NOTE / NOTE

Caution on isotopic model use for analyses of consumer diet

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Abstract: Isotopic models are increasingly used to determine the relative contribution of different food sources to an animal's diet. However, these models are based on restrictive assumptions and provide estimates rather than exact values of contributions to consumer diet. The sources of inaccuracies in isotopic models are not well understood and laboratory experiments may be useful to evaluate model performance. In this paper we assess the accuracy of the three main isotopic models in controlled laboratory experiments, involving a breed of Norway rats (*Rattus norvegicus* (Berkenhout, 1769)), in which the isotopic values of resources are known. At the same time, we measure errors resulting from the use of fixed or specific discrimination factor values for each resource and tissue. We show that the results of the three main isotopic models deviate considerably from the correct values in some cases. Estimations obtained using specific discrimination factors (corresponding to each experimental diet and tissue) were more accurate than those obtained using fixed discrimination factors (obtained from the literature). In addition, estimations varied depending on the tissue used, with the liver giving more accurate results than muscle or hair. We discuss the assumptions and limitations of isotopic models and highlight the importance of taking these assumptions into account when the most accurate results are sought. Finally, we propose some recommendations for the correct use of isotopic models, emphasizing the need to use specific discrimination factors for each species, tissue, and diet isotopic value.

Résumé : On utilise de plus en plus fréquemment des modèles isotopiques pour déterminer les contributions relatives des différentes sources de nourriture au régime alimentaire des animaux. Ces modèles sont, cependant, basés sur des présuppositions restrictives et ils fournissent des estimations plutôt que des valeurs exactes des contributions au régime du consommateur. On connaît mal les sources des imprécisions dans les modèles isotopiques; des expériences en laboratoire peuvent donc être utiles pour évaluer la performance de ces modèles. Dans notre étude, nous évaluons la précision de trois modèles isotopiques courants dans des expériences contrôlées en laboratoire avec une race de rats surmulots (*Rattus norvegicus* (Berkenhout, 1769)), dans lesquelles les valeurs isotopiques des ressources sont connues. En même temps, nous mesurons les erreurs produites par l'utilisation de valeurs de facteurs de discrimination fixes ou spécifiques pour chaque ressource et tissu. Nous démontrons que les résultats obtenus dans les trois modèles isotopiques courants peuvent s'éloigner considérablement des valeurs réelles dans certains cas. Les estimations obtenues par l'usage de facteurs de discrimination spécifiques (correspondant à chaque régime et tissu de l'expérience) sont plus précises que celles obtenues par l'utilisation de facteurs de discrimination fixes (tirés de la littérature). De plus, les estimations varient en fonction des tissus, car le foie permet des résultats plus précis que les muscles ou les poils. Nous discutons des présuppositions et des limites des modèles isotopiques et nous insistons sur l'importance de tenir compte de ces présuppositions pour l'obtention des résultats les plus précis. Nous faisons, enfin, des recommandations sur l'usage approprié des modèles isotopiques en soulignant la nécessité d'utiliser des facteurs de discrimination spécifiques pour les valeurs isotopiques de chaque espèce, chaque tissu et chaque régime alimentaire.

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Introduction

Stable isotope analyses are frequently used to determine the relative contributions of different food sources to an animal's diet (Gannes et al. 1997; Hobson 1999; Post 2002). Recently, a number of studies have used geometric procedures to reconstruct such diets (Kline et al. 1993; Ben-David et al. 1997*a*, 1997*b*; Szepanski et al. 1999; Phillips 2001; Phillips and Koch 2002; Phillips and Gregg 2003). These isotopic models typically use the mean $\delta^{13}C$ and $\delta^{15}N$ values for each type of diet corrected for the discrimination factor of the consumer (the increase in consumer isotopic ratio compared with that of the consumer's diet). Since the 1950s, when the first isotopic models were used, an increas-

