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Authors: Bond, Alexander L., and Hobson, Keith A.

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Reporting Stable-isotope Ratios in Ecology: Recommended Terminology, Guidelines and Best Practices

ALEXANDER L. BOND[1,2,*] AND KEITH A. HOBSON[2]

[1]Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan, S7N 5E2, Canada

[2]Environment Canada, 11 Innovation Boulevard, Saskatoon, Saskatchewan, S7N 3H5, Canada

[*]Corresponding author; E-mail: alex.bond@usask.ca

Abstract.—The application of stable-isotope analysis (SIA) in ecology has increased exponentially in the last 20 years. As with any novel field of inquiry, there has been inconsistent (and sometimes confusing) use of terminology and great variation in how the results of SIA are presented in the scientific literature. Recently, guidelines and recommendations for the consistent use of terminology, the expression of results, and presentation of symbols were prepared and published at the request of the Commission on Isotopic Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC). Here, key components of the CIAAW recommendations pertinent to ecologists are summarized, along with suggestions for best practices in reporting results of SIA not covered by the CIAAW guidelines. A set of universally adopted and consistently used terminology and practices will minimize ambiguity, especially in the overlap of different fields, such as analytical chemistry and ecology. *Received 17 January 2012, accepted 7 March 2012.*

Key words.—guidelines, reference material, stable isotopes, terminology.

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The Commission on Isotopic Abundances and Atomic Weights (CIAAW) is a commission of the International Union of Pure and Applied Chemistry (IUPAC) charged with maintaining scientific clarity and consistency in areas of chemistry concerned with relative measures of amounts of elements, including atomic weights (De Bièvre and Peiser 1992) and isotopic compositions (Berglund and Weiser 2011). Recently, Coplen (2011) summarized the CIAAW recommendations and guidelines for the reporting of the results of stable-isotope analysis (SIA), though from an analytical chemistry perspective. The use of SIA in ecology in general, and ornithology in particular, is growing rapidly (Inger and Bearhop 2008; Boecklen *et al.* 2011; Hobson 2011), but so is the disconnection between end-users of SIA and the origins of SIA in analytical chemistry. Many users of stable isotopes in ecology rely on fee-for-service laboratories or have little direct interaction with the analytical side of isotope analysis. Recognizing this, pertinent IUPAC CIAAW guidelines and recommendations for reporting SIA results are presented here in a manner geared towards ecologists, biologists and ornithologists as end-users. Further, "best reporting practices" are recommended to ensure a high level of scientific rigor to enable readers to assess critically SIA results reported in the literature. The goal of both Coplen (2011) and this review is to "improve the global exchange of scientific information in different disciplines that measure or make use of variations in isotopic abundance" (Coplen 2011: 2538) by establishing clear and consistent standards and terminology in studies using SIA. Additionally, this paper is intended to act as a reference for end-users of stable isotopes in ecology. Those wishing greater detail are directed to the comprehensive summary by Coplen (2011) and the other references therein.

Recommendations are divided into two sections: guidelines concerning general concepts (Table 1), and those concerning specific terminology (Table 1). A third recommendation section for "best reporting practices" in the ecological literature, particularly as it pertains to isotopic reference materials, is also presented (Table 2).

General Concepts

Symbols and Notation

Symbols (either Roman or Greek symbols) that indicate quantities in the Système international d'unités (SI) must be in italics,

Table 1. A summary of the CIAAW-IUPAC guidelines for reporting the results of stable-isotope analysis (SIA) pertinent to ecologists using SIA. Examples of the frequent incorrect usage, and correct usage, as well as a brief explanation are provided. For further detail, see text.

