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Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis

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Abstract Measurements of δ^{15}N of consumers are usually higher than those of their diet. This general pattern is widely used to make inferences about trophic relationships in ecological studies, although the underlying mechanisms causing the pattern are poorly understood. However, there can be substantial variation in consumer-diet δ^{15}N enrichment within this general pattern. We conducted an extensive literature review, which yielded 134 estimates from controlled studies of consumer-diet δ^{15}N enrichment, to test the significance of several potential sources of variation by means of meta-analyses. We found patterns related to processes of nitrogen assimilation and excretion. There was a significant effect of the main biochemical form of nitrogenous waste: ammonotelic organisms show lower δ^{15}N enrichment than ureotelic or uricotelic organisms. There were no significant differences between animals feeding on plant food, animal food, or manufactured mixtures, but detritivores yielded significantly lower estimates of enrichment. δ^{15}N enrichment was found to increase significantly with the C:N ratio of the diet, suggesting that a nitrogen-poor diet can have an effect similar to that already documented for fasting organisms. There were also differences among taxonomic classes: molluscs and crustaceans generally yielded lower δ^{15}N enrichment. The lower δ^{15}N enrichment might be related to the fact that molluscs and crustaceans excrete mainly ammonia, or to the fact that many were detritivores. Organisms inhabiting marine environments yielded significantly lower estimates of δ^{15}N enrichment than organisms inhabiting terrestrial or freshwater environments, a pattern that was influenced by the number of marine, ammonotelic, crustaceans and molluscs. Overall, our analyses point to several important sources of variation in δ^{15}N enrichment and suggest that the most important of them are the main biochemical form of nitrogen excretion and nutritional status. The variance of estimates of δ^{15}N enrichment, as well as the fact that enrichment may be different in certain groups of organisms should be taken into account in statistical approaches for studying diet and trophic relationships.

Keywords Stable isotopes · Fractionation · Trophic level · Nitrogen · Nitrogen excretion

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Introduction

DeNiro and Epstein (1981) launched an extremely fruitful line of inquiry when they showed that trophic relationships among organisms could be inferred from comparisons of the natural abundances of stable nitrogen isotopes. Measurements of δ^{15}N (the ratio of ^{15}N/^{14}N relative to atmospheric nitrogen) of a consumer's tissues are usually higher than those of its diet, and the magnitude of the difference is relatively consistent among organisms (Gaebler et al. 1966; DeNiro and Epstein 1981; Minagawa and Wada 1984; Owens 1987; Smit 2001). This difference is generally referred to as enrichment, denoted by the symbol $\triangle$, where

$$\Delta = \delta^{15}\mathrm{N}_{\mathrm{consumer}} - \delta^{15}\mathrm{N}_{\mathrm{diet.}} \quad (1)$$

Ecologists have taken advantage of the relative consistency (and so predictability) of $\triangle$ in several ways (Robinson 2001). In one application, estimates of $\triangle$ are used to calculate the trophic positions of organisms (e.g. Hobson and Welch 1992; Hobson 1993; Post et al. 2000; Vander Zanden et al. 2000; Post 2002). In this application, the logic is that, if one knows the δ^{15}N of a 'base'

trophic level and the $\delta^{15}N$ of a consumer, the number of increments of size $\triangle$ that fit between them is a measure of height in the food web (e.g. Hobson and Welch 1992). The same logic can be applied in a hypothesis-testing framework: for example, Ponsard and Arditi (2000) tested whether differences in $\delta^{15}N$ between detritivores and predators matched the expected enrichment for one trophic step, and whether the variability of $\delta^{15}N$ within each of these groups was compatible with the hypothesis that they were homogeneous trophic levels (Eggers and Jones 2000; Ponsard and Arditi 2000; 2001). In another application, estimates of $\triangle$ are used to 'correct' source (i.e. food) $\delta^{15}N$ values prior to incorporating them into mixing-models aimed at estimating the relative contribution of several potential food sources (Ben-David et al. 1997; Szepanski et al. 1999; Phillips and Gregg 2001; Phillips and Koch 2002).

In all these applications, conclusions rely on the assumption that $\triangle$ values are transferable across a broad range of organisms. However, while $\triangle$ values are relatively consistent, there is nevertheless some variation, and it remains unclear whether this variation is random or is due to specific, predictable, influences (Gannes et al. 1997). This variation can hamper conclusions, particularly from studies that have used average Δ values without incorporating estimates of variability. Calculations have often been based either on a single $\triangle$ value derived from an identical or taxonomically related consumer species (e.g. Hobson 1993; Szepanski et al. 1999; Vander Zanden et al. 2000), or on the unweighted mean and variance of Δ values measured for a group of unrelated organisms, usually from Minagawa and Wada's (1984) review (e.g. Ponsard and Arditi 2000; Post et al. 2000; Vander Zanden and Rasmussen 2001; Post 2002).

There are two main avenues for improving the robustness of conclusions. First, incorporate estimates of variability into calculations. Second, choose estimates of $\triangle$ most appropriate to the organisms being studied. A number of mathematical methods incorporating variability have already been developed to improve mixing models (Phillips 2001; Phillips and Gregg 2001; Phillips and Koch 2002), and estimates of trophic position (Vander Zanden and Rasmussen 2001), but "the weakest link in the application of mixing models to a dietary reconstruction relates to the estimation of appropriate Δ values" (Phillips and Koch 2002). The same statement applies to calculations of trophic position (Vander Zanden and Rasmussen 2001). Both estimation and choice of appropriate $\triangle$ values requires a better knowledge and understanding of the sources of variation in $\delta^{15}N$ enrichment.

There are multiple potential sources of variation. For example, Δ might vary with type of food (Webb et al. 1998; Vander Zanden and Rasmussen 2001), among consumer species (Macko et al. 1982; Minagawa and Wada 1984), among tissues and organs within an organism (Yoneyama et al. 1983; Hobson and Clark 1992), and due to physiological stresses such as lack of proteins (Hobson et al. 1993; Scrimgeour et al. 1995; Webb et al. 1998; Ponsard and Averbuch 1999; Adams and Sterner 2000; Dittel et al. 2000; Oelbermann and Scheu 2002; but see Schmidt et al. 1999) and/or lack of drinking water (Schoeninger and DeNiro 1984; Ambrose and DeNiro 1986; 1987; Cormie and Schwartcz 1996; Sealy et al. 1987). It has also been hypothesized that Δ might vary among organisms with different forms of nitrogen excretion, although this assertion has never been tested with any significant amount of empirical data (Minagawa and Wada 1984; Ponsard and Averbuch 1999).

Several authors have called for controlled laboratory experiments to enable sources of variation in $\delta^{15}N$ enrichment to be identified (Owens 1987; Gannes et al. 1997). Our aim is to examine some potential sources of variation through meta-analyses of such experiments. Over the past 20 years, a substantial corpus of data has been collected and now offers opportunities to do so without a large risk of committing a β-type error (i.e. of not detecting an existing effect). However, no comprehensive compilation has been published since Minagawa and Wada (1984) compiled 27 estimates, of which 16 were from laboratory studies. Recently there have been several partial compilations, but only for aquatic species (Vander Zanden and Rasmussen 2001, based on 35 estimates, of which 24 were from laboratory studies; Post 2002, based on 56 estimates, of which the number of laboratory studies was not stated).

Our review is based on 134 estimates of $\delta^{15}N$ enrichment from the literature, focusing exclusively on controlled studies. We tested the hypotheses that there were consistent differences in estimates of $\triangle$ among:

1. Organisms that differ in the primary biochemical form of nitrogen excretion
2. Organisms that feed on different types of diet
3. Organisms from different taxonomic classes
4. Organisms from different environments (terrestrial, marine, freshwater), and
5. Different tissues or organs within organisms.