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ing number of studies have employed these models to quantify the contribution of multiple sources in a mixture, such as the proportions of different types of food sources in an animal's diet (Phillips 2001). As is the case with traditional methods of estimating consumer diet, isotopic models are not exact and provide estimates only (Ben-David and Schell 2001; Phillips 2001). Previous studies on isotopic models have highlighted this problem and called for laboratory studies to assess the extent of inaccuracies and to ascertain the reasons why the errors occur (Ben-David and Schell 2001; Phillips 2001). Laboratory studies offer opportunities to obtain accurate isotopic values for both a consumer and the consumer's resources, allowing tests of model performance. In addition, the use of a fixed discrimination factor has been suggested as a possible source of error in the determination of mixed diet composition. Whereas it is difficult to design field studies to demonstrate this assertion, several studies continue to use fixed discrimination factors without taking into account the taxon under study, the tissue examined, or the type of diet (Hicks et al. 2005; Inger et al. 2006; Lepoint et al. 2006; Reich and Worthy 2006). For example, in some cases the fixed discrimination factor used in the model has been earlier associated with a different species or, even if the selected factor was earlier used with the same species, the factor is now used for work with different tissues or different diets. Ecologists and zoologists working with species in the wild need to know the accuracies of isotopic models in diet reconstruction, as well as the implications of choosing given discrimination factors.

The purpose of this paper is therefore (*i*) to assess the accuracy of the three main isotopic models by back-calculating isotopic values of resources that are known beforehand as controlled laboratory experiments are performed and (*ii*) to assess the errors resulting from using fixed instead of specific discrimination factors for each resource and tissue. We then discuss the assumptions of isotopic models and propose some recommendations on how to apply these models with greater accuracy.

Materials and methods

Experimental procedures

We conducted a controlled feeding study to measure isotopic discrimination in experimental Sprague Dawley rats (a breed of Norway rats (*Rattus norvegicus* (Berkenhout, 1769)); 48 male rats, ~6 weeks of age) fed four synthetic diets differing in isotopic values (A, B, C, and M; Fig. 1). Diet A was fish meal and corn flour; diet B was corn flour and casein; diet C was alfalfa meal and corn flour (for the isotopic composition of each diet component see Caut et al. 2008*a*); and diet M was 60% of diet A, 20% of diet B, and 20% of diet C (percentages based on the mass of each diet). All diets had the same energy value (3.5–4 kcal/g, where 1 cal = 4.1868 J), and the same proportions of lipids (9% by mass), proteins (4% by mass), and carbohydrates (4% by mass). We added a mixture of minerals (UAR205b), a mixture of vitamins (UAR200), and vegetable oil to each diet. Rats were randomly assigned to four groups corresponding to the four diets; there were six rats per group, distributed into two cages with three rats each. The experiment lasted 8 weeks. At the end of the experiment, animals were humanely euthanized and tissue samples of liver, muscle, and hair were collected for isotopic analysis. For more details on the experimental design see Caut et al. (2008*a*).

Stable isotope analysis

Samples for stable isotope analyses (from both animals and diets) were freeze-dried, ground to a fine powder, weighed in tin capsules, and stored in a desiccator until measurement. Lipids were not removed from samples. We verified that the possible effects of lipids on $\delta^{13}C$ were minimal, based on two considerations. First, Post et al. (2007) recommends that $\delta^{13}C$ values should be normalized if C/N ratios are >4, which was not the situation in our study; second, simple regressions between $\delta^{13}C$ and corresponding C/N ratios were not significant (all p values >0.05) (see Caut et al. 2008*a*). Isotopic analyses were performed using an IsoPrime (MicroMass) spectrometer coupled with a EuroEA 3024 (EuroVector) analyser. Stable C ($\delta^{13}C$) and N ($\delta^{15}N$) isotope ratios are expressed as $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The standard for C is IAEA-NBS 21 (graphite, 28.13‰) and the standards for N are IAEA-N1 (+0.4‰) and IAEA-N2 (+20.3‰). Ten replicate assays of internal laboratory standards indicated maximum measurement errors (SDs) of ±0.15‰ and ±0.2‰ for stable $\delta^{13}C$ and $\delta^{15}N$ measurements, respectively. A discrimination factor between a food resource (X) and a consumer (Y) is described in terms of the difference in delta (δ) values ($\delta Y - \delta X$). A positive value indicates a relatively greater concentration of the isotope with higher mass in Y, and Y therefore has a lower isotopic value than X. Preliminary analysis showed that there were no differences in $\delta^{13}C$ and $\delta^{15}N$ between cages; hence, for each diet we pooled data obtained from the six rats in the two cages.

