

Discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) in an omnivorous consumer: effect of diet isotopic ratio

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Summary

1. Naturally occurring stable isotopes in resources and their consumer allow the estimation of nutritional flows between the two and have been much used to improve our understanding of the nutritional ecology of free-living animals.
2. The difference in isotopic composition between an animal and its diet is represented by a discrimination factor. Carbon and nitrogen flows are estimated by calculating the discrimination factors in stable isotope ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$), which are presumed to be *c.* 3‰ and 1‰ heavier in the consumer tissues than those in their resources, respectively.
3. The discrimination factor is known to vary according to species, tissue, age, growth rates and food quality, but the estimation of discrimination factors is difficult and a fixed discrimination factor is usually used in diet reconstruction. It has also been suggested that discrimination factors could vary linearly with the diet isotopic ratio. If this linear relationship could be demonstrated using regression, this would provide an adequate method for the estimation of discrimination factors. In order to understand how diet isotopic ratios affect the discrimination factor, we investigated the pattern of its change in nitrogen ($\Delta^{15}\text{N}$) and carbon ($\Delta^{13}\text{C}$) in different tissues (liver, muscle and hair) of an omnivore species, the rat *Rattus rattus*. We fed captive rats with diets of the same nutritional quality but on different isotopic ratios.
4. First, discrimination factors for $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ showed great variability, ranging from -1.46‰ to 4.59‰ and from -8.79‰ to 0.64‰ , respectively. Discrimination factors depended on both diet isotopic ratio and tissue.
5. We also show that isotope ratios in shaved hairs showed a turnover during the first month, and then stabilized during the second month. Using shaved hairs has the potential to be an effective non-lethal method for determining resource shifts in non-specialist consumers.
6. Finally, we demonstrated, for all tissues, a decrease of $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ with an increased values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively. These relationships allow us to propose a framework to estimate discrimination factors from diet isotopic ratios by means of regression models.

Key-words: diet–tissue relationship, fractionation factor, mixing models, rat, stable isotope

Introduction

There are several techniques for assessing the diet of organisms, including traditional methods (foraging observations, stomach and faeces analysis), and examination of chemical constituents such as stable isotopes or nutrients within the tissues of organisms compared with their potential food sources. Direct observation and gut analysis are difficult to interpret and restrictive. Behavioural observations can show the manner and location of feeding, but there are observational biases because it is often easier to see animals feeding on one

type of prey or habitat than another. Stomach and a faeces content analysis show what a species has recently eaten and in what quantity, but retention times and digestibility of dietary components differ, biasing the results (Levey & Karasov 1994; Afik & Karasov 1995). Moreover, they cannot distinguish undigested material, thus providing incorrect estimates of the percentages of various materials assimilated by the individual (Kasai, Horie & Sakamoto 2004). In contrast, stable isotopes offer an alternative method to reconstruct diets and to evaluate the relative importance of the dietary components to the consumer (Szepanski, Ben-David & Van Ballenberghe 1999). This approach is based on the fact that stable isotope ratios of nitrogen ($^{15}\text{N}/^{14}\text{N}$, noted $\delta^{15}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$, noted

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$\delta^{13}\text{C}$) in the consumer tissues reflect those in their resources in a predictable manner (DeNiro & Epstein 1978, 1981). Stable isotopes offer advantages over traditional methods because stable isotopes provide information on assimilated foods (not just ingested foods) as well as time-integrated information (Boutton, Arshad & Tieszen 1983).

Recently, different isotopic models have been used to reconstruct diets from isotopic ratios in animal tissues (Ben-David, Flynn & Schell 1997a; Ben-David *et al.* 1997b; Szepanski *et al.* 1999; Phillips & Gregg 2001, 2003; Brooks *et al.* 2002). A difficulty in using isotopic models for evaluating incorporation of resources from mixed diets is that consumer metabolic processes may discriminate between different isotopes; their isotopic ratio may not exactly correspond to the isotopic ratio of the food resource. The difference in isotopic composition between any tissue of an animal (e.g. muscle, liver and hair) and its diet is represented by a discrimination factor (Δ , also called trophic shift or enrichment). Interpretation of stable isotope output of isotopic model requires knowledge of the discrimination factors between diet and consumer. Discrimination may vary depending on a consumer's nutritional status, lipid content, the quality of the diet consumed, size, age, dietary ontogeny, and the tissue and elemental composition of both consumer and diet (Minagawa & Wada 1984; Ben-David & Schell 2001; Vanderklift & Ponsard 2003).

The discrimination factor is usually assumed to be low for $\delta^{13}\text{C}$ but significant in $\delta^{15}\text{N}$ (Hullar, Fry & Peterson 1996). Because there is little carbon enrichment between an organism and its diet, the isotopic $\delta^{13}\text{C}$ is largely indicative of dietary sources of carbon (C_3 , C_4 or marine plants) (Vander Zanden & Rasmussen 2001; Post 2002). Thus, the carbon isotopic composition of whole animal tissues typically reflects the isotopic ratio of the food eaten, with only a slight (0.5‰–1‰) enrichment at each trophic level. Currently, there is little knowledge on how $\Delta^{13}\text{C}$ varies between individuals of a given species under different food conditions. However, Oelbermann & Scheu (2002) found that a generalist predatory spider (*Pardosa lugubris*) had a consistently higher $\delta^{13}\text{C}$ when fed high quality prey. Nitrogen isotopes behave in a different manner compared to carbon isotopes. DeNiro & Epstein (1981) were the first to demonstrate that animal tissues are enriched in $\delta^{15}\text{N}$ compared with their diets. Subsequently, Minagawa & Wada (1984) proposed an enrichment ranging between 1.3‰ and 5.3‰, but in practice a constant discrimination factor of 3.5‰ is often used for the back calculation of diets (Herrera *et al.* 2002). However, levels of $\Delta^{15}\text{N}$ have been shown to vary considerably according to age (Overman & Parrish 2001), tissue type and species (Kelly 2000; Pearson *et al.* 2003), as well as food type, quantity and quality represented by %N or the ratio %C : %N (Hays *et al.* 2000; Gaye-Siessegger *et al.* 2004; Robbins, Felicetti & Sponheimer 2005).

