KNAUER, G. A., D. G. REDALJE, W. G. HARRISON, AND D. M. KARL. 1990. New production at the VERTEX time-series site. Deep-Sea Res. **37:** 1121–1134.

KÖRZINGER, A., D. E. SCHULZ-BULL, G. PETRICK, AND J. C. DUINKER. 1994. Evidence for dissolution of fatty acids in sediment traps: Impact on flux estimates. J. Geophys. Res. **99:** 3407–3415.

KREMLING, K., U. LENTZ, B. ZEITZSCHEL, D. E. SCHULTZ-BULL, AND J. C. DUINKER. 1996. New type of time-series sediment trap for the reliable collection of inorganic and organic trace chemical substances. Rev. Sci. Instrum. **67:** 4360–4363.

LEE, C., J. I. HEDGES, S. G. WAKEHAM, AND N. ZHOU. 1992. Effectiveness of various treatments in retarding microbial activity in sediment trap material and their effects on the collection of swimmers. Limnol. Oceanogr. **37:** 117–130.

———, S. G. WAKEHAM, AND J. I. HEDGES. 1988. The measurement of oceanic particle flux—are ''swimmers'' a problem? Oceanography **1:** 34–36.

MARTIN, J. H., G. A. KNAUER, D. M. KARL, AND W. W. BROENKOW. 1987. VERTEX: Carbon cycling in the northeast Pacific. Deep-Sea Res. **34:** 267–286.

NOJI, T. T., K. Y. BØRSHEIM, F. REY, AND R. NORTVEDT. 1999. Dissolved organic carbon associated with sinking particles can be crucial for estimates of vertical carbon flux. Sarsia **84:** 129–135.

SAMBROTTO, R. N., AND OTHERS. 1993. Elevated consumption of carbon relative to nitrogen in the surface ocean. Nature **363:** 248–250.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry, 3rd ed. W. H. Freeman.

SUESS, E. 1980. Particulate organic carbon flux in the oceans—surface productivity and oxygen utilization. Nature **288:** 260–263.

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Stable isotope values of lotic invertebrates: Sources of variation, experimental design, and statistical interpretation

Abstract—In a subset of a stream food web, whole-body isotope values of $\delta^{13}C$ and $\delta^{15}N$ were measured for eight populations of lotic invertebrates. Observed isotopic differences among species corresponded broadly to their trophic status, as also revealed by gut content analysis, but with some exceptions. Species within a guild of grazer/scraper mayflies differed significantly in $\delta^{13}C$; a predatory caddisfly (*Rhyacophila dorsalis*) and a collector/gatherer stonefly (*Leuctra inermis*) had statistically indistinguishable values of $\delta^{13}C$ and $\delta^{15}N$. The variation associated with the mean isotope value of each population was partitioned into the variation among individuals and the variation that arises from analysis by isotope ratio mass spectrometry. For some taxa, within-population variance was lower than or equal to the variance attributable to the measurement error of the mass spectrometer. The highest but conservative estimate of within-population variation was a mean coefficient of variation of 11% for $\delta^{15}N$ in a predator, *R. dorsalis.* The minimum detectable difference between two populations was negatively associated with the number of replicate samples and the number of individual animals combined in each replicate. The optimum number of replicate samples, therefore, varies depending on the hypotheses of interest.

Stable isotope analysis (SIA) has become an important technique for examining trophic interactions and elucidating energy flow pathways through food webs and ecosystems. In freshwater systems, the $^{13}C:^{12}C$ ratio ($\delta^{13}C$) can indicate the relative importance of autochthonous versus allochthonous sources of carbon (e.g., Jones et al. 1998). The dissolved inorganic carbon pool exploited by freshwater photosynthetic organisms is typically more depleted in ^{13}C than in atmospheric CO_2, which is the carbon source of terrestrial plants. Thus, the isotope value of allochthonous material in freshwater systems is usually more similar to that of terrestrial plants than to that of aquatic algae. Fractionation of isotopes along biochemical pathways can result in isotopic enrichment in animal tissues relative to their food source and thus indicate trophic status (e.g., Doucett et al. 1996). Nitrogen isotopes, $^{15}N:^{14}N$ ($\delta^{15}N$), are especially useful indicators of trophic status, because the loss of isotopically lighter ^{14}N via excretion results in a 3–5‰ enrichment in $\delta^{15}N$ of consumers relative to their food (Peterson and Fry 1987). In studies of freshwater food webs, SIA has often been used to characterize the mean stable isotope enrichment of different populations or taxa in a particular system at a particular time. Hitherto, the interpretation of stable isotope values in multispecies food webs has been based predominantly on verbal descriptions of isotope biplots; statistical analyses for hypothesis testing are less common. Further, the focus has been largely on the mean isotopic value of different populations, and less attention has been given to the ecological significance of within-population variation in isotope value. In part, this may relate to the large number of samples (many species and many replicates) required for food web studies and to the financial cost of SIA. Nevertheless, numerical analyses are required in order to exploit this valuable tool to its full potential. The success of such analyses will be restricted, in part by error and variation in the data, so the sources and magnitude of variation need to be identified and careful consideration given to the number of replicates required to test hypotheses.

There are two main sources of variation associated with estimates of the mean isotope value of a population: (1) variation among individuals within the population, and (2) variation that arises from analysis by isotope ratio mass spec-

Table 1. List of species used for stable isotope analysis, their mean (SD) body length and head capsule width ($n = 10$ for all measurements). Items in gut contents based on examination of 10 individuals. Numbers after food item codes indicate how many individuals had each food item in their guts. Codes for food items: B, *Baetis*; TC, cased caddis; TP, Polycentropodidae; P, Plecoptera; C, Chironomidae; S, Simuliidae; LF, leaf fragments; FD, fine detritus; FA, filamentous algae; PY, Periphyton (including diatoms, desmids, chrysophytes, etc.).

