models with those of the simpler one-compartment model are similar between the time and mass models. The mass model makes the additional assumption that the organism is growing exponentially. For obvious reasons, the model cannot be applied to organisms that are not growing, whereas the time model can, and has, been used in animals at steady state such as fully-grown determinate growers (Carleton & Martinez del Rio 2005). Indeed, the time model seems to be remarkably robust to a variety of growth trajectories (Martínez del Rio, unpublished data).

If the purpose of a study is to partition the relative contribution of growth and catabolism to isotopic incorporation the mass model has distinct advantages. It estimates the ratio of k_c/k_g directly, and allows diagnosing whether one can use the simple linear approximation to estimate the isotopic composition of newly deposited tissue (δ_∞) by using equation 4 ($\delta_t = \delta_0 + (\delta_\infty - \delta_0)\frac{\Delta w}{w_t}$, see Kelly & Martínez del Rio 2010). Under our experimental conditions, the use of this equation (i.e. $k_g >>> k_c$) was only satisfied for the muscle of fish

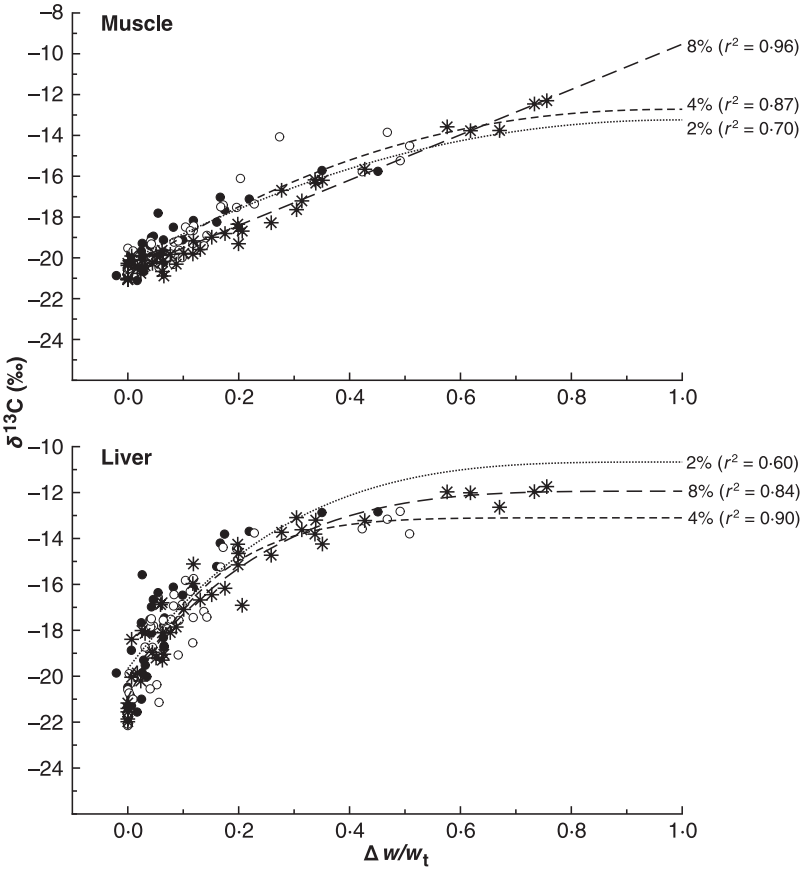

Fig. 3. The incorporation of ^{13}C into muscle (A) and liver (B) after a diet shift was described well by the equation $\delta_t = \delta_\infty - (\delta_\infty - \delta_0)(1 - \frac{\Delta w}{w_t})^{(1+\frac{k_c}{k_g})}$. Note that the relationship between δ^{13}C and $\Delta w/w_t$ in the muscle of fish fed on the highest ration is linear indicating the increased contribution of growth to isotopic incorporation.

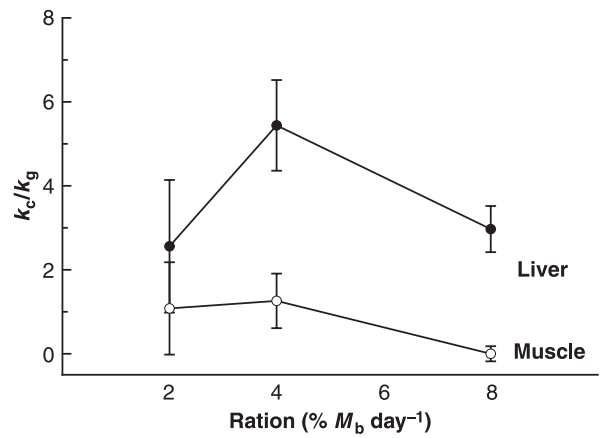

Fig. 4. The ratio of catabolic turnover to growth rate (k_c/k_g) was consistently higher for liver (solid circles) than muscle (open circles). Ration size seemed to have a nonlinear effect on this ratio, with maxima at intermediate ratios.