Discrimination factor may be affected by diet isotopic values. Only two studies have indirectly noted a diet isotopic effect on discrimination factors; Hilderbrand *et al.* (1996) found that carbon and nitrogen isotope ratios in bear plasma were linearly related to the carbon and nitrogen isotope ratios of single-item and mixed diets. Felicetti *et al.* (2003) showed

similar result with a significant relationship between diet and plasma stable isotope values (carbon, nitrogen and sulphur) for three species of bears. If discrimination factors vary not only between species and tissues but also according to the isotopic value of the diet eaten, the use of a constant discrimination factor for a species/tissue in isotopic models would be inadequate. However, the existence of a possible linear relationship between diet isotopic values and discrimination factors (such as the proposed by Hilderbrand *et al.* (1996) and Felicetti *et al.* (2003)) would allow adequate estimation of discrimination factors for different diets or mixed diets. This would be possible if equations describing this relationship could be derived for different species and tissues, and based on a wide range of diet isotopic values. These equations would provide researchers with a simple method to estimate diet composition with correct discrimination factors without having to go through difficult and costly experiments with the target animals (this would be especially useful when investigating endangered species). Experimental studies with omnivores could enable these equations to be derived, as the range of dietary components is wide and discrimination factors of different tissues can be obtained.

The $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ have been evaluated experimentally for a wide range of taxa of different trophic groups (Gannes, Del Rio & Koch 1998; Kelly 2000). However, estimating variation in discrimination factors for omnivore species would provide indispensable information on how a wide range of resources (C_3 and C_4 plants, birds, reptiles, arthropods, etc) with diverse isotope ratios are assimilated differently within a species. More detailed information is needed on stable discrimination in the omnivore food chain, as well as more laboratory research to interpret data gathered in the field.

In this paper, we investigate the variability of the discrimination factors of different tissues of an omnivorous rodent, *Rattus rattus* (L.) when fed with diets of known and variable isotopic ratios in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. We used three very different monospecific diets, three mixes of two of these, one mix of the three diets and one diet with two monospecific diets in turn. Through this experimental setting, we evaluated the effect of diet quality (%N and C/N) on discrimination factors. The long duration of the experiment used allowed us to detect diet turnover rates, through changes of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in shaved hairs, thereby providing a test of the effectiveness of this tissue to be used as a non-invasive sampling. However, the two main goals of our paper were to: (i) alert ecologists that the current practice of using a standard discrimination value in isotopic model is inadequate, as it can vary according to the isotopic ratio of the diet; and (ii) provide a simple and accurate method using regression models between discrimination factor and diet isotopic ratios to obtain a more appropriate value.

Materials and methods

EXPERIMENTAL DESIGN

The experimental Sprague–Dawley rats were obtained from the 'Elevage Janvier' in France; all came from the same batch and were

Table 1. Mean values (\pm SD) of isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), and percentages of carbon (%C) and nitrogen (%N) for (A) the standard diets and (B) the major constituents. Each diet was prepared independently with the detailed constituents, and the isotopic values and percentages were calculated using a sample of each prepared diet

	<i>n</i>	%C	%N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
(A) Diet					
A: fish meal (fish meal, corn flour, mineral premix, vitamin premix, vegetal oil)	6	38.9 \pm 3.3	2.4 \pm 0.2	-15.7 \pm 0.2	10.9 \pm 0.1
B: corn flour (corn flour, casein, mineral premix, vitamin premix, vegetal oil)	6	39.8 \pm 0.4	1.4 \pm 0.4	-12.6 \pm 0.2	4.5 \pm 0.2
C: alfalfa meal (alfalfa meal, corn flour, mineral premix, vitamin premix, vegetal oil)	6	41.1 \pm 0.3	1.6 \pm 0.1	-22.6 \pm 0.4	-0.8 \pm 0.1
D: A + B	4	38.7 \pm 3.9	2.1 \pm 0.4	-13.8 \pm 0.5	9.1 \pm 0.4
E: A + C	4	38.4 \pm 5.4	2.6 \pm 0.4	-18.7 \pm 0.5	7.9 \pm 0.7
F: B + C	4	40.1 \pm 2.5	1.5 \pm 0.2	-18.1 \pm 0.4	1.4 \pm 0.8
G: A + B + C	4	40.9 \pm 1.4	1.9 \pm 0.3	-16.6 \pm 0.6	6.9 \pm 0.7
(B) Constituents					
Fish meal		41.4	12.0	-20.1	11.6
Corn flour		40.5	1.3	-11.7	6.4
Alfalfa meal		39.3	2.7	-29.1	-0.7
Casein		48.3	14.2	-18.0	5.0