Species	Length (cm)	Head capsule width (mm)	Feeding habit	Items in gut contents
Gammarus pulex	0.78 (0.09)		detritivore	C × 1, LF × 10, FD × 10, FA × 7, PY × 10
Baetis rhodani	0.86 (0.08)	1.2 (0.10)	scraper/collector	LF × 1, FD × 10, FA × 10, PY × 10
Ecdyonurus torrentis	1.28 (0.20)	4.5 (0.55)	scraper/collector	LF × 10, FD × 10, FA × 10, PY × 10
Rhithrogena semicolorata	0.92 (0.13)	2.5 (0.16)	scraper/collector	LF × 5, FD × 10, FA × 10, PY × 10
Leuctra inermis	0.71 (0.07)	0.92 (0.05)	collector	LF × 10, FD × 10, FA × 10, PY × 10
Large *Dinocras cephalotes*	2.78 (0.21)	5.5 (0.76)	predator	B × 4, TC × 1, C × 1
Medium *D. cephalotes*	1.75 (0.25)	4.0 (0.32)	predator	B × 3, C × 2
Rhyacophila dorsalis		1.2 (0.10)	predator	TP × 1, P × 1, C × 2, S × 6

trometry (IRMS) (assuming no error in sample preparation prior to SIA). Variation also may occur among tissues of the same organism (DeNiro and Epstein 1978, 1981). In studies of invertebrates, samples are usually prepared from whole organisms (Ponsard and Arditi 2000), because the organisms are simply too small for a specific tissue to be isolated. Thus, the focus of this paper is on variation among whole individuals, and any tissue-dependent differences in the isotope value of whole individuals will be manifest as within-population variance (*see* further discussion below). Advances in IRMS can result in high precision, e.g., for $\delta^{13}C$ as low as $\pm 0.1‰$ for samples 1 mg in size (e.g., Marra et al. 1998). The ability to analyze small samples makes it possible to measure stable isotope values of individual lotic invertebrates, instead of combining several individuals to achieve sufficient mass. Analyses of individuals are essential to quantify within-population variance, but there may be a concomitant increase in the number of samples required to estimate the population mean with an acceptable level of error.

How much variation associated with population estimates of isotope ratios is acceptable? That depends on the hypotheses being tested. Ideally, the acceptable level of variation should be determined a priori to any hypothesis testing, although this is rarely done. For example, establishing the trophic status of different taxa may require detecting $\leq 3‰$ differences in $\delta^{15}N$, the often-cited enrichment between consumer and resource (Peterson and Fry 1987). If, however, some taxa are omnivorous (i.e., feed on more than one trophic level) (Kling et al. 1992) or if the objective is to test for ontogenetic changes in diet that may be reflected in isotope values (Yoshioko et al. 1994; Hentschel 1998; Branstrator et al. 2000), then the power to detect smaller differences may be required. Further, attempts to quantify the relative contribution of different food sources to the diet of a consumer using mixing models may be sensitive to the degree of variation associated with the estimated mean isotope value of every species or energy source in the food web.

In a subset of a stream food web, we measured whole-body isotope values of $\delta^{13}C$ and $\delta^{15}N$ for eight populations of lotic invertebrate and characterized their diet by gut content analysis. Our objective was to examine how the number of sample replicates influences the relative magnitude of the variation around the population mean that is attributable to interindividual differences and that attributable to the reproducibility of measurements by IRMS. Further, we wanted to quantify the minimum detectable difference among pairs of species and to examine how our ability to detect statistically significant differences among species changes with replication and with the number of individual animals combined in each replicate. Ultimately, this will provide guidance on how to make the most efficient use of resources in future SIA studies of trophic interactions.

Methods and materials—Selected species of benthic invertebrates (Table 1), representing a subset of the total food web, were collected from the headwaters of Whiteadder Water, southeast Scotland (55°53′36″N, 2°35′31″W), on 5 May 1999. Species were chosen to encompass a range of feeding habits yet were chosen to include different taxa with similar feeding habits (e.g., Ephemeroptera). Within each species, we took care to collect individuals of the same size (i.e., the same developmental stage) to minimize variance associated with ontogenetic changes in isotope value. For one species, *Dinocras cephalotes*, we sampled two different age classes in order to examine this possibility of ontogenetic variation directly. Specimens were all late instar larvae or nymphs; mayfly and stonefly nymphs did not have black wing buds, which indicate imminent emergence and animals that are unlikely to feed, i.e., guts empty of any food items. Specimens were frozen immediately and stored at −22°C. Later, specimens were thawed, cleaned of any attached detritus, and measured, and guts were removed by dissection. Gut contents of 10 individuals of each species were mounted in glycerol and viewed at a minimum of ×100 magnification on a phase-contrast microscope, and the presence/absence of potential food items was recorded. The remaining body parts were oven dried (40°C for 24 h) and homogenized with a mortar and pestle for SIA. One individual was used per sample replicate, with the exception of *Leuctra inermis*, where several individuals were pooled to achieve sufficient biomass for SIA.

SIA: Approximately 1 mg of tissue from each sample replicate was loaded into a 4- × 6-mm tin capsule and combusted in a Carlo Erba C/N/S analyzer (Thermoquest) interfaced with a Finnigan Tracer Matt continuous flow isotope ratio mass spectrometer (CF-IRMS). All stable isotope ratios

are reported in permil (‰) using the δ notation according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1{,}000$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio $^{13}C:{}^{12}C$ or $^{15}N:{}^{14}N$. $R_{standard}$ for $\delta^{13}C$ is the Pee Dee Belemnite, and for $\delta^{15}N$, it is atmospheric nitrogen. The precision with which $\delta^{13}C$ and $\delta^{15}N$ can be measured is discussed below.