We further tested whether there were significant relationships between estimates of $\triangle$ and

6. The % nitrogen content of the food source, and
7. The C:N ratio of the food source.

To test the significance of these potential sources of variation, we used meta-analysis (Laird and Mosteller 1990; Gurevitch and Hedges 1993; 1999; Hedges et al. 1999). Meta-analysis was well-suited for our purpose, as it provides a statistical framework for detecting trends and synthesising overall estimates from a collection of studies done using different methods, with different numbers of replicates, and that yield very different estimates of variability.

Materials and methods

Data selection

Because the aim of this study was to synthesise data from experiments in which diets were known and controlled, rather than inferred or assumed, we constrained the literature review to studies of animals in captivity (in laboratories or zoos). We searched widely, using web-based search engines and reference lists of published articles. If data in a paper were only presented in graphs, we first attempted to get the necessary information from the author(s); when this was not possible, the graphs were scanned, and we obtained the data using the free software DataThief (http://www.nikhef.nl/~keeshu/datathief). We excluded studies in which the numbers of replicates and/or variances were unavailable and could not be calculated, because they are needed for the meta-analyses (see Meta-analysis of variation among groups below).

Compiling the meta-data

Meta-analysis requires an estimate of 'effect size' for each study: typically this is a standardised metric (such as Hedge's *d*), used to convert the results of studies using different units of measurement to a single unitless metric (Laird and Mosteller 1990). The effect size metric we adopted was simply δ^{15}N enrichment ($\triangle_{ij}$). We preferred this because all the results that we reviewed were expressed using the same units (i.e. δ^{15}N), and we wished to express results using the same measure. For studies including one consumer and one diet, $\triangle_{ij}$ was simply calculated following Eq. 1, while the variance $v(\triangle_{ij})$ was calculated following Hedges and Olkin (1985) as

$$v(\Delta_{ij}) = \frac{(n^{\mathrm{f}} - 1)(s^{\mathrm{f}})^2 + (n^{\mathrm{c}} - 1)(s^{\mathrm{c}})^2}{n^{\mathrm{f}} + n^{\mathrm{c}} - 2} \quad (2)$$

where n^{f} and n^{c} are the sample sizes of the food and consumer, respectively and $(s^{\mathrm{f}})^2$ and $(s^{\mathrm{c}})^2$ are the variances for the food and consumer, respectively.

For studies in which multiple analyses were performed on males and females fed the same diet, we used pooling techniques to calculate a common mean and variance, because the differences in $\triangle$ between genders were negligible. Pooling followed the procedure given by Zar (1996) for situations in which only means and variances are provided. With the pooled data, the enrichment $\triangle_{ij}$ and the variance $v(\triangle_{ij})$ were calculated as in Eqs. 1 and 2.

Meta-analysis of variation among groups

The suite of means and variances compiled in this way formed the meta-data. To test whether estimates of $\triangle_{ij}$ varied consistently according to different sources of variation, a mixed model meta-analysis was used (Gurevitch and Hedges 1993; 1999). Here, the effect of each of the pre-defined categories was considered fixed, with observations (i.e., the $\triangle_{ij}$ values) within each category considered random. Calculations were done in Excel spreadsheets (available from M.V. on request), following methods outlined by Gurevitch and Hedges (1993) and Hedges et al. (1999).

The focus of tests for differences between categories was the estimate of between-group heterogeneity Q_B (Hedges and Olkin 1985; Gurevitch and Hedges 1993). The Q_B statistic has a χ^2 distribution with $m-1$ degrees of freedom, where m is the number of categories (Gurevitch and Hedges 1993). If the Q_B yielded by comparing categories is greater than the critical value of the χ^2 distribution, we can thus conclude that the categories are significantly different. To identify which categories were different from each other, 95% confidence intervals were compared: pairs of categories were considered significantly different when 95% confidence intervals around the means did not overlap. (This latter approach results in a slightly increased possibility of type I errors, but we elected not to adjust the confidence intervals).

During the analyses, and during interpretation of results, we faced two constraints. First, often several estimates of Δ_{ij} were yielded by a single study. The estimates of Δ_{ij} used in the analyses may therefore not have been totally independent (Gurevitch and Hedges 1999); for example, estimates for different tissues are often made from the same individual organism. One way to remove this potential bias would be to randomly select just one estimate per study per analysis. However, the relatively small number of available data made the cost of such a practice in terms of power of the analysis too high compared with the small gain in rigor (Hedges et al. 1999). Instead, as other authors have done (Gurevitch and Hedges 1993; Curtis and Wang 1998; Downing et al. 1999; Hughes et al. 2002), we elected to include all estimates that otherwise met our criteria.

Second, there was the potential for uneven distribution of estimates from different groups to confound some interpretations about differences. For example, some tissue/organ categories were represented in few taxonomic groups. For invertebrates, whole bodies were always used. For vertebrates, whole body analyses occurred only for some fish; more typically, individual tissues or organs were used (these methods mirror those normally used in field-based studies of trophic relationships). In order to generate results for which interpretations were not confounded, we did a series of comparisons of categories that were directly comparable.

To explore the possible influence of food quality, we conducted regressions of estimates of Δ_{ij} against the % nitrogen content and the C:N ratio of the food sources. These two variables have been used as measures of food quality (and therefore indicators of nutritional stress) by several authors (Hobson and Clark 1992; Hobson et al. 1993; Adams and Sterner 2000), and can be used as surrogates for protein content, a factor that has been suggested to influence δ^{15}N enrichment.

Results

The literature review yielded 134 estimates of $\triangle_{ij}$ from 32 publications (Table 1). Mammals (39 estimates, 9 publications), birds (25 estimates, 3 publications), crustaceans (21 estimates, 7 publications), insects (19 estimates, 7 publications) and fishes (14 estimates, 7 publications) were relatively well represented. Molluscs (9 estimates, 2 publications) and spiders (4 estimates, 1 publication) were less well represented. The overall mean δ^{15}N enrichment was 2.54‰ (±0.11‰ SE). Other studies were found that could not be used in the meta-analyses (these are also listed in Table 1); the estimates yielded by these studies were all within the range of estimates used in the meta-analyses.

Excretion

We classified organisms according to the main biochemical form in which they excrete nitrogenous wastes, following Rieutord (1999). There were five categories: organisms excreting mainly urea (ureotelic), organisms excreting mainly uric acid (uricotelic), organisms excreting mainly ammonia (ammonotelic), organisms excreting mainly guanine (guanicotelic), and organisms excreting mainly amino acids. There were significant differences in enrichment among categories (Q_B =38.35, df=4, P<0.001; Fig. 1). Ureotelic organisms (3.11‰) and uricotelic organisms (2.73‰) yielded significantly higher enrichment than ammonotelic organisms (2.00‰), guanicotelic

Table 1 Estimates of δ^{15}N enrichment, compiled from published studies in which diet was known or controlled. △, mean δ^{15}N enrichment, calculated as in Eq. 1; *Var (△)*, the variance of the mean δ^{15}N enrichment, and was calculated as in Eq. 2. Abbreviations for classes: *M* mammal, *I* insect, *MO* mollusc, *C* crustacean,*F* fish,*B* bird. Abbreviations for trophic position: *H* herbivore,*C* carnivore, *M* manufactured diet,*D* detritivore. Abbreviations for excretion: *U* urea, *UA* uric acid,*A* ammonia, *G* guanine, *AA* amino acids. Data that were not used in the meta-analyses (because not all the necessary information was available) are marked with an asterisk

