Calibration chain transformation improves the comparability of organic hydrogen and oxygen stable isotope data

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Abstract
1. Stable hydrogen and oxygen isotopic compositions (δ2H and δ18O, respectively) of animal tissues have been used to infer geographical origin or mobility based on the premise that the isotopic composition of tissue is systematically related to that of local water sources. Isotopic data for known-origin samples are required to quantify these tissue–environment relationships. Although many of such data have been published and could be reused by researchers, differences in the standards used for calibration and analytical procedures for different datasets limit the comparability of these data.
2. We develop an algorithm that uses results from comparative analysis of secondary standards to transform data among reference scales and estimate the uncertainty inherent in these transformations. We apply the algorithm to a compilation of known-origin keratin data published over the past ~20 years.
3. We show that transformation improves the comparability of data from different laboratories, and that the transformed data suggest ecophysiological meaningful differences in keratin–water relationships among different animal groups and taxa.
4. The compiled data and algorithms are freely available in the ASSIGNR R-package to support geographical provenance research, and more generally offer a methodology overcoming several challenges in geochemical data integration and reuse.

KEYWORDS
- calibration
- comparative equilibration
- database
- isotopic exchange
- keratin
- known-origin
- reference materials
- standards

1 | INTRODUCTION

The stable hydrogen and oxygen isotope compositions (δ2H and δ18O, respectively) of animal tissues have found widespread use as a geographical marker in wildlife, archaeological and forensic applications (reviewed by Carter & Chesson, 2017; Hobson & Wassenaar 2019; Meier-Augenstein, 2018). The underlying premise of these applications is that tissue isotopic composition is linked...
to that of local water sources (i.e. precipitation) through relatively predictable relationships (e.g. Chamberlain et al., 1996; Ehleringer et al., 2008; Hobson & Wassenaar, 1997). The isotopic composition of precipitation varies predictably across space and time (Bowen & Revenaugh, 2003; Bowen, Wassenaar, et al., 2005; Craig, 1961), and thus tissue isotope ratios derived ultimately from precipitation-driven local food webs can be compared to environmental water isoscapes (predictive models of spatio-temporal isotope patterns) to infer tissue origin (Ma et al., 2020; Vander Zanden et al., 2014; Wunder, 2010). Keratinous tissues, such as feather, hair or nail, are metabolically inert once formed and so preserve an isotopic composition characteristic of the location of tissue growth (Hobson, 1999; Macko et al., 1999; West et al., 2004), and are the focus here and in many published studies.

Because tissue–environment relationships vary among taxa and regions (Magozzi et al., 2019 and references therein), samples of known origin are needed to quantify such relationships (e.g. Chamberlain et al., 1996; Ehleringer et al., 2008; Hobson et al., 2012; Hobson & Wassenaar, 1997). The collection and analysis of known-origin samples, however, is resource-intensive and in some cases prohibitive, reducing the efficiency and applicability of the approach. Although many known-origin datasets have already been published, and in theory could be reused to quantify tissue–environment relationships in new studies, different sample preparation, analytical and calibration practices used at different laboratories (or even at a single laboratory over time) have generated data that are not directly comparable (e.g. Bowen, Chesson, et al., 2005; Meier-Augenstein et al., 2013; Soto et al., 2017; Wassenaar & Hobson, 2003). As a result, responsible users of published data have thus far focused on measurements made in a single laboratory or obtained with identical protocols.