Data analysis: Computer randomizations were used to explore how the number of replicate samples in a sample set influences the perceived mean isotope value of a population, in terms of $\delta^{13}C$ and $\delta^{15}N$, and to quantify the relative magnitude of the variation around the population mean that is attributable to interindividual differences and to the measurement technique. Randomization procedures, designed specifically for this project, were programmed in THINK Pascal Version 4.0. Virtual sample replicates were selected at random from populations of a ''known'' or ''true'' mean and standard deviation for $\delta^{13}C$ and for $\delta^{15}N$—i.e., for each of the eight invertebrate species, the true mean and standard deviation were estimated from the maximum number, *n*, of sample replicates available for each species. During the randomization procedures, each virtual sample replicate (VR) in a virtual sample set (VS) was calculated as

$$VR = \bar{X}_{true} \pm (SD_{true} \times \text{deviate})$$

where a normally distributed deviate (mean = 0 and unit variance) was generated at random for each VR. The mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ of the VRs in a VS were calculated for each VS. After 1,000 such randomizations, we calculated the mean, the 5th and 95th percentile of the 1,000 VS means, and the standard deviations. Given that it is possible, albeit risky, to estimate 0.1 and 99.9 percentiles from 1,000 randomizations, we are confident that our estimates of 5th and 95th percentiles are reasonable. This procedure was carried out for levels of replication ranging from 2 to 15 VRs per VS. The variation attributable to the measurement technique was characterized by similar computer randomizations using data from internal laboratory standards (mix of urea and sucrose) of known $\delta^{13}C$ and $\delta^{15}N$ and with standard deviations estimated from 51 sample replicates of laboratory standards analyzed during this project (known mean ± 1 SD of the laboratory standards = -16.9 ± 0.25‰ for $\delta^{13}C$; -1.1 ± 0.35‰ for $\delta^{15}N$).

For each species and each level of replication, within-population variation was quantified as the coefficient of variation once the variation attributable to IRMS had been subtracted from the total sample variation, using the following formula:

$$CV = \frac{\sqrt{(SD^2_{VS\ animal} - SD^2_{VS\ standard})}}{\bar{X}_{true\ animal}} \times 100$$

(Note that virtual animal and virtual standard deviations are based on the same number of VRs per VS but that $\bar{X}$, the true mean of a species, is based on the maximum number of replicates available for each species.) Thus, we assume that the variances attributable to interindividual differences and to the measurement are additive and that the precision of the CF-IRMS (as indicated by the SDs of the standards) does not scale with the magnitude of $\delta^{13}C$ and $\delta^{15}N$. This provides a conservative estimate of within-population variation. Coefficients of variation were calculated for the average scenario (e.g., SDs based on the mean of 1,000 randomizations: mean SD_{animal} − mean $SD_{standard}$) and for two extremes (e.g., 5% SD_{animal} − 95% $SD_{standard}$ and 95% SD_{animal} − 5% $SD_{standard}$).

The minimum detectable difference between the means of two sample sets (*t*-test at $\alpha = 0.05$, $\beta = 0.10$) was calculated (Zar 1984) for different numbers of replicates, *r*, from $r = 1$ to *n*, for $\delta^{13}C$ and $\delta^{15}N$ of each species. Further, we explored how combining several individual animals in each replicate submitted for SIA influences the minimum detectable difference. In essence, each new replicate was the mean of *g* individuals chosen at random, without replacement, from the *n* samples. On average, such combinations reduce the population variance (based on one individual per replicate) by $1/g$, where g = number of individuals combined in each replicate.

Stable isotope values—Species separation was possible with either the carbon or nitrogen isotope value, or both (Fig. 1). Isotopic values of $\delta^{13}C$ and $\delta^{15}N$ were compared among species using multivariate analysis of variance (MANOVA; Wilks $\lambda = 0.0758$, $F_{14,138} = 24.4$, $P < 0.0001$; $\delta^{13}C$: $F_{7,70} = 34.1$, $P < 0.0001$; $\delta^{15}N$: $F_{7,70} = 17.5$, $P < 0.0001$), with a posteriori comparisons among species (Bonferroni tests, a conservative procedure in which the error rate is divided by the number of tests performed). The observed differences among species corresponded broadly to predictions regarding the trophic status of these taxa (Table 1), but SIA also provided some unexpected insights.

Nitrogen isotope value differentiated between some predators and prey. Predatory nymphs of the stonefly *D. cephalotes* were significantly more ^{15}N-enriched than all other species examined (Fig. 1B) and ~2‰ higher than the mayfly *Baetis rhodani,* which is one of its main prey (Table 1). Age or size-specific differences in isotopic enrichment have been observed in other aquatic invertebrates (Hentschel 1998; Branstrator et al. 2000), but no significant difference was apparent between medium and large-sized *D. cephalotes.* Although clearly predatory (Table 1), *Rhyacophila dorsalis* contained less ^{15}N than *D. cephalotes.* It is possible that different ^{15}N baselines exist between sources in the same site, e.g., between allochthonous and autochthonous sources, as is the case for ^{13}C. Consequently, carnivores in different food chains within the same food web may have $\delta^{15}N$ values that reflect different baseline resources more strongly than trophic position. For example, *D. cephalotes* prey mainly on baetid mayflies that graze algae, whereas *R. dorsalis* prey mainly on larval Simuliidae that filter feed on particles of diverse, but largely terrestrial, origin. Simuliidae were unavailable for SIA in this study, but it is difficult to hypothesize why they would be very ^{15}N-depleted relative to *B. rhodani* when detritivores in this system (e.g., *G. pulex*) are not. Indeed, Doucett et al. (1996, 1999*a*) recorded *Simulium* that appeared to be ^{15}N-enriched relative to other primary consumers including *Baetis.* Why *R. dorsalis* showed such

