

Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: Implications for aquatic food web studies

Abstract—Use of stable isotope techniques to quantify food web relationships requires a priori estimates of the enrichment or depletion in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between prey and predator (known as trophic fractionation; hereafter $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$). We conducted a broad-scale analysis of $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ from aquatic systems, including three new field estimates. Carnivores had significantly higher $\Delta\delta^{15}\text{N}$ values than herbivores. Furthermore, carnivores, invertebrates, and lab-derived estimates were significantly more variable than their counterparts (*f*-test, $p < 0.00001$). $\Delta\delta^{13}\text{C}$ was higher for carnivores than for herbivores ($p = 0.001$), while variances did not differ significantly. Excluding herbivores, the average $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ were 3.4‰ and 0.8‰, respectively. But even with unbiased fractionation estimates, there is variation in isotopic fractionation that contributes to error in quantitative isotope model outputs. We simulated the error variance in $\delta^{15}\text{N}$ -based estimates of trophic position and two-source $\delta^{13}\text{C}$ diet mixing models, explicitly considering the observed variation in $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$, along with the other potential error sources. The resultant error in trophic position and mixing model outputs was generally minor, provided that primary consumers were used as baseline indicators for estimating trophic position and that end member $\delta^{13}\text{C}$ values in dietary mixing models were sufficiently distinct.

Measurement of carbon ($^{13}\text{C}/^{12}\text{C}$; $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$; $\delta^{15}\text{N}$) stable isotope ratios has enhanced our understanding of food webs and energy flow in aquatic ecosystems. Nitrogen isotope ratios become enriched at successive trophic levels, thereby allowing estimates of consumer trophic position (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999). Stable carbon isotopes can indicate feeding and carbon flow pathways because there is little fractionation from prey to predator, and different energy sources can have distinct $\delta^{13}\text{C}$ values (Hecky and Hesslein 1995).

Traditionally, food web studies using stable isotopes have provided qualitative depictions of trophic structure of single systems. Yet major advances in the use of stable isotopes have involved *quantitative* estimates of food web parameters, allowing examination of food web patterns and treatment of applied problems. Such quantitative applications require that the investigator assume the behavior of the isotopic tracer, in particular, the change in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values from prey to predator (hereafter called trophic fractionation; $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$). Provided $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ are known, variables such as omnivory, trophic position, food chain length, and energy flows and sources can be calculated. It is often assumed that the $\delta^{15}\text{N}$ value of a consumer is enriched by 3.4‰ over that of its diet based on the results of Minagawa and Wada (1984). This average $\Delta\delta^{15}\text{N}$ value was, in fact, the mean of 16 individual estimates with a standard deviation (hereafter 1 SD) of 1.1‰, although a

close examination of this paper reveals that consumer diets were poorly characterized. Similarly, the common assumption that $\delta^{13}\text{C}$ exhibits little or no trophic enrichment is based on early studies (Fry and Sherr 1984; Peterson and Fry 1987). For both ^{15}N and ^{13}C , fractionation estimates have sometimes deviated from these values (Pinnegar and Polunin 1999). No studies have explicitly examined variation in trophic fractionation and the resultant error in quantitative stable isotope model outputs.

In this study we address a few fundamental questions relevant to the application of stable isotopes to quantitative food web studies: Do the mean and variance in trophic fractionation vary systematically with taxon, habitat, or food type? How much error does the observed variation in trophic fractionation introduce into isotope-based trophic position estimates and $\delta^{13}\text{C}$ mixing model outputs? To address these questions, we synthesize field and laboratory $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ estimates from aquatic systems and examine patterns of variation in isotopic fractionation. Using our estimates of variation in trophic fractionation and the known error in the other variables used in these models, we quantify the resultant error in $\delta^{15}\text{N}$ -based trophic position estimates and $\delta^{13}\text{C}$ mixing model outputs.