Different sample treatment and analysis methods are one source of inconsistency in published data. Exchange of H atoms from protein carboxyl and hydroxyl groups with ambient atmospheric water vapour molecules affects measured δ²H values and must be corrected for (Bowen, Chesson, et al., 2005; Chesson et al., 2009; Schimmelmann, 1991; Wassenaar & Hobson, 2000, 2003), most commonly through comparative analysis against keratin standards for which non-exchangeable δ²H values have been established (Kelly et al., 2009; Sauer et al., 2009; Wassenaar & Hobson, 2003). Water is also tightly adsorbed by keratin and adheres to sample capsules, contributing to the measured δ²H and δ¹⁸O values (Wortmann et al., 2001) unless samples are thoroughly dried prior to combustion (Bowen, Chesson, et al., 2005; Coplen & Qi, 2012; Soto et al., 2017). Analytical methods have themselves evolved since the advent of online thermal conversion/elemental analysis isotope ratio mass spectrometry (TC/EA-IRMS) for keratin H and O isotope analysis ~20 years ago. The use of different pyrolysis reactor fillings (e.g. chromium vs. glassy carbon; Gehre et al., 2015; Nair et al., 2015) and chromatographic conditions (Hunsinger et al., 2013; Qi et al., 2011) has been shown to affect measured δ²H and δ¹⁸O values, respectively.

Fortunately, isotope ratio analysis is performed as a comparative analysis, wherein the sample values are calibrated to the accepted values for co-analysed standards. Under ideal circumstances conforming to the principle of identical treatment (PIT; Werner & Brand, 2001), which requires the preparation and analysis of matrix-matched (chemically and physically equivalent) standards alongside the unknown samples, analytical biases should affect the samples and standards similarly and have little effect on sample values reported relative to the standard values (i.e. on a ‘reference scale’ defined by the standards used and their assigned values). However, ideal circumstances are not possible in many situations. Even where samples and standards are compositionally similar (i.e. keratin), differences in preparation (i.e. grinding/powdering) and biochemistry (i.e. amino acid profile) can affect H exchange and may impart bias to otherwise PIT-compliant comparisons (Alibardi, 2017; Bowen, Chesson, et al., 2005; Robbins, 2012). Additionally, the internationally accepted reference scale (here the Vienna Standard Mean Ocean Water, ‘VSMOW’, scale) for δ²H and δ¹⁸O data is defined by primary standards that are water, meaning that calibrating organic secondary standards to the VSMOW scale using PIT procedures is impossible. As methodologies have advanced, a set of ‘optimal’ procedures minimizing bias in these non-PIT comparisons has been developed, but in the interim many different secondary standard calibrations have been produced, leading to published known-origin data that are laboratory-specific and not robustly traceable to the VSMOW scale.

Here, we leverage cross-calibration studies, in which one set of secondary standards is analysed alongside and calibrated to a reference scale defined by a second set, to develop a method that transforms data between reference scales. Transformation from the original scale to a target scale proceeds along a chain of linked calibrations, ideally with each link consisting of a PIT-based cross-calibration or a non-PIT calibration using optimal methods (Qi et al., 2011; Soto et al., 2017; see also Coplen & Qi, 2016). The algorithm propagates uncertainty, and permits comparison of data reported on more than two dozen reference scales. We apply and test the method using a compilation of data for >4,000 keratin samples, showing that the method reduces, but does not always eliminate, discrepancies among data from different laboratories. The data and transformation algorithm are available in the ASSIGNR R-package (Ma et al., 2020) to support their open reuse.

2 | MATERIALS AND METHODS

2.1 | Secondary standard calibration history

We compile information on widely used keratin secondary standards. Our list is not exhaustive, but includes standards calibrated by five stable isotope laboratories (United States Geological Survey [USGS] Denver and Reston, the University of Ottawa, Environment Canada [Saskatoon] and the University of Utah) that have been used to generate a large fraction of the published keratin isotope data. Each reference scale is defined by two secondary standards and their assigned values (Dunn & Carter, 2018). In a typical laboratory application, a linear model relating instrument-reported values for the secondary standards to their assigned values is applied to the
measured values for unknowns to calibrate them to the reference scale.