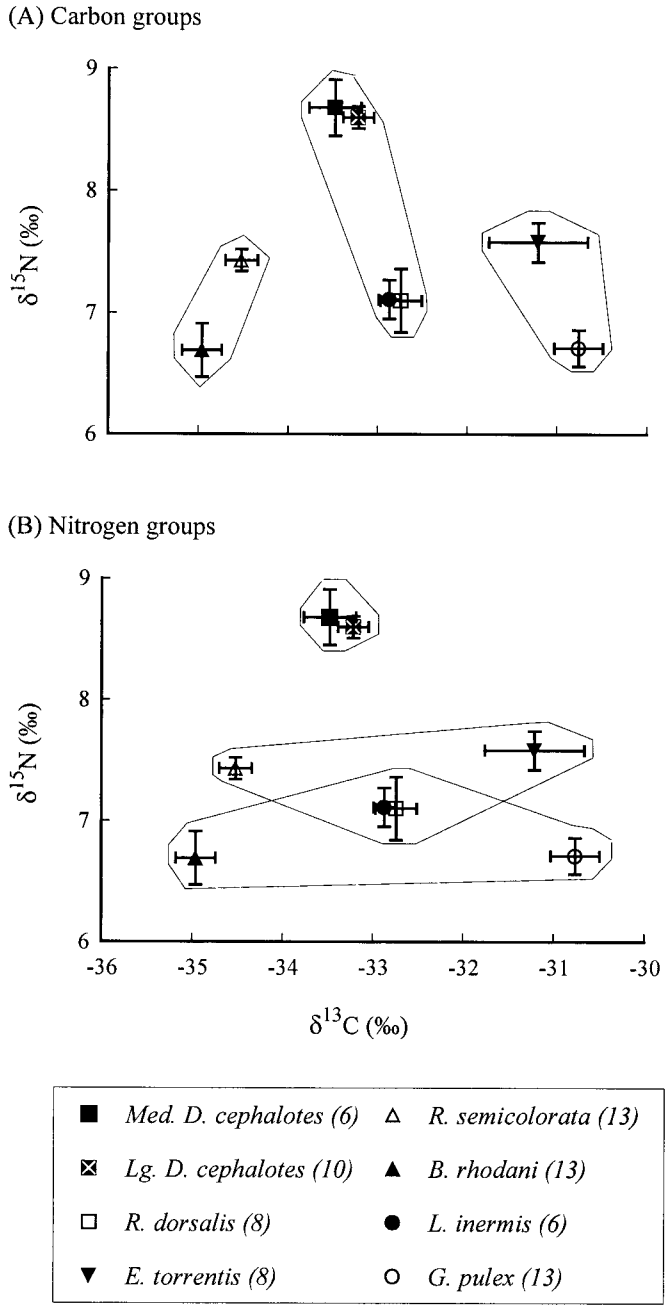

Fig. 1. Dual isotope biplots of mean (±1 SE) $\delta^{15}N$ vs $\delta^{13}C$ for invertebrate species in the Whiteadder Water. Square symbols represent predators, triangular symbols represent grazers/collectors, and circular symbols represent detritivores/collectors (*see also Table 1*). Numbers in the legend indicate the number of samples analyzed for each species. Polygons group species that are not significantly different (Bonferroni a posteriori tests; $P \leq 0.05$) with respect to (A) carbon, and (B) nitrogen.

little enrichment in the Whiteadder Water awaits further investigation.

The primary consumers (*Gammarus pulex*, *B. rhodani*, *Ecdyonurus torrentis*, *Rhithrogena semicolorata*, and *L. inermis*) had distinctly different isotopic values with respect to C and N (Fig. 1). This is surprising, given the high overlap in the diet (Table 1), although our quantification of gut contents is coarse. There are at least two possible explanations (not mutually exclusive) for this apparent mismatch between diet and isotope values.

First, species-specific differences in the fraction of the food uptake that is actually digested and assimilated could result in different isotope values. The species of primary consumer analyzed in this project appeared to be unselective in their food intake (*see* also Jones 1950), or the method of food selection also results in the ''passive'' consumption of many other potential food items. For example, *G. pulex* that consume shredded leaf fragments are likely to consume also any periphyton and fine detritus clinging to the leaf surface. Those species that were most depleted in ^{13}C are likely to digest a high proportion of autochthonous carbon (e.g., periphyton) from the total material consumed; less depleted species are likely to digest allochthonous sources of carbon (e.g., detritus of terrestrial origin) from the total material consumed. *G. pulex* sp. were the most ^{13}C-enriched, which is consistent with their strongly detritivorous habits (Graça et al. 1994), despite the presence of periphyton in the gut contents. That *E. torrentis* was more positive in ^{13}C than the two other mayflies (*B. rhodani* and *R. semicolorata*) was unexpected (Fig. 1A). Perhaps nymphs of *E. torrentis* digest less algae and more detritus than usually expected. Likewise, ^{15}N enrichment in *R. semicolorata* and *E. torrentis* compared with *B. rhodani* is surprising. Such differences may reflect species-specific isotopic fractionation between consumer and resource, but this is speculative in the absence of any empirical data on isotopic enrichment factors.

Second, species-specific differences in the proportional contribution of different compounds or tissue types (which may differ in isotopic value) to the total dry mass could produce different whole-body isotope values for different species with the same diet. For example, lipid synthesis discriminates against ^{13}C in favor of ^{12}C (DeNiro and Epstein 1977); thus, whole-body isotope values of species with high lipid loads may be more depleted in ^{13}C than species with low lipid loads. The lipid content of stream invertebrates varies among and within species (Meier et al. 2000), as does the proportional contribution of other tissues, such as cuticle that is ^{15}N-enriched, so this possibility cannot be ruled out. Some researchers advocate routine lipid extraction prior to SIA, especially of vertebrate tissues, whereas others are more cautious, because the procedure can increase variance and reduce statistical power (*see* discussions in Pinnegar and Polunin 1999; Ponsard and Arditi 2000). Alternatively, isotope values for whole organisms may be corrected or normalized for variable lipid loads among samples (McConnaughey and McRoy 1979). Extracting or normalizing lipids, however, will not alleviate tissue-dependent differences in the isotope value of whole organisms unless the proportional contribution of all other tissues is constant among individuals. Whether extraction or normalization of lipids, or any other tissue or compound, is appropriate for a particular study will be depend on the organisms, tissues, and hypothesis of interest.