obtained at the same time. A total of 48 rats were used, all males, weighing on average 192 SD = 1.0 g (*c.* 6-weeks-old). Rats were randomly assigned to eight groups: six rats per group, distributed in two cages of three rats. Each group was fed with a specific synthetic diet (A–H) in the form of flour, hereafter called diets or diet treatments. Diet A was constituted by fish meal and corn flour; diet B was constituted by corn flour and casein; diet C constituted by alfalfa meal and corn flour; diet D was constituted by a mixture of diets A and B; diet E was constituted by a mixture of diets A and C; diet F was constituted by a mixture of diets B and C; diet G was constituted by a mixture of diets A, B and C; for diet H, rats were fed with diet A during the first 8 weeks and with diet B during the following 8 weeks in order to estimate isotopic turnover when switching between two different known resources (Table 1A). Mixed diets (D–G) were a mix of the principal constituents of the main diets (A or B or C); for example, we mixed the constituents of diets A and B to obtain diet D. Isotopic values of these diets were gathered from analysing a sample of each mixed diet (Table 1A). All diets had the same energetic value (3.5–4 kcal/g), with a 9 : 4 : 4 ratio of lipids, proteins and carbohydrates. A mixture of minerals (UAR205b), vitamins (UAR200) and vegetal oil were added to each diet (Table 1B).

At the beginning of the experiment one rat per box was randomly selected and its back was shaved (*c.* 4 cm²). This procedure was repeated four times during the experiment (six times for diet H), in the same area on each rat, to obtain a sample of hairs grown between shaves, at intervals as regular as possible, determined by the availability of new hairs (on average every 20 days). Thus, we obtained samples of two types of hair: hair shaved on the back during the experiment in one specific rat per box (hereafter shaved hair); and hair shaved elsewhere on the body of this rat (and of the other rats) at the end of the experiment (hereafter unshaved hair).

The experiment lasted 8 weeks for the rats assigned to diet treatments A–G, and 16 weeks for the rats assigned to diet treatment H. At the end of the experiment, all rats were sacrificed, shaved, dissected and a sample of liver, muscle and hair was obtained.

ISOTOPIC ANALYSES

Samples for stable isotope analyses (animals and diets) were freeze-dried and grounded to a fine powder. Dried samples were weighed in tin capsules and stored in a desiccator until measurement. Isotopic analyses were performed by a spectrometer IsoPrime (MicroMass,

Institut de Biotechnologie des Plantes, Université Paris Sud, France) coupled with analyser EuroEA 3024 (EuroVector). Stable C : N isotope ratio are expressed as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where *R* is ¹³C/¹²C or ¹⁵N/¹⁴N for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. The standard for C is the IAEA-NBS 21 (graphite: -28.13‰) and for N the IAEA-N1 (+0.4‰) and IAEA-N2 (+20.3‰). Ten replicate assays of internal laboratory standards indicate measurement maximum errors (SD) of $\pm 0.15\%$ and $\pm 0.2\%$ for stable carbon and nitrogen isotope measurements, respectively.

Discrimination factors between a food resource (*X*) and a consumer (*Y*) is described in terms of the difference in delta (δ) values using the Δ notation, where $\Delta = \delta Y - \delta X$.

STATISTICAL ANALYSES

We first tested whether the rats, randomly distributed in the different diet treatments, were similar in their isotopic ratios at the beginning of the experiment. We performed one-way ANOVAs using either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of shaved hair of the two shaved rats for each diet treatment as the dependent variable, and the diet treatment (A–H) was treated as the independent variable. In each diet treatment we had six rats distributed in two cages, which could create an extra source of variability. We performed preliminary analyses to test whether rats in the two cages were significantly different within each diet treatment. We performed nested ANOVAs in which we tested the effect of the cage nested to each diet treatment for all the dependent variables: isotopic ratios and discrimination factors of carbon and nitrogen.

We performed factorial ANOVAs to test the effect of diets on isotopic ratios and on the discrimination factor of carbon and nitrogen of each tissue type. Dependent variables were either $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\Delta^{13}\text{C}$ or $\Delta^{15}\text{N}$, and independent variables were the diet treatment (A–G) and the tissue type (liver, muscle and unshaved hair). Because rats assigned to diet H were fed on two different diets consecutively, they were not included in these analyses. However, isotopic ratios and discrimination factor of carbon and nitrogen of rats fed on diet H (16-weeks-old) were compared to rats fed on diets A and B (8-weeks-old); we performed a one-way ANOVA for each tissue type in order to determine whether diet H produced different results from

Table 2. Effect of diet treatments on isotope ratio and discrimination factors of carbon and nitrogen of different tissues (muscle, liver and unshaved hair). Values represent *MS/F* statistics of ANOVA ($P < 0.001$ in all cases)

	df	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$
(A) Differences between diet treatments A–G (factorial ANOVA, Diet \times Tissue)					
Effect					
Diet	6	66.61/118.74	157.14/423.20	57.30/102.15	42.22/112.67
Tissue	2	38.75/69.09	86.03/231.69	38.75/69.09	77.17/205.95
Diet \times Tissue	12	7.15/12.74	2.06/5.55	7.15/12.74	1.83/4.88
(B) Differences between diet treatments A, B and H (one-way ANOVA for each tissue)					
Diet effect					
Muscle	2	2.44/27.01	61.13/562.27	18.10/200.43	45.70/420.32
Liver	2	5.54/52.61	66.71/595.69	8.05/76.46	27.19/200.10
Unshaved Hair	2	40.34/59.39	72.79/84.88	72.84/107.25	83.26/97.09

diets A or B (the independent variable was diet, with these three levels). Thus, a post-hoc Tukey test was performed to compare the mean of diet H to those of A and B.