Concept	Incorrect	Correct	Explanation
Isotope names	C^{13}, 15-nitrogen	^{13}C, nitrogen-15	Mass number comes before the element, or after the full name.
Notation	$\delta^{13}C = -19.3‰$	$\delta^{13}C = -19.3$ ‰	δ is in italics, en-dash rather than hyphen, space between value and ‰.
Hydrogen isotope ratios	δD	$\delta^{2}H$	D is not an acceptable substitute for ^{2}H.
Enriched/depleted	Enriched in $\delta^{13}C$	Enriched in ^{13}C	δ values can not be enriched or depleted; samples can be enriched or depleted in an isotope (e.g., ^{13}C).
Isotope ratio (*R*, not δ)	$\delta^{15}N$ isotope ratio	$\delta^{15}N$ value	"Isotope ratio" describes the ratio of heavy to light isotopes in a single sample (not relative to an international measurement standard).
Isotopic difference (Δ)	Fractionation	Isotopic discrimination	Fractionation refers to changes in δ values between a single substrate and product. Biological processes involve many rate-limited biochemical reactions, and so "isotopic discrimination" is preferred.
Per mil notation (‰)	Equation 2 in text, plus "× 1000"	Equation 2 in text	δ values are expressed in ‰, and including "× 1000" inflates values by 10^3.
Precision	$\delta^{15}N = +12.74‰$	$\delta^{15}N = +12.7‰$	Report experimental values only to the number of decimal places of the precision established using secondary isotopic reference materials.

Table 2. A checklist of recommendations concerning the selection and reporting of results from secondary isotopic reference materials. The same applies to "in-house" isotopic reference materials. For further detail, see text. Authors should report these details in the Methods section of papers.

Checklist
Report SD of secondary isotopic reference materials within and among analytical runs
Measured values of secondary isotopic reference materials should be reported, and where possible, should span the range expected in unknown samples
The mass and matrix of secondary isotopic reference materials should match those of the unknown samples where possible
Secondary isotopic reference materials for δ^2H should be calibrated to non-exchangeable hydrogen

and their superscripts and subscripts in normal type (BIPM 2006). This applies to the lowercase δ (as in δ^{15}N) and the uppercase Δ (as in Δ^{15}N). Single isotopes are written by placing the mass number in superscript before the element (e.g. ^{2}H, ^{18}O), but when written out, the mass number follows the name of the element and is separated with a hyphen (e.g. "sulfur-34", "nitrogen-15").

Because delta values are relative differences in isotope ratios of a sample and an internationally-recognized standard, they are difference values and both positive and negative values are possible. All delta values should be preceded by either "+" or "–" (en-dash, not a hyphen). Delta values are expressed in parts per thousand (per mil with symbol ‰). A space is printed between a numerical value and the term or SI unit. For example: "+12.5 ‰" (not "12.5 ‰"), and "-28.3 ‰" (not "-28.3 ‰").

A solidus (oblique stroke, or forward slash, "/") can be used to separate isotopes (e.g. "^{13}C/^{12}C") or to separate an unknown from a standard (e.g., "$\delta\,^{13}C_{r/VPDB}$").

Terminology

Hydrogen has two stable isotopes: ^{1}H and ^{2}H (not "D"). These are named "protium" and "deuterium", and the isotopic ratio (^{2}H/^{1}H) is written as "$\delta\,^2$H" (not "δ D"), and described as "stable isotopes of hydrogen" or similar phraseology.

The terms "enriched" and "depleted" are relative comparisons of the heavier isotope in two or more analytical samples and should not be used to describe relative magnitude of delta values. For example: "primary feathers were enriched in ^{13}C relative to tail feathers" (not "primary feathers were enriched in δ^{13}C relative to tail feathers").