From these results, it is clear that functional feeding groups may describe food intake and feeding behavior, but they do not necessarily correspond closely to food assimi-

lation, as indicated by SIA. Species from the same functional feeding group and with similar diets can have very different isotope values (e.g., *B. rhodani* and *E. torrentis*); species whose isotope values are statistically indistinguishable could have similar diets (e.g., large and medium *D. cephalotes*) or very different diets (e.g., *L. inermis* and *R. dorsalis*). It is common for researchers studying freshwater food webs (e.g., Doucett et al. 1996; Thorp et al. 1998) to analyze the stable isotopes of selected taxa that are perceived to be ''representative'' of particular functional feeding groups, presumably to limit financial and temporal investments. Such economies may yield erroneous results, however, when testing some hypotheses. Differences in the isotopic value of taxa within the same functional feeding group and in the same system have been described previously (e.g., Rounick and Hicks 1985; Junger and Planas 1994), but few isotope studies of freshwater food webs have considered how such differences might influence the conclusions.

Sources of variation—Some species are inherently more variable in their isotope value than others. The distance between the sample and standard lines in Fig. 2 reflects the within-population variation in isotope value for four representative species, encompassing the range of patterns in our data. An increase in sample replication decreases the range of possible sample means (Fig. 2), but the 5th and 95th percentiles eventually converge on asymptotes. The precision with which isotopic ratios can be measured, using either CF-IRMS or more conventional techniques, can vary (e.g., Marra et al. 1998; cf. Doucett et al. 1996, 1999*b;* this study) and will, in turn, influence the number of replicates needed before adequate convergence occurs. For the taxa analyzed in this study, convergence on an asymptote occurred at approximately seven replicates, so a meaningful discussion of sources of error should be restricted to the patterns evident at larger sample sizes. (Note that the number of replicates needed for convergence will vary among data sets and must be calculated for each study.) For some elements and some taxa, such as δ^{13}C in *E. torrentis,* within-population variation was apparent, over and above the variation attributable to the IRMS. For others, such as δ^{15}N in *D. cephalotes,* there was very little within-population variation. These patterns are clearer in Fig. 3, where the variation attributable to the CF-IRMS has been removed, and within-population variation is quantified as a coefficient of variation. In our set of species, *E. torrentis* had the most variable δ^{13}C value (CV = 5%), and *R. dorsalis* and *B. rhodani* had the most variable δ^{15}N value (CV = 11 and 9%, respectively). Large *D. cephalotes* were isotopically most homogenous, i.e., the coefficient of variation was ~1.5% for δ^{13}C and 0% for δ^{15}N. Where within-population variance is detectable (i.e., over and above the variance attributable to the measurement technique), high replication may be desirable in order to quantify that variation. Where within-population variance is very low (within the variance attributable to IRMS), relatively few replicate samples may be adequate to characterize the population mean, the exact number being determined by the required statistical power.

Why do conspecific individuals differ in isotope value in some situations? Samples from a population that includes

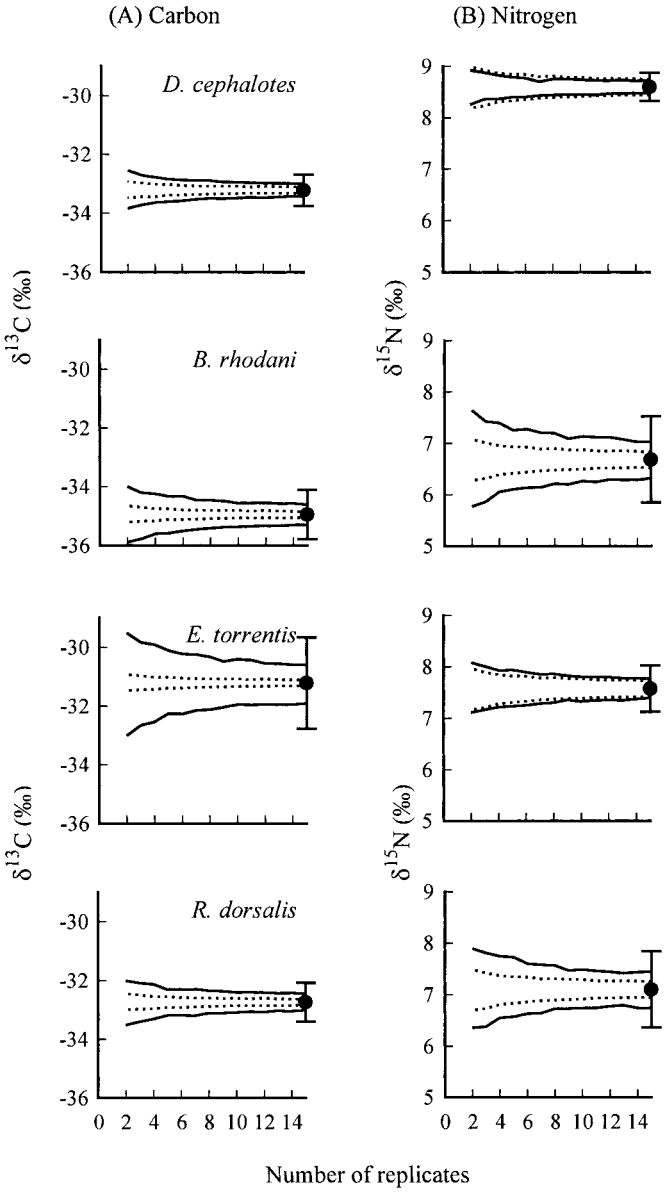

Fig. 2. Estimates of mean (A) δ^{13}C, and (B) δ^{15}N values of VSs in relation to the number of VRs of four representative species and of laboratory standards. Solid lines are 5 and 95 percentiles of animal samples; dashed lines are 5th and 95th percentiles of laboratory standard samples (based on 1,000 randomizations). The closed circle indicates the ''true'' mean (±1 SD) of all animal samples.