Table 3. The mass model can be used to estimate the parameters of the time model, including average retention time. Using the mass model to estimate retention time leads to large standard errors as a result of error propagation in arithmetic operations. Using parameters estimated by the mass model in the time model, leads to an adequate fit as judged by r^2 values

Ration (%)	Tissue	Mean retention time ± SE	r^2
2	Muscle	205·8 ± 121·4	0·86
	Liver	120·7 ± 63·7	0·78
4	Muscle	54·6 ± 16·9	0·67
	Liver	19·2 ± 4·2	0·72
8	Muscle	93·1 ± 26·6	0·92
	Liver	23·4 ± 26·6	0·87

growing at a rate of ≈1·2% per day when fed on the 8% ration. We emphasize that tilapia are capable of much faster growth than that experienced in our experiment. Typically, commercially farmed Tilapia grow at an average rate of 2% per day on diets with 40–45% fish meal protein (Lovell 1989; Wilson 1991, Ogunji *et al.* 2008). Our diet had lower protein content (≈32%) and the protein quality of corn gluten is relatively low (Robaina *et al.* 1997).

The mass model might have another advantage. Many isotope incorporation studies are conducted for less time than needed for tissues to reach an asymptotic ('equilibrium') value. This is especially true in ectotherms, which typically have lower incorporation rates than endotherms (Miller 2000; Dalerum & Angerbjörn 2005). Martínez del Rio *et al.* (2009) have argued that the nonlinear routines regularly used to fit the values of the time model are notoriously unreliable if the data set does not include values of δ_t that are close to the function's asymptote (Bates & Watts 1988; Caut, Angulo & Courchamp 2008). Because the estimates of δ_∞ and fractional incorporation rate λ obtained by the nonlinear fitting procedure are positively correlated, the overestimation of δ_∞ leads

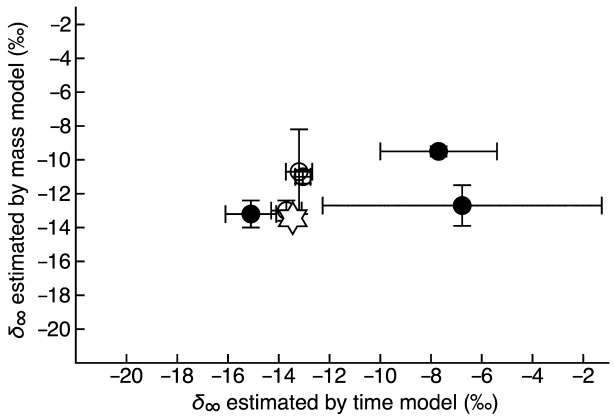

Fig. 5. The mass model estimates a smaller (and biologically more realistic) range of δ_∞ values than the time model for both liver (open circles) and muscle (closed circles). The star symbol is the value of the diet.

to the overestimation of λ (Martínez del Rio and Carleton, unpublished data). Figure 5 shows the values of δ_∞ estimated by the time and the mass model. Note that the time model's estimates of δ_∞ fall in a broad range that includes unrealistically high values. In contrast, the mass model estimated a narrower range of δ_∞ values. We speculate that the mass model gives more accurate estimates of δ_∞ for the same range of time values. However, this conjecture requires further theoretical exploration and experimental testing.

FEEDING RATE, GROWTH, CATABOLISM AND ISOTOPIC INCORPORATION

Our experiments were guided by several assumptions, some of which proved to be correct, others that did not. We expected liver to have a faster incorporation rate than muscle, and we expected the contribution of catabolic turnover to be higher than that of growth in liver than in muscle (de la Higuera *et al*. 1999, Peragón *et al*. 2001). Our predictions stemmed from the fundamental functional differences between liver and muscle. The liver is a homeostatic organ that receives, processes, and redistributes assimilated and catabolized macromolecules (Peragón *et al*. 2001). In contrast, muscle serves primarily a mechanical function, and therefore reacts more slowly in metabolic terms than does the liver (Peragón *et al*. 2001). In rats and trout, for example, the magnitude of total protein turnover is several times higher in liver than in muscle (Waterlow 2006).