Assigned values for secondary standards are the product of calibration (through co-analysis) relative to a reference scale defined by a different set of primary or secondary standards. These calibrations have used a wide range of methodologies, and for most keratin standards multiple calibrations have been generated using different methods. In general, these can be classified as calibrations based on (1) PIT methods, with full reporting of methods and uncertainty, (2) measurement against non-matrix-matched standards using optimal methods to minimize matrix effects or (3) measurements that are neither PIT-based nor optimal. For category 2, we consider optimal methods to include thorough sample drying (e.g., using a sealed, evacuated and heated carousel such as the Uni-Prep™ device; Wassenaar et al., 2015; Soto et al., 2017), correction for exchangeable H via equilibration with multiple waters and use of a Cr-filled (vs. glassy carbon) pyrolysis reactor to avoid bias caused by HCN-producing reactions (Soto et al., 2017; Soto et al., 2017; Wassenaar et al., 2015; L.I. Wassenaar, pers. comm.) EC_H_7 and 8 offered uncertainty-quantified, PIT-based calibrations to the OldEC_1_H_1 and OldEC_2_H_1 reference scales, respectively (Soto et al., 2017; L.I. Wassenaar, pers. comm.). EC_H_9 represented certified values for the KHS and CBS secondary standards based on calibration to VSMOW using optimal methods (https://isotopes.usgs.gov/lab/referencematerials/KHS.pdf; https://isotopes.usgs.gov/lab/referencematerials/CBS.pdf; Soto et al., 2017).

The USGS Denver laboratory produced two hair standards designated AK and LA (Alaska and Louisiana bear hair). These were initially calibrated through offline zinc-reduction and dual-inlet analyses against non-matrix-matched secondary standards (DEN_H_1; C. Stricker, pers. comm.). A round-robin experiment in 2005 produced a PIT-based calibration to the OldEC_1_H_1 reference scale (DEN_H_2; C. Stricker, pers. comm.).

In 2005, the SIRFER laboratory (University of Utah) prepared the FH and UH (Florida and Utah horse hair) standards. Seven calibrations exist, including a series of calibrations to primary standards using non-optimal methods (US_H_1-4 and 6; Coplen & Qi, 2012, 2016; Qi & Coplen, 2011). Seven calibrations exist, including a series of calibrations to primary standards using non-optimal methods (US_H_1-4 and 6; Coplen & Qi, 2012, 2016; Qi & Coplen, 2011). In 2012, IsoForensics Inc., introduced the DS and ORX (Dalil sheep and oryx antelope horns) standards to replace FH and UH. Five calibrations for these are available (UT_H_1-5; L.A. Chesson & J.D. Howa, pers. comm.; Table S3). A PIT calibration to the US_H_1 reference scale (see below) was published in 2011 (OldUT_H_2; Coplen & Qi, 2012; Qi & Coplen, 2011). In 2012, IsoForensics Inc., introduced the DS and ORX (Dalil sheep and oryx antelope horns) standards to replace FH and UH. Five calibrations for these are available (UT_H_1-5; L.A. Chesson & J.D. Howa, pers. comm.; J.D. Howa, pers. comm.; Table S3). Each of these is either non-PIT or lacking uncertainty, however, and therefore considered a floating calibration.

In 2011–2012, the USGS Reston isotope laboratory introduced the USGS42 and USGS43 (Tibetan and Indian human hair) standards. Seven calibrations exist, including a series of calibrations to primary standards using non-optimal methods (US_H_4-6; Coplen & Qi, 2012, 2016; Qi & Coplen, 2011; Wassenaar et al., 2015; summarized by Soto et al., 2017) and one using optimal methods (US_H_7; Soto et al., 2017). The certified values for these materials comprise the US_H_6 scale (https://isotopes.usgs.gov/lab/referencematerials/USGS42.pdf; https://isotopes.usgs.gov/lab/referencematerials/USGS43.pdf; Coplen & Qi, 2016), and, although this calibration used non-optimal drying methods, the values obtained are indistinguishable from US_H_7 (Soto et al., 2017) and thus adopted here as the authoritative calibration to VSMOW. USGS42 and USGS43 were also calibrated to the UT_H_2 reference scale (US_H_5; Table S3).