Author	Consumer	Diet	△	Var (△)	Class	Tissue	Diet	Excretion	%N	C:N
Steele and Daniel 1978	*Bos taurus* (cow)	Silage	4.20	0.00	M	Blood	H	U		
DeNiro and Epstein 1981	*Melanoplus sanguinipes* (grasshopper)	Corn seedlings	1.69	1.09	I	Whole body	H	UA		
DeNiro and Epstein 1981	*Melanoplus sanguinipes* (grasshopper)	Wheat seedlings	–0.75	2.94	I	Whole body	H	UA		
DeNiro and Epstein 1981	*Sitophilus granarius* (weevil)	Wheat seeds	5.89	3.98	I	Whole body	H	UA		
DeNiro and Epstein 1981	*Sitophilus oryzae* (weevil)	Wheat seeds	2.79	0.35	I	Whole body	H	UA		
DeNiro and Epstein 1981	*Desmia funeralis* (moth)	Grape leaves	4.21	7.08	I	Whole body	H	UA		
DeNiro and Epstein 1981	*Helix aspersa* (snail)	Romaine lettuce leaves	–0.36	1.89	MO	Whole body	H	A		
DeNiro and Epstein 1981	*Calliphora vicina* (blow fly)	Horsemeat	1.43	0.01	I	Whole body	C	UA		
DeNiro and Epstein 1981	*Calliphora vicina* (blow fly)	Pork	1.82	0.24	I	Whole body	C	UA		
DeNiro and Epstein 1981	*Musca domestica* (house fly)	Horsemeat	4.54	0.61	I	Whole body	C	UA		
DeNiro and Epstein 1981	*Musca domestica* (house fly)	Pork	3.36	0.61	I	Whole body	C	UA		
DeNiro and Epstein 1981	*Oncopeltus fasciatus* (milkweed bug)	Milkweed seeds	2.79	1.35	I	Whole body	H	UA		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Wayne Lab-Blox F6	5.83	1.18	M	Brain	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Wayne Lab-Blox F6	3.05	1.20	M	Hair	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Wayne Lab-Blox F6	2.11	1.21	M	Kidney	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Wayne Lab-Blox F6	4.22	1.20	M	Liver	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Purina Rat Chow	3.84	0.40	M	Brain	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Purina Rat Chow	1.52	0.23	M	Hair	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Purina Rat Chow	0.89	0.27	M	Kidney	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	Purina Rat Chow	2.62	0.31	M	Liver	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	JAX 911A	5.74	0.74	M	Brain	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	JAX 911A	3.20	0.77	M	Hair	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	JAX 911A	0.85	0.81	M	Kidney	M	U		
DeNiro and Epstein 1981	*Mus musculus* (mouse)	JAX 911A	4.67	0.77	M	Liver	M	U		
Macko et al. 1982	*Amphithoe valida* (amphipod)	*Ulva*	–0.70	0.20	C	Whole body	H	A		
Macko et al. 1982	*Amphithoe valida* (amphipod)	*Gelidium*	–0.20	0.20	C	Whole body	H	A		
Macko et al. 1982	*Paryhale hawaiensis* (amphipod)	*Ulva*	2.30	0.20	C	Whole body	H	A		
Macko et al. 1982	*Paryhale hawaiensis* (amphipod)	*Gelidium*	2.20	0.20	C	Whole body	H	A		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	3.43	0.46	M	Liver	M	U		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	2.30	1.75	M	Lung	M	U		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	3.90	1.44	M	Heart	M	U		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	1.73	0.20	M	Spleen	M	U		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	1.63	0.82	M	Kidney	M	U		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	3.87	1.56	M	Brain	M	U		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	2.03	0.72	M	Red blood cells	M	U		
Yoneyama et al. 1983	*Rattus* sp. (rat)	MF	3.93	0.76	M	Plasma	M	U		
Minagawa and Wada 1984	*Lebistes* sp. (guppy)	Tetramin	3.20	1.02	F	Whole body	M	A		
Minagawa and Wada 1984	*Mus musculus* (mouse)	Oriental yeast	2.90	0.18	M	Muscle	H	U		
Minagawa and Wada 1984	*Artemia* sp. (brine shrimp)	Yeast	4.90	0.20	C	Whole body	H	A		
Checkley and Entzeroth 1985	*Temora* sp. * (copepod)	Suspended particulate matter	5.80							
Estep and Vigg 1985	*Chasmistes cujus*	Hatchery feed	3.40	0.08	F	Muscle	M	A		
Toda and Wada 1990	*Neomysis intermedia* (mysid)	Frozen cladocerans	3.18	0.11	C	Whole body	C	A		
Hare et al. 1991.	*Sus scrofa* (pig) *	C_4 plants	1.80							
Hare et al. 1991.	*Sus scrofa* (pig) *	C_3 plants	0.90							
Mizutani et al. 1992	*Larus crassirostris* (black-tailed gull)	Saurel	5.30	1.33	B	Feather	C	UA		3.3

Table 1 (continued)

Author	Consumer	Diet	△	Var (△)	Class	Tissue	Diet	Excretion	%N	C:N
Mizutani et al. 1992	*Nycticorax caledonicus* (nankeen night heron)	Saurel	4.20	1.52	B	Feather	C	UA		3.3
Mizutani et al. 1992	*Egretta alba* (great white egret)	Saurel	3.90	1.74	B	Feather	C	UA		3.3
Mizutani et al. 1992	*Ardea cinerea* (grey heron)	Saurel	4.30	1.54	B	Feather	C	UA		3.3
Mizutani et al. 1992	*Spheniscus humboldti* (Humboldt's penguin)	Anchovy	4.80	0.39	B	Feather	C	UA		3.3
Mizutani et al. 1992	*Phalacrocorax carbo* (common cormorant)	Mackerel	3.70	0.61	B	Feather	C	UA		3.9
Mizutani et al. 1992	*Eudocimus ruber* (scarlet ibis)	Mixture	4.50	0.24	B	Feather	M	UA		5.1
Mizutani et al. 1992	*Eudocimus albus* (white ibis)	Mixture	4.30	0.32	B	Feather	M	UA		5.5
Hobson and Clark 1992	*Gallus gallus* (chicken)	Turkey starter	1.70	0.01	B	Liver	M	UA	4.1	
Hobson and Clark 1992	*Gallus gallus* (chicken)	Turkey starter	0.20	0.03	B	Muscle	M	UA	4.1	
Hobson and Clark 1992	*Gallus gallus* (chicken)	Turkey starter	1.50	0.01	B	Collagen	M	UA	4.1	
Hobson and Clark 1992	*Gallus gallus* (chicken)	Turkey starter	1.10	0.01	B	Feather	M	UA	4.1	
Hobson and Clark 1992	*Coturnix japonica* (quail)	Turkey starter	2.20	0.04	B	Blood	M	UA	4.0	
Hobson and Clark 1992	*Coturnix japonica* (quail)	Turkey starter	2.30	0.04	B	Liver	M	UA	4.0	
Hobson and Clark 1992	*Coturnix japonica* (quail)	Turkey starter	1.00	0.03	B	Muscle	M	UA	4.0	
Hobson and Clark 1992	*Coturnix japonica* (quail)	Turkey starter	2.50	0.10	B	Collagen	M	UA	4.0	
Hobson and Clark 1992	*Coturnix japonica* (quail)	Turkey starter	1.60	0.03	B	Feather	M	UA	4.0	
Hobson and Clark 1992	*Larus delawarensis* (gull)	Perch	3.10	0.04	B	Blood	C	UA	11.8	
Hobson and Clark 1992	*Larus delawarensis* (gull)	Perch	2.70	0.02	B	Liver	C	UA	11.8	
Hobson and Clark 1992	*Larus delawarensis* (gull)	Perch	1.40	0.02	B	Muscle	C	UA	11.8	
Hobson and Clark 1992	*Larus delawarensis* (gull)	Perch	3.10	0.04	B	Collagen	C	UA	11.8	
Hobson and Clark 1992	*Larus delawarensis* (gull)	Perch	3.00	0.04	B	Feather	C	UA	11.8	
Hobson and Clark 1992	*Falco peregrinus* (falcon)	Quail	3.30	0.11	B	Blood	C	UA		
Hobson and Clark 1992	*Falco peregrinus* (falcon)	Quail	2.70	0.15	B	Feather	C	UA		
Hesslein et al. 1993	*Coregonus nasus* (broad whitefish)	Commercial trout food	3.77	0.16	F	Muscle	M	A		
Hesslein et al. 1993	*Coregonus nasus* (broad whitefish)	Experimental trout food	2.18	0.19	F	Muscle	M	A		
Hesslein et al. 1993	*Coregonus nasus* (broad whitefish)	Experimental trout food	1.71	0.42	F	Liver	M	A		
Ben-David 1996	*Mustelus vison* (mink)	Salmon	2.20	0.09	M	Blood	C	U		
Ben-David 1996	*Mustelus vison* (mink)	Beef	4.30	0.05	M	Blood	C	U		
Ben-David 1996	*Mustelus vison* (mink)	Mixed	2.70	0.04	M	Blood	C	U		
Hilderbrand et al. 1996	*Ursus americanus* (black bear)	Salmon	2.25	0.16	M	Plasma	C	U		
Hilderbrand et al. 1996	*Ursus americanus* (black bear)	Deer	4.08	0.06	M	Plasma	C	U		
Hilderbrand et al. 1996	*Ursus americanus* (black bear)	Apples	4.21	0.03	M	Plasma	H	U		
Hobson et al. 1996	*Pagophilus groenlandicus* (harp seal)	Herring	2.28	0.23	M	Skin	C	U		
Dittel et al. 1997	*Penaeus vannamei* (shrimp)	*Artemia*	1.05	1.19	C	Whole body	C	A		9.3
Dittel et al. 1997	*Penaeus vannamei* (shrimp)	Zooplankton	2.76	1.56	C	Whole body	C	A		7.1
Dittel et al. 1997	*Penaeus vannamei* (shrimp)	Detritus-plus-meiofauna	–0.52	5.50	C	Whole body	D	A		18.4
Dittel et al. 1997	*Penaeus vannamei* (shrimp)	Detritus-plus-meiofauna	2.19	5.21	C	Whole body	D	A		19.6
Dittel et al. 1997	*Penaeus vannamei* (shrimp)	Detritus	–0.51	6.73	C	Whole body	D	A		17.2
Ostrom et al. 1997	*Hippodamia variegata* (ladybird beetle)	Aphids	2.90	0.27	I	Whole body	C	UA		
Yoneyama et al. 1997	*Rhopalosiphum padi* (aphid)	Phloem	–0.10	3.61	I	Whole body	H	UA	8.3	20.0
Webb et al. 1998	*Locusta migratoria* (locust)	Wheat	2.20	1.70	I	Whole body	H	UA		
Webb et al. 1998	*Locusta migratoria* (locust)	Maize	5.00	0.80	I	Whole body	H	UA		
Bearhop et al. 1999	*Phalacrocorax aristotelis* (cormorant)	Sprat	4.90	0.19	B	Feather	C	UA		
Gorokhova and Hanson 1999	*Mysis mixta* (mysid)	*Artemia*	3.56	0.71	C	Whole body	C	A	8.0	6.0
Gorokhova and Hanson 1999	*Mysis mixta* (mysid)	*Enteromorpha*	2.69	0.15	C	Whole body	H	A	1.0	26.0
Ponsard and Amlou 1999	*Drosophila melanogaster* (fruit fly)	Nutritive medium	2.75	0.05	I	Whole body	H	UA		
Pinnegar and Polunin 1999	*Oncorhynchus mykiss* (rainbow trout)	Trout food	2.55	0.01	F	Muscle	M	A		