Shaved hairs of rats were used to study the turnover of isotopic ratios during the experiment of different diet treatments. We performed GLMM, which allows for a distinction between fixed and random effects (Littel *et al.* 1996): individual rats were treated as the random effect and period of time and diet treatment as the fixed effects. The period of time had five categories corresponding to the five times when hair was shaved in the experimental rats during the 8 weeks. Diet treatment had seven categories corresponding to the diets A–G, and data of rats assigned to diet H were added to diet A data (rats assigned to diet H were fed on diet A for the first 8 weeks of the experiment, hence the second 8 weeks of diet H are not considered in this analysis). Dependent variables were the contents of either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of shaved hairs.

We were also interested in testing the differences between shaved and unshaved hairs to evaluate the possible benefits of a non-lethal method to survey turnover of isotopic values in the field. We performed one-way ANOVAs in which dependent variables were either $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\Delta^{13}\text{C}$ or $\Delta^{15}\text{N}$, and the independent variable was the type of hair (shaved or unshaved hair). Only the data obtained from hairs shaved during the last sampling day (the day of death) were compared with the unshaved hairs obtained on the same day from the same individual.

To investigate whether relationships exist between diet $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ or diet $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ in each tissue type, we performed general regression models. We fitted simple or polynomial regressions to each pair of variables using the average values of discrimination factors for each diet (A–G) in each tissue: for both carbon and nitrogen, we performed eight regression models corresponding to four tissues (liver, muscle, unshaved and shaved hair) allowing two types of regression (simple or polynomial) for the relationships between each diet isotopic ratio and their corresponding discrimination factor. The best fit (simple or polynomial) was chosen on the basis of the regression coefficient (R^2), and the F and P statistics associated with the whole model. We used similar general regression models to investigate the relationship between $\Delta^{15}\text{N}$ and (i) percentage of nitrogen in the diet (%N); and (ii) the ratio between percentage of nitrogen and percentage of carbon in the diet (noted C : N), for each tissue.

Normality of all dependent variables was tested before fitting models. Computations were performed with STATISTICA 6.0 (StatSoft Inc. 2001) and SAS package (procedure MIXED, v. 8.2, SAS Institute Inc. 2004).

Results

EFFECT OF DIETS ON ISOTOPIC RATIOS AND DISCRIMINATION FACTORS

At the beginning of the experiment, the rats used in this experiment did not differ in their isotopic ratios of shaved hairs. A one-way ANOVA resulted in non-significant differences in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of shaved hairs between assigned diet treatments, that is, variability within each diet treatment was similar to variability between treatments ($F = 2.0$, $P = 0.172$ and $F = 0.81$, $P = 0.600$ for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, respectively, $n = 16$). At the end of the experiment, rats in the two cages of the same diet treatment were not significantly different in their isotopic ratios or discrimination factors (nested ANOVAs, $P > 0.1$ in all cases). This allowed us to mix the six rats of each diet treatment for the rest of statistical analyses.

The effects of diet treatments were highly significant for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$, the effects being significantly variable among tissues (Table 2A). This result shows that the interaction between diet treatments and tissues was significant; however, we were more interested in the main effect of the tissue, because the diet treatment was expected, as it was created by the Experimental design. Thus, even if some diets responded differently, in general unshaved hairs had the lowest isotopic ratios and discrimination factors, followed by muscle and liver (Fig. 1). Post-hoc Tukey tests showed significant differences among tissues in all the parameters examined ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$; $P < 0.005$ in all cases).

As diet H was made of 8 weeks of diet A followed by 8 weeks of diet B, we also analysed potential differences in isotopic ratios and discrimination factors between rats fed on these three diets. We detected significant differences for all tissue types (Table 2B). Exploring such differences by means of post-hoc Tukey tests, we found that diet H was different from diet treatment A or B for all the variables and tissue types, except for $\delta^{15}\text{N}$ for unshaved hair, for which diet H was not significantly different to diet treatment A ($P = 0.894$), meaning it had not significantly reflected its diet shift for $\delta^{15}\text{N}$. However, isotopic values in the liver and muscle for diet H rats