Field estimates of trophic fractionation—During detailed dietary and isotopic studies of more than 20 lakes in Ontario and Quebec lakes, we identified three lakes (Lake Memphremagog [central basin], Lake Opeongo, and Victoria Lake) that could be used to provide field estimates of $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ because (1) the top predator (lake trout, *Salvelinus namaycush*) was known from gut contents to feed exclusively (>95% by volume) on a single prey species (either cisco [*Coregonus artedii*] or rainbow smelt [*Osmerus mordax*]), and (2) both predator and prey were relatively large and long lived, thereby minimizing temporal isotopic variation. Adult lake trout, cisco, and rainbow smelt were collected during the summer of 1995 and prepared for stable isotope analysis as described elsewhere (Vander Zanden et al. 1999a,b, 2000). Stable isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PDB limestone for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$); $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1000$, where $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The standard error of the mean for replicates of the same tissue was 0.13‰ for $\delta^{13}\text{C}$ and 0.15‰ for $\delta^{15}\text{N}$.

The mean $\delta^{15}\text{N}$ of lake trout varied by more than 6‰ among lakes (Table 1). Enrichment in $\delta^{15}\text{N}$ from prey to predator ($\Delta\delta^{15}\text{N}$) was relatively consistent across lakes, with an average of $3.49\text{‰} \pm 0.23$ ($\Delta\delta^{15}\text{N} \pm 1$ SD). Trophic fractionation for $\delta^{13}\text{C}$ averaged $0.05\text{‰} \pm 0.63$. ANOVA comparing $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of lake trout and their prey

Table 1. Trophic fractionation for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for adult lake trout from three lakes in which lake trout feed on a single prey species (>95% by volume). Values are means \pm 1 SE.

Lake	$\delta^{15}\text{N}$		$\Delta\delta^{15}\text{N}$	$\delta^{13}\text{C}$		$\Delta\delta^{13}\text{C}$
	(\pm 1 SE) lake trout	Prey		(\pm 1 SE) lake trout	Prey	
Opeongo	9.46 \pm 0.17	5.74 \pm 0.22*	3.72	-26.12 \pm 0.24	-26.14 \pm 0.31*	0.02
<i>N</i> =	24	8		23	7	
Memphremagog	16.13 \pm 0.11	12.86 \pm 0.33†	3.26	-29.31 \pm 0.24	-28.75 \pm 0.25†	-0.56
<i>N</i> =	19	8		19	8	
Victoria	9.83 \pm 0.17	6.34 \pm 0.13*	3.49	-27.31 \pm 0.25	-28.01 \pm 0.22*	0.70
<i>N</i> =	15	8		13	5	
Mean (\pm 1 SE)			3.49 \pm 0.13			0.05 \pm 0.36

* Prey item is cisco (*Coregonus artedii*).

† Prey item is rainbow smelt (*Osmerus mordax*).

indicated that fractionation did not differ significantly from 3.4‰ and 0‰.

Patterns of isotopic fractionation—A literature review produced 35 $\Delta\delta^{15}\text{N}$ estimates and 42 $\Delta\delta^{13}\text{C}$ estimates from 22 studies and 20 species (Web Appendix 1: http://www.aslo.org/lo/toc/vol_46/issue_8/2061al.pdf).