We found that catabolic turnover in tilapia was also several times higher in liver than in muscle (Table 2). Furthermore, in liver, catabolic turnover was the primary determinant of isotopic incorporation at all rations, whereas in muscle, growth also plays an important role and at the highest ration it plays the predominant role (Table 2, Fig. 4).We wrongly assumed a strong linear effect of ration size on growth. Instead, we found a decelerating effect (Table 2). The effect of ration size on catabolic turnover was nonlinear as well. Catabolic turnover appeared to be highest in fish fed on the 4% ration. The consequence of these two observations was that instead of observing the monotonically decreasing relationship between k_c/k_g that we had predicted, we observed a more complex pattern (Fig. 4).

The higher magnitude of catabolic turnover in liver than muscle seems to be a general feature of ectotherms (Hesslein, Hallard & Ramlal 1993; Miller 2000; Logan *et al*. 2006) thathas potentially useful implications for isotopic research. It suggests that ecologists can use liver and muscle as ingredients of isotopic clocks (Phillips & Eldrige 2006) to time diet/habitat shifts (Herzka & Holt 2000; McIntyre & Flecker 2006; Gulenix *et al*. 2007). It also suggests that liver and muscle may provide different information in an ecological study (Martínez del Rio *et al*. 2009). In fish with discontinuous seasonal growth patterns, the isotopic composition of muscle reflects that of food consumed only during periods of growth, whereas the isotopic composition of liver responds to diet changes even if fish are not growing (Perga & Gerdeaux 2005). The temporal variation in the isotopic composition of a slow tissue, such as muscle, might greatly underestimate the amplitude of an animal's trophic niche (Newsome *et al*. 2007). Ecologists should exercise caution when studying growing organisms as differential patterns of growth and catabolism can have confounding effects on interpretation of field isotopic data in diet studies.

Acknowledgements

We thank Joe Bobbit and Steve DeVries for their assistance setting up the experiment. Leona Kelly and Teresa Tibbets assisted in care of animals. Our research was funded by NSF (IBN-0114016).

References

Bates, D.M. & Watts, D.G. (1988) *Nonlinear Regression Analysis and its Applications*. John Wiley and Sons Inc., New York, NY.

Bearhop, S., Hilton, G.M., Votier, S.C. & &Waldron, S. (2004a) Stable isotope ratios indicate that body condition in migrating passerines is influenced by winter habitat. *Proceedings of the Royal Society of London B*, **271**, 215–218.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & MacLeod, H. (2004b) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, **73**, 1007–1012.

Carleton, S.A. & Martinez 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.

Carleton, S.A., Kelly, L., Anderson-Sprecher, R. & Martinez del Rio, C. (2008) Should we use one- or multi-compartment models to describe ^{13}C incorporation into animal tissues? *Rapid Communications in Mass Spectrometry*, **22**, 3008–3014.

Caut, S., Angulo, E. & Courchamp, F. (2008) Caution on isotopic model use for analysis of consumer diet. *Canadian Journal of Zoology*, **86**, 438–445.

Cerling, T.E., Ayliffe, L.K., Dearing, M.D., Ehleringer, J.R., Passey, B.H., Podlesak, D.W., Torregrossa, A.M. & West, A.G. (2007) Determining biological tissue turnover using stable isotopes: the reaction progress variable. *Oecologia*, **151**, 175–189.

Dalerum, F. & Angerbjörn, A. (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia*, **144**, 647–658.

Ebert, T.A. (1999) *Plant and Animal Populations: Methods in Demography*. Academic Press, San Diego, CA.

Feynman, R. (1967) *The Character of Physical Law*. MIT press, Cambridge, MA.

Fry, B. & Arnold, C. (1982) Turnover of $^{13}C/^{12}C$ during growth in brown shrimp. *Oecologia*, **54**, 200–204.

Gaye-Siessegger, J., Focken, U., Abel, H.-J. & Becker, K. (2003) Feeding level and diet quality influencetrophic shift of C and N isotopes in Nile Tilapia (*Oreochromisniloticus*). *Isotopes in Environmental and Health Studies*, **39**, 125–134.