Two human hair standards (AND and CAL-CAN) were prepared at the University of Ottawa G.G. Hatch (now Jan Veizer) stable isotope laboratory in 2011. Four non-PIT calibrations have been presented (CAN_H_1-3 and 5; Coplen & Qi, 2012; Meier-Augenstein et al., 2011). Although CAN_H_5 represents a keratin-to-keratin calibration, the two sets of secondary standards were not uniformly powdered prior to analysis (T.B. Coplen, pers. comm.); therefore, this calibration did not meet PIT requirements. A single PIT-based calibration to the US_H_1 reference scale was reported by Meier-Augenstein et al. (2011; CAN_H_4).


**TABLE 1** Calibrated $\delta^2$H values for most commonly used secondary and primary standards for H isotope analysis of keratins

| Calibration | Standard | $\delta^2$H (mean) | $\delta^2$H (SD) | n | Reference scale | Citation | Methods |
|-------------|----------|--------------------|------------------|---|----------------|----------|---------|
| DEN_H_1     | LA       | −63                |                  |   |                 | C. Stricker (pers. comm.) | Equilibration with waters; Zn-reduction and dual inlet-IRMS; calibration to non-keratin organic standards (PEF-1) |
|             | AK       | −164               |                  |   |                 |          |         |
| DEN_H_2     | LA       | −78.1              | 3                | 29 | OldEC.1_H_1    | C. Stricker (pers. comm.) | Co-analysis with OldEC.1_H_1; TCEA-IRMS; calibration to OldEC.1_H_1 |
|             | AK       | −171.5             | 3.3              | 30 |                 |          |         |
| OldEC.1_H_1 | BWB      | −108               |                  |   | OldEC.1_H_1    | Wassenaar and Hobson (2003) | Equilibration with waters at high temperature; Zn-reduction and dual inlet-IRMS method described by Wassenaar and Hobson (2000); calibration to water standards |
|             | CHS      | −187               |                  |   |                 |          |         |
| OldEC.2_H_1 | BWB      | −108               |                  |   | OldEC.2_H_1    | Wassenaar and Hobson (2003) | Equilibration with waters at high temperature; Zn-reduction and dual inlet-IRMS method described by Wassenaar and Hobson (2000); calibration to water standards |
|             | CFS      | −138               |                  |   |                 |          |         |
| EC_H_1      | KHS      | −37.1              |                  |   | EC_H_1         | Soto et al. (2017) | Equilibration with waters at room temperature for 6 days; dried using different methods, assumed $\varepsilon = 0\%$; TCEA-IRMS with glassy C-filled reactor; calibration to water standards |
|             | CBS      | −166.3             |                  |   |                 |          |         |
| EC_H_2      | KHS      | −41                |                  |   | EC_H_2         | Soto et al. (2017) | Equilibration with waters at 120°C, evacuated using different methods, assumed $\varepsilon = 0\%$ per mil; TCEA-IRMS with glassy C-filled reactor; calibration to water standards |
|             | CBS      | −177.9             |                  |   |                 |          |         |
| EC_H_3      | KHS      | −35.5              |                  |   | EC_H_3         | Soto et al. (2017) | Equilibration with waters at 70°C for 2 hr, evacuated and flushed with He online, assumed $\varepsilon = 80\%$; TCEA-IRMS with glassy C-filled reactor; calibration to water standards |