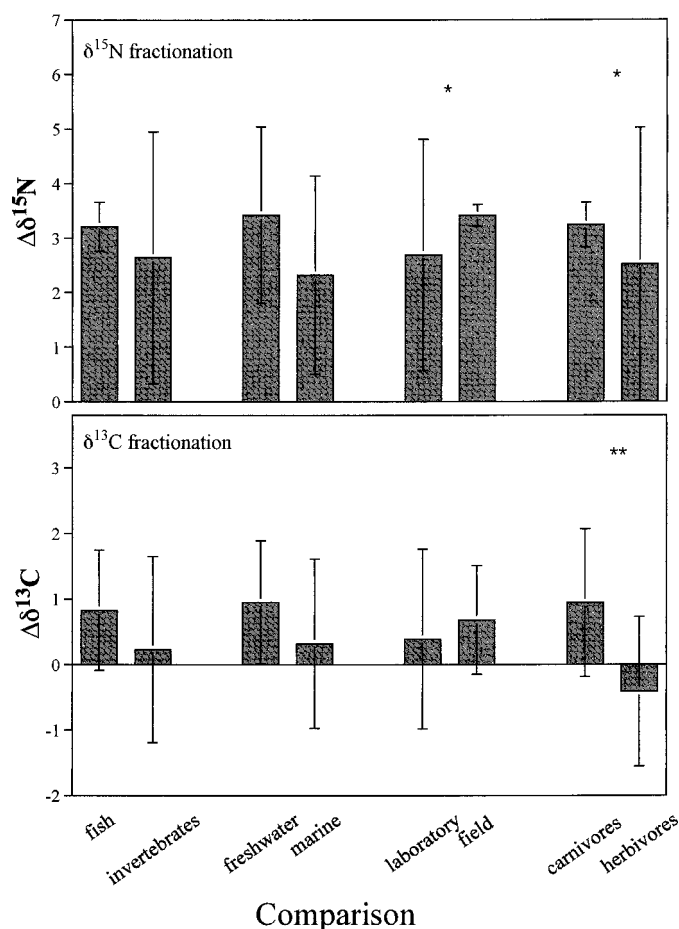


Fig. 1. Comparison of mean $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ (‰) (trophic fractionation; error bars represent 1 SD) values for taxon, habitat, estimate type, and diet. Significance based on Mann-Whitney *U* statistic for $\Delta\delta^{15}\text{N}$ and ANOVA for $\Delta\delta^{13}\text{C}$. One asterisk indicates mean difference significant at $p < 0.05$. Two asterisks indicate difference significant at $p < 0.01$.

org/lo/toc/vol_46/issue_8/2061al.pdf). Fractionation estimates were based on isotopic analysis of nonacid treated whole organisms for small invertebrates and larval fish, and white muscle tissue for larger consumers, corresponding with the suggestions of Pinnegar and Polunin (1999). When a single study provided multiple fractionation estimates, estimates were pooled if the same food type was fed to the same consumer species. Estimates were not included if consumer diets were not specifically known (Minagawa and Wada 1984) or if the duration of laboratory experiments was not sufficient to allow for isotopic equilibrium between predator and prey. A few studies estimated the average fractionation across an entire food chain; these estimates are considered separately as whole food chain estimates. Analysis of variance ($\Delta\delta^{13}\text{C}$) and two-tailed Mann-Whitney tests ($\Delta\delta^{15}\text{N}$) were used to examine differences in trophic fractionation as a function of taxon (fish vs. invertebrates), habitat (freshwater vs. marine), estimate type (laboratory vs. field), and diet (herbivore vs. carnivore). Mixed model ANOVA was used to further examine random species effects. Two-tailed *F*-tests were used to test differences between population variances.

$\Delta\delta^{15}\text{N}$ values ranged from -0.7‰ to 9.2‰ , with an overall mean (\pm 1 SD) of $2.92\text{‰} \pm 1.78\text{‰}$. Field $\Delta\delta^{15}\text{N}$ estimates were significantly higher than estimates from the laboratory (3.41 ± 0.20 vs. 2.69 ± 2.11), and estimates for carnivores were significantly higher than for herbivores (3.23 ± 0.41 vs. 2.52 ± 2.5 ; Mann-Whitney; $p < 0.05$; Fig. 1). Mixed model ANOVA indicated a significant random species effect ($p < 0.0001$). Invertebrates were significantly more variable than fish, laboratory estimates were more variable than field estimates, and herbivores were more variable than carnivores (*F*-test; all comparisons significant at $p < 0.00001$). Some of the significant differences in means and variance were driven by unbalanced data and the tremendous variation in $\Delta\delta^{15}\text{N}$ among herbivores, which were almost exclusively laboratory-reared invertebrates. The elevated variance for herbivores can be seen by comparing frequency histograms for herbivores, carnivores, and whole food chains (Fig. 2).