Gulenix, J., Maes, J., Van Den Driessche, P., Geysen, B., Dehairs, F. & Ollevier, F. (2007) Changes in δ^{13}C and δ^{15}N in different tissues of juvenile sand goby *Pomatoschistusminutus*: a laboratory diet-switch experiment. *Marine Ecology Progress Series*, **341**, 205–215.

Herzka, S.Z. & Holt, G.J. (2000) Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. *Canadian Journal of Fisheries and Aquatic Science*, **57**, 137–147.

Hesslein, R.H., Hallard, K.A. & Ramlal, P. (1993) Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonusnasus*) in response to a change in diet traced by δ^{34}S, δ^{13}C, and δ^{15}N. *Canadian Journal of Fisheries and Aquatic Science*, **50**, 2071–2076.

de la Higuera, M., Akharbach, H., Hidalgo, M.C., Peragón, J., Lupiáñez, J.A. & García-Gallego, M. (1999) Liver and white muscle protein turnover rates in the European eel (*Anguilla anguilla*): effects of dietary protein quality. *Aquaculture*, **179**, 203–216.

Kelly, L.J. & Martínez del Rio, C. (2010) The fate of carbon in growing fish: an experimental study of isotopic routing. In Review. *Physiological and Biochemical Zoology*, In press, DOI: 10.1086/649628.

Logan, J., Haas, H., Deegan, L. & Gaines, E. (2006) Turnover rates of nitrogen stable isotopes in the salt marsh mummichog, *Fundulusheteroclitus*, following a laboratory diet switch. *Oecologia*, **147**, 391–395.

Lovell, T. (1989)*Nutrition and Feeding of Fish*. Van Nostrand Reinhold, New York.

Martínez del Rio, C., Wolf, N., Carleton, S.A. & Gannes, L.Z. (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews*, **84**, 91–111.

Martínez del Rio, C. & Anderson-Sprecher, R. (2008) Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. *Oecologia*, **156**, 765–772.

McIntyre, P.B. & Flecker, A.S. (2006) Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. *Oecologia*, **148**, 12–21.

Miller, T. (2000) Tissue-specific response of δ^{15}N in adult pacific herring (*Culpeapallasi*) following an isotopic shift in diet. *Environmental Biology of Fishes*, **76**, 177–189.

Newsome, S., Martinez del Rio, C., Phillips, D.L. & Bearhop, S. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, **5**, 429–436.

Norris, D.R., Marra, P.P., Kyser, T.K. & Ratcliffe, L.M. (2005) Tracking habitat use of a long-distance migratory bird, the American redstart *Setophagaruticilla*, using stable-carbon isotopes in cellular blood. *Journal of Avian Biology*, **36**, 164–170.

Ogunji, J., Toor, R., Schulz, C. & Kloas, W. (2008) Growth performance, nutrient utilization of Nile Tilapia, *Oreochromisniloticus* fed housefly maggot meal (magmeal) diets. *Turkish Journal of Fisheries and Aquatic Sciences*, **8**, 141–147.

Peragón, J., Barroso, J.B., Garcia-Salguero, L., de la Higuera, M. & Lupiáñez, J.A. (2001) Growth, protein-turnover rates and nucleic-acid concentrations in the white muscle of rainbow trout during development. *International Journal of Biochemistry and Cell Biology*, **33**, 1227–1238.

Perga, M.E. & Gerdeaux, D. (2005) Are fish what they eat' all year round. *Oecologia*, **144**, 598–606.

Phillips, D.L. & Eldrige, P.M. (2006) Estimating the timing of diet shifts using stable isotopes. *Oecologia*, **147**, 195–203.

Reich, K.J., Bjorndal, K.A. & Martinezdel Rio, C. (2008) Effects of growth and tissue type on the kinetics of ^{13}C and ^{15}N incorporation in a rapidly growing ectotherm. *Oecologia*, **155**, 651–663.

Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M. & Montero, J.M. (1997) Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. *Aquaculture*, **157**, 347–359.

Waterlow, J.C. (2006) *Protein Turnover*. CABI, Wallingford, UK.

Wilson, R.P. (1991) *Handbook of Nutrient Requirements of Finfish*. CRC Press, Boca Raton, FL.

Wolf, N., Carleton, S.A. & Martinez del Rio, C. (2009) Ten years of experimental isotopic ecology. *Functional Ecology*, **23**, 17–26.

Received 2 September 2009; accepted 9 February 2010
Handling Editor: David Raubenheimer