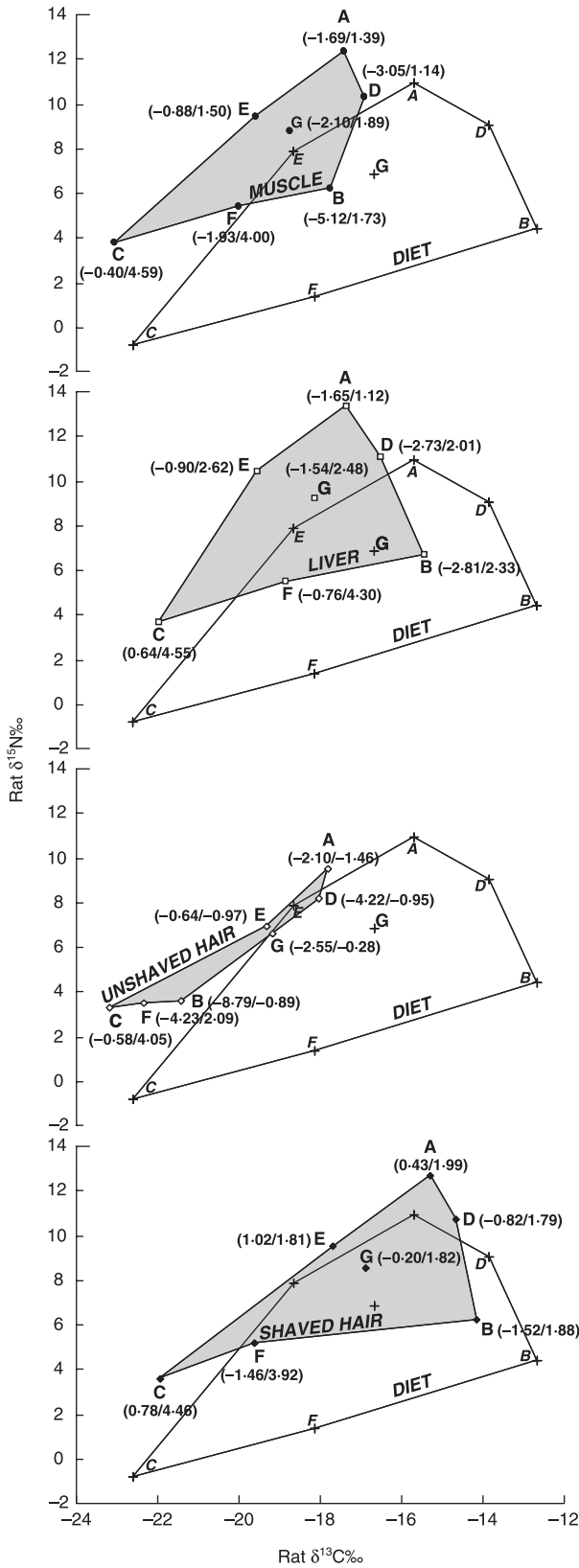


Fig. 1. Mean values of isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) for the seven diet treatments (A–G; white triangle) and for three different tissues (grey polygon; muscle, liver, unshaved hair and shaved hair). Discrimination factors for shaved hair with diets C and F were represented, although they have been calculated after only 26 days. Between parenthesis, discrimination factor values of carbon and nitrogen ($\Delta^{13}\text{C}/\Delta^{15}\text{N}$).

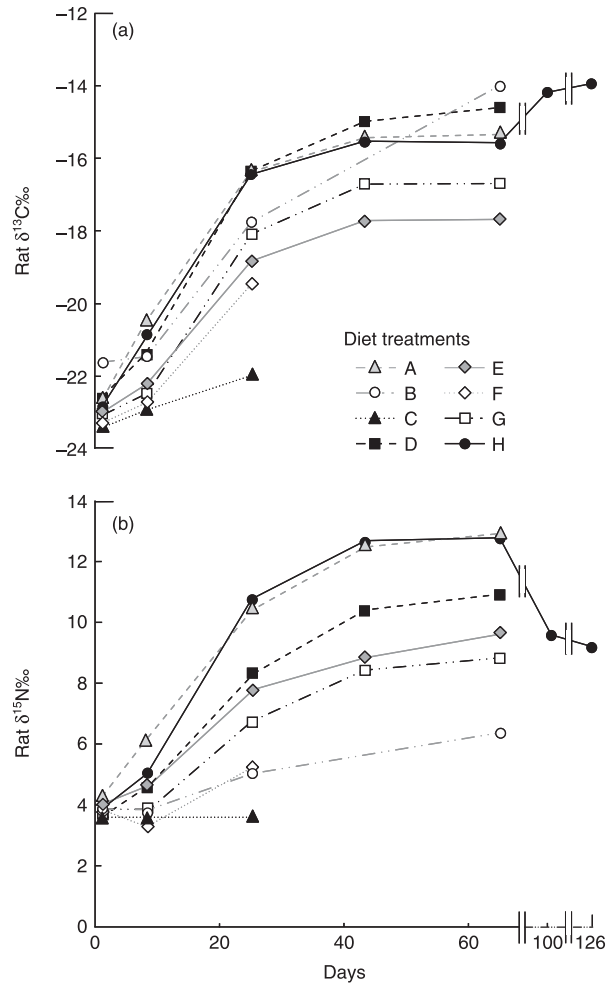


Fig. 2. Evolution of averaged isotopic ratios of (a) carbon and (b) nitrogen of shaved hair for each diet treatment during the 16 weeks of experimentation, showing stabilisation after a time for all diets, and a shift after the diet change for diet H.

approached those values of rats in treatment B, reflecting the diet shift, except for $\delta^{13}\text{C}$ in muscle (but diets A and B values were very close, see Fig. 1).

Isotopic ratios of shaved hairs varied significantly during the 8 weeks of experiment ($F = 1348.4$ for $\delta^{13}\text{C}$ and $F = 501.78$ for $\delta^{15}\text{N}$; $n = 7$, $P < 0.001$) and this variation differed significantly between diet treatments ($F = 58.50$ for $\delta^{13}\text{C}$ and $F = 49.05$ for $\delta^{15}\text{N}$; $n = 7$, $P < 0.001$). Due to different growth rates, shaved hairs were not always available in sufficient quantity in all the intervals, thus resulting in missing values for the last two periods in diet treatments C and F (Fig. 2). Isotope ratios showed a turnover during the first month and appeared to stabilize during the second month (Fig. 2). In fact, the switch in the isotope ratios in the hairs of rats fed with diet H is visible on Fig. 2; expectedly, values similar to those measured for diet A were recorded for the first period, followed by values tending to those of diet B for the second period.