individuals of different ages, developmental stages, or nutritional states may have high variance, because all these factors can influence isotope values (e.g., Focken and Becker 1998; Branstrator et al. 2000). Such variation can be minimized by controlling for these factors, as in this study, where we took care to collect animals from only one size class. Invertebrates are ideal study organisms in this respect, because they often have highly synchronized cohorts and do not develop social hierarchies, with the consequent influence on physiological condition, as found in some fish. For some species in this study (e.g., *D. cephalotes*), interindividual

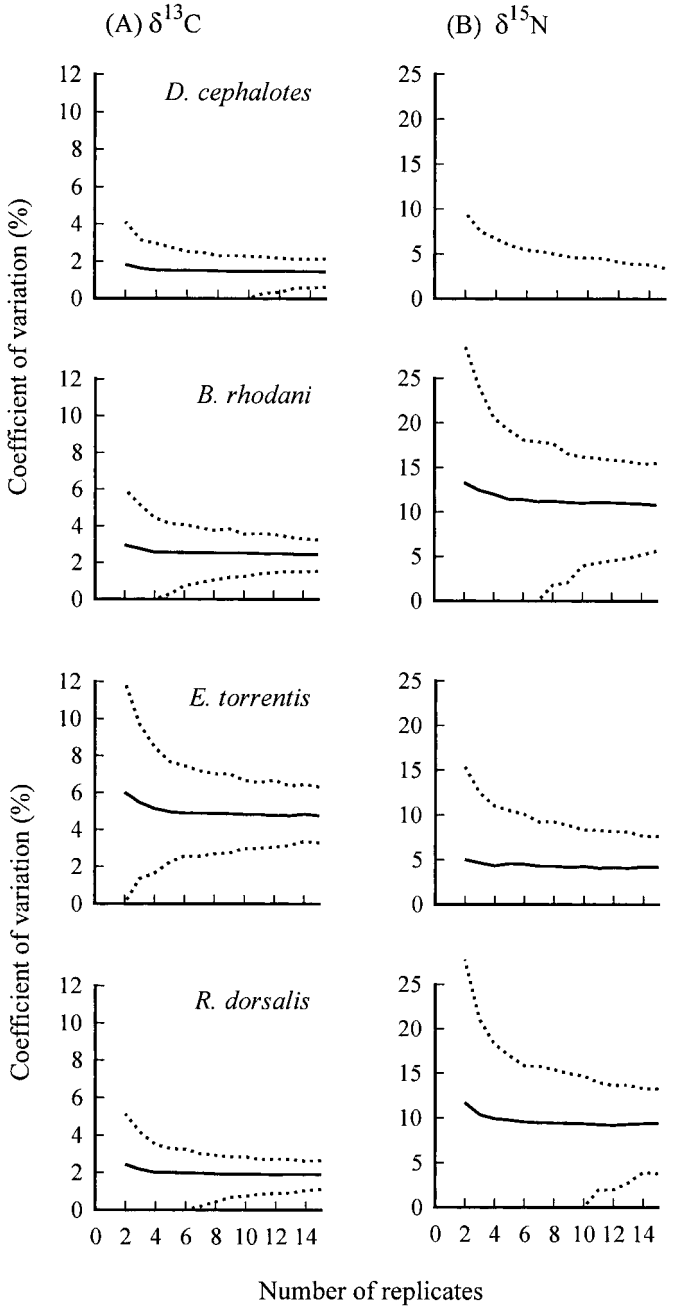

Fig. 3. Conservative estimates of the coefficient of variation attributable to within-population variation for (A) δ^{13}C, and (B) δ^{15}N values of four representative species. Solid lines indicate the mean; dashed lines the best- and worst-case scenarios.

variation was very low, almost unmeasurable against the variance attributable to the measurement technique.

Why does interindividual variation in isotope value differ among species? There is no obvious association between functional feeding group and the magnitude of the within-population variation. Likewise, high variation in δ^{13}C is not necessarily accompanied by high variation in δ^{15}N, or vice versa. Given that stable isotope values integrate food consumed over a long period and that most lotic invertebrates are generalist feeders in some respect (Lancaster and Robertson 1995), this interindividual variation is unlikely to reflect individualistic feeding preferences. It may, however, relate to the mobility of each species, their propensity to wander, and the patchy (or homogeneous) distribution of their food. Individuals with low mobility are likely to spend long periods in a small patch of stream bed, and their isotope values may reflect the patch-specific availability of food resources. Finlay et al. (1999) found differences in the δ^{13}C values of epilithic diatoms among microhabitats that differed in water velocity (riffle vs. pool) and suggested that this may have contributed to differences in δ^{13}C among invertebrates (*see* also Hecky and Hesslein 1995). If there is high among-patch variation in food resources within the stream, then one might expect high within-population variation in isotope value for that species, provided that residence time in a patch is long enough for tissue turnover. Conversely, highly mobile species that wander throughout the stream habitat should have little within-population variation in isotope values, because each individual will encounter roughly the same range and relative abundance of food, and tissue-turnover time is likely to be high relative to movement rates. Unfortunately, the species-specific movement behaviors of lotic invertebrates are poorly understood, so these ideas remain speculative.

