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REVIEW

Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction

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Summary

1. The use of stable isotopic techniques to study animal diets and trophic levels requires a priori estimates of discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$, also called fractionation factors), which are the differences in isotopic composition between an animal and its diet. Previous studies have shown that these parameters depend on several sources of variation (e.g. taxon, environment, tissue) but diet as a source of variation still needs assessment.

2. We conducted an extensive review of the literature (66 publications) concerning estimates of animal-diet $\Delta^{13}C$ (n = 290) and $\Delta^{15}N$ (n = 268). We analysed this data set to test the effect of diet isotopic ratio on the discrimination factor, taking into account taxa, tissues, environments and lipid extraction treatments. Our results showed differences among taxonomic classes for $\Delta^{13}C$, but not for $\Delta^{15}N$, and significant differences among tissues for both $\Delta^{13}C$ and $\Delta^{15}N$. We found a significant negative relationship between both, $\Delta^{13}C$ and $\Delta^{15}N$, with their corresponding diet isotopic ratios. This relationship was found also within taxonomic classes for mammals ($\Delta^{13}C$ and $\Delta^{15}N$), birds ($\Delta^{13}C$), fishes ($\Delta^{13}C$ and $\Delta^{15}N$) and invertebrates ($\Delta^{13}C$ and $\Delta^{15}N$). From these relationships, we propose a method to calculate discrimination factors based on data on diet isotope ratios (termed the 'Diet-Dependent Discrimination Factor', DDDF).

3. To investigate current practice in the use of discrimination factors, we reviewed studies that used multi-resource isotopic models. More than 60% of models used a discrimination factor coming from a different species or tissues, and in more than 70% of models, only one $\Delta^{13}C$ or $\Delta^{15}N$ was used for all resources, even if resources had very different isotopic ratios. Also, we estimated DDDFs for the studies that used isotopic models. More than 40% used $\Delta^{15}N$ values and more than 33% used $\Delta^{13}C$ values differing > 2‰ from estimated DDDFs.

4. *Synthesis and applications*. Over the last decade, applied ecologists have discovered the potential of stable isotopes for animal diet reconstruction, but the successful adoption of the method relies on a good estimation of discrimination factors. We draw attention to the high variability in discrimination factors, advise caution in the use of single discrimination factors in isotopic models, and point to a method for obtaining adequate values for this parameter when discrimination factors cannot be measured experimentally. Future studies should focus on understanding why discrimination factors vary as a function of the isotopic value of the diet.

Key-words: carbon, discrimination, fractionation, nitrogen, diet isotopic value, isotopic model

Introduction

Stable isotopic analyses are becoming widespread as a tool for studies of community structure and ecosystem function (Post 2002). In trophic studies, heavier isotopes of any given element increase in abundance compared with lighter isotopes, through the process of isotope discrimination. Early laboratory studies showed that for carbon (C) the isotopic ratio values of consumers are usually similar to those of their diets (DeNiro & Epstein 1978a,b). Since the ratio of carbon

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isotopes changes little (about +1‰; DeNiro & Epstein 1981; Peterson & Fry 1987; France & Peters 1997) as carbon moves through food webs, this ratio is commonly used to evaluate the source of the carbon, typically to distinguish carbon fixed by terrestrial C_3 plants from that fixed by C_4 plants or marine C_3 plants (Peterson & Fry 1987). In contrast, consumers are typically enriched by 3–4‰ (DeNiro & Epstein 1981; Minagawa & Wada 1984; Peterson & Fry 1987) in isotopic ratios of nitrogen (N) relative to their diets. The isotopic ratio of nitrogen is thus commonly used to estimate trophic positions.

Based on the assumption that 'you are what you eat', two major types of studies have used differences in isotopic ratios between consumers and their resources: (i) trophic relationship studies and (ii) animal diet reconstruction studies. Each type uses the difference between isotopic ratios of an animal and its diet, called discrimination factor or trophic enrichment ($\Delta^{13}C$ and $\Delta^{15}N$ for carbon and nitrogen, respectively). Recent approaches in the use of isotopic mixing models to derive quantitative estimates of dietary contributions from isotopically distinct components specifically require precise estimates of diet discrimination factors (Phillips & Gregg 2001). Small variations in the values used for the discrimination factor may lead to important differences in the output of isotopic-mixing models (Ben-David & Schell 2001).

Recent reviews of the factors affecting discrimination factors have predominantly focused on the effects of dietary quality (Vander Zanden & Rasmussen 2001; Post 2002; Vanderklift & Ponsard 2003; Robbins, Felicetti & Sponheimer 2005). Discrimination factor estimates are subject to uncertainty because discrimination may vary depending on a consumer's nutritional status, lipid extraction, diet quality, size, age, dietary ontogeny, and tissue and elemental composition (Minagawa & Wada 1984; Ben-David & Schell 2001; Vanderklift & Ponsard 2003). However, the isotopic value of the diet also affects discrimination factors. Hilderbrand *et al.* (1996) showed significant relationships between the C and N isotopic ratios of diets and in the discrimination factors of the animals fed on these diets, and similar results have been found in bears (*Ursus* sp.) (Felicetti *et al.* 2003) and rats *Rattus rattus*, the latter fed on diets of different isotopic value but similar energetic value (Caut, Angulo & Courchamp 2008a). These studies have had limited impact on the application of stable isotopes to ecology, as studies on diet reconstruction and trophic relationships have continued to use fixed discrimination factors independently of the diet isotopic values (e.g. Kasai & Nakata 2005; Lepoint *et al.* 2006; Reich & Worthy 2006), probably because it is difficult to obtain species-specific discrimination factors for different diets.

In this study, we assess the importance of diet isotopic values on discrimination factors. To this end, we examined some sources of variation potentially affecting discrimination factors: consumer class, environment, type of tissue, presence or absence of lipid extraction treatment, and finally diet isotopic ratios. This was done through analysis of estimates of $\Delta^{15}N$ and $\Delta^{13}C$ values in the literature. Based on the extensive set of studies analysed, we propose a method (the Diet-Dependent Discrimination Factor method) for obtaining a baseline for appropriate isotope discrimination factors calculated from the diet isotope values, controlling for other sources of variation. This provides suitable discrimination factors for each consumer class and tissue, and can be used to infer discrimination factor values in cases where data are lacking. In addition, to investigate current pratice in the use of discrimination factors, we reviewed 32 multi-resource isotope model studies. Finally, we make recommendations for the use of discrimination factors in isotope models.

Literature compilation

We searched the ISI Web of Knowledge electronic data base (1983–2007, http://portal.isiknowledge.com) for literature involving stable carbon and nitrogen isotopic discrimination factors for any species, using the keywords stable isotope, stable carbon, stable nitrogen, carbon-13, nitrogen-15, discrimination factor, isotopic fractionation, isotopic enrichment and trophic enrichment. References cited in each of the resulting studies were reviewed for the presence of any additional studies (especially prior to 1983) that could have been missed in the previous search step. Because the focus of this review was the relationship between discrimination factor and diet isotopic ratio, estimates were not included where the diet was a mixture or was not controlled for (i.e. wild studies). If a study provided multiple discrimination factors estimated for one diet fed to one species of consumer, we pooled the data. If a study involved the same diet fed to different consumer species in the same taxon, the result for each species was considered one estimate. If a study involved different diets fed to the same species, the result for each diet was considered one estimate (see Supporting Information, Table S1). Estimates were not included if consumer diets were not specified, or if the authors explicitly stated that the duration of laboratory experiments was insufficient to allow for isotopic equilibrium between the consumer and its diet (see the 'time' column in Supporting Information, Table S1). Data available only in a graphical form were converted to a numerical form following fourfold enlargement of the graphs involved (estimated error = 0·05‰).

The literature search identified 66 references involving discrimination factors concerning 86 different species (Supporting Information, Table S1). To examine the use of discrimination factors in isotope models, we included only the 32 studies involving the four most used isotope models (Supporting Information, Table S2): (i) the geometric dual-isotope mixing model (Kline *et al.* 1993; Ben-David, Flynn & Schell 1997a; Ben-David *et al.* 1997b); (ii) the linear mixing model (Phillips 2001); (iii) the concentration-weighted linear mixing model (Phillips & Koch 2002); and (iv) the IsoSource partitioning mixing model (Phillips & Gregg 2003).

Statistical analysis

To examine variables affecting discrimination factors of carbon and nitrogen, we applied general linear mixed models

(GLMM) in which the dependent variable was the carbon or the nitrogen discrimination factor. The normality of dependent variables was confirmed prior to analysis. We used mixed models because data from the same literature reference were correlated, and this covariance structure was handled by introducing the reference as a random effect into the GLMM.

To determine whether discrimination factors of carbon and nitrogen differed among consumer taxonomic classes, environments in which the species live, lipid extraction treatment of samples, or the analysed tissues, we performed independent GLMMs for each one of these independent variables. We distinguished four consumer classes (fishes, birds, mammals and invertebrates), three environments (terrestrial, marine and freshwater), and nine tissues (blood, collagen, feather, hair, liver, muscle, plasma, red blood cells and whole body). We performed a similar analysis to determine whether there was any relationship between discrimination factor and diet isotopic ratio for both carbon and nitrogen.

In analyses of the effect of tissues, we also investigated whether discrimination factors of carbon and nitrogen for each tissue differed among consumer classes by performing independent GLMMs for each type of tissue, using the consumer class as the independent variable.

After testing the general effects of consumer taxonomic classes, environment, lipid extraction treatment, tissue and diet isotopic ratios on the discrimination factors, we tested which of these variables significantly affected discrimination factors within each consumer class. To this end, we ran independent GLMMs for each consumer class in which the dependent variable was the carbon or the nitrogen discrimination factor, and the main independent variable was the diet isotopic ratio (carbon or nitrogen). Depending on the data available, three more independent categorical variables were added to the models: the environment of the species (as noted above), the tissue analysed (as noted above), and lipid extraction treatment (yes or no). We used these categorical variables when more than one category were available and valid (each category had more than five data points and/or the sample sizes of different categories were equilibrated). We ran a full model to identify the significant variables. However, the full model is unsatisfactory for prediction because it includes variables that are nonsignificant (Whittingham *et al.* 2006); and because one of the goals of this study was to develop a method for estimating discrimination factors when diet isotopic values are available, we searched for significant regression equations between discrimination factors and diet isotopic ratio, through simple general linear models (GLMs) within consumer classes (and within tissues, lipid extraction categories or environments, when these variables were significant in the full model). As these regressions do not consider random effects, GLM results could be slightly different from GLMM results with respect to the significance of the diet isotopic ratio. Discrimination factors ($\delta Y - \delta X$) and diet isotopic values (δX) are not totally independent variables as one is partially derived from the other. Although the use of related variables (such as ratios) in regression is controversial as it could lead to spurious relationships, differences seem to not be so problematic (Hills 1978). Differences are frequently used, for example, in sexual size dimorphism (Szekely, Freckleton & Reynolds 2004) or community ecology (Cerda, Retana & Cros 1998) studies. Indeed, using the residuals of the relationship between diet and tissues should yield the same results. Computations were performed with STATISTICA 6·0 (StatSoft Inc. 2001) and SAS package (procedure MIXED, version 8·2, SAS Institute Inc. 2004).

Variation in discrimination factors

The literature review yielded 290 animal-diet discrimination factor estimates of carbon and 268 animal-diet discrimination factor estimates of nitrogen from 66 publications (Supporting Information, Table S1) distributed (for $\Delta^{13}C$ and $\Delta^{15}N$, respectively) as follows: mammals (95 and 89), birds (61 and 52), fishes (41 and 47), reptiles (3 and 3), and invertebrates (90 and 77). The overall mean estimates for $\Delta^{13}C$ and $\Delta^{15}N$ were 0·75‰ (SE = 0·11) and 2·75‰ (SE = 0·10), respectively.

EFFECT OF LIPID EXTRACTION TREATMENT

Samples are sometimes treated to extract lipids before isotope analysis, and this can affect the values obtained (Murry *et al.* 2006; Sweeting, Polunin & Jennings 2006; Bodin, LeLoch & Hily 2007). We separated discrimination factor values into two categories according to whether consumer and diet samples were subject to lipid extraction. If lipids were extracted from one but not the other sample type, data were not included in the analysis. Discrimination factors of carbon and nitrogen did not differ with lipid extraction ($F_{1,196} = 1{\cdot}22$, $P = 0{\cdot}271$ and $F_{1,187} = 0{\cdot}89$, $P = 0{\cdot}348$, for $\Delta^{13}C$ and $\Delta^{15}N$, respectively).

EFFECT OF ENVIRONMENT

We found significant differences among environments for the carbon discrimination factor but not the nitrogen discrimination factor ($F_{2,229} = 3{\cdot}10$, $P = 0{\cdot}047$ and $F_{2,209} = 0{\cdot}09$, $P = 0{\cdot}912$, for $\Delta^{13}C$ and $\Delta^{15}N$, respectively). Higher mean estimates of $\Delta^{13}C$ were obtained for organisms inhabiting freshwater environments (1·33‰, SE = 0·07, $n = 42$) than for those inhabiting marine (0·96‰, SE = 0·18, $n = 87$) or terrestrial (0·32‰, SE = 0·17, $n = 158$) environments.

EFFECT OF TAXON

Discrimination factors differed among the consumer classes for carbon ($F_{3,228} = 2{\cdot}96$, $P = 0{\cdot}033$) but not for nitrogen ($F_{3,207} = 1{\cdot}51$, $P = 0{\cdot}214$, Fig. 1). However, interpretation of the effect of taxon difference was somewhat confounded by other sources of variation that may have influenced the discrimination factor values, in particular that the data represented different tissues, environments, diets, or treatment of samples (i.e. lipid extraction or not) before isotopic analysis.

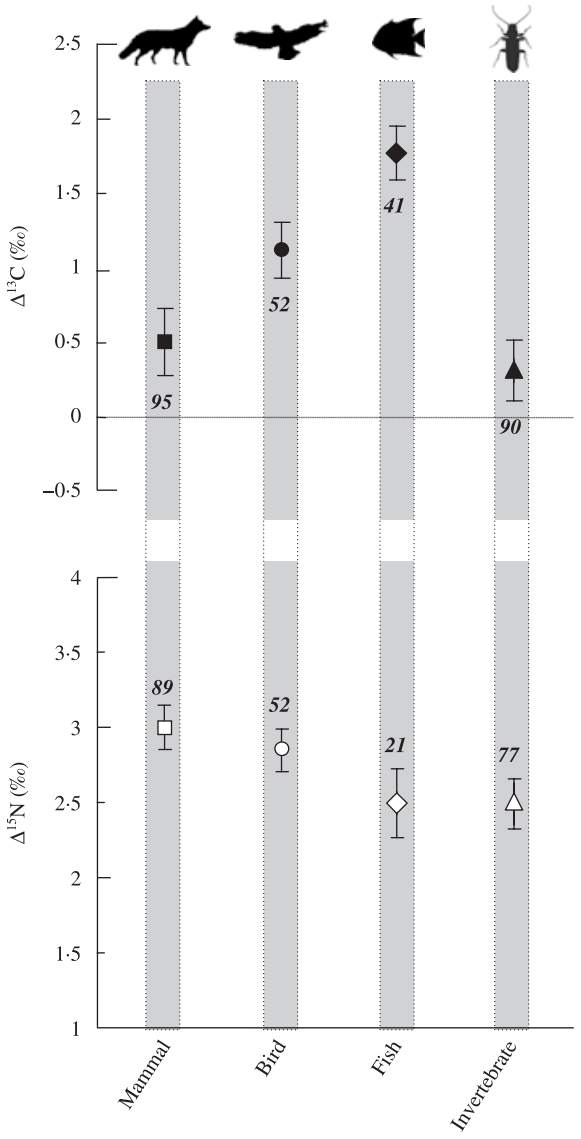

Fig. 1. Mean (± SE) $\Delta^{13}C$ and $\Delta^{15}N$ values among taxonomic classes. Numbers inside the bars indicate the sample size. Each pictogram represents a taxonomic class: mammals ( ), birds ( ), fishes ( ) and invertebrates ( ).

EFFECT OF TISSUE

We initially analysed all taxa combined. Discrimination factors of carbon and nitrogen differed among tissues ($F_{9,220} = 1{\cdot}93$, $P = 0{\cdot}049$, and $F_{8,198} = 2{\cdot}71$, $P = 0{\cdot}007$, respectively). Consequently, we analysed differences among the consumer classes for muscle, plasma, liver, blood and the whole body (Fig. 2); other tissues did not have sufficient data to carry out this analysis. The carbon discrimination factor for muscle was significantly different among birds, fishes and mammals ($F_{2,21} = 9{\cdot}15$, $P = 0{\cdot}001$), but the differences were not significant for the nitrogen discrimination factor ($F_{2,18} = 1{\cdot}86$, $P = 0{\cdot}184$). For plasma, both the carbon and the nitrogen discrimination factors were not significantly different between birds and mammals ($F_{1,16} = 3{\cdot}52$, $P = 0{\cdot}079$ and $F_{1,16} = 2{\cdot}53$, $P = 0{\cdot}131$, respectively). The carbon discrimination factor for liver did not differ significantly among birds, fishes and mammals ($F_{2,16} = 2{\cdot}92$, $P = 0{\cdot}083$), but did differ significantly for the nitrogen discrimination factor ($F_{2,16} = 6{\cdot}67$, $P = 0{\cdot}008$, Fig. 2). For blood, the carbon discrimination factor was no different between birds and mammals ($F_{1,13} = 0{\cdot}05$, $P = 0{\cdot}834$), but the difference was significant for the nitrogen discrimination factor ($F_{1,11} = 5{\cdot}52$, $P = 0{\cdot}039$). For the whole body, the carbon discrimination factor was significantly different between invertebrates and fishes ($F_{1,80} = 9{\cdot}14$, $P = 0{\cdot}003$), but no significant differences were found for the nitrogen discrimination factor ($F_{1,68} = 0{\cdot}13$, $P = 0{\cdot}721$).

EFFECT OF DIET

Our initial analysis of all taxa combined showed significant negative relationships between discrimination factors and their diet isotopic ratios ($F_{1,230} = 51{\cdot}58$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}19$ and $F_{1,210} = 50{\cdot}54$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}16$, for ΔC and ΔN, respectively). We then performed independent GLMMs for these relationships within each consumer class (birds, mammals, fishes and invertebrate) and, taking into account where possible the type of tissue, the environment and the lipid extraction treatment (we used one of these variables when more than one category was available and valid, as described in the Statistical analysis section). In general, we found the same trend as in the initial analysis (all combined taxa) of significant negative relationships between discrimination factors and their corresponding isotopic ratios (Table 1). However, in some consumer classes, the relationship was not significant, as described below.

For mammals, only two categorical independent variables were added to the GLMM: the lipid extraction treatment and the type of tissue (six categories: blood, red blood cells, hair, liver, muscle and plasma). The carbon discrimination factor was negatively correlated with the diet carbon isotopic ratio, but none of the categorical variables was significant in the full model (Table 1). The nitrogen discrimination factor showed differences among tissues and a significant negative correlation with the diet nitrogen isotopic ratio (Table 1). The GLM on the relationships between discrimination factors and their corresponding diet isotopic ratios confirmed these results, showing significant relationships for both carbon and nitrogen ($\Delta^{13}C$: $F_{1,88} = 91{\cdot}44$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}51$; and $\Delta^{15}N$: $F_{1,78} = 14{\cdot}25$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}15$; Fig. 3a,b). For the nitrogen discrimination factor and within tissues, the GLM between $\Delta^{15}N$ and diet isotopic values were only significant for muscle, liver and plasma ($F_{1,13} = 17{\cdot}74$, $P = 0{\cdot}001$, $R^2 = 0{\cdot}58$; $F_{1,14} = 8{\cdot}17$, $P = 0{\cdot}013$, $R^2 = 0{\cdot}37$; and $F_{1,17} = 19{\cdot}17$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}53$, respectively).

For birds, three categorical independent variables were added to the GLMM: the lipid extraction treatment, the environment of the bird (three categories: terrestrial, marine and freshwater), and the type of tissue (five categories: blood, feather, liver, muscle and plasma). The carbon discrimination factor was positively correlated with the diet carbon isotopic ratio, and there were significant differences among tissues

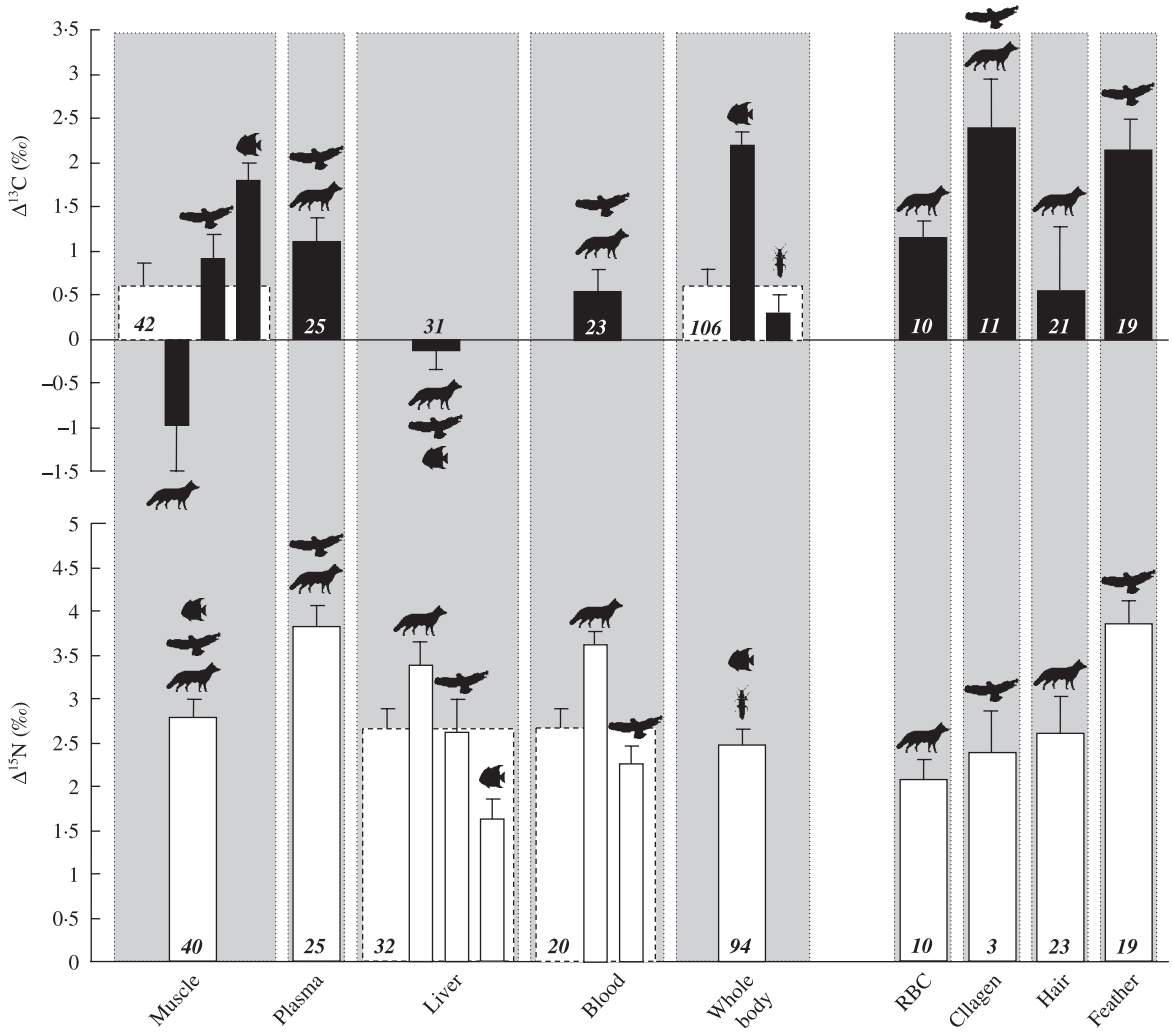

Fig. 2. Mean (± SE) $\Delta^{13}C$ and $\Delta^{15}N$ within animal consumer classes among different tissues. Each pictogram above the bar indicates the classes included in each tissue analysis (symbols as in Fig. 1). Numbers inside the bar indicate the sample size. When a tissue had significant differences within classes, the mean for all classes is represented by a bar with a dotted line, and the mean of each class is represented inside by a bar with a solid line. Four tissues (hair, feather, collagen and red blood cells) had no categories or insufficient data for assessment of differences within animal consumer classes.

Table 1. Factors affecting the carbon and nitrogen discrimination factors in general linear mixed models. The analysed variables are presented for each consumer class and in italics are the ones significant in the full model

	$\Delta^{13}C$				$\Delta^{15}N$			
Classes	Variables	dfn, dfd	*F*	*P*	Variables	dfn, dfd	*F*	*P*
Mammal	*Diet $\delta^{13}C$*	*1, 64*	*47·70*	*< 0·001*	*Diet $\delta^{15}N$*	*1, 64*	*90·23*	*< 0·001*
	Tissue	5, 64	0·23	0·949	*Tissue*	*5, 64*	*9·72*	*< 0·001*
	Lipid	1, 64	0·02	0·901	Lipid	1, 64	0·23	0·632
Bird	*Diet $\delta^{13}C$*	*1, 24*	*5·32*	*0·030*	Diet $\delta^{15}N$	1, 22	1·45	0·242
	Environment	2, 24	1·83	0·183	*Environment*	*2, 22*	*5·39*	*0·012*
	Tissue	*4, 24*	*6·67*	*< 0·001*	*Tissue*	*4, 22*	*11·82*	*< 0·001*
	Lipid	1, 24	3·08	0·092	*Lipid*	*1, 22*	*11·30*	*0·003*
Fish	*Diet $\delta^{13}C$*	*1, 18*	*8·60*	*0·009*	*Diet $\delta^{15}N$*	*1, 21*	*9·08*	*0·007*
	Tissue	*2, 18*	*13·39*	*< 0·001*	*Tissue*	*2, 21*	*14·48*	*< 0·001*
	Environment	1, 18	1·12	0·304	Environment	1, 21	0·01	0·937
	Lipid	1, 18	1·31	0·267	Lipid	1, 21	2·01	0·171
Invertebrate	*Diet $\delta^{13}C$*	*1, 67*	*5·85*	*0·018*	*Diet $\delta^{15}N$*	*1, 55*	*30·36*	*< 0·001*
	Environment	1, 67	0·82	0·368	Environment	1, 55	0·18	0·675

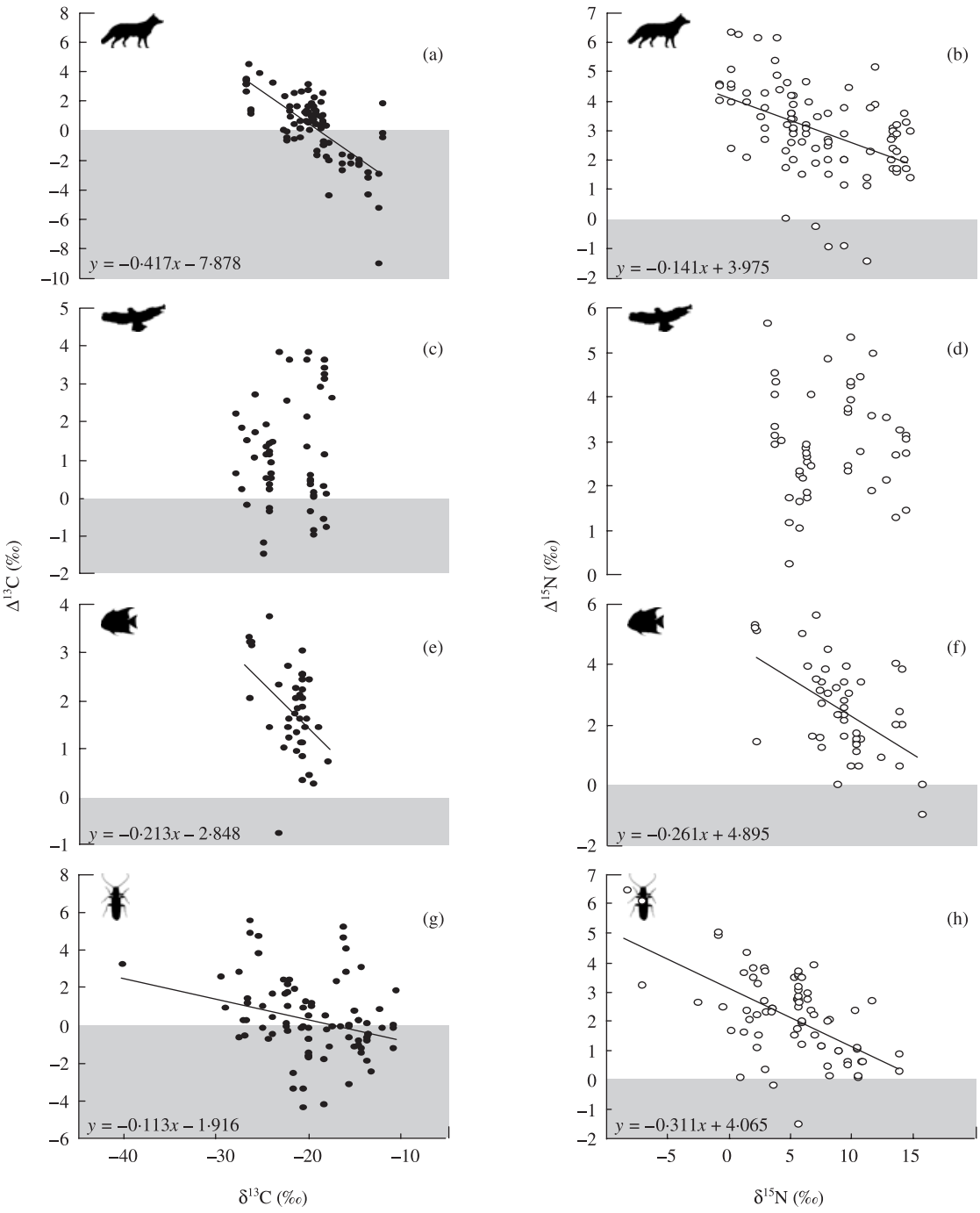

Fig. 3. Relationship for each taxonomic class between estimates of $\Delta^{13}C$ and carbon diet isotopic ratio $\delta^{13}C$, and estimates of $\Delta^{15}N$ and nitrogen diet isotopic ratio $\delta^{15}N$. Each taxonomic class is represented by a pictogram as in Fig. 1. Regressions are only shown when significant.

(Table 1). The nitrogen discrimination factor showed differences among tissues, environments and lipid extraction treatments, but there was no significant relationship with the diet nitrogen isotopic ratio (Table 1). The GLM showed no significant relationships between the carbon discrimination factor and carbon diet isotopic ratio ($\Delta^{13}C$: $F_{1,53} = 0{\cdot}64$, $P = 0{\cdot}425$, $R^2 = 0{\cdot}012$; Fig. 3c); within tissues the GLM between $\Delta^{13}C$ and the carbon diet isotopic value was only significant negative for blood ($F_{1,14} = 8{\cdot}92$, $P = 0{\cdot}010$, $R^2 = 0{\cdot}39$). For nitrogen the GLM showed no significant relationships between the discrimination factor and diet isotopic ratio ($\Delta^{15}N$: $F_{1,46} = 0{\cdot}009$, $P = 0{\cdot}924$, $R^2 = 0{\cdot}00$; Fig. 3d). Within tissues and lipid extraction categories, the GLMs between $\Delta^{15}N$ and nitrogen diet isotopic value were not significant, and for environment, the relationships were only significant for marine and terrestrial environments ($F_{1,16} = 18{\cdot}91$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}54$ and $F_{1,15} = 18{\cdot}88$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}56$, respectively).

For fishes, three categorical independent variables were added to the GLMM: the lipid extraction treatment, the type of tissue (three different tissues: liver, muscle or whole body)

and the environment of the fish (two categories: marine and freshwater). The GLMM for the carbon and nitrogen discrimination factors showed negative relationships with the diet isotopic ratios, and there were also differences among tissues (Table 1). The GLM analysis showed significant relationships between both discrimination factors and the corresponding diet isotopic ratios ($\Delta^{13}C$: $F_{1,39} = 10{\cdot}69$, $P = 0{\cdot}002$, $R^2 = 0{\cdot}22$; and $\Delta^{15}N$: $F_{1,45} = 19{\cdot}28$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}30$; Fig. 3e,f). For the carbon and nitrogen discrimination factors and within tissues, the GLM between discrimination factors and diet isotopic values were only significant for whole body and muscle (carbon: $F_{1,14} = 8{\cdot}34$, $P = 0{\cdot}012$, $R^2 = 0{\cdot}37$, and $F_{1,16} = 7{\cdot}01$, $P = 0{\cdot}018$, $R^2 = 0{\cdot}30$, respectively; nitrogen: $F_{1,15} = 16{\cdot}65$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}53$, and $F_{1,17} = 7{\cdot}85$, $P = 0{\cdot}012$, $R^2 = 0{\cdot}32$, respectively).

For invertebrates, one categorical variable was added to the GLMM model that was not significant in the full model: the environment of the invertebrate (three categories: terrestrial, marine and freshwater). The discrimination factors for nitrogen and carbon were negatively correlated with their corresponding diet isotopic ratios (Table 1). The same trend was evident in the GLM analysis (Fig. 3g,h), which was significant for carbon ($F_{1,84} = 7{\cdot}97$, $P = 0{\cdot}006$, $R^2 = 0{\cdot}09$) and nitrogen ($F_{1,71} = 39{\cdot}50$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}36$) .

Use of discrimination factors for dietary reconstruction

The literature review identified 32 publications that used one of the four main model types (Supporting Information, Table S2): 10 involving the geometric dual-isotope mixing model, 11 involving the linear mixing model, 3 involving the concentration-weighted linear mixing model, and 9 involving the *IsoSource* source partitioning mixing model. All taxa were represented: mammals (8 publications), birds (8 publications), fishes (6 publications), invertebrates (11 publications) and reptiles (1 publication).

We first investigated the source of $\Delta^{13}C$ and $\Delta^{15}N$ used in the isotopic models in the reviewed papers (i.e. the study cited in each paper), and examined the concordance between the consumer classes and tissues of the discrimination factor in the publication source, and the discrimination factor used in the isotopic models. In most cases (20 of 35 publications for carbon and 23 of 38 publications for nitrogen), the discrimination factor for the consumer class and tissue in the publication source differed from that of the model (references marked with *D* in Supporting Information, Table S2). In addition, in 16 cases for carbon and 22 cases for nitrogen, the discrimination factors used in the isotopic models came from published reviews (references marked with an asterisk in Supporting Information, Table S2). These reviews included both field and laboratory studies. Therefore, it is likely that the values of discrimination factors coming from these reviews (i.e. the values used in 19 of the 32 studies) strongly biased the results of the isotopic models. However, these were probably the best values available at the time of publication.

Secondly, we examined the number of discrimination factors used in the isotopic models in relation to the number of different diets and the range of diet isotopic ratio values. The discrimination factors used in the isotopic models ranged from –2·6 to 3·4 for ΔC, and from 0 to 5 for ΔN (ΔC and ΔN columns in Supporting Information, Table S2). However, 23 publications used only one nitrogen or carbon discrimination factor for all diets, 2 publications used the same nitrogen and carbon discrimination factors for all diet items but used different values for comparison (Gauthier, Bety & Hobson 2003; Grey, Waldron & Hutchinson 2004, see Supporting Information, Table S2), and only 7 of the 32 publications used different discrimination factors according to the diet type (Ben-David *et al.* 1997a,b; Szepanski, Ben-David & Van-Ballenberghe 1999; Drever *et al.* 2000; Ben-David, Titus & Beier 2004; Morrissey, Bendell-Young & Elliott 2004; Caut *et al.* 2006, see Supporting Information, Table S2). Across the isotopic models, we found a very wide range ($8 \pm 0{\cdot}96$‰ on average) and up to 19·7‰ (Bunn, Davies & Winning 2003; see Supporting Information, Table S2) for isotopic ratio values of diet inputs ($\delta^{13}C$ and $\delta^{15}N$ columns in Supporting Information, Table S2).

To assess the potential inappropriate use of discrimination factor values in isotopic models for diet reconstruction, we calculated new nitrogen and carbon discrimination factors for all the reviewed papers using a Diet-Dependent Discrimination Factor method (DDDF, see Fig. 4). We constructed a decision diagram (Fig. 4) using the quantifications of the effects of diet isotopic values and type of tissues within different consumer classes. We used the regression equations for each consumer class when only the relationships between discrimination factors and their corresponding diet isotopic ratios were significant (e.g. invertebrates, see Table 1). When both the diet isotopic ratio and the tissue significantly affected discrimination factors, we used the regression equations between discrimination factors and diet isotopic ratios for each tissue (e.g. muscle for fishes), or the mean for the tissue when this regression was not significant (e.g. liver for fishes). In the case of birds, the diet isotopic ratios were not significantly related to their corresponding discrimination factors, and hence, we took the mean for each tissue (except for blood and $\Delta^{13}C$, where we used the regression equation). In the case of mammals, the tissue was not a significant variable affecting carbon discrimination factor but we were interested to search for regression equations among tissues (although main equation for all tissues together was also included).

For both carbon and nitrogen, we calculated two DDDFs corresponding to the minimum and maximum values of the isotopic ratios of the diet (except where there was only one diet isotopic value). We found that 18 of 39 $\Delta^{13}C$, and 29 of 42 $\Delta^{15}N$ used in the isotopic models were different from estimated DDDFs (e.g. outside of our interval). Moreover, some of the used discrimination factors were included in the estimated DDDF range but this range was very wide.

To illustrate the possible deviation between the range of the estimated DDDFs and the discrimination factors used in the isotopic models, we calculated a parameter called the deviation coefficient (C_D). This parameter was the maximum deviation

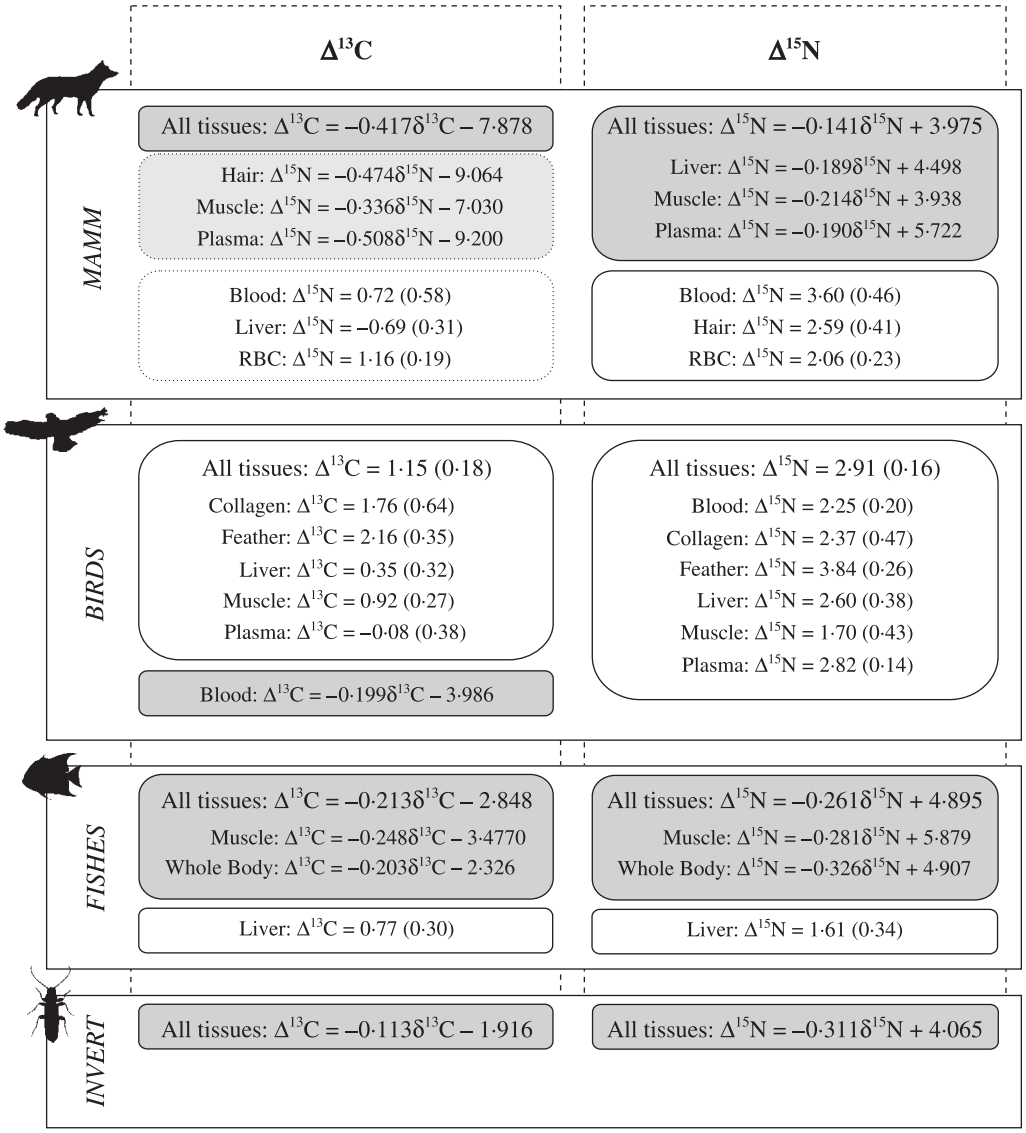

Fig. 4. Decision diagram to calculate estimates of carbon and nitrogen discrimination factors from diet isotopic ratios for each animal consumer class (and type of tissue, marked in grey when significant). Averaged means (± SE) are given as estimates when the diet isotopic ratio was not significantly related to the discrimination factors (in white area). Estimates for carbon discrimination factors from diet isotopic ratios for mammal tissues were represented for information, even if not significant in full model.

between the discrimination factor value used in the isotopic study and the discrimination factor of DDDFs for each diet item. Thus, C_D represents the deviation (in ‰) of discrimination factors used in the isotopic models from DDDFs. Based on these criteria, we found 11 used $\Delta^{13}C$ and 15 used $\Delta^{15}N$ within [0–1‰ C_D] (‰ of deviation from DDDFs), 16 $\Delta^{13}C$ and 11 $\Delta^{15}N$ within [1–2‰ C_D], 6 $\Delta^{13}C$ and 13 $\Delta^{15}N$ within [2–3‰ C_D], 3 $\Delta^{13}C$ and 3 $\Delta^{15}N$ within [3–4‰ C_D], 3 $\Delta^{13}C$ within [4–5‰ C_D] and 1 $\Delta^{13}C$ and 1 $\Delta^{15}N$ > 5‰ C_D, for carbon and nitrogen respectively (see Supporting Information Table 2A). Averaged C_D of used values were 1·78‰ (SD: 1·36) for $\Delta^{13}C$ and 1·69‰ (SD: 1·09) for $\Delta^{15}N$. In summary, 40% used $\Delta^{15}N$ and 33% $\Delta^{13}C$ differed more than 2‰ from estimated DDDFs. This means that more than 35% of the values used in isotopic model studies could have an error ≥ 2‰ leading to incorrect results, and that an appropriate value should be used instead.

Discussion

Two key points emerge from the thorough review of the literature reported here. First, there is high variability in both $\Delta^{13}C$ and $\Delta^{15}N$ values, and this is mainly dependent on the consumer class, the tissue and the diet isotopic ratio. Indeed, we show a significant negative relationship between both $\Delta^{13}C$ and $\Delta^{15}N$ and their corresponding diet isotopic ratios. Secondly, most studies have used inappropriate discrimination factor values, or averages from data that should not have been

combined, both of which could generate inaccuracies in isotope model outputs, and hence, in interpretation in diet reconstruction studies. Although these were probably the best values available at the time the studies involved were published, we stress that using inappropriate values for discrimination factors potentially leads to large errors or meaningless results. We propose the DDDF method to generate values when the data for use in isotope models are lacking.

In our study, the overall mean of the 268 estimates of $\Delta^{15}N$ across the reviewed papers was 2·75 ± 0·10‰, which is lower than estimates reported by Minagawa & Wada (1984; 3·40 ± 0·27‰, n = 16), Post (2002; 3·40 ± 0·13‰, n = 56) and Robbins *et al.* (2005; 3·21 ± 0·2‰, n = 33), similar to the estimate of Vander Zanden & Rasmussen (2001; 2·9 ± 0·3‰, n = 35), and higher than the estimates of Vanderklift & Ponsard (2003; 2·54 ± 0·11‰, n = 134) and McCutchan *et al.* (2003; 2·00 ± 0·20‰, n = 83). Our mean estimate differs only by 0·7‰ from these reviews. This could be due to the greater sample size and the inclusion of some laboratory-based studies. The overall mean of the 290 estimates of $\Delta^{13}C$ was 0·75 ± 0·11‰, which is higher than reported by Post (2002; 0·4 ± 1·3‰, n not specified), McCutchan *et al.* (2003; 0·4 ± 0·12, n = 111) and Vander Zanden & Rasmussen (2001; 0·47 ± 0·19‰, n = 42).

Most previous studies have suggested that there is less variability in carbon than in nitrogen isotopic discrimination (Minagawa & Wada 1984; Vander Zanden & Rasmussen 2001; Post 2002; McCutchan *et al.* 2003; Vanderklift & Ponsard 2003; Robbins *et al.* 2005). In fact, the range of discrimination factors in these papers is very important (–8·79 to 6·1‰ for $\Delta^{13}C$, and –3·22 to 9·2‰ for $\Delta^{15}N$). The large variability can be explained by differing variables that can be grouped at two different scales: (i) the individual scale, which includes the consumer class and species (Minagawa & Wada 1984; Vanderklift & Ponsard 2003), the tissues and organs examined (Hobson & Clark 1992a,b; Vanderklift & Ponsard 2003), the physiological stress (Adams & Sterner 2000; Oelbermann & Scheu 2002) and the form of nitrogen excretion (Minagawa & Wada 1984; Vanderklift & Ponsard 2003); and (ii) the diet scale, which includes the diet protein quality (Post 2002; McCutchan *et al.* 2003; Vanderklift & Ponsard 2003; Robbins *et al.* 2005), the type of food (Webb, Hedges & Simpson 1998; Vander Zanden & Rasmussen 2001) and the diet isotopic ratio (Felicetti *et al.* 2003; Caut *et al.* 2008a).

In this study, we have shown that discrimination factors are significantly affected by the consumer taxonomic group and the consumer tissue. Indeed, we found differences between taxonomic classes in $\Delta^{13}C$ but not in $\Delta^{15}N$, although these results could be partly confounded by the use of different tissues. Previous studies have noted that $\Delta^{15}N$ may vary among species (e.g. DeNiro & Epstein 1981; Vanderklift & Ponsard 2003). Differences may be partly due to the excretion mode of each class (Vanderklift & Ponsard 2003). The results also revealed consistent differences in nitrogen discrimination factor among tissues that could be explained by different metabolic properties characterizing organs and tissues within the body that are similar across taxa (e.g. turnover rates, biochemical composition). In this aspect, several studies have found contrasting results (e.g. DeNiro & Epstein 1981; Hobson & Clark 1992a; Hilderbrand *et al.* 1996).

Stable isotope models are used to quantify the contributions of multiple sources to a mixture, such as the proportions of different types of food sources in an animal's diet. The use of isotope models in ecology has dramatically increased in the last several decades (see references in Supporting Information, Table S2). Mean signature values are calculated for each of the sources, and based on this, the fractional contribution of each source to the mixture is calculated. However, the most important parameter in the isotope model is the discrimination factor, which can considerably modify the model output with respect to the difference in isotopic composition between an animal and its diet (Ben-David & Schell 2001). Despite the large variability in nitrogen and carbon discrimination factors highlighted here, most isotope model studies have used a single discrimination factor for carbon and nitrogen, often obtained from a published review. Although these were probably the best values available at the time the studies involved were published, this has created two problems. Firstly, the estimates in the reviews were derived from inappropriate combinations of laboratory studies (which represent discrimination factors) and field studies (which represent trophic enrichment), and included different consumer classes, tissues and other variables. Secondly, a common assumption has been that discrimination factors are independent of the diet isotope value. We have shown that diet-dependent discrimination factors calculated using the method we have proposed differ markedly from those used in the reviewed studies (see Supporting Information, Table S2), with the consequence that most models generate incorrect results. In fact, as isotope models are very sensitive to changes (> 1‰) in discrimination factors (Ben-David & Schell 2001), the use of diet-dependent discrimination factors could significantly change the results and hence the interpretation (Caut *et al.* 2008b).

Based on an extensive literature review, the trend between discrimination factors and diet isotopic ratios is consistent among consumer classes and explains between 9% and 51% of the variation in discrimination factors. Caut *et al.* (2008a) showed higher percentages in a controlled experiment: diet isotopic values explained 60–98% of the variation of discrimination factors in different tissues in the rat (*Rattus* sp.). Similarly, Felicetti *et al.* (2003) showed that diet isotopic values explained 88% of $\Delta^{13}C$ and 98% of $\Delta^{15}N$ of bear plasma. Here, we have provided a decision diagram (Fig. 4) for estimating the discrimination factors for different animal consumer classes (rows) and two isotopic elements (columns) when using isotope models. For each class and isotopic element, ecologists can easily estimate a discrimination factor relevant to the particular field situation, according to the significant variables in each case. The decision diagram includes the three most significant variables (taxon, tissue and diet isotopic value) from the five studied in this review, but other variables not considered here could also play a role (e.g. quality of diet, type of excretion, trophic level). Moreover, the data in Supporting

Information, Table S1 could be used to construct and test regression equations for specific situations (e.g. for a researcher working with insects, where only data for this consumer class can be used).

We do not have a functional explanation for this relationship, and future effort should be focused on laboratory-based studies to quantify the involvement of factors including C : N ratio, amino acid composition and nutritional state. However, knowledge of this relationship provides researchers with a tool to reduce model output errors created by use of inappropriate discrimination factors. To date, the lack of an easy method to quantify species-specific discrimination factors for different diets and tissues has led ecologists to use an idiosyncratic collection of procedures mainly relying on fixed discrimination factors, particularly in studies of elusive, rare or endangered species. Although we concur with the note of caution expressed by Ben-David & Schell (2001), that regressions of this type cannot be used as surrogates for mixing models, such regressions should be very useful in providing estimates of discrimination factors when no field data are available. We strongly recommend using the decision diagram when the diet sources used in the isotope model have significantly different isotope ratios (e.g. in omnivore species). Finally, field ecologists should recognize that discrimination factor values are estimated with error and that this error propagates in the use of mixing models. For example, some of the relationships we present are noisy and this noise is ignored when running computer programs to estimate sources in animal diets. Future studies should devise models that incorporate errors in discrimination factors.

In summary, understanding and estimating discrimination factors remains problematic. Stable isotope methods are currently among the most powerful tools for the study of trophic relationships and animal diets. However, the assumptions underpinning isotope models, such as the potential sources of variation in discrimination factors, should not be overlooked. In particular, researchers using isotope models should consider the diet-dependent discrimination factor as a tool for obtaining more accurate results.

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References

Adams, T.S. & Sterner, R.W. (2000) The effect of dietary nitrogen content on trophic level N-15 enrichment. *Limnology and Oceanography*, **45**, 601–607.

Ben-David, M. & Schell, D.M. (2001) Mixing models in analyses of diet using multiple stable isotopes: a response. *Oecologia*, **127**, 180–184.

Ben-David, M., Flynn, R.W. & Schell, D.M. (1997a) Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia*, **111**, 280–291.

Ben-David, M., Hanley, T.A., Klein, D.R. & Schell, D.M. (1997b) Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, **75**, 803–811.

Ben-David, M., Titus, K. & Beier, L.R. (2004) Consumption of salmon by Alaskan brown bears: a trade-off between nutritional requirements and the risk of infanticide? *Oecologia*, **138**, 465–474.

Bodin, N., LeLoch, F. & Hily, C. (2007) Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *Journal of Experimental Marine Biology and Ecology*, **341**, 168–175.

Bunn, S.E., Davies, P.M. & Winning, M. (2003) Sources of organic carbon supporting the food web of an arid zone floodplain river. *Freshwater Biology*, **48**, 619–635.

Caut, S., Roemer, G.W., Donlan, C.J. & Courchamp, F. (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. *Ecological Applications*, **16**, 1893–1900.

Caut, S., Angulo, E. & Courchamp, F. (2008a) Discrimination factors (Δ15N and Δ13C) in an omnivorous consumer: effect of diet isotopic ratio. *Functional Ecology*, **22**, 255–263.

Caut, S., Angulo, E. & Courchamp, F. (2008b) Caution on isotopic model use for analyses of consumer diet. *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, **86**, 438–445.

Cerda, X., Retana, J. & Cros, S. (1998) Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. *Functional Ecology*, **12**, 45–55.

DeNiro, M.J. & Epstein, S. (1978a) Carbon isotopic evidence for different feeding patterns in two hydrax species occupying the same habitat. *Science*, **201**, 906–908.

DeNiro, M.J. & Epstein, S. (1978b) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495–506.

DeNiro, M.J. & Epstein, S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341–351.

Drever, M.C., Blight, L.K., Hobson, K.A. & Bertram, D.F. (2000) Predation on seabird eggs by Keen's mice (*Peromyscus keeni*): using stable isotopes to decipher the diet of a terrestrial omnivore on a remote offshore island. *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, **78**, 2010–2018.

Felicetti, L.A., Schwartz, C.C., Rye, R.O., Haroldson, M.A., Gunther, K.A., Phillips, D.L. & Robbins, C.T. (2003) Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, **81**, 763–770.

France, R.L. & Peters, R.H. (1997) Ecosystem differences in the trophic enrichment of C-13 in aquatic food webs. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1255–1258.

Gauthier, G., Bety, J. & Hobson, K.A. (2003) Are greater snow geese capital breeders? New evidence from a stable-isotope model. *Ecology*, **84**, 3250–3264.

Grey, J., Waldron, S. & Hutchinson, R. (2004) The utility of carbon and nitrogen isotope analyses to trace contributions from fish farms to the receiving communities of freshwater lakes: a pilot study in Esthwaite Water, UK. *Hydrobiologia*, **524**, 253–262.

Hilderbrand, G.V., Farley, S.D., Robbins, C.T., Hanley, T.A., Titus, K. & Servheen, C. (1996) Use of stable isotopes to determine diets of living and extinct bears. *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, **74**, 2080–2088.

Hills, M. (1978) On ratios – a response to Atchley, Gaskins, and Anderson. *Systematic Zoology*, **27**, 61–62.

Hobson, K.A. & Clark, R.G. (1992a) Assessing avian diets using stable isotopes 2. Factors influencing diet-tissue fractionation. *Condor*, **94**, 189–197.

Hobson, K.A. & Clark, R.G. (1992b) Assessing avian diets using stable isotopes 1. turnover of C-13 in tissues. *Condor*, **94**, 181–188.

Kasai, A. & Nakata, A. (2005) Utilization of terrestrial organic matter by the bivalve *Corbicula japonica* estimated from stable isotope analysis. *Fisheries Science*, **71**, 151–158.

Kline, T.C., Goering, J.J., Mathisen, O.A., Poe, P.H., Parker, K.L. & Scalan, R.S. (1993) Recycling of elements transported upstream by runs of Pacific salmon: II. Δ15N and Δ13C evidence in the Kvichak river Watershed, Bristol Bay, Southwestern Alaska. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 2350–2365.

Lepoint, G., Cox, A.S., Dauby, P., Poulicek, M. & Gobert, S. (2006) Food sources of two detritivore amphipods associated with the seagrass *Posidonia oceanica* leaf litter. *Marine Biology Research*, **2**, 355–365.

McCutchan, J.H., Lewis, W.M., Kendall, C. & McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378–390.

Minagawa, M. & Wada, E. (1984) Stepwise enrichment of N-15 along food-chains – further evidence and the relation between delta-N-15 and animal age. *Geochimica et Cosmochimica Acta*, **48**, 1135–1140.
Morrissey, C.A., Bendell-Young, L.I. & Elliott, J.E. (2004) Linking contaminant profiles to the diet and breeding location of American dippers using stable isotopes. *Journal of Applied Ecology*, **41**, 502–512.
Murry, B.A., Farrell, J.M., Teece, M.A. & Smyntek, P.M. (2006) Effect of lipid extraction on the interpretation of fish community trophic relationships determined by stable carbon and nitrogen isotopes. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 2167–2172.
Oelbermann, K. & Scheu, S. (2002) Stable isotope enrichment (delta N-15 and delta C-13) in a generalist predator (*Pardosa lugubris*, Araneae : Lycosidae): effects of prey quality. *Oecologia*, **130**, 337–344.
Peterson, B.J. & Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, **18**, 293–320.
Phillips, D.L. (2001) Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia*, **127**, 166–170.
Phillips, D.L. & Gregg, J.W. (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**, 171–179.
Phillips, D.L. & Gregg, J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261–269.
Phillips, D.L. & Koch, P.L. (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, **130**, 114–125.
Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.
Reich, K.J. & Worthy, G.A.J. (2006) An isotopic assessment of the feeding habits of free-ranging manatees. *Marine Ecology-Progress Series*, **322**, 303–309.
Robbins, C.T., Felicetti, L.A. & Sponheimer, M. (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia*, **144**, 534–540.
SAS Institute Inc. (2004) *SAS, 9·1·3, Help and Documentation*. Cary, NC, USA.
StatSoft Inc. (2001) Data analysis software system, version 6. http://www.statsoft.com
Sweeting, C.J., Polunin, N.V.C. & Jennings, S. (2006) Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry*, **20**, 595–601.
Szekely, T., Freckleton, R.P. & Reynolds, J.D. (2004) Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 12224–12227.
Szepanski, M.M., Ben-David, M. & Van Ballenberghe, V. (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. *Oecologia*, **120**, 327–335.
Vander Zanden, M.J. & Rasmussen, J.B. (2001) Variation in delta N-15 and delta C-13 trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061–2066.
Vanderklift, M.A. & Ponsard, S. (2003) Sources of variation in consumer-diet delta N-15 enrichment: a meta-analysis. *Oecologia*, **136**, 169–182.
Webb, S.C., Hedges, R.E.M. & Simpson, S.J. (1998) Diet quality influences the delta C-13 and delta N-15 of locusts and their biochemical components. *Journal of Experimental Biology*, **201**, 2903–2911.
Whittingham, M.J., Stephens, P.A., Bradbury, R.B. & Freckleton, R.P. (2006) Why do we still use stepwise modeling in ecology and behaviour? *Journal of Animal Ecology*, **75**, 1182–1189.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Description of discrimination factors obtained in the literature search, sorted by taxonomic class and reference year

Table S2. Description of discrimination factors (Δ) used in each of the 32 reviewed isotopic model papers

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