Isotope ratios and discrimination factors were significantly higher for shaved hairs than unshaved hairs ($F = 13.11$, $P = 0.002$; $F = 5.01$, $P = 0.040$; $F = 10.44$, $P = 0.004$; $F = 6.15$,

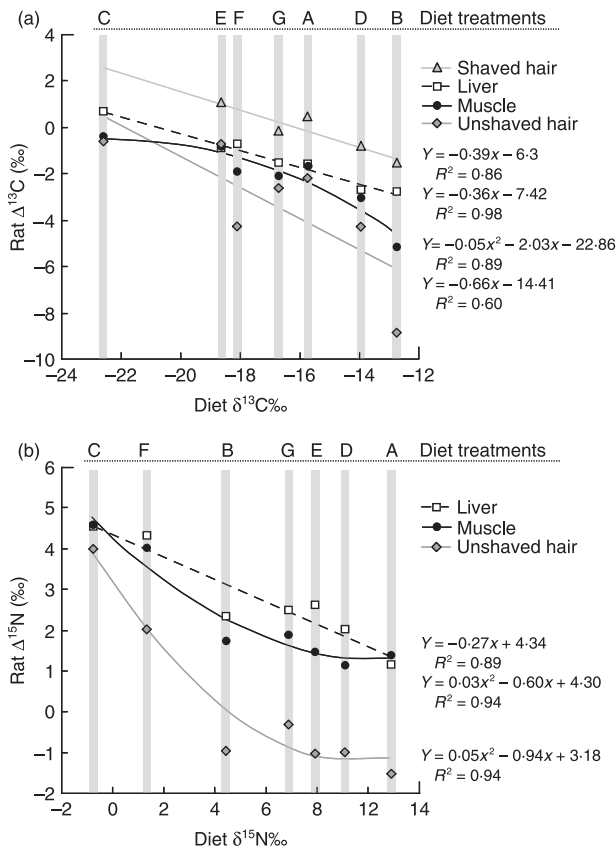


Fig. 3. Relationship between mean values of (a) isotopic ratio of carbon ($\delta^{13}\text{C}$) and discrimination factor of carbon ($\Delta^{13}\text{C}$), and (b) isotopic ratio of nitrogen ($\delta^{15}\text{N}$) and discrimination factor of nitrogen ($\Delta^{15}\text{N}$), for the different sampled tissues. Capital letters at the top of each graph correspond to each diet treatment. Equations (and associated regression coefficients) and fits are shown for significant models and allow an estimate of the discrimination factor when the diet isotopic value is known.

$P = 0.021$ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$, respectively; $n = 22$). Both individuals from diet treatments C and F, and one individual of diet treatment B could not be included in this analysis due to the unavailability of sufficient quantity of shaved hairs to perform isotopic analyses.

RELATIONSHIPS BETWEEN DIET ISOTOPIC RATIOS AND DISCRIMINATION FACTORS

We found significant relationships between the diet $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ for all tissue types (muscle, liver, unshaved and shaved hairs, Fig. 3a). In addition, we found a significant relationship between the diet $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ for all tissue except shaved hairs (Fig. 3b). For muscle, the best fits were quadratic fits ($F = 15.70$, $P = 0.013$ and $F = 34.68$, $P = 0.003$ for carbon and nitrogen, respectively); for the liver the best fits were simple regressions ($F = 261.59$, $P < 0.0001$ and $F = 40.25$, $P = 0.001$ for carbon and nitrogen, respectively); for the unshaved hairs the best fits were a simple regression for the carbon and a quadratic fit for the nitrogen ($F = 7.41$, $P = 0.042$ and $F = 33.96$, $P = 0.003$, respectively); in the case of shaved hairs

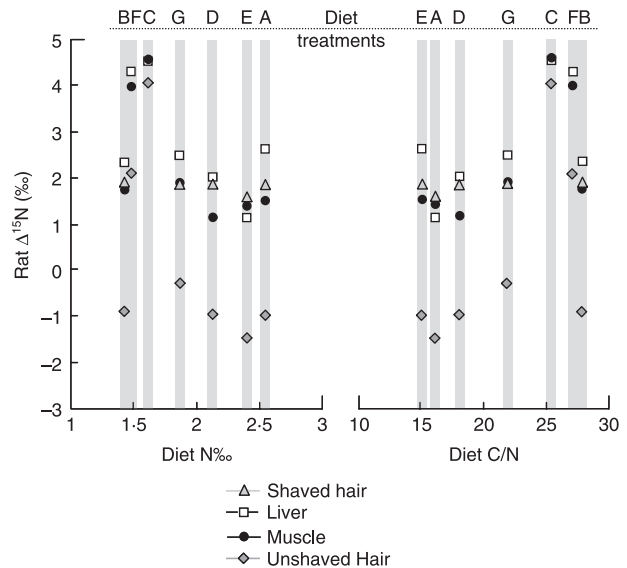


Fig. 4. Relationship between mean values of N‰ (a) and C/N (b) and discrimination factor of nitrogen ($\Delta^{15}\text{N}$) for the different sampled tissues. Capital letters at the top of each graph correspond to each diet treatment.

the significant model for the carbon was a simple regression ($F = 19.31$, $P = 0.022$). However, sample size for shaved hairs was reduced in comparison to the other tissues (see Fig. 3).