Table 1 (continued)

Author	Consumer	Diet	△	Var (△)	Class	Tissue	Diet	Excretion	%N	C:N
Pinnegar and Polunin 1999	*Oncorhynchus mykiss* (rainbow trout)	Trout food	2.09	0.04	F	Red muscle	M	A		
Pinnegar and Polunin 1999	*Oncorhynchus mykiss* (rainbow trout)	Trout food	1.97	0.01	F	Heart	M	A		
Pinnegar and Polunin 1999	*Oncorhynchus mykiss* (rainbow trout)	Trout food	1.61	0.06	F	Liver	M	A		
Pinnegar and Polunin 1999	*Oncorhynchus mykiss* (rainbow trout)	Trout food	2.33	0.08	F	Whole body	M	A		
Adams and Sterner 2000	*Daphia magna* (anomopod)	*Scennedesmus acutus*	2.95	0.51	C	Whole body	H	A		9.6
Adams and Sterner 2000	*Daphia magna* (anomopod)	*Scennedesmus acutus*	5.28	0.15	C	Whole body	H	A		24.7
Dittel et al. 2000	*Callinectes sapidus* (blue crab)	*Artemia*	1.50	0.07	C	Whole body	C	A		6.2
Dittel et al. 2000	*Callinectes sapidus* (blue crab)	*Littoraria irrorata*	0.80	0.08	C	Whole body	C	A		4.6
Dittel et al. 2000	*Callinectes sapidus* (blue crab)	*Uca pugnax*	0.90	0.09	C	Whole body	C	A		5.9
Dittel et al. 2000	*Callinectes sapidus* (blue crab)	Detritus-plus-meiofauna	3.10	0.98	C	Whole body	D	A		16.6
Dittel et al. 2000	*Callinectes sapidus* (blue crab)	Detritus	2.30	0.09	C	Whole body	D	A		23.2
Dittel et al. 2000	*Callinectes sapidus* (blue crab)	Zooplankton	0.10	0.17	C	Whole body	C	A		5.6
Markow et al. 2000	*Drosophila nigrospiracula* (fruit fly)	Yeast	3.50	0.01	I	Whole body	H	UA		
Roth and Hobson 2000	*Vulpes vulpes* (red fox)	Pellet food	2.60	0.12	M	Plasma	M	U		
Roth and Hobson 2000	*Vulpes vulpes* (red fox)	Pellet food	3.20	0.12	M	Red blood cells	M	U		
Roth and Hobson 2000	*Vulpes vulpes* (red fox)	Pellet food	3.30	0.11	M	Liver	M	U		
Roth and Hobson 2000	*Vulpes vulpes* (red fox)	Pellet food	3.40	0.11	M	Muscle	M	U		
Roth and Hobson 2000	*Vulpes vulpes* (red fox)	Pellet food	3.60	0.11	M	Hair	M	U		
Herzka and Holt 2000	*Sciaenops ocellatus* (red drum)	Artificial diet	1.67	0.02	F	Whole body	M	A		
Focken 2001	*Oreochromis niloticus*, (Nile tilapia)	Pellet food	1.00	0.12	F	Whole body	M	A		
Kurata et al. 2001	*Assiminea japonica* (snail)	Litter	–0.76	0.09	MO	Whole body	D	A	1.6	27.2
Kurata et al. 2001	*Assiminea japonica* (snail)	Soil	0.65	0.16	MO	Whole body	D	A	0.2	14.0
Kurata et al. 2001	*Assiminea japonica* (snail)	Deposited seston	0.09	0.10	MO	Whole body	D	A	0.2	9.3
Kurata et al. 2001	*Assiminea japonica* (snail)	Diatom	5.73	0.11	MO	Whole body	H	A	0.3	4.8
Kurata et al. 2001	*Angustassiminea castanea* (snail)	Litter	–0.66	0.09	MO	Whole body	D	A	1.6	27.2
Kurata et al. 2001	*Angustassiminea castanea* (snail)	Soil	0.67	0.06	MO	Whole body	D	A	0.2	14.0
Kurata et al. 2001	*Angustassiminea castanea* (snail)	Deposited seston	–0.07	0.20	MO	Whole body	D	A	0.2	9.3
Kurata et al. 2001	*Angustassiminea castanea* (snail)	Diatom	4.59	0.10	MO	Whole body	H	A	0.3	4.8
Thoman et al. 2001	*Sciaenops ocellatus* (red drum) *	Pelleted diet	2.30							
Oelbermann and Scheu 2002	*Pardosa lugubris* (spider)	*Drosophila melanogaster*	2.16	0.04	S	Whole body	C	G	9.8	
Oelbermann and Scheu 2002	*Pardosa lugubris* (spider)	*Heteromurus nitidus*	2.53	0.90	S	Whole body	C	G	11.3	
Oelbermann and Scheu 2002	*Pardosa lugubris* (spider)	*Folsomia candida*	–3.22	1.10	S	Whole body	C	G	12.5	
Oelbermann and Scheu 2002	*Pardosa lugubris* (spider)	*Rhopalosiphum padi*	1.50	0.10	S	Whole body	C	G	8.3	
Oelbermann and Scheu 2002	*Heteromurus nitidus* (collembolan)	Yeast 1	5.19	0.49	I	Whole body	H	UA		
Oelbermann and Scheu 2002	*Folsomia candida* (collembolan)	Yeast 2	4.54	0.57	I	Whole body	H	UA		
Oelbermann and Scheu 2002	*Drosophila melanogaster* (fruit fly)	Banana	3.25	0.04	I	Whole body	H	UA		
Oelbermann and Scheu 2002	*Rhopalosiphum padi* (aphid)	Wheat	–1.16	0.14	I	Whole body	H	UA		
Harvey et al. 2002	*Salvelinus namaycush* (lake trout)	Mixture	3.60	0.11	F	Muscle	M	A	7.73	5.73
Harvey et al. 2002	*Salvelinus namaycush* (lake trout)	Mixture	3.37	0.16	F	Muscle	M	A	8.93	4.96
Kurle 2002	*Callorhinus ursinus* (northern fur seal)	Fish (herring and capelin)	3.92	0.04	M	Red blood cells	C	U		
Kurle 2002	*Callorhinus Ursinus* (northern fur seal)	Fish (herring and capelin)	5.05	0.04	M	Plasma	C	U		
Lesage et al. 2002	*Phoca vitulina* (harbor seal)	Herring	2.89	0.55	M	Serum	C	U		
Lesage et al. 2002	*Phoca vitulina* (harbor seal)	Herring	1.80	0.40	M	Red blood cells	C	U		
Lesage et al. 2002	*Phoca vitulina* (harbor seal)	Herring	2.30	1.14	M	Hair	C	U		
Lesage et al. 2002	*Halichoerus grypus* (gray seal)	Herring	3.26	0.51	M	Serum	C	U		
Lesage et al. 2002	*Halichoerus grypus* (gray seal)	Herring	1.67	0.45	M	Red blood cells	C	U		