$\Delta\delta^{13}\text{C}$ values ranged from -2.1‰ to $+2.8\text{‰}$, with an overall mean (\pm 1 SD) of $0.47\text{‰} \pm 1.23\text{‰}$. There were no significant differences in $\Delta\delta^{13}\text{C}$ based on habitat, taxon, or estimate type, although herbivores exhibited significantly lower fractionation than nonherbivores (mean \pm 1 SD;

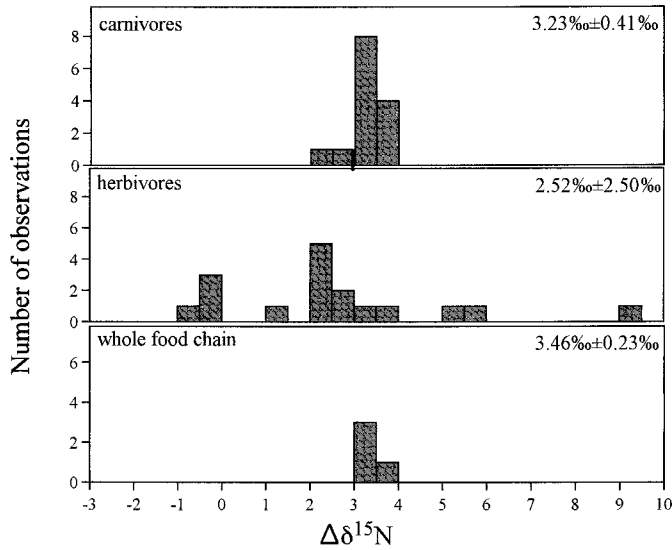


Fig. 2. Frequency distributions of nitrogen fractionation ($\Delta\delta^{15}\text{N}$) values for carnivores, herbivores, and whole food chains, based on a literature survey of fractionation estimates. Values in upper right corner are means ± 1 SD.

$-0.41\text{‰} \pm 1.14$ vs. $0.91\text{‰} \pm 1.04$; ANOVA; $p = 0.003$; Fig. 1). As with $\Delta\delta^{15}\text{N}$, a random species variable was highly significant for $\Delta\delta^{13}\text{C}$ ($p = 0.0003$). There were no systematic effects of taxon, habitat, estimate type, or diet on $\Delta\delta^{13}\text{C}$ variances (F -tests, $p < 0.05$).

Estimating error in trophic position—One quantitative application of stable isotopes has been the estimation of trophic position of aquatic consumers. Trophic position is defined as a noninteger value that represents the energy-weighted number of trophic energy transfers leading to a consumer (Vander Zanden et al. 1997; Vander Zanden and Rasmussen 1999). Using primary consumers as the baseline trophic level, consumer trophic position can be calculated using the formula

$$\text{Trophic position}_{\text{consumer}} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})/3.4 + 2 \quad (1)$$

Note that estimating trophic position requires an a priori assumption of a trophic fractionation constant (typically 3.4‰ is assumed based on the seminal paper, Minagawa and Wada 1984). Because the variance in fractionation for an individual consumer-resource link is known, we can quantify how much error is introduced into trophic position estimates when we assume a constant $\Delta\delta^{15}\text{N}$ value. Furthermore, error associated with the other terms of the trophic position equation ($\delta^{15}\text{N}_{\text{consumer}}$ and $\delta^{15}\text{N}_{\text{baseline}}$) can also be estimated, allowing quantification of the overall error in stable isotope-based trophic position estimates.

Fractionation estimates presented thus far are based on a single prey-predator linkage, while real food webs consist of multiple trophic links. Because estimating trophic position uses a single fractionation value for an entire food web, a fractionation value derived from a single prey-predator linkage (mean ± 1 SD; $3.4\text{‰} \pm 0.4\text{‰}$ for carnivores and 2.5‰