Discrimination factors have been shown to vary considerably with changes in various parameters, including the consumer taxonomic class, the tissue or organ examined, the age and physical condition of the individual, and the type, quantity, quality, and isotopic value of the diet (Adams and Sterner 2000; Van der Zanden and Rasmussen 2001; Oelbermann and Scheu 2002; Felicetti et al. 2003; Vanderklift and Ponsard 2003; Robbins et al. 2005). In fact, Caut et al. (2008*a*) showed significant relationships between $\delta^{13}C$ and $\delta^{15}N$ values of diets and their corresponding carbon and nitrogen discrimination factors in different rat tissues. At the beginning of our experiment, age, isotopic values, and physical condition were similar for all rats, and during the experiment rats were fed ad libitum with diets of similar energetic values but different isotopic values. Thus, we determined the discrimination factors corresponding to each monospecific diet (A, B, C) for each tissue (Table 1); we term these values diet-dependent discrimination factors (DDDFs).

Stable isotopic models

To test the performance of different isotopic models, we selected the three most commonly used models. Model 1 is the linear mixing model, which is based on mass balance

Fig. 1. Isotopic values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for different diets and in different tissues of Norway rats (*Rattus norvegicus*). A, B, and C are the three main diets, whereas M is the mixed diet. Mean diet values ($\delta^{13}C/\delta^{15}N$) are in parentheses and percentages of carbon and nitrogen (C/N) are in brackets.

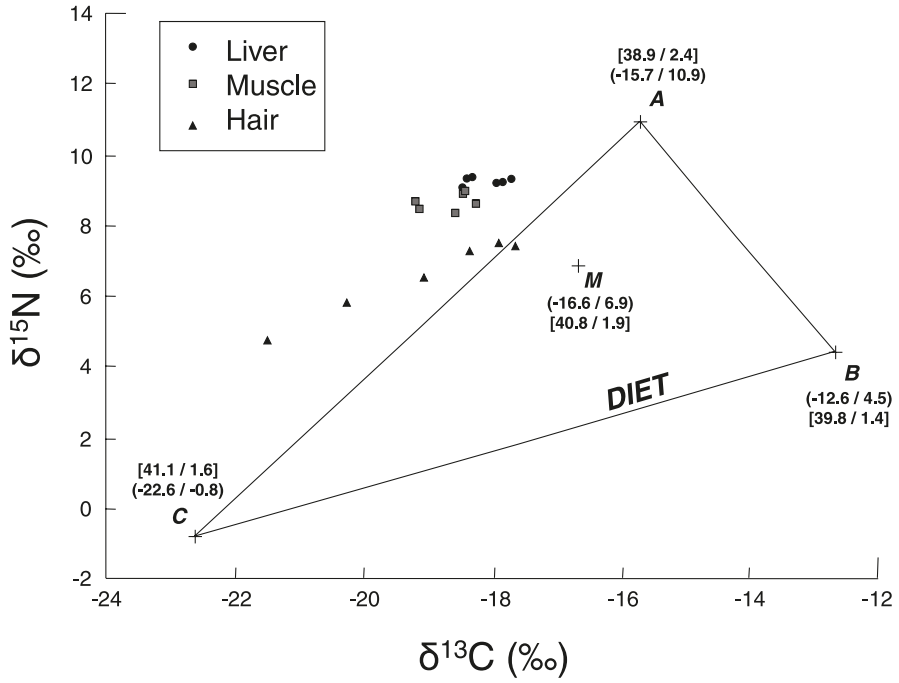


Table 1. Discrimination factors for carbon (ΔC) and nitrogen (ΔN) in the three basic diets (A, B, and C), for muscle, liver, and hair of Norway rats (*Rattus norvegicus*).

	Diet A		Diet B		Diet C	
Tissue	ΔC (‰)	ΔN (‰)	ΔC (‰)	ΔN (‰)	ΔC (‰)	ΔN (‰)
Muscle	–1.69 (0.10)	1.39 (0.08)	–5.12 (0.14)	1.73 (0.07)	–0.40 (0.04)	4.59 (0.07)
Liver	–1.65 (0.07)	1.12 (0.14)	–2.81 (0.19)	2.33 (0.07)	0.64 (0.11)	4.55 (0.41)
Hair	–2.10 (0.39)	–1.46 (0.51)	–8.79 (0.29)	–0.89 (0.07)	–0.58 (0.07)	4.05 (0.10)