Detectable differences—While high precision analysis can render small changes in isotopic composition more meaningful, many replicates may be required to estimate the ''true'' population mean; thus, the trade-off is increased analytical expense. Where the primary ecological interest is in comparing the mean isotope value of populations that differ taxonomically, spatially, or temporally, there may be an advantage in combining several individuals per replicate. The minimum detectable difference between the mean isotope value of two populations is negatively associated with the number of replicates and the number of individuals per replicate (Fig. 4). Using the variance structure of *G. pulex,* for example, to detect differences of ~2‰ in δ^{13}C and 1‰ in δ^{15}N (i.e., to compare diet or infer partial trophic differences) would require six replicates per population at one individual animal per replicate, or three replicates at three individuals per replicate. In a project requiring SIA of 10 populations, this represents a difference of 30 versus 60 samples for SIA and could be financially significant. (Note that the number of replicates and the number of individuals per replicate required to detect a particular difference between means will vary among data sets and must be calculated for every study using the variance structure of those particular data.) The drawback to combining individuals, of course, is that attempts to characterize the interindividual variance of isotope values are no longer feasible. Such projects will always require many samples. Combining individuals in a sample replicate may not be feasible in all situations, e.g., whole-body analyses of large organisms, but neither is tissue-specific analysis feasible in all cases, e.g., isolating muscle tissues from mayflies.

Conclusions—SIA is a potentially powerful tool to elucidate trophic interactions and energy flow pathways in freshwater ecosystems. There are still many facets of the

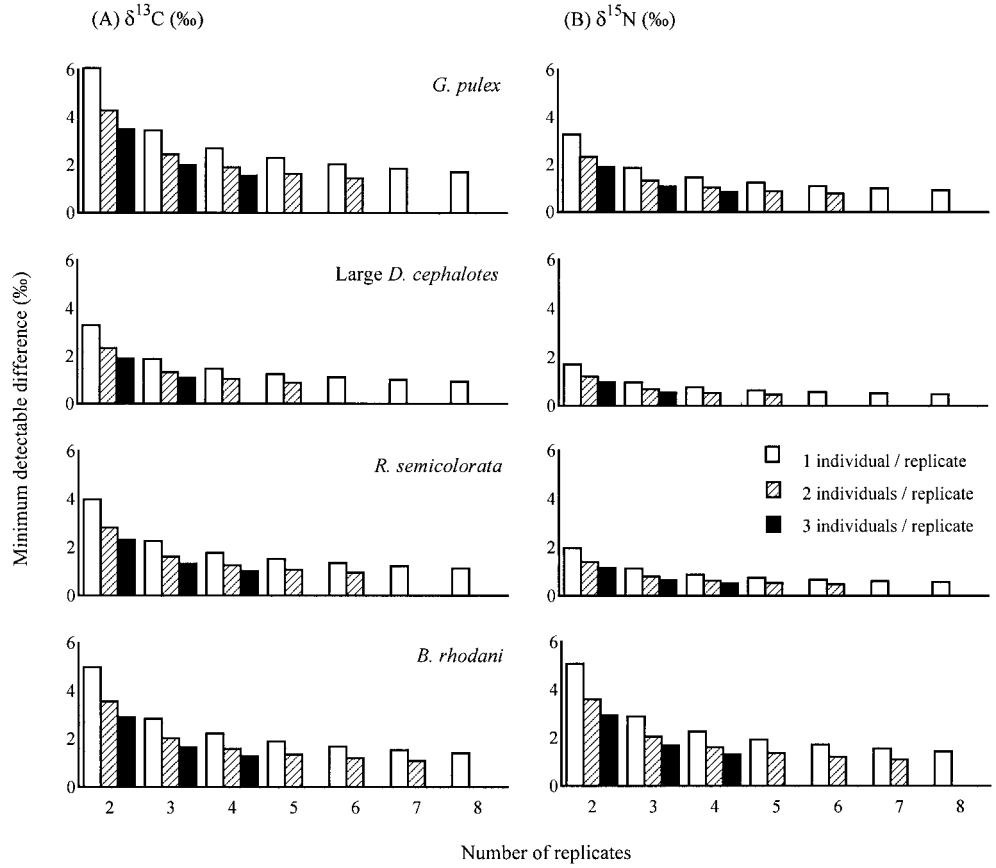

Fig. 4. On average, the minimum detectable difference of (A) $\delta^{13}C$, and (B) $\delta^{15}N$ between two sample means (*t*-test at $\alpha = 0.05$, $\beta = 0.10$) in relation to the number of replicate samples per mean value and the number of individuals combined in each replicate, based on the variance structure of four representative species.

technique that need refining and exploring, but it is timely that researchers adopt more quantitative and statistical approaches when designing sampling programs and analyzing data. There is a cost to SIA, as with all sample analyses, so elegant and simple research designs have benefits over and above the satisfaction of providing a clear answer to a clear question. A carefully designed hypothesis will dictate whether the primary interest is in the population mean and/or variance of whole organisms or of certain tissues and, consequently, whether sample replicates should contain individual organisms, more than one organism, or only certain tissues or compounds. The most appropriate approach will depend on the question. It is important to determine a priori the potential sources of variation, whether they are important to the hypothesis, and whether they can be controlled, e.g., select animals of the same age or size class. This paper illustrates the utility of characterizing and examining the variance structure of the data a priori and using this information to determine the appropriate number of sample replicates and the number of individuals to be combined per replicate. Most importantly, it provides information on the power of statistical tests to detect biologically meaningful patterns and processes. This is, after all, our primary objective.

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References

BRANSTRATOR, D. K., G. CABANA, A. MAZUMDER, AND J. B. RASMUSSEN. 2000. Measuring life-history omnivory in the opossum shrimp, *Mysis relicta*, with stable nitrogen isotopes. Limnol. Oceanogr. **45:** 463–467.