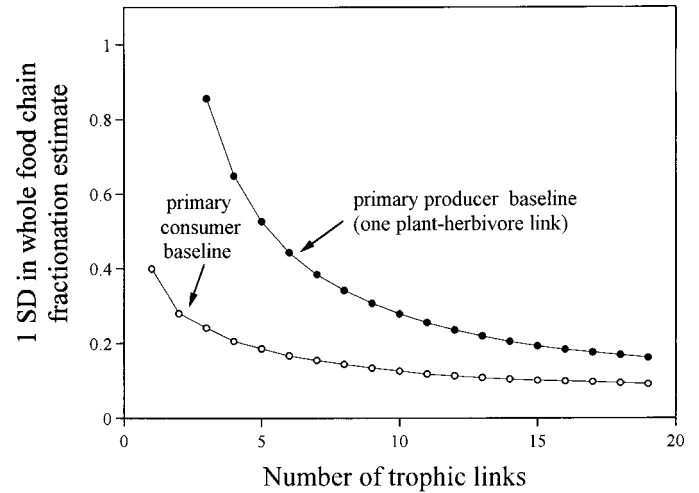


Fig. 3. Error variance (1 SD) in trophic fractionation decreases with increasing numbers of trophic links contributing to the whole food chain fractionation estimate. Filled circles are for a primary consumer baseline (no plant-herbivore links). Open circles are for a primary producer baseline (includes one plant-herbivore link).

$\pm 2.5\text{‰}$ for herbivores) cannot be used. Instead, the whole food chain fractionation can be calculated based on the number of trophic links leading to a given consumer. For example, assuming two carnivore links (mean ± 1 SD; $3.4\text{‰} \pm 0.4\text{‰}$), the sum of the two links is $6.8\text{‰} \pm 0.57\text{‰}$ (to sum variances, calculate the variance from the SD, $0.4^2 = 0.16$; multiply by two because there are two links, $0.16 \times 2 = 0.32$; calculate the pooled standard deviation, $\sqrt{0.32} = 0.57$), or $3.4\text{‰} \pm 0.28\text{‰}$ per trophic link. Note that the error variance (1 SD) declines as we average over an increasing number of links, reaching an asymptotic value of 0.1‰ if no herbivores are included (i.e., primary consumers are used as the baseline trophic level; Fig. 3). Adding a third, plant-herbivore link ($2.5\text{‰} \pm 2.5\text{‰}$) to our previous example produced a mean (± 1 SD) of $9.3\text{‰} \pm 2.55\text{‰}$ or $3.1\text{‰} \pm 0.85\text{‰}$ per link. Figure 3 indicates that inclusion of even a single plant-herbivore trophic link dramatically increased the error variance in whole food chain fractionation.

If we know the error variances of the other components of Eq. 1 we can combine them to estimate the error variance associated with trophic position estimates based on ^{15}N . We repeatedly generated values of trophic position from Eq. 1 by combining estimates of $\delta^{15}\text{N}_{\text{consumer}}$, $\delta^{15}\text{N}_{\text{baseline}}$, and the fractionation factor drawn from normal distributions with the appropriate error variances. The error variance for $\delta^{15}\text{N}_{\text{consumer}}$ was the mean standard error (SE) for lake trout populations from Table 1 (0.15‰). Note that our error simulations are performed at the level of the population, rather than the individual. Error at the level of the individual (i.e., analytical or measurement error) is negligible when considered at the population level. The error variance term for $\delta^{15}\text{N}_{\text{baseline}}$ was taken from Vander Zanden and Rasmussen (1999; 1 SE = 0.55‰ ; average sample size = 6). One standard error was used because lake-specific baseline estimates were based on an average of six measurements. Error variance estimates for fractionation are as described above; we assumed the food

Table 2. Estimated error variance (1 SD) in $\delta^{15}\text{N}$ and consumer trophic position as a result of the observed variation in trophic fractionation, $\delta^{15}\text{N}_{\text{consumer}}$, and $\delta^{15}\text{N}_{\text{baseline}}$.

Source of variation	Error variance in $\delta^{15}\text{N}$	Error variance in trophic position
Individual sources		
Fractionation; primary consumer baseline*	$\pm 0.18\%$	± 0.11
Fractionation; primary producer baseline†	$\pm 0.52\%$	± 0.34
$\delta^{15}\text{N}_{\text{consumer}}$	$\pm 0.15\%$ §	± 0.045
$\delta^{15}\text{N}_{\text{baseline}}$	$\pm 0.55\%$ §	± 0.16
Minagawa and Wada (1984)‡	$\pm 0.49\%$	± 0.33
Pooled sources		
$\delta^{15}\text{N}_{\text{baseline}}$ $\delta^{15}\text{N}_{\text{consumer}}$ fractionation (primary consumer baseline) ¹	—	± 0.20
$\delta^{15}\text{N}_{\text{baseline}}$ $\delta^{15}\text{N}_{\text{consumer}}$ fractionation (primary producer baseline) ²	—	± 0.37

* Assumes five carnivore links.