|             | CBS      | −153.3             |                  |   |                 |          |         |
| EC_H_4      | KHS      | −47.5              |                  |   | EC_H_4         | Soto et al. (2017) | Equilibration with waters at 105°C for 2 hr, evacuated and flushed with He online, assumed $\varepsilon = 80\%$; TCEA-IRMS with glassy C-filled reactor; calibration to water standards |
|             | CBS      | −178.8             |                  |   |                 |          |         |
| EC_H_5      | KHS      | −54.1              |                  |   | EC_H_5         | Soto et al. (2017) | Co-analysis with OldEC.1_H_1; TCEA-IRMS; calibration to OldEC.1_H_1; no uncertainty reported |
|             | CBS      | −197               |                  |   |                 |          |         |
| EC_H_6      | KHS      | −54.1              |                  |   | EC_H_6         | Soto et al. (2017) | Co-analysis with OldEC.2_H_1; TCEA-IRMS; calibration to OldEC.2_H_1; no uncertainty reported |
|             | CBS      | −197               |                  |   |                 |          |         |
| EC_H_7      | KHS      | −54.7              |                  |   | OldEC.1_H_1    | L.I. Wassenaar (pers. comm.) | Co-analysis with OldEC.1_H_1; TCEA-IRMS; calibration to OldEC.1_H_1; data until 2012 |
|             | CBS      | −197.2             |                  | 711|                 |          |         |
|             |          | 4.34              |                  | 689|                 |          |         |
| EC_H_8      | KHS      | −54.7              |                  |   | OldEC.2_H_1    | L.I. Wassenaar (pers. comm.) | Co-analysis with OldEC.2_H_1; TCEA-IRMS; calibration to OldEC.2_H_1; data until 2012 |
|             | CBS      | −197.2             |                  | 711|                 |          |         |
|             |          | 4.34              |                  | 689|                 |          |         |
| EC_H_9*     | KHS      | −35.3              |                  |   | VSMOW_H        | Soto et al. (2017) | Equilibration with waters over a range of temperatures (25–105°C) and drying procedures, assumed $\varepsilon = 0\%$; TCEA-IRMS with Cr-filled reactor; calibration to water standards (optimal method) |
|             | CBS      | −157               |                  | 5 |                 |          |         |
|             |          | 0.9               |                  | 5 |                 |          |         |
| OldUT_H_1   | FH       | −76.3              |                  |   | OldUT_H_1      | Bowen, Chesson, et al. (2005), L.A. Chesson and J.D. Howa (pers. comm.) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (cellulose and PEF-1) |
|             | UH       | −141.6             |                  |   |                 |          |         |
| OldUT_H_2   | FH       | −52.9              |                  | 6 | US_H_1         | Qi and Coplen (2011), Coplen and Qi (2012) | Co-analysis with US_H_1; TCEA-IRMS; calibration to US_H_1 |
|             | UH       | −117               | 1.7              | 6 |                 |          |         |
| OldUT_H_3   | FH       | −58.8              |                  |   | OldUT_H_3      | L.A. Chesson and J.D. Howa (pers. comm.) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (dual inlet calibrated n-C24 and n-C28) |
|             | UH       | −128.7             |                  |   |                 |          |         |