DENIRO, M. J., AND S. EPSTEIN. 1977. Mechanism of carbon frac-

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tionation associated with lipid synthesis. Science **197:** 261–263.
———, AND ———. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta **42:** 495–506.
———, AND ———. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta **42:** 341–351.
DOUCETT, R. R., D. J. GIBERSON, AND G. POWER. 1999*a*. Parasitic association of *Nanocladius* (Diptera: Chironomidae) and *Pteronarcys bilboa* (Plecoptera: Pteronarcyidae): Insights from stable isotope analysis. J. North Am. Benthol. Soc. **18:** 514–523.
———, G. POWER, D. R. BARTON, R. J. DRIMMIE, AND R. A. CUNJAK. 1996. Stable isotope analysis of nutrient pathways leading to Atlantic salmon. Can. J. Fish. Aquat. Sci. **53:** 2058–2066.
———, M. POWER, G. POWER, F. CARON, AND J. D. REIST. 1999*b*. Evidence for anadromy in a southern relict population of Arctic charr from North America. J. Fish. Biol. **55:** 84–93.
FINLAY, J. C., M. E. POWER, AND G. CABANA. 1999. Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. Limnol. Oceanogr. **44:** 1198–1203.
FOCKEN, U., AND K. BECKER. 1998. Metabolic fractionation of stable carbon isotopes: Implications of different proximate compositions for studies of the aquatic food webs using δ^{13}C data. Oecologia **115:** 337–343.
GRAÇA, M. A. S., L. MALTBY, AND P. CALOW. 1994. Comparative ecology of *Gammarus pulex* (L.) and *Asellus aquaticus* (L.). 2. Fungal preferences. Hydrobiologia **281:** 163–170.
HECKY, R. E., AND R. H. HESSLEIN. 1995. Contribution of benthic algae to lake food webs as revealed by stable isotope analysis. J. North Am. Benthol. Soc. **14:** 631–653.
HENTSCHEL, B. T. 1998. Intraspecific variations in δ^{13}C indicate ontogenetic diet changes in deposit-feeding polychaetes. Ecology **79:** 1357–1370.
JONES, J. R. E. 1950. A further ecological study on the River Rheidol; the food of the common insects in the main-stream. J. Anim. Ecol. **19:** 159–174.
JONES, R. I., J. GREY, D. SLEEP, AND C. QUARMBY. 1998. An assessment, using stable isotopes, of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. Proc. R. Soc. Lond. B **265:** 105–111.
JUNGER, M., AND D. PLANAS. 1994. Quantitative use of stable carbon isotope analysis to determine the trophic base of invertebrate communities in a boreal forest lotic system. Can. J. Fish. Aquat. Sci. **51:** 52–61.
KLING, G. W., B. FRY, AND W. J. O'BRIEN. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology **73:** 561–566.
LANCASTER, J., AND A. L. ROBERTSON. 1995. Microcrustacean prey and macroinvertebrate predators in a stream food web. Freshwater Biol. **34:** 123–134.
MARRA, P. P., K. A. HOBSON, AND R. T. HOLMES. 1998. Linking winter and summer events in a migratory bird by using stable carbon isotopes. Science **282:** 1884–1886.
MCCONNAUGHEY, T. A., AND C. P. MCROY. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar. Biol. **53:** 257–262.
MEIER, G. M., E. I. MEIER, AND S. MEYNS. 2000. Lipid content of stream macroinvertebrates. Arch. Hydrobiol. **147:** 447–463.
PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18:** 293–320.
PINNEGAR, J. K., AND N. V. C. POLUNIN. 1999. Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: Implications for the study of trophic interactions. Funct. Ecol. **13:** 225–231.
PONSARD, S., AND R. ARDITI. 2000. What can stable isotopes (δ^{15}N and δ^{13}C) tell us about the food web of soil macro-invertebrates? Ecology **81:** 852–864.
ROUNICK, J. S., AND B. J. HICKS. 1985. The stable carbon isotope ratios of fish and their invertebrate prey in four New Zealand rivers. Freshwater Biol. **15:** 207–214.
THORP, J. H., M. D. DELONG, K. S. GREENWOOD, AND A. F. CASPER. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. Oecologia **117:** 551–563.
YOSHIOKO, T., E. WADA, AND H. HAYASHI. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. Ecology **75:** 835–846.
ZAR, J. H. 1984. Biostatistical analysis. Prentice.

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Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters

Abstract—Heterotrophic bacteria are a key component driving biogeochemical processes in aquatic ecosystems. In 1998, we examined the role of heterotrophic bacteria by quantifying plankton biomass and bacterial and planktonic respiration across a trophic gradient in several small Minnesota lakes as well as Lake Superior. The contribution of bacteria (<1-μm fraction) to total planktonic respiration ranged from ~10 to 90%, with the highest contribution occurring in the most oligotrophic waters. The bacterial size fraction constituted a substantial reservoir of planktonic carbon, nitrogen, and phosphorus (14–58%, 10–49%, and 14–48%, respectively), being higher in oligotrophic than in eutrophic waters. However, we saw no clear evidence for the selective enrichment of either nitrogen or phosphorus in the bacteria size fraction relative to total plankton. Carbon : nitrogen and carbon : phosphorus ratios in both the total particulate matter and <1-μm fractions were similar and above Redfield values in oligotrophic waters, but approached them in eutrophic waters. Carbon-based bacterial growth efficiencies (BGE) were variable (4–40%) but were lowest in oligotrophic systems and increased in eutrophic systems. BGE varied negatively with carbon : nitrogen : phosphorus ratios, suggesting increased maintenance costs in low-nutrient waters. In oligotrophic waters most of the organic matter is dissolved, supporting a predominantly microbial food web, whereas in eutrophic waters there is an increased abundance of particulate organic matter supporting a food web consisting of larger autotrophs and phagotrophic heterotrophs.

Autotrophs and heterotrophs constitute two of the most