GUIDELINES FOR REPORTING SIA RESULTS, AND TERMINOLOGY

International measurement standards and isotopic reference material

Each sample's stable-isotope ratio is expressed relative to the isotopic ratio in an *international measurement standard*. Currently, these are Vienna Standard Mean Ocean Water (VSMOW) for δ^2H and δ^{18}O, Vienna Peedee belemnite (VPDB) for δ^{13}C, atmospheric N_2 (Air) for δ^{15}N, and Vienna Cañon Diablo Troilite (VCDT) for δ^{34}S (Mariotti 1983; Coplen 1994; Krouse and Coplen 1997). Previous international measurement standards (Standard Mean Ocean Water, (SMOW), Peedee belemnite (PDB), and Cañon Diablo Troilite (CDT)) are no longer used (Coplen 1995). VSMOW has been replaced by VSMOW2; nevertheless, it is recommended that δ^2H and δ^{18}O values continue to be reported relative to VSMOW (IAEA 2009).

In reality, due to limited supplies, most international measurement standards are not run routinely, and *secondary isotopic reference materials* (SIRMs) with known isotopic compositions relative to international measurement standards are analyzed instead. Isotopic reference materials may be developed in-house at an analytical lab provided they are sufficiently homogenous and stable in their isotopic composition (e.g. for δ^2H in keratins, see below). Alternatively, they may be obtained from an international body such

as the International Atomic Energy Agency (IAEA), a national measurement institute, such as the National Institute of Standards and Technology (NIST), a government agency such as the U.S. Geological Survey (USGS) or a private company. Developing in-house reference materials is a time-consuming and laborious process involving well-designed and executed inter-laboratory comparisons, should be done only in close collaboration with expert stable-isotope laboratory personnel, and only when another suitable reference material is not available (Wassenaar 2008). Calibration of in-house isotopic reference materials is available from the USGS Reston Stable Isotope Laboratory (http://isotopes.usgs.gov/lab/referencematerials.html). Isotopic reference materials obtained from national and international bodies (e.g. IAEA, NIST, USGS) are often provided with documentation on storage, use and isotopic composition (including uncertainty) of the material. SIRMs have undergone considerable inter-laboratory testing and verification before being released for general use and application, and they generally are preferred over in-house reference materials, but may not necessarily be available in quantities or composition needed to satisfy analytical requirements (see Best Practices below). Ecologists should discuss reference materials with their analytical laboratory personnel, and laboratories should offer a range of appropriate biological SIRMs.

Regardless of the isotopic reference materials used, the resulting isotope ratios are expressed relative to the isotope ratios of international measurement standards. Authors should always define the international standard used in the Methods section of papers. Ambiguity can be avoided through the use of a subscript (e.g. $\delta\,^{2}H_{VSMOW}$ $\delta\,^{13}C_{VPDB}$, $\delta\,^{15}N_{Air}$, $\delta\,^{18}O_{VSMOW}$, $\delta\,^{34}S_{VCDT}$). Following this definition, authors may use the δ notation without the subscript (e.g. $\delta\,^{2}H$, $\delta\,^{13}C$, etc.). Many international measurement standards are available only in finite quantities, and as happened with PDB and CDT, can be exhausted (Coplen 1995; Krouse and Coplen 1997). Appending the international measurement standard used in a study ensures that published values of unknowns can be adjusted for future changes in values of international measurement standards as analytical techniques improve and measurement uncertainty is lowered.

Terminology

Isotope ratio, *R*. The isotope ratio is the mathematical expression of the number of atoms of one elemental isotope relative to another, with the isotopes separated by a solidus (e.g. "R ($^{2}H/^{1}H$)"). The heavier (and more rare) isotope is written first. The term "*R*" has been used in the ecological literature to refer to relative differences of isotope ratios (expressed as δ values), but this is incorrect; see "relative differences of isotope ratios" below.

Isotopic composition. Isotopic composition refers to a general observation based on isotopic information (e.g. "The isotopic composition of heron feathers varied between sites"). It should not be used to refer to relative differences in isotope ratios (see below).