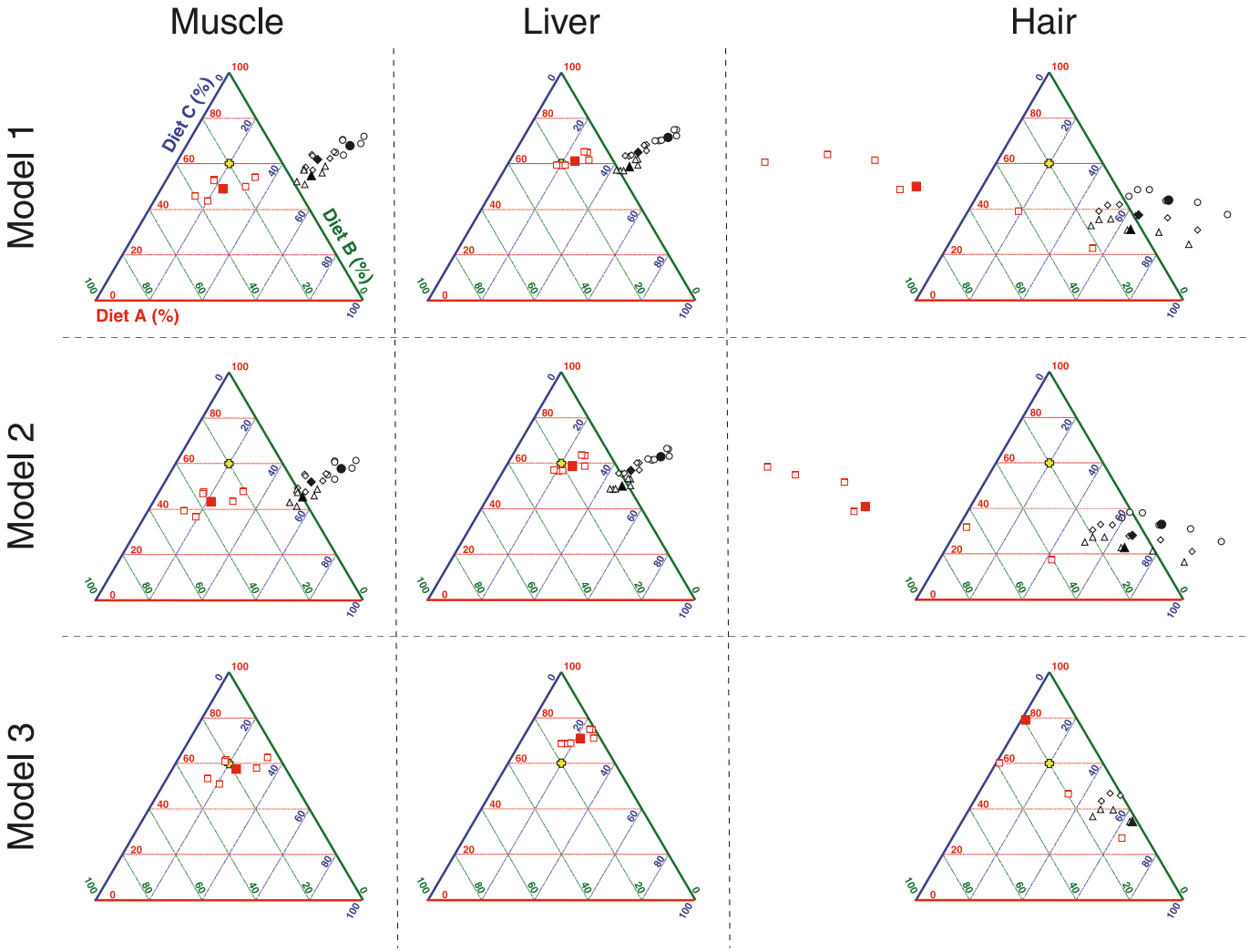
Note: Values are means (SE).

equations (Phillips 2001). This model estimates the proportion of each source regardless of whether all sources are utilized. The number of isotope ratios (e.g., $\delta^{13}C$, $\delta^{15}N$) utilized (n) allows the determination of a unique solution of the relative contributions of at most $n + 1$ sources to the mixture (Phillips and Gregg 2001). For example, a two-isotope system can be used to determine a unique solution of the relative contributions of three diet items. In this case, the linear mixing model is a system of three equations with three unknowns (the fractional contribution of each of the three food sources to the consumer's diet), which can be solved for a unique solution of the unknowns.