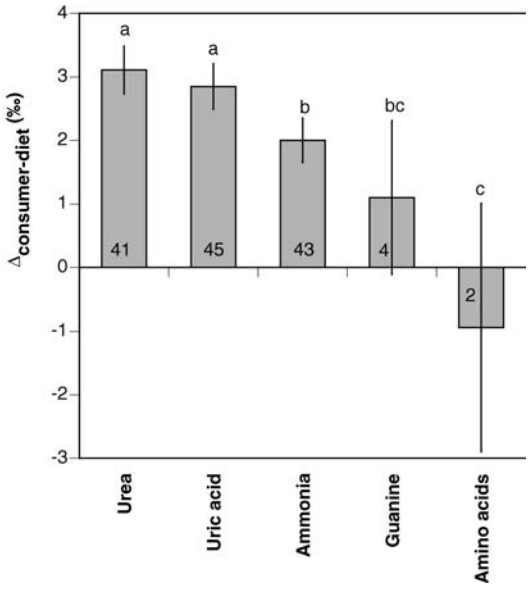

Fig. 1 Mean δ^{15}N enrichment (△±95% confidence intervals) among organisms classified according to the primary form of nitrogen excretion. Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*, *c*) are not significantly different

organisms (1.09‰) and organisms excreting mainly amino acids (-0.93‰). If organisms excreting mainly guanine (n=4, all spiders, all from one publication) or amino acids (n=2, all aphids) were excluded, estimates for ammonotelic organisms remained significantly lower than ureotelic and uricotelic organisms (Q_B =18.33, df=2, P<0.001).

Diet

We classified organisms according to the diet they were fed in the study, regardless of what their 'natural' diet is. There were four categories: organisms fed on animal matter (carnivorous), organisms fed on plant matter (herbivorous), organisms fed on food that was manufactured, either commercially or specifically for the study from a mixture of food (mixed diet), and organisms fed on detritus, soil, litter or seston (detritivorous). There were significant differences among the estimates of Δ yielded by each group (Q_B =30.93, df=3, P<0.001; Fig. 2). Organisms with carnivorous, herbivorous and mixed diets yielded similar estimates of Δ (2.69‰, 2.98‰ and 2.56‰ respectively) while organisms consuming detritus yielded significantly lower estimates of Δ (0.53‰).

There was no apparent relationship between estimates of Δ and the % nitrogen of the food source (r^2=0.008, $F_{1, 29}$=0.22, P>0.6; Fig. 3A). However, there was a significant positive relationship between estimates of Δ and the C:N ratio of the food source (r^2=0.222, $F_{1, 32}$=9.13, P<0.01; Fig. 3B).

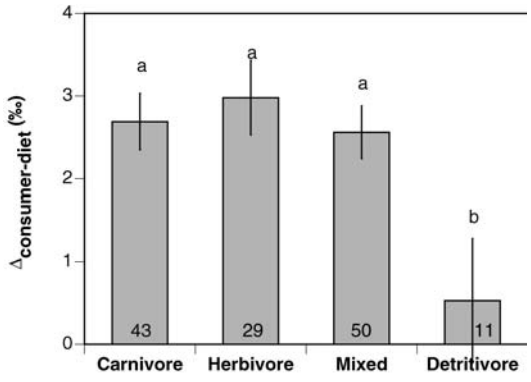

Fig. 2 Mean δ^{15}N enrichment (△±95% confidence intervals) among organisms classified according to diet used in the experiments. Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*) are not significantly different

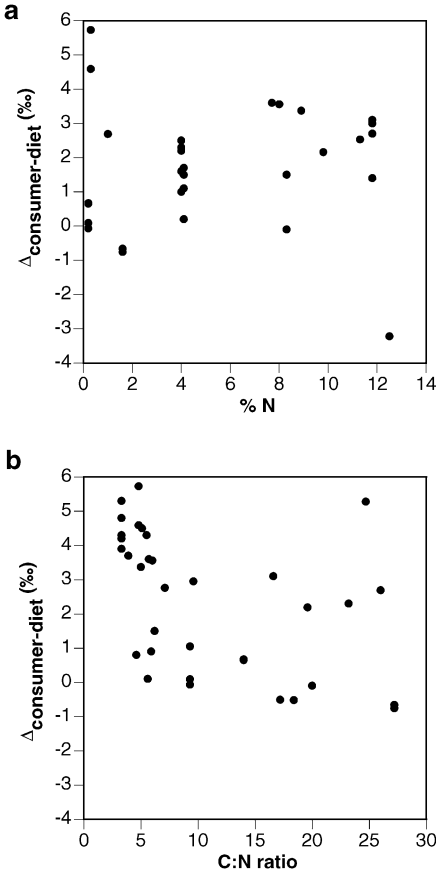

Fig. 3a, b Relationship between estimates of △ and **a** the % nitrogen and **b** the C:N ratios of the diets

Excretion × diet

There were sufficient data to perform comparisons on nine of the possible excretion-diet combinations (detritivores and organisms excreting mainly guanine and amino acids were excluded). Whether on carnivorous or herbiv-

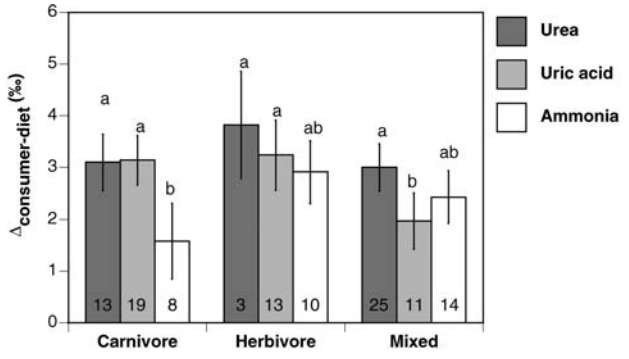

Fig. 4 Mean δ^{15}N enrichment (△±95% confidence intervals) among organisms classified according to all nine possible combinations of the primary form of excretion (excluding guanine and amino acids) and diet. Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*) are not significantly different