Isotopic difference Δ. The term "isotopic difference" is most frequently used to describe differences in δ values between species (e.g. prey and consumer) or tissues (e.g. blood and albumen). The acceptable ecological term is *discrimination factor* or *isotopic discrimination factor*. As an example, $\Delta^{15}N = \delta\,^{15}N_{consumer} - \delta\,^{15}N_{prey}$ (not $\Delta\delta\,^{15}N$). "Fractionation factor" is not appropriate, unless it describes the change in isotopic composition between a pure substrate and resulting product during a single chemical step (e.g. measuring $\delta\,^{2}H$ in water and the resulting water vapor). Biological processes that result in an isotopic difference between tissues or species are typically the result of multiple rate-limited biochemical steps and so should be referred to as *isotopic discrimination*. Similarly, "trophic enrichment factor" implies that any change in δ values is 1) positive, and 2) attributed solely to a trophic process, neither of which is necessarily accurate.

Per mil, ‰. For a given element, the heavier of the two isotopes being compared (e.g. ^{18}O to ^{16}O) is much more scarce (Berglund and Weiser 2011), meaning that the relative abundance of the heavier to the lighter iso-

tope commonly is of the magnitude of 10^{-2} to 10^{-3}. To facilitate reading and interpretation, the relative difference is presented in per mil (‰) notation (or one part in one thousand). Thus, instead of "$\delta^2H = -0.0257$", write "$\delta^2H = -25.7‰$." This non-SI term is dimensionless and does not imply any quantity. To date, the correct use of per mil notation has been common practice in ecology. Relative difference of isotope ratios, δ

SIA results are reported and defined as:

$$\delta^{j/i}X = \frac{({}^jX/{}^iX)_{sample} - ({}^jX/{}^iX)_{standard}}{({}^jX/{}^iX)_{standard}} \qquad \text{(Equation 1)}$$

and can be rewritten as:

$$\delta^{j/i}X = \frac{({}^jX/{}^iX)_{sample}}{({}^jX/{}^iX)_{standard}} - 1 \qquad \text{(Equation 2)}$$

where ${}^jX$ is the heavier isotope (e.g. ^{15}N), and ${}^iX$ the lighter isotope (e.g. ^{14}N) in the analytical sample (numerator) and international measurement standard (denominator). Commonly, $\delta^{j/i}X$ is shortened to δ^jX (e.g. $\delta^{18}O$). Hydrogen, carbon and nitrogen, each has only two stable isotopes; oxygen has three (^{16}O, ^{17}O and ^{18}O), and sulfur has four (^{32}S, ^{33}S, ^{34}S and ^{36}S). When reporting relative differences in sulfur isotope ratios, specify which of the four isotopes are being compared: "We measured $^{34}S/^{32}S$ or $\delta^{34}S$ in tern blood plasma." $^{34}S/^{32}S$ and $^{18}O/^{16}O$ are the most commonly measured ratios, and unless specified, these are the ratio assumed when one writes $\delta^{34}S$ and $\delta^{18}O$. In general, δ^jX refers to the comparison of isotope j to the most abundant isotope unless stated otherwise. If there is any ambiguity (e.g. reporting multiple isotopes of lead, mercury, iron, etc.), provide the ratio explicitly each time (e.g. $\delta^{202/200}Hg$). Note the absence of "× 1000" in the equations above, though it is often included in publications. This is because δ^jX is expressed in per mil notation, and including both per mil notation (see above) and "× 1000" technically results in values being inflated by a factor of 10^3.

"Relative difference of isotope ratios" is often shortened to "isotope ratios" but this is incorrect, as isotope ratios (R) are the simple ratio of the number of atoms of two isotopes in a material (see above), and the δ value is a mathematical manipulation of a ratio of isotope ratios (equation 2). "Relative difference of isotope ratios" is cumbersome, and will not likely be universally adopted, so authors should use the term "values" or "δ values" (e.g. "$\delta^{34}S$ values differed between years" or "the measured δ values are presented relative to the following international reference materials"). Avoid the term "isotope signatures" unless referring to an unvarying quantity (e.g. endpoint in an isotopic model).