(Continues)
| Calibration | Standard | \(\delta^{2}H\) (mean) | \(\delta^{2}H\) (SD) | n | Reference scale | Citation | Methods |
|-------------|----------|----------------------|-------------------|---|----------------|----------|---------|
| OldUT_H_4   | FH       | -60.2                |                   |   | OldUT_H_4      | Table S3 | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (continuous flow calibrated n-C24 and n-C28) |
|             | UH       | -130.1               |                   |   |                |          |         |
| UT_H_1      | ORX      | -34                  | -172.7            |   | UT_H_1         | L.A. Chesson and J.D. Howa (pers. comm.) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (dual inlet calibrated n-C24 and n-C28) |
|             | DS       |                      |                   |   |                |          |         |
| UT_H_2      | ORX      | -35.4                | -174.1            |   | UT_H_2         | Table S3 | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (continuous flow calibrated n-C24 and n-C28); calibrated at IsoForensics Inc. |
|             | DS       |                      |                   |   |                |          |         |
| UT_H_3      | ORX      | -51.2                | -200              |   | UT_H_3         | J.D. Howa (pers. comm.) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (continuous flow calibrated n-C24 and n-C28) |
|             | DS       |                      |                   |   |                |          |         |
| UT_H_4      | ORX      | -47.2                | -195.9            |   | UT_H_4         | J.D. Howa (pers. comm.) | Co-analysis with EC_H_5; TCEA-IRMS; calibration to EC_H_5; calibrated at UC Davis stable isotope facility; no uncertainty reported |
|             | DS       |                      |                   |   |                |          |         |
| UT_H_5      | ORX      | -43.1                | -191.7            |   | UT_H_5         | J.D. Howa (pers. comm.) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (continuous flow calibrated n-C24 and n-C28) |
|             | DS       |                      |                   |   |                |          |         |
| CAN_H_1     | AND      | -71.6                | -106.8            |   | CAN_H_1        | Meier-Augenstein et al. (2011) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (PEF-1 and KGa-1); calibrated at University of Ottawa Jan Veizer stable isotope laboratory; mean values obtained with different desiccators |
|             | CAL-CAN  |                      |                   |   |                |          |         |
| CAN_H_2     | AND      | -70.6                | -103.2            |   | CAN_H_2        | Meier-Augenstein et al. (2011) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (PEF-1 and KGa-1); calibrated at University of Ottawa Jan Veizer stable isotope laboratory; values obtained with plastic desiccator with lubricant |
|             | CAL-CAN  |                      |                   |   |                |          |         |
| CAN_H_3     | AND      | -72.9                | -105.9            |   | CAN_H_3        | Meier-Augenstein et al. (2011) | Equilibration with waters at room temperature; TCEA-IRMS; calibration to non-keratin organic standards (PEF-1 and coumarin); calibrated at James Hutton Institute stable isotope unit in Dundee (UK); values obtained with glass desiccator evacuated and filled with Sicapent |
|             | CAL-CAN  |                      |                   |   |                |          |         |
| CAN_H_4     | AND      | -70.4                | 1.1               | 10| US_H_1         | Meier-Augenstein et al. (2011) | Equilibrated with waters at high temperature; TCEA-IRMS; calibration to US_H_1; calibrated at USGS Reston stable isotope laboratory |
|             | CAL-CAN  | -109.8               | 0.8               | 10|                |          |         |
| CAN_H_5     | AND      | -67.9                | -103              |   | CAN_H_5        | Qi and Coplen (2011), Coplen and Qi (2012) | Co-analysis with US_H_1; TCEA-IRMS; calibration to US_H_1; PIT not met |
|             | CAL-CAN  |                      |                   |   |                |          |         |
| US_H_1      | USGS43   | -50.3                | -78.5             |   | US_H_1         | Qi and Coplen (2011), Coplen and Qi (2012), Soto et al. (2017) | Equilibration with waters at room temperature for 6 days, evacuated using different methods, assumed \(\varepsilon = 0\%\); TCEA-IRMS with glassy C-filled reactor; calibration to water standards |
|             | USGS42   |                      |                   |   |                |          |         |
| US_H_2      | USGS43   | -58.5                | -88.3             |   | US_H_2         | Qi and Coplen (2011), Soto et al. (2017) | Equilibration with waters at 120°C, evacuated using different methods, assumed \(\varepsilon = 0\%\); TCEA-IRMS with glassy C-filled reactor; calibration to water standards |
|             | USGS42   |                      |                   |   |                |          |         |
| US_H_3      | USGS43   | -48.7                | -75.7             |   | US_H_3         | Wassenaar et al. (2015), Soto et al. (2017) | Equilibration with waters at 70°C for 2 hr, evacuated and flushed with He, assumed \(\varepsilon = 80\%\); TCEA-IRMS with glassy C-filled reactor; calibration to water standards |
|             | USGS42   |                      |                   |   |                |          |         |
A smaller number of secondary standards have been widely used for keratin O isotope analysis (Table 2). The International Atomic Energy Agency (IAEA) prepared two benzoic acid (IAEA-601 and IAEA-602) standards for use in organic O analysis. Values based on a series of calibrations to water primary standards and inorganic secondary standards at numerous laboratories have been published (IAEA_O_1 and 3–8; Brand et al., 2009; Schimmelmann, 2002), and a consensus value has been adopted based on an inter-laboratory average (IAEA_O_2; Brand et al., 2009, 2014). Although we include the benzoic acids here, we note that these are not matrix-matched standards for keratins and are not calibrated to VSMOW following the criteria that we have accepted as optimal. Thus, we discourage their use in keratin analysis and suggest caution in the interpretation of transformations involving these standards.