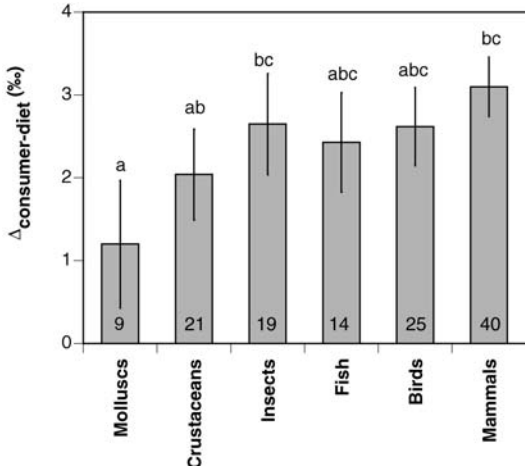

Fig. 5 Mean δ^{15}N enrichment (△±95% confidence intervals) among organisms classified according to taxonomic class. Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*, *c*) are not significantly different

orous diets, ammonotelic organisms yielded lower estimates of enrichment than ureotelic and uricotelic organisms – the difference was significant for carnivores (Q_B =31.89, *df*=8, *P*<0.001; Fig. 4). For organisms on mixed diets, ammonotelic organisms did not yield lower estimates, but uricotelic organisms did.

Taxon

There were significant differences among taxonomic classes (Q_B =24.67, *df*=5, *P*<0.001; Fig. 5). This result was heavily influenced by molluscs, which showed significantly lower enrichment values than most other groups. When molluscs were removed from the analyses, differences remained significant (Q_B =13.26, *df*=4, *P*=0.02), with crustaceans yielding lower estimates of enrichment than mammals.

Overall, invertebrates yielded lower estimates of enrichment than vertebrates (Q_B =8.93, *df*=1, *P*<0.01; 2.08‰ for invertebrates, 2.88‰ for vertebrates). However, interpretation of the among-taxon comparisons is somewhat confounded by the fact that data for crustaceans, insects and molluscs represent whole-body analyses, while data for vertebrates mainly represent analyses of different tissues or organs, as well as by the fact that taxonomic classifications are partly confounded with diet and form of excretion. To avoid the former confounding effect, separate analyses were performed in which only similar data were compared. Comparisons of the four classes for which there was whole-body data (the three classes of invertebrates and some fish) yielded no significant differences (Q_B =4.69, *df*=3, *P*>0.1). There were very few data to enable valid comparisons of all three vertebrate classes once restricted to a single tissue type. The only tissue that was measured in each vertebrate class was muscle: bird muscle yielded significantly lower estimates of Δ than mammal and fish muscle (Q_B =28.87, *df*=2, *P*<0.01; 0.87‰ for birds, 3.05‰ for mammals, and 2.96‰ for fish), but the number of samples was low (*n*=3 for birds, *n*=2 for mammals, *n*=7 for fish). Taxonomic classifications are partly confounded with diet and form of nitrogen excretion, but these confounding effects cannot be entirely disentangled. For our results, diet and form of excretion should be seen as possible explanations for differences between taxa rather than confounding factors that need to be studied independently.

Excretion × taxon

We separated studies into the possible combinations of main form of nitrogen excretion and taxon (vertebrates vs invertebrates). Because urea as the major nitrogen excretion product is restricted to vertebrates and guanine and amino acid excretion are restricted to some invertebrates, only ammonotelic and uricotelic organisms were included in analyses. Ammonotelic invertebrates yielded significantly lower, and more variable, estimates of δ^{15}N enrichment (Q_B =99.17, *df*=3, *P*<0.001; Fig. 6). The same trend could be found in vertebrates, with fish (ammonotelic) tending to have lower values than birds (uricotelic), although the difference was not significant.

Diet x taxon

We separated studies into the contributions of diet and taxon (again, at the level of vertebrate vs. invertebrate). Only carnivores and herbivores had representatives in both taxa, and so analyses did not include organisms with mixed diets or detritivores. Invertebrates yielded lower estimates for both carnivores and herbivores, but the difference was significant only for carnivores (Q_B =16.33, *df*=3, *P*<0.001; Fig. 7).

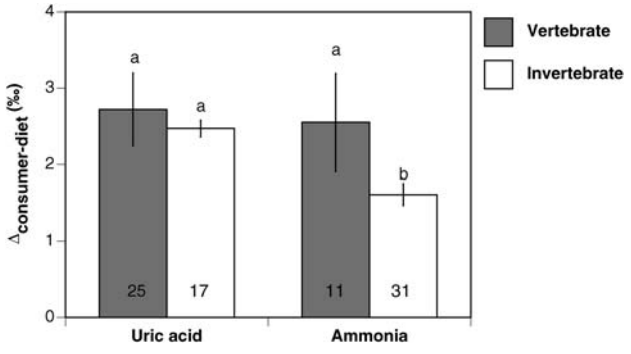

Fig. 6 Mean δ^{15}N enrichment (△±95% confidence intervals) among organisms classified according to the primary mode of excretion and taxon (not including animals excreting urea, which were all mammals). Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*) are not significantly different

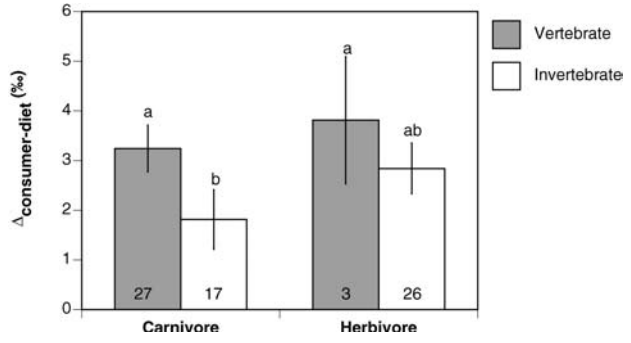

Fig. 7 Mean δ^{15}N enrichment (△±95% confidence intervals) among organisms classified according to taxonomic class and diet. Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*) are not significantly different

Environment

We separated estimates into three categories according to the environment that the organisms inhabit (i.e. terrestrial, marine, freshwater). Seals and seabirds were classified as 'terrestrial' because they breed, and spend a large proportion of resting time, on land. There were significant differences between environments (Q_B =19.07, *df*=2, *P*<0.001; Fig. 8); organisms inhabiting marine environments yielded significantly lower estimates of δ^{15}N enrichment (1.48‰), than organisms inhabiting terrestrial (2.82‰) or freshwater (2.98‰) environments.

Tissue

Comparisons of tissues were conducted separately for birds and mammals, because different tissues were used in studies of these classes. For example, feathers were only present for birds and fur for mammals (rather obviously!), while some tissues were only recorded for mammals (brain, blood cells, plasma, kidney).