† Assumes five carnivore links and two herbivore links.

‡ Assumes five links, each with a mean ± 1 SD of $3.4\% \pm 1.1\%$.

§ One standard error (SE) is used since calculations are for populations rather than individuals.

web to be comprised of five trophic links, which is a conservative estimate of food web complexity.

The error variance in consumer trophic position (1 SD) was threefold greater when using primary producers (rather than primary consumers) as baseline indicator organisms. Variation in $\delta^{15}\text{N}_{\text{consumer}}$ contributed little to the error variance in consumer trophic position. $\delta^{15}\text{N}_{\text{baseline}}$ was a more important source of error in trophic position (Table 2). Considering all three sources of error and using a primary consumer baseline, the error variance (1 SD) associated with trophic position estimates was approximately 0.2 trophic levels.

Estimating error in $\delta^{13}\text{C}$ mixing models—As with $\delta^{15}\text{N}$, the error variance associated with use of $\delta^{13}\text{C}$ mixing models can be estimated using stochastic simulation. Two-source $\delta^{13}\text{C}$ mixing models are frequently used to quantify contributions of two potential food sources (prey *a* and prey *b*) to a predator population (pred):

$$\%_{\text{preya}} = (\delta^{13}\text{C}_{\text{pred}} - \delta^{13}\text{C}_{\text{preyb}}) / (\delta^{13}\text{C}_{\text{preya}} - \delta^{13}\text{C}_{\text{preyb}}) \times 100 \quad (2)$$

and

$$\%_{\text{preyb}} = 100 - \%_{\text{preya}} \quad (3)$$

Error variance for $\delta^{13}\text{C}$ trophic fractionation was estimated assuming two trophic links (since the model assumes a predator feeding on two prey items). One standard deviation for carnivore $\delta^{13}\text{C}$ trophic fractionation was 1.13‰, which produces a pooled SD of 1.6‰, or 0.8‰ per trophic link. The error variance associated with model end members ($\delta^{13}\text{C}$ prey *a*, $\delta^{13}\text{C}$ prey *b*) and $\delta^{13}\text{C}$ pred is 1 SE of the population mean (0.25‰; Table 1). Variation in $\delta^{13}\text{C}$ fractionation contributed the majority of the error variance to mixing model outputs (Fig. 4). Variance in predator and prey $\delta^{13}\text{C}$ values

were minor contributors to variance in mixing model outputs (Fig. 4). In both cases, the error variance declines as the absolute difference between the two isotopic endpoints increases.

Discussion—Stable isotopes have most often been used to provide qualitative summaries and diagrammatic representations of food webs. Quantitative uses of stable isotopes represents an important advance, allowing estimates of important and difficult-to-measure food web variables such as omnivory, trophic position, food chain length, and energy flow pathways (Cabana and Rasmussen 1996; Vander Zanden et al. 1999b). With a quantitative approach, stable isotope techniques can be used more effectively to address specific questions and problems, such as the modeling of contaminant bioaccumulation (Cabana and Rasmussen 1994), food web impacts of species invasions (Vander Zanden et al. 1999a), and the incorporation of sewage derived nitrogen into food webs (Van Dover et al. 1992). While quantitative use greatly broadens the applicability of this tool, it must also be realized that such applications require an explicit assumption of trophic fractionation. Even if the commonly assumed values of 3.4‰ and 0‰–1‰ approximate the mean fractionation, the variability in fractionation has not been considered, and it is not known how much error the observed variation imparts to quantitative stable isotope model outputs.

The analysis of whether trophic fractionation varies with habitat, taxa, estimate type, and diet was complicated by a relatively unbalanced study design. Note that of the 16 (i.e., 2⁴) potential combinations of these four variables (habitat, taxa, estimate type, and diet), $\Delta\delta^{15}\text{N}$ estimates were available for only eight of these, and three had a single observation. Nevertheless, differences between laboratory and field estimates and between carnivores and herbivores were statistically significant (Fig. 1). Furthermore, all carnivore estimates (except two experimental values from Pinnegar and Polunin 1999) had $\Delta\delta^{15}\text{N}$ values $>3.0\%$, while herbivores exhibited highly variable $\Delta\delta^{15}\text{N}$ values (Fig. 2).