Several calibrations for the Environment Canada standard materials have been reported based on non-optimal, non-PIT methods (OldUT_O_1; Bowen, Chesson, et al., 2005; L.A. Chesson & J.D. Howa, pers. comm.). They were later PIT calibrated to the US_O_1 reference scale (see below; OldUT_O_2; Coplen & Qi, 2012), and a calibration to the IAEA_O_1 scale was also conducted (OldUT_O_4; Table S4). The ORX and DS standards have been calibrated to the IAEA_O_1 scale at two laboratories (UT_O_2 and 4; J.D. Howa, pers. comm.). Because the UT_O_4 and UT_O_5 calibrations lack uncertainty estimates, we treat them as floating calibrations. Although they reflect neither optimal protocols nor PIT conditions, we accept the OldUT_O_4 and UT_O_2 calibrations as the best available calibrations between the IAEA benzoic acids and keratin secondary standards, and use them for transformations involving the benzoic acid standards.

The FH and UH standards were initially calibrated using non-optimal, non-PIT methods (OldUT_O_1; Bowen, Chesson, et al., 2005; L.A. Chesson & J.D. Howa, pers. comm.). They were later PIT calibrated to the US_O_1 reference scale (see below; OldUT_O_2; Coplen & Qi, 2012), and a calibration to the IAEA_O_1 scale was also conducted (OldUT_O_4; Table S4). The ORX and DS standards have been calibrated to the IAEA_O_1 scale at two laboratories (UT_O_2 and 4; J.D. Howa, pers. comm.), and to the EC_O_11 scale at Texas A&M University (UT_O_5; J.D. Howa, pers. comm.). Because the UT_O_4 and UT_O_5 calibrations lack uncertainty estimates, we treat them as floating calibrations. Although they reflect neither optimal protocols nor PIT conditions, we accept the OldUT_O_4 and UT_O_2 calibrations as the best available calibrations between the IAEA benzoic acids and keratin secondary standards, and use them for transformations involving the benzoic acid standards.

The AND and CAL-SAL standards were calibrated to waters using non-optimal, non-PIT methods (OldUT_O_1; Bowen, Chesson, et al., 2005; L.A. Chesson & J.D. Howa, pers. comm.). They were later PIT calibrated to the US_O_1 reference scale (see below; OldUT_O_2; Coplen & Qi, 2012), and a calibration to the IAEA_O_1 scale was also conducted (OldUT_O_4; Table S4). The ORX and DS standards have been calibrated to the IAEA_O_1 scale at two laboratories (UT_O_2 and 4; J.D. Howa, pers. comm.), and to the EC_O_11 scale at Texas A&M University (UT_O_5; J.D. Howa, pers. comm.). Because the UT_O_4 and UT_O_5 calibrations lack uncertainty estimates, we treat them as floating calibrations. Although they reflect neither optimal protocols nor PIT conditions, we accept the OldUT_O_4 and UT_O_2 calibrations as the best available calibrations between the IAEA benzoic acids and keratin secondary standards, and use them for transformations involving the benzoic acid standards.

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| Calibration | Standards | $\delta^{18}O$ (mean) | $\delta^{18}O$ (SD) | n | Reference scale | Citation | Methods |
|-------------|-----------|----------------------|-------------------|---|----------------|----------|---------|
| OldEC.2.O_2 | BWB       | 12.82                | 0.13              | 3 | VSMOW.O       | Qi et al. (2011) | HTC-IRMS with new GC column; calibration to water standards sealed in silver tubes (optimal method) |
|             | CFS       | 5.24                 | 0.02              | 3 |               |           |         |
| OldEC.3.O_1 | CHS       | 5.59                 |                   |   | OldEC.3.O_1   | Qi et al. (2011) | HTC-IRMS with new GC column, peak detected on mass 28; calibration to water standards sealed in silver tubes |
|             | CFS       | 5.38