BEST PRACTICES IN REPORTING SIA RESULTS

The following are not based on CIAAW recommendations or guidelines, but come from existing ecological literature, and mainly concern the use and reporting of secondary isotopic reference materials (SIRMs); the same applies to in-house isotopic reference materials (Table 2). In cases where the isotopic reference material is not distributed widely (e.g. available from NIST, USGS, etc.), authors should cite the reference describing the reference material's properties (e.g. matrix, expected value, variation within/among runs or instruments). Including these data is as important as detailing statistical or field procedures, and journal editors and reviewers should require them for publication. Furthermore, as values of SIRMs are refined (and eventually replaced), reporting their measured value will allow future researchers to fully compare results obtained using different calibration curves (Coplen and Qi 2012). Adopting these practices will result in greater clarity and confidence in SIA results presented in the literature.

Reporting instrument precision and accuracy

Jardine and Cunjak (2005) presented recommendations for the presentation of analytical error in SIA studies; and their points bear repeating. Researchers should report accuracy as the mean ± S.D. of SIRMs. Precision should be reported as the

mean ± S.D. of SIRMs *within each analytical run* and over all analytical runs. These can be presented as "IAEA-CH6, δ^{13}C mean within runs = -10.49‰ to -10.42‰, mean among runs = -10.43 ± 0.07‰", as a table in the manuscript, or as supplemental material. When homogenous tissues are analysed, present mean ± S.D. of replicate samples within runs. Note that feathers and other solid phase biological tissues are not usually homogenous (Wassenaar and Hobson 2006). Once the appropriate measurement precision has been established, δ values of samples should be quoted to a precision that does not exceed the minimum decimal place. So, if the precision of measurement for δ^{13}C is ± 0.1‰, then a sample value can only be reported to one decimal place (e.g. -21.3‰ not -21.32‰).

SIRMs should span the range of experimental values

In each analytical run, SIRMs (and/or in-house lab standards) are included, and their measured δ values compared with accepted values to create at minimum a two-point linear regression used to correct the measured values of unknowns (see Fig. 1 in Jardine and Cunjak 2005). In order for this calibration equation to accurately adjust the relative difference in isotope ratios of samples, the samples should ideally fall within or close to the range of the values covered by the SIRMs. For most routine isotopic analyses, especially of δ^{13}C and δ^{15}N, measurement calibration remains linear over a substantial range (and beyond the range defined by the SIRMs used). However, in general, calibration curves should, where possible, span the range of samples being measured (Jardine and Cunjak 2005). In cases where δ^{13}C and δ^{15}N are determined simultaneously, there should be CIRMs spanning a sufficient range to calibrate both elements. Jardine and Cunjak (2005) also provided recommendations for the placing of SIRMs (and replicates) within runs to account for instrumental drift and performance (Merritt and Hayes 1994; Ohlsson and Wallmark 1999).

Secondary isotopic reference materials should match the unknowns

Under the "principle of identical treatment" (IT principle), any treatment (chemical or physical) of SIRMs and unknowns should be as similar as possible (Werner and Brand 2001). The two main areas where end users may have some control are in matching the mass and the matrix of SIRMs and unknowns. Wassenaar (2008) provided general guidelines on the amount of sample required for determination of each isotope ratio, but researchers should seek detailed instructions from their analytical laboratory as specific requirements will vary. Importantly, the elemental mass yield of all samples and SIRMs should be very close (i.e. a maximum ± 5% of the target. Thus, if the target weight was 1.00 mg then all samples and SIRMs should be weighed accordingly to obtain masses of 0.95 to 1.05 mg. Most importantly, mass-dependent differences in isotope ratios can result from variation in gas pressures during analysis. These differences generally are small for C and N isotope analysis, but can be significant when determining δ^{2}H or δ^{18}O (Wassenaar 2008).