With the exception of herbivores, +3.4‰ and +0.8‰ approximate the mean trophic fractionation for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ respectively. A more important issue is the variability associated with these mean values. For $\delta^{15}\text{N}$, the variance for herbivores was much greater than for carnivores. Numerical simulations indicated that the observed variance in fractionation at the plant–herbivore level produced substantial amounts of error in trophic position estimates, while carnivore $\Delta\delta^{15}\text{N}$ was much less variable and a relatively minor source of error to trophic position estimates (Table 2).

Simulations of $\delta^{13}\text{C}$ mixing models indicated that error in model output was most sensitive to the magnitude of the difference between the two isotopic end members. Thus, investigators should ensure that isotopic end members are distinct when using isotopic mixing models. Error variance resulting from the observed variation in fractionation could be an important source of error, depending on the spread between the isotopic end members. Error variance associated with isotopic end member and predator $\delta^{13}\text{C}$ signatures was a relatively minor source of error variance to mixing model outputs, al-

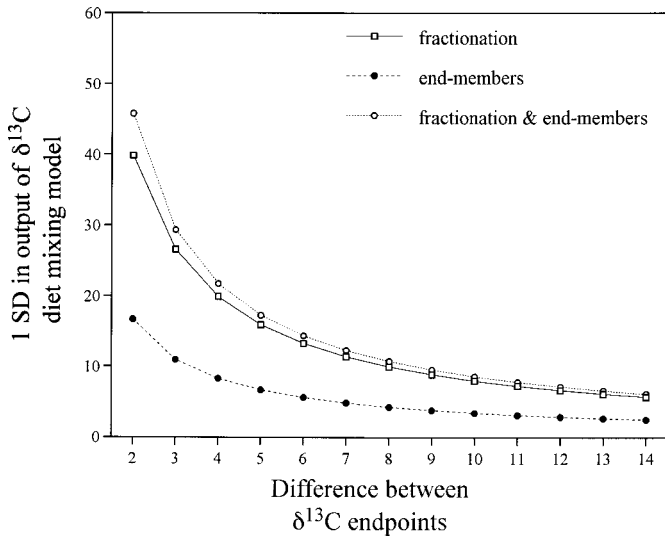


Fig. 4. Error variance (1 SD) in the $\delta^{13}\text{C}$ mixing model output decreases as the difference between the two $\delta^{13}\text{C}$ end members increases. Variation in $\delta^{13}\text{C}$ trophic fractionation is the major source of error, while error associated with the end member and consumer $\delta^{13}\text{C}$ values are relatively minor sources of error.

though this could be an important source of error if end members were more heterogeneous and variable (Fig. 4).

Patterns of ^{15}N fractionation—Among carnivores, $\Delta\delta^{15}\text{N}$ fell within a narrow range and was less variable than previously reported in the literature (DeNiro and Epstein 1981; Minagawa and Wada 1984; Owens 1987). In contrast, trophic $\delta^{15}\text{N}$ enrichment from plants to herbivores was highly variable. Trophic enrichment of $\delta^{15}\text{N}$ is generally attributed to fractionation during amino acid deamination and transamination, whereby ^{14}N amine groups are preferentially removed to produce isotopically light metabolites, leaving the remaining nitrogen pool enriched in ^{15}N (referred to as metabolic fractionation) (Macko et al. 1986; Gannes et al. 1997). Alternatively, fractionation could result from isotopic discrimination during nitrogen *assimilation* or isotopic differences between assimilated and unassimilated nitrogen pools (referred to as assimilative fractionation). Metabolic fractionation is expected to be the dominant process for carnivores because the animal nitrogen is more biochemically homogenous and dominated by proteins. As a result, we would expect assimilated and ingested material to be isotopically similar, and feces and tissues should have similar $\delta^{15}\text{N}$ values.

For herbivores, both assimilative and metabolic factors are likely to affect trophic fractionation. Aquatic plants can contain nitrogenous defense compounds that are not readily assimilated by herbivores (Van Donk 1997), and isotopic differences among biochemical fractions of the plant nitrogen pool, coupled with highly variable nitrogen contents and assimilation efficiencies, create the potential for herbivore $\Delta\delta^{15}\text{N}$ to be highly variable. Isotopic mass balance studies comparing isotopic signatures of food, fecal material, metabolites, and body tissues would discern whether variability in fractionation for herbivores results from metabolic or assimilative processes.

Adams and Sterner (2000) found that the $\Delta\delta^{15}\text{N}$ of herbivorous *Daphnia magna* was highly variable and decreased with increasing algal N content. Similarly, starving animals exhibit elevated $\delta^{15}\text{N}$ enrichment (Hobson et al. 1993), presumably because animals catabolize their own bodily proteins, producing isotopic enrichment analogous to that for ingested food (Gannes et al. 1998). Nitrogen limitation and starvation appear to have similar isotopic consequences, and the low (and variable) nitrogen content of plants may contribute to the wide variation in ^{15}N fractionation. Conversely, under normal conditions, carnivores should exhibit relatively consistent fractionation due to the high nitrogen content of their food, although starvation, migration, and other prolonged metabolic expenditures are likely to affect isotopic interpretations (Doucett et al. 1999).

There are other mechanisms by which nitrogen-limited herbivores might exhibit variable fractionation. Urea recycling, whereby urea is made available for synthesis of non-essential amino acids, occurs among many herbivores as a mechanism for nitrogen conservation (Gannes et al. 1998). The dynamics of individual amino acids may be another factor, since individual amino acids exhibit characteristic $\delta^{15}\text{N}$ signatures, spanning 9‰ within an individual tissue. While essential amino acid should differ little from the dietary source, synthesis of nonessential amino acids may involve isotopic fractionation (Gaebler et al. 1966).

Patterns of ^{13}C fractionation—Previous laboratory studies of trophic fractionation typically reported little change or slight enrichment in $\delta^{13}\text{C}$ from prey to predator (Fry and Sherr 1984; Peterson and Fry 1987). We report an overall mean of value $+0.47\text{‰}$, with $\Delta\delta^{13}\text{C}$ values being lower for herbivores than for carnivores (Fig. 2). Efforts to understand the difference between carnivores and herbivores must consider that major biochemical components (lipids, proteins, carbohydrates) differ in $\delta^{13}\text{C}$ signatures (DeNiro and Epstein 1978; Focken and Becker 1998), and that consumers assimilate dietary components with varying efficiencies. In particular, lipids are depleted in ^{13}C due to fractionation occurring during lipid synthesis (DeNiro and Epstein 1978). Because plants have low lipid content, herbivores may be more likely than carnivores to synthesize lipids from dietary carbohydrates, thus producing lower $\Delta\delta^{13}\text{C}$ values. Another factor is isotopic routing, whereby animals allocate dietary elements differentially to specific tissues (Schwarcz 1991; Gannes et al. 1997). The isotopic composition of a consumer's tissue should reflect that of the biochemical fraction used to build that particular tissue, rather than that of the bulk diet. For example, animals fed high-protein diets reflected the $\delta^{13}\text{C}$ of dietary proteins, while animals fed low-protein diets reflected the $\delta^{13}\text{C}$ of bulk diet, presumably because amino acids were synthesized from nonprotein dietary components (Ambrose and Norr 1993). If dietary proteins are enriched in $\delta^{13}\text{C}$ relative to bulk diet, the origin of amino acid carbon skeletons might explain $\delta^{13}\text{C}$ differences between herbivores and carnivores.

In summary, while quantitative uses of stable isotopes greatly extend the potential applications to studies of aquatic food web processes, the issue of error associated with model outputs has not been addressed. Is the *variability* in trophic

fractionation significant? And what are the *implications* of the described variance for quantitative stable isotope models? Incorporating error into quantitative stable isotope models is critical to their proper interpretation, and that variability should be routinely considered in quantitative stable isotope analyses. Simulations presented here suggest that error in model output tends to be minor, provided that primary consumers are used as the baseline trophic level and that mixing model end members are sufficiently distinct.

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