Model 1 assumes that the proportional contributions of C and N to the diet are similar for a given food source (Phillips and Koch 2002). This assumption is reasonable if the elemental concentrations of each food source are similar and if they are equally digestible (Koch and Phillips 2002). In the omnivore diet, however, food items can have dramatically different elemental concentrations. For example, terrestrial plants generally have low concentrations of N relative to the concentration of C, and hence may provide much lower amounts of N relative to C in the diet. This discloses the need to incorporate both isotopic composition and elemental concentration in a model designed to determine proportional dietary sources. To solve this problem, the concentration-weighted linear mixing model (hereinafter Model 2) of Phillips and Koch (2002) assumes that the contribution of each diet item is proportional to the contributed mass multiplied by the elemental concentration in the food source.

Models 1 and 2 remained too limited, however, because the number of food sources of omnivorous consumers often exceeds the number of isotopes needed to differentiate among sources. To cope with this problem, Phillips and Gregg (2003) developed the source-partitioning mixing model IsoSource (hereinafter Model 3). IsoSource evaluates all biomass combinations from each food source (from 0% to 100%) to identify source combinations that sum to the known isotopic signature of the mixture within a small prescribed tolerance. The output of this model is a distribution of the frequency and range of potential source contributions instead of a unique solution. In our study, we had only three sources and two tracers ($\delta^{13}C$ and $\delta^{15}N$); a unique solution therefore exists using either Model 1 or Model 2. It is also possible, however, to run Model 3, resulting in a distribution of frequency of source contributions of each diet. This

Fig. 2. Proportions of each component (A, B, and C) of diet M (60% of diet A, 20% of diet B, and 20% of diet C; all by mass) estimated by the three models (1–3) for different tissues (muscle, liver, and hair) of Norway rats (*Rattus norvegicus*). Four discrimination factors were used for comparison and are represented by different shapes: red squares for DDDFs (for values see Table 1), circles for 2/3 of ΔC/ΔN, diamond for 1/3 of ΔC/ΔN, and triangles for 0.8/3.4 of ΔC/ΔN. For each tissue and model, solid shapes represent the averaged proportions from the six individuals and open shapes are values for each of the six individual rats. Shapes were represented only when the isotopic mixing model provided any results, in which case they were inside the triangle when positive and outside the triangle when negative. The actual percentage of diet M is shown with a yellow cross. Model 3 gives a frequency distribution of each diet instead of a unique solution; we have shown the average of this distribution for each individual.



model can be applied even if the number of resources does not require application of a model of this complexity. Moreover, this model has also been used when isotopic values of the food sources were only weakly distinct (e.g., in Lepoint et al. 2006) or when a distribution is required for the estimation of other parameters (e.g., in Caut et al. 2006).

We used Models 1–3 to back-calculate the contributions of the three food sources in the diets used in our experiments, using the isotopic values of three different tissues (muscle, liver, and hair) after correction for discrimination factors. When the consumer is a mammal, discrimination factors of 0.8‰, 1‰, and 2‰ for $\delta^{13}C$ and 3.4‰, 3‰, and 3‰ for $\delta^{15}N$ have been commonly used in isotopic model studies (DeNiro and Epstein 1978; Ben-David 1996; Ben-David et al. 1997*a*, 1997*b*; Drever et al. 2000; Kasai et al. 2004). We first ran each model using these three fixed discrimination factors and compared the outputs with a run of each model using the DDDFs. For each of these four conditions, the model outputs (proportion of each resource in the consumer diet) were compared with the original, known, values of diet M (60%, 20%, 20% by mass of diets A, B, and C, respectively).

Results

We found considerable variations in dietary proportion estimates given by the three models. Among the models, results with the discrimination factors calculated from our experiment for each tissue and diet (the DDDFs) were generally closer to the actual values than were results obtained when other discrimination factors were used (except for hair, which yielded nonsensical results with DDDFs in Models 1

Table 2. Assumptions made, and limitations resulting from such assumptions, in different steps of isotopic model use, and recommendations for optimal choices in the use of isotopic models for diet reconstruction studies.