We did not find any significant relationships between $\Delta^{15}\text{N}$ and the percentage of nitrogen (%N) or between $\Delta^{15}\text{N}$ and the ratio between %N and %C (C/N) in any tissue ($P > 0.1$ in all cases, Fig. 4).

Discussion

We showed that discrimination factors vary accordingly to the diet of the consumer, and to its tissue, demonstrating the inadequacy of using a single, constant value for this important variable. In addition, we have shown significant relationships between the diet isotopic ratio and the discrimination factor for all tissues tested (except for shaved hairs for $\Delta^{15}\text{N}$). These enabled us to provide regressions models to estimate adequate values of discrimination factors by using only diet isotopic ratio values. Moreover, isotopic ratios of tissues followed during the whole course of the experiment (shaved hair) showed a turnover during the first month, stabilising thereafter. This experiment allowed us to test how using hair could be used as a non-lethal method to assess diet.

VALUES OF THE DISCRIMINATION FACTORS

It is often assumed that the $\delta^{15}\text{N}$ value of a consumer is enriched by 3.4‰ (this assumption being based on the results of Minagawa & Wada 1984 and Post 2002) and that the $\delta^{13}\text{C}$ value exhibits little or no trophic enrichment (this assumption being mainly based on the studies of Hullar *et al.* (1996)).

These assumptions have important consequences for omnivorous species. The foraging behaviour and feeding

ecology of omnivores are generally characterized by trophic adaptability, enabling them to feed on a variety of resources according to environmental conditions and resource availability. As a consequence, the use of stable isotope analysis for these species is very complicated. For instance, it is especially difficult to determine the relative contribution of different resources to the diet with mixing isotopic models, because the diversity of resources potentially generates a wide range of isotopic ratios. Our results show that diet isotopic values affect discrimination factors and that therefore they can be used neither as constants nor as unique values for all diet items.

Felicetti *et al.* (2003) fed three bear species (*Ursus arctos horribilis*, *U. americanus* and *U. maritimus*) single- and mixed-species diets. They observed plasma $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ enrichment ranging from 3.2‰ to 5‰ and from -4.5‰ to -0.5‰, respectively. In our study, we found enrichment in $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ ranging from -1.46‰ to 4.59‰ and from -8.79‰ to 0.64‰, respectively, when considering all tissue types and diets together. Post (2002) observed values in $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ ranging from 0.5‰ to 5‰ and from -3‰ to 3.5‰ based on a literature review of aquatic and terrestrial organisms ranging in size from copepods to polar bears. Vander Zanden & Rasmussen (2001) observed ranges from -0.7‰ to 9.2‰ and from -2.1‰ to 2.8‰ for discrimination factors of nitrogen and carbon, respectively, based in a literature survey of 22 studies and 20 different species. The range of variation observed in our study (in only one species) is thus considerable in relation to these previous studies. It further stresses the importance of using adequate values of discrimination factors in the future.

VARIABLES AFFECTING DISCRIMINATION FACTORS

Discrimination factors are known to be affected by variables such as omnivory, trophic position, food chain length and energy flows and sources (Minagawa & Wada 1984; Hobson & Clark 1992; Vander Zanden & Rasmussen 2001). Recently, the relationship between discrimination factor and the diet quality represented by %N or C/N has been investigated (review by Vanderklift & Ponsard 2003; Robbins *et al.* 2005). Vanderklift & Ponsard (2003) found a weak positive relationship between $\delta^{15}\text{N}$ discrimination factor and the nutritional quality of the food, as measured by the C/N ratio of the diet. Nutritional stress (influenced by the quality of the diet or by starvation) has been suggested as possibly affecting $\delta^{15}\text{N}$ enrichment, but results have been contradictory: some studies determining this to be greater under conditions of nutritional stress (Hobson & Welch 1992; Hays *et al.* 2000), while others have shown it to be lower (Oelbermann & Scheu 2002), or to have no effect at all (Schmidt, Scrimgeour & Curry 1999). In our study, we found no relationship between discrimination factors and %N or C/N ratio. This result may be explained by the low variability of the %N and the C/N ratio between diets, and was expected as our study design aimed at testing the influence of the diet isotopic ratio, with diets of similar quality. In addition, the differential metabolic routing of

elements from diet to tissues can contribute to variation in derived diet-tissue-discrimination factors (Dalerum & Angerbjorn 2005; Podlesak & McWilliams 2006). For example, protein C tends to be preferentially incorporated into protein in the animal's tissues, and lipid C tends to be preferentially incorporated into lipid C. If the lipid, protein and carbohydrate components of the diet have different isotopic signatures, different animal tissues may show different apparent dietary proportions. The macromolecular differences could play a role in the resulting discrimination values but we focused on isotopic ratio differences and our analyses show that these explain 60%–98% of the variation. Thus the discrimination factor is not a constant and ecologists should be warned that the current practice of using a standard discrimination factor should be avoided (Dalerum & Angerbjorn 2005).

Hilderbrand *et al.* (1996) and Felicetti *et al.* (2003) showed that isotope ratios in bear plasma were linearly related to diet isotopic ratios. In both studies bears were fed with a wide range of diet items (0‰–18‰ for $\delta^{15}\text{N}$ and -17‰ to -27‰ for $\delta^{13}\text{C}$). Although only plasma was examined, the ranges of the values obtained were similar to ours. When we compare regression equations of these two studies with ours, we observe that the data on carbon bear plasma fits very well with our regression lines (especially the shaved hair). For nitrogen, the regression line of bear plasma of the Felicetti *et al.* (2003) study fits well with the rat liver regression line, although with higher discrimination factor values. Pearson *et al.* (2003) conducted a captive feeding experiment using an omnivorous songbird testing different diets (fruits and insects). They found that the isotopic ratio of plasma increased linearly with the isotopic ratio of the diet plus the discrimination factor for both N and C. However, the different diets in Pearson *et al.*'s (2003) study had a low range in the variation of diet isotopic ratios (6.01‰–6.15‰ for $\delta^{15}\text{N}$ and -24.21‰ to -27.90‰ for $\delta^{13}\text{C}$). We designed our study so that the different diets would have a large range of isotopic ratios (-0.8‰ to 10.9‰ for $\delta^{15}\text{N}$ and -12.6‰ to -22.6‰ for $\delta^{13}\text{C}$), in order to simulate the potentially important (yet realistic) variability of rodent diet. For example, it has been shown that the three main species of commensal rats (*R. exulans*, *R. rattus* and *R. norvegicus*) may have a diverse diet including amphibians (Donlan, Townsend & Golden 2004), reptiles (Cree *et al.* 1999; Bruno *et al.* 2000), birds (Atkinson 1985), plants and even mammals (Atkinson 1985).

Here, we provide equations that can be used to calculate an approximate value for the discrimination factor when the isotopic value of the diet is known. This should help future studies working with omnivorous species, especially invasive rats, in the estimation of discrimination factors based on the isotopic value of their diet.

HAIR AS A USEFUL TISSUE FOR ECOLOGICAL STUDIES

Dietary information over several time-scales can be obtained by measuring several tissues within an individual. However, different tissues (muscle, liver) can be highly invasive and even

result in the death of an individual (especially sampling the liver); thus making it impossible to undertake a long-term study of individual dietary shifts. Fortunately, other tissue types such as feathers, skin, nails or hair may be appropriate for this goal. Stable isotopes of most elements in these metabolically inactive tissues do not turn over, therefore reflecting only the diet of individuals during a limited period of tissue growth (Tieszen *et al.* 1983). However, these tissues could be sampled with limited negative impact on individuals. In order to determine the time required for a dietary shift to be reflected in the isotopic composition of the consumer's tissue, we followed the isotopic values of hairs shaved during the experimental period. In our study, carbon and nitrogen isotope ratios of shaved hair stabilized after *c.* 40 days. This is consistent with the results of a previous study on gerbils in which authors found a half-life of carbon components in hair of 47.5 days (Tieszen *et al.* 1983). The small difference between these results could respond to differences in experimental protocols, species, environmental conditions, initial isotopic values of animal and diet or diet quality. We have shown that hair provides an alternative tissue for the studying the diet of mammals (as do feathers for avian diets). Such a method would benefit functional ecology studies because it represents a non-lethal source of tissue while allowing satisfying individual determinations of dietary and resource shifts.

In our study, we assumed that each tissue was in isotopic equilibrium with its diet at the beginning of the experiment (the laboratory rats had grown on identical diets before the start of the experiment). The experiment lasted 8 weeks and during this time isotopic values of shaved hair arrived to equilibrium (Fig. 3), thus liver and muscle, alleged to reach equilibrium faster than hair, should also have reached equilibrium (Tieszen *et al.* 1983). Hair showed the same dynamics as other tissues, although it has to be taken into account that our 'shaved' and 'unshaved' hair presented significant differences in their isotopic ratios and discrimination factors. The difference in the polygon shapes (Fig. 1) between unshaved hair and the other tissues was probably due to the difference in setting: the values of this tissue corresponded to the old diet (during the first 6 weeks of rat's life) plus the experimental diets (8 weeks). However, at the end of the experiment, there was a significant variation in unshaved hair rat isotopic ratios between each diet treatment (Fig. 1); this showed that unshaved hair was not completely inert (the hair grew during the experiment).

We observed also that the rate of hair growth (shaved hair) was reduced after the shift from diet A to diet B; this could explain why we have similar values between unshaved hairs of rats treated with diet A and those treated with diet H. In addition, other variables can affect these dynamics such as the physical condition (Williams *et al.* 2007); in fact at the end of the diet H experiment, the differences in the isotopic values (muscle and liver) of rats in diets H and B may have been affected by the higher mean weight of rats when beginning the diet B component of treatment H (501 g), compared to the mean weight of rats at the beginning of diet B (190 g).

Conclusion

Major advances in the use of stable isotopes have involved quantitative estimates of food web relationships, but they require a good estimation of the changes in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ (especially with respect to the discrimination factor, $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$). This study is the first to measure tissue discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) in relation to diet isotopic ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) with the same diet quality. We showed a decrease of $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ with an increase of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively, these relationships differing significantly for each tissue type. This allowed us to propose a method for estimating discrimination factors when diet isotopic values are known. However, we do not have a functional explanation of this relationship, and future studies should be focused on understanding why discrimination factors vary as a function of the isotope value of the diet. Although we agree with Ben-David & Schell (2001) that linear regression equations should be used with caution as an alternative to mixing models, they should be very useful in providing values for discriminating factors when no data are available from the field. While previous studies had similar objectives (Ben-David & Schell 2001; Felicetti *et al.* 2003), our experimental approach in the laboratory allowed a more solid and convincing demonstration.

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