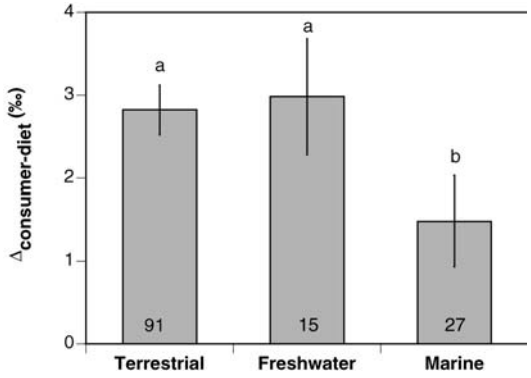

Fig. 8 Mean δ^{15}N enrichment (△±95% confidence intervals) among organisms classified according to the environment within which they live. Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*) are not significantly different

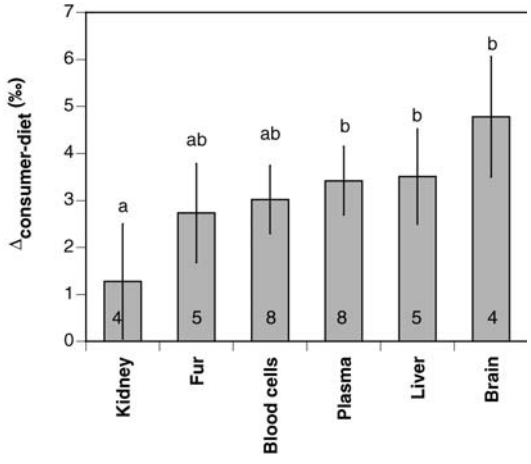

Fig. 9 Mean δ^{15}N enrichment (△±95% confidence intervals) among different tissues and organs (mammals only). Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*) are not significantly different

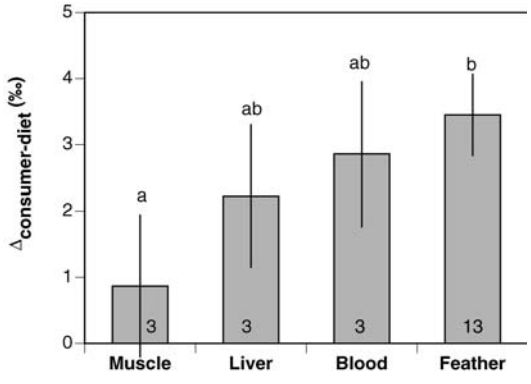

Fig. 10 Mean δ^{15}N enrichment (△±95% confidence intervals) among different tissues and organs (birds only). Numbers within the *bars* indicate the sample size *n*. Groups sharing the same superscript letter (*a*, *b*) are not significantly different

For mammals, variation among tissues was significant (Q_B =16.78, *df*=5, *P*=<0.01; Fig. 9). Brain tissue yielded the highest estimates of enrichment (4.78‰), while kidney tissue yielded the lowest (1.28‰). For birds, variation among tissues was significant (Q_B =17.77, *df*=3, *P*<0.01; Fig. 10). Muscle yielded the lowest estimates of enrichment (0.87‰). Estimates from similar tissues did not tend to rank in the same order for birds and mammals. For example, for birds, feathers and blood tended to yield higher estimates than liver and muscle, while for mammals liver tended to yield high estimates.

Discussion

The overall mean of the 134 estimates of Δ compiled in the present study was 2.54‰ (±0.11 SE) which is lower than the previous mean enrichment estimates reported in Minagawa and Wada (1984; 3.4±0.27‰ SE, *n*=16), Vander Zanden and Rasmussen (2001; 2.9±0.30‰ SE, *n*=35) and Post (2002; 3.4±0.13‰ SE, *n*=56). Our average Δ estimate was significantly lower than the estimates of Minagawa and Wada (1984) and Post (2002) (Welch's *t*-test, both *P*<0.01), but not the estimate of Vander Zanden and Rasmussen (2001; *P*>0.15). The weighting process that we used greatly reduced the variability around the estimate: however, variance ratio tests using unweighted data indicated that the variability in our dataset was significantly higher than those of Minagawa and Wada (1984) and Post (2002) (*F*-test, *P*<0.05), but not Vander Zanden and Rasmussen (2001; *P*>0.25). However, more importantly, we identified several sources of variation that yield important differences in δ^{15}N fractionation between a consumer and its diet.

Excretion

Our results show that there are large and consistent differences in enrichment according to the main biochemical form of nitrogen excretion—the first published quantitative evidence for this pattern. Comparisons of groups of organisms that excrete nitrogen in different forms yielded some of the largest differences in enrichment of any of the sources of variation that we tested; means ranged from –0.93‰ for aphids excreting amino acids and 1.09‰ for spiders excreting guanine to 3.11‰ for organisms excreting urea. These differences were generally preserved in orthogonal comparisons with trophic position (i.e. carnivores vs herbivores vs omnivores) and taxon (i.e. vertebrates vs invertebrates).

These differences might be due to the number of 'steps' involved in synthesis of different biochemical forms of nitrogenous waste products. When proteins are catabolized, ammonia (NH_4^+) is produced, but cannot be stored in the body because it is highly toxic (Rieutord 1999). Most aquatic animals continuously excrete ammonia as it is produced. Most terrestrial animals have developed a series of additional biochemical reactions that bind the $-NH_3$ group into either uric acid (a poorly soluble molecule that precipitates very easily) or urea (which is more soluble). Animals that, when embryos, eliminate nitrogenous waste via the maternal body (mammals) excrete mainly urea. Animals that develop in a closed egg (birds, insects) excrete mainly uric acid. The additional reactions to transform ammonia into urea or uric acid might involve further nitrogen fractionation. If there are differential rates of reactions for ^{15}N and ^{14}N at each step, the result should be that urea and uric acid have proportionally more ^{14}N than ammonia. All other things being equal we would then expect organisms excreting urea and uric acid to yield greater consumer-diet δ^{15}N enrichment (Ponsard and Averbuch 1999). Data with which this can be verified are scarce. Mammals excrete nitrogen mainly as urea, but a small amount of ammonia is produced as well, and in humans it is commonly observed that ammonia is isotopically 'heavier' than urea (Tom Preston, personal communication).

High nitrogen use efficiency (i.e. low nitrogen excretion compared with the amount ingested) may also contribute to low fractionation. High nitrogen use efficiency may be a genuine adaptation to feeding on extremely low-nitrogen foods (such as for aphids feeding on sap) or dead vegetation (the food of certain detritivores). Apparent nitrogen use efficiency may occur in organisms that store unexcreted nitrogen in special organs, as for example with spiders which store excreted nitrogen in the opistosoma. In both cases δ^{15}N enrichment is expected to be closer to zero than for species with lower nitrogen use efficiency, which is consistent with our observations. However, more data are needed to clarify the respective influence of nitrogen use efficiency and main form of nitrogenous waste in setting the value of ^{15}N fractionation, especially for species excreting guanine or amino acids which are poorly represented in the data we compiled.

Diet

We explored the possible influence of diet on the consumer-diet fractionation in several ways. First, we compared organisms that were fed on different diets. Classifications were made according to the diet that they were fed in the study, not according to their diet in natural conditions. We found no difference between organisms fed on animal matter, plant matter or manufactured mixtures. This result contrasts with that of Vander Zanden and Rasmussen (2001), who found δ^{15}N enrichment to be lower (and more variable) for herbivores, but is consistent with the result of Post (2002), who found no differences between carnivores and herbivores. We did however find that organisms consuming detritus yielded significantly lower estimates of △. It is difficult to think of any reason why consuming detritus should lead to lower enrichment. A higher nitrogen use efficiency might explain a low value of △ for detritivores feeding on decaying plant

parts. However, detritivores feeding mainly on dead animals are unlikely to show a higher nitrogen-use efficiency than, say, predators. It is possible that $\triangle$ was less accurately estimated in studies where the food consisted of detritus in which microorganisms were active during the experiment. Indeed, the activity of microorganisms may lead to a progressive shift in the isotopic composition of the bulk detritus. For instance, Ponsard and Amlou (1999) observed that the δ^{15}N of rotten *Drosophila* was significantly different from that of fresh *Drosophila*. Therefore, if the detritus δ^{15}N reported in the studies analysed here was measured before the food was offered to the consumer, or after the feeding had taken place, while microorganisms were continuously active, the measured value might not reflect accurately the δ^{15}N value of the food at the time when it was ingested, thus leading to inaccurate Δ estimates. However, as there is hardly any knowledge on ^{15}N fractionation by micro-organisms, interpreting the δ^{15}N enrichment patterns of detritivores and other animals whose diet consists of a large proportion of microorganisms remains difficult.