Secondly, the matrix of SIRMs should match that of the unknowns as closely as possible (Jardine and Cunjak 2005). Isotope ratios are measured by combusting samples at high temperature (typically >1000°C), and measuring the isotopic composition of the resultant purified analyte gases (e.g. CO_2 or N_2). Regardless of the isotope ratio of an SIRM, its composition might affect chemical isotope fractionation during combustion (Werner and Brand 2001). Using SIRMs of a similar matrix to unknowns (e.g. keratin SIRMs when measuring δ^{2}H in feather keratins) will minimize differential isotope fractionation of resultant gases and adhere to the IT principle (Gentile *et al.* 2011). Using inorganic SIRMs to calibrate organic unknowns can result in decreased analytical accuracy (though no observable difference in precision), though this is less of an issue when measuring δ^{13}C, δ^{15}N, and/or δ^{34}S, and more critical for δ^{2}H and δ^{18}O measurements (Werner and Brand 2001; Coplen and Qi 2012).

Oxygen and exchangeable hydrogen

The δ^2H analyses of avian tissue are most frequently conducted on keratins, which contain two types of hydrogen: exchangeable and non-exchangeable. Non-exchangeable hydrogen is bound to carbon, whereas hydrogen bound to nitrogen, oxygen or sulfur can exchange with hydrogen in the ambient environment (Wassenaar and Hobson 2000). Because ambient water vapor in laboratories can undergo dramatic seasonal oscillations in isotopic composition (especially at mid-continental locations), researchers should determine the δ^2H value of non-exchangeable hydrogen, which requires SIRMs of a similar matrix for which the δ^2H value of the non-exchangeable hydrogen is known (Wassenaar and Hobson 2003), or the use of steam-calibrated unknowns and standards (Schimmelmann and DeNiro 1986; Schimmelmann 1991). There are now a few options for SIRMs used in δ^2H and δ^{18}O analysis of complex organic materials with exchangeable H. Wassenaar and Hobson (2003) have developed two homogenous keratin isotopic reference materials for δ^2H that span the range of -54.1 to -197 ‰ for measurement and calibration of unknowns to non-exchangeable δ^2H values, and Coplen and Qi (2012) give details of two other SIRMs for δ^2H and δ^{18}O analysis (USGS42 and USGS43). Using SIRMs that contain only non-exchangeable hydrogen in order to calibrate materials containing exchangeable hydrogen can lead to errors and result in erroneous interpretations. There have also been recent summaries of best analytical practices for δ^2H and δ^{18}O analysis of keratins (Qi and Coplen 2011; Qi *et al.* 2011).

Authors should provide information on SIRMs used

There are several compelling reasons for authors to provide information on the SIRMs used in their measurements. In order for readers to assess the quality assurance/quality control (QA/QC) of the analysis, and to assure editors and reviewers that data are trustworthy, the name and measured values of all SIRMs should be provided. This will demonstrate that the calibration curve used to generate data for unknowns is appropriate. As Coplen and Qi (2012) demonstrated, calibrating δ^2H and δ^{18}O results using different isotopic reference materials can affect the comparability of results among analytical labs and studies; reporting the isotopic reference materials used, and their values, can alleviate this problem. Furthermore, it will allow an assessment of the suitability of the SIRMs matrix, especially in the case of publishing δ^2H and δ^{18}O values.

CONCLUSIONS

The IUPAC is the authoritative international body on chemical nomenclature, terminology and measurement. The growing separation of ecologists from the analytical chemistry foundations of SIA has resulted in inconsistent (and in some cases, incorrect) terminology becoming entrenched in the ecological literature. The authors are just as at fault in this regard, and this review is not meant to chastise any group or individuals. Indeed, until Coplen (2011), there were no universal recommendations (but see Jardine and Cunjak 2005; Wassenaar 2008). The unique cross-disciplinary nature of SIA is seldom acknowledged, but as a technique firmly embedded in the chemical sciences, the use of proper chemical terminology and notation, and consistent reporting of results should be strongly encouraged, if not required.

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