Steps	Assumptions and limitations	Recommendations
Isotopic model choice	Depends on the number of isotopic tracers (n) and resources	Model 1 and 2 for $n + 1$ resources. Model 3 for more than $n + 1$ resources
Resource choice	The correct diet items should be used (the value of the consumer must be inside the area bounded by the corrected values of resources)	Use of traditional methods (faeces, gut contents, direct observations) as the basis for the diet choice
	Diet items should have different isotopic values	Group sources with similar isotopic values, (e.g., coastal prey, herbivorous insects)
Tissue choice	Tissue should be in equilibrium with the diet	Requires information on diet changes through time. Try to use a tissue with a known turnover
	Tissue turnover rate (the time tissues need to reflect isotopic value of the diet) should correspond with the period of diet study	Difficult to assess for each species and tissue but need to be assessed (e.g., under experimental conditions). In general, liver and plasma have higher turnover rates than blood cells or muscle. Hair or feathers reflect the diet during the period of tissue growth
	Tissues chosen are a trade-off between accuracy and invasiveness	Hair would be less invasive but less accurate, liver would be invasive but more accurate. Blood would be in an intermediate level of invasiveness (but not evaluated here)
Discrimination factor choice	The adapted discrimination factor should be used. Models are sensitive to modifications of >1‰ in discrimination factors	Use the most correct discrimination factor: specific for each species, age, tissue, etc. Use (*i*) diet-specific discrimination factors (e.g., obtained with experimental data) and (*ii*) linear regression equations to estimate diet-dependent discrimination factors

and 2). Indeed, estimations with fixed discrimination factors either gave no data (Model 3), negative estimates (Models 1 and 2), or greatly underestimated one dietary component (Models 1 and 2; Fig. 2).

When comparing outputs obtained with the DDDFs, the accuracies of tissue data differed substantially. Hair systematically gave the least precise results, whereas liver data were almost always the most accurate. When comparing models using DDDFs in the liver, Model 2 provided the best results with only 3% difference from the actual percentages, whereas Model 1 resulted in a 6% error and Model 3 with a 13% error. When comparing models using DDDFs in the muscle, Model 3 gave the best results with only a 2% error with respect to the actual percentages, whereas Model 1 resulted in a 10% error and Model 2 with a 15% error.

Discussion

Stable isotope models (Models 1–3) are increasingly used in field studies to assess the relative contribution of different food sources to a consumer (Caut et al. 2006, 2008*b*; Gao et al. 2006; Inger et al. 2006; Lepoint et al. 2006; Lusseau and Wing 2006) and recently to define ecological niche (Newsome et al. 2007). A general weakness of applying isotopic models in ecological studies has been the difficulty of assessing whether the models accurately calculate dietary compositions. Although isotopic models are unquestionably very helpful, they have restrictive assumptions and inevitably have mathematical and biological limitations (Ben-David and Schell 2001; Phillips 2001; Phillips and Gregg 2001), especially if they are not applied in their optimal form. In this paper, we assessed the accuracy of the three main isotopic models in a controlled laboratory experiment with diets of known isotopic values; different tissues (muscle, liver, and hair) of rats fed with a mixed diet M (60% of diet A, 20% of diet B, and 20% of diet C; all by mass) were sampled and percentages of each diet were back-calculated using isotopic models. We tested the accuracies of fixed discrimination factors and specific discrimination factors (species-specific, tissue-specific, and diet-dependent; the DDDFs). Our study shows that model choice is important, but tissue and discrimination factors also require consideration. The use of DDDFs offered performances better than those obtained when fixed discrimination factors were employed. Model 3 was the most accurate (Model 1 was in second place), and liver was the most suitable tissue (with hair proving to be the least adequate choice). In particular, our results disclose the crucial effect of discrimination factor choice on the accuracy of isotopic models for diet reconstruction. To help researchers choose appropriate isotopic models, we present below the key assumptions of the models and give some recommendations for their correct use (Table 2).

The choice of sources is an early, essential, and delicate stage that precedes the use of isotopic models. Stable isotopes offer advantages over traditional methods (direct observations, gut content analysis, or faecal analysis) because they provide information on assimilated foods (not just ingested foods), as well as time-integrated information (Boutton et al. 1983). Conventional methods are, however, complementary to isotopic models, because they provide a taxonomic resolution of diet that is necessary before choosing the diet composition of the consumer (Hecky and Hesslein 1995; Van der Zanden et al. 1997). Indeed, isotopic models do not permit discrimination between foods either "consumed" or "not consumed", as all resources added to the isotopic model will have a percentage of occurrence (or there will be no solution offered by the model). This shows

the importance of good selection of resources (using traditional methods) when using isotopic models (Table 2).

Indeed, the lack of independent tracers generally limits the choice to only a few possible resources, although some isotopic models accept a greater number of resources than the number of independent tracers available (Table 2). This limitation, together with the requirement that resources have significantly different isotopic values, can be solved by grouping resources with similar isotopic values (e.g., marine mammals; benthic or coastal prey). This problem is especially relevant when working with omnivore species (see, e.g., Caut et al. 2008*b*). In addition, the isotopic value of the consumer must be inside the area bounded by the corrected value of consumer resources (Phillips 2001). When the isotopic value is outside this area, the isotopic mixing models provide meaningless, negative values, or do not offer any results (Fig. 2), suggesting either that an important food source was not identified (Ben-David and Schell 2001; Phillips 2001) or that various parameters were not appropriately estimated (e.g., discrimination factors, tissue turnover).

Tissue choice depends both upon the organism under study and the scientific question being examined. When animals undergo dietary shifts, each tissue needs a period of time to become accustomed to the new diet and to reach isotopic equilibrium because of isotopic "memory" of the previously assimilated diet. Isotopic models assume that consumers are in equilibrium with their diet. This is an important problem in field studies where the notion of equilibrium is difficult to estimate. Our experiment lasted 8 weeks and during this time we assumed that isotopic values of all tissues attained equilibrium (for more information see Caut et al. 2008*a*).

Dietary information over several time scales can be obtained by measuring several tissues within an individual depending on specific tissue turnover rates (Table 2). Access to some tissues can be highly invasive, however, and may even result in the death of an individual, thus making it impossible to undertake a long-term study of individual dietary shifts. Fortunately, tissues such as feathers, skin, nails, or hair may be appropriate for long-term studies. In these metabolically inactive tissues, there is little turnover of stable isotopes of most elements; hence, the levels of these isotopes reflect the diet of individuals during only a limited period of tissue growth (Tieszen et al. 1983). The poor performance of the models with hair in our experiment was perhaps because this tissue offered data values corresponding to both an old diet (during the first 6 weeks of life) and the experimental diets (8 weeks). At the end of the experiment, however, there was significant variation in hair isotopic ratios between each diet treatment (Table 1), showing that hair was not completely inert (the hair grew during the experiment). Even though this tissue yielded low experimental performance, we find the results interesting, especially because hair is a nonlethal source of tissue.

One fundamental feature in the successful application of a stable isotopic model is the establishment of diet–tissue discrimination factors (Hobson and Bairlein 2003). Discrimination factor estimates are subject to uncertainty, because discrimination may vary depending on a consumer's nutritional status, diet quality, size, age, dietary ontogeny, tissue, and dietary elemental composition (Minagawa and Wada 1984; Ben-David and Schell 2001). In addition, the differential metabolic routing of elements from diet to tissues can contribute to variations in derived diet–tissue discrimination factors. Hilderbrand et al. (1996) showed significant relationships between C and N isotopic ratios of diets and of the animals fed on such diets; Felicetti et al. (2003) showed similar results in bears (genus *Ursus* L., 1758). Caut et al. (2008*a*) obtained analogous data in black rats (*Rattus rattus* (L., 1758)) fed diets of different isotopic values. Currently, most isotopic model studies for diet reconstruction use a single discrimination factor for C and N, which (*i*) usually comes from a review study that mixed up different consumer species or tissues and (*ii*) does not take into account the dependence of discrimination factors on dietary isotopic values. Here, we have shown that using fixed discrimination factors, and (or) discrimination factors that are not diet-dependent, leads to incorrect results with most models (Fig. 2). The existence of a possible linear relationship between dietary isotopic values and discrimination factors (such as that proposed by Felicetti et al. 2003 and Caut et al. 2008*a*) allows the estimation of adequate diet discrimination factors. This can be a good solution when the identification of the exact discrimination factor (for the target species, tissue, and resource) is costly or simply not possible (e.g., in studies of endangered species).

In summary, understanding and estimating a consumer diet with a stable isotope model is still complex. Stable isotope methods are currently among the most powerful tools for the study of trophic relationships and animal diets. The performances of the three models examined in the present work differed only to a small degree; hence, model choice will depend on the number of isotopic tracers available, as well as experimental resources. The assumptions of isotopic models, such as the potential sources of variation in discrimination factors, should not be overlooked. In particular, users of future work on isotopic models should consider the dependence of discrimination factors on diet isotopic values.

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