The second way we explored the influence of diet was through examining relationships between $\triangle$ and the %N or C:N of the diet. We found a weak positive relationship between δ^{15}N enrichment and the nutritional quality of the food as measured by the C:N ratio of the diet (but not as measured by the %N of the diet). Several authors have suggested that nutritional stress, influenced by the quality of the diet or by starvation, might affect δ^{15}N enrichment (Hobson et al. 1993; Fantle et al. 1999; Adams and Sterner 2000). However, the mechanisms causing these patterns, and even the direction of change, remain unclear. Some studies have found that enrichment may be greater under conditions of nutritional stress—that is, when growth rates are higher, δ^{15}N enrichment may be lower (Hobson and Clark 1992; Hobson et al. 1993; Adams and Sterner 2000). Others have found the opposite—lower δ^{15}N enrichment for organisms experiencing nutritional stress (Oelbermann and Scheu 2002), or no effect at all (Schmidt et al. 1999). The consequences of nitrogen stress on nitrogen metabolism and, more specifically, on nitrogen fractionation, may vary with the intrinsic nitrogen use efficiency of the species. Our results for C:N, but not those for %N, support the idea that better quality food (i.e. with lower C:N) tends to be related with lower δ^{15}N enrichment. It must be kept in mind that most of our data come from laboratory studies in which animals are a priori fed according to their needs and severe nutritional stresses were probably avoided. Nutritional stresses were (in most cases) probably mild, so thathere the risk of not detecting an effect is high.

Taxon

Taxonomically-similar organisms usually have similar physiological processes: we might therefore expect some patterns in δ^{15}N enrichment related to taxonomic identity. Indeed, we found some differences related to taxonomic identity, although conclusions are partly confounded by the use of different body components for invertebrates and vertebrates. Other researchers have noted that δ^{15}N enrichment may vary among species (e.g. DeNiro and Epstein 1981; Minagawa and Wada 1984; Hobson and Clark 1992), but this is the first quantitative evidence that differences may vary consistently among taxonomic classes. Differences may be partly due to the excretion mode of each class, because the two classes yielding the lowest enrichment (crustaceans and molluscs) both excrete ammonia. One consequence of this pattern is that, when using literature-derived estimates of $\triangle$ in ecological studies, those estimates should represent values of physiologically related and/or taxonomically related organisms.

Environment

A consequence of the pattern we found for lower estimates of $\triangle$ in ammonotelic organisms is that organisms from aquatic environments should tend to show slightly smaller δ^{15}N enrichment than terrestrial organisms, because most fully-aquatic organisms excrete mainly ammonia. Our analyses supported this prediction for organisms inhabiting marine environments, but not for organisms from freshwater environments. Schoeninger and DeNiro (1984) reported a slight difference between marine and terrestrial food chains, but in the other direction (enrichment tended to be higher in marine food chains). Our results might have been biased by the presence of detritivorous crustaceans and molluscs in the marine category, because they yielded low estimates of $\triangle$. If they were excluded from the analysis, estimates of $\triangle$ by marine organisms remained lower than estimates from terrestrial or freshwater organisms, but the differences were only marginally significant (P<0.1). Consequently, we have little confidence that our results reflect true differences between freshwater and marine food chains.

Tissue

The results also revealed consistent differences in δ^{15}N enrichment among tissues within both birds and mammals. Several researchers have found significant variation among tissues of vertebrates (e.g. DeNiro and Epstein 1981; Hobson and Clark 1992; Yoneyama et al. 1983; Hobson et al. 1996; Hilderbrand et al. 1996), albeit with contrasting results. Hobson et al. (1996) found that the blood of seals was less enriched than other tissues, while Hildebrand et al. (1996) found that only fat was significantly different from a range of other mammal tissues. In birds, Hobson and Clark (1992) found only muscle tissue significantly different from blood, liver, collagen and feathers.

Because certain metabolic properties characterizing various organs and tissues within the body are similar

across taxa (e.g. relative turnover rates, types of biochemical reactions, biochemical composition), we might expect some consistent differences in δ^{15}N enrichment. No such consistency could be seen in our data set: however, the data available for comparisons of tissues were limited, so we remain cautious about drawing conclusions.

Other sources of variation

Other sources of variation might have influenced the estimates of enrichment. One potential source of error is the time-lag before the δ^{15}N of a consumer's tissues have equilibrated following introduction to a new diet. Generally, on introduction to a new diet, the $\Delta_{\text{consumer-diet}}$ will change asymptotically until a new equilibrium value is reached. Potentially, the estimates of δ^{15}N enrichment for some studies may be overestimates or underestimates—a longer time period may have yielded a different Δ.

Another potential source of variation is the degree of success in controlling the animal's diets. We selected only studies of animals in captivity so that the diet would be known and controlled; however in some instances animals might have supplemented the diet provided from other sources (e.g. birds in zoological gardens might forage for arthropods in addition to their given diet), or microorganism activity might have changed the isotopic composition of detritus used as food (see above). This behaviour could lead to incorrect estimates of Δ.

A further, related source of variation is the possibility for selective feeding by animals from the diet they have been presented. This is possible, for example, for organisms feeding on detritus, carnivores feeding on parts of a carcass, or herbivores feeding only on parts of a plant. Because the δ^{15}N of the diet is a 'bulk' measure (that is, averaged over all nitrogen-containing molecules), it might not be a good measure of the specific components of the diet targeted by these 'fussy' eaters.

Uses of meta-data

The results of syntheses such as ours could be used to explore uncertainty in the outcomes of mixing-models and estimates of trophic positions. When possible, it will be better to use estimates of enrichment that are known to apply to the consumer-diet combination in question, because the outcomes of calculations can be very sensitive to changes in fractionation factors (Phillips and Koch 2002). Nevertheless, there are likely to be many situations in which the real enrichment is unknown or poorly known—the results given here may help researchers to explore outcomes and to allow calculation of estimates of variability. However, we emphasise that because the mechanisms leading to δ^{15}N enrichment are poorly known, and because food web studies often include a large variety of species, it is not wise practice to apply estimates yielded by syntheses such as ours without also incorporating estimates of uncertainty. Methods for doing so have been developed for mixing models (Phillips and Gregg 2001; Phillips and Koch 2002), calculations of trophic position (Vander Zanden and Rasmussen 2001) and tests of hypotheses about food webs (Ponsard and Arditi 2000; 2001).

When it is not possible or not appropriate to use empirically derived, species-specific, estimates, the most relevant set of estimates should be determined by the characteristics of the study. Sometimes, hypotheses are relevant to one species or a group of taxonomically related species. Other studies will involve a broad range of taxonomically dissimilar species. In the former case, we suggest that the main criteria for compiling estimates be the biochemical form of nitrogenous waste excretion, taxonomic class (providing species within the class excrete nitrogenous wastes in the same form, which will usually, but not always, be true), and estimates relevant to the parts of the body used. Our results suggest that environment and diet (except possibly detritivory) are comparatively minor influences. In the latter case, a more complete set of estimates incorporating all the relevant taxa should be compiled.

Fruitful avenues for future research

The variability surrounding estimates of $\triangle$ will consist of systematic variation due to physiological processes, and of more stochastic variation due to, for example, measurement errors. We have attempted to identify some of the systematic sources of variation. However, the results presented here should be considered exploratory: meta-analyses cannot replace well-designed factorial experiments or larger amounts of data, but can help identify where they are most needed. We suggest more data is needed for highly variable groups (e.g. crustaceans, molluscs) to help identify whether there are important sources of variation within those groups. Further, more data is needed for taxa with 'rare' biochemical forms of nitrogen excretion (e.g. spiders excreting guanine, aphids excreting amino acids, elasmobranchs excreting allantoin) in order to more completely determine the effect of different modes of nitrogen excretion on δ^{15}N enrichment. Experiments are also needed to help clarify the impact of fasting, water stress and nitrogen use efficiency on δ^{15}N fractionation. Further sources of variation may be identified as more data become available, but the process could be further accelerated by developing and testing hypotheses about why some groups appear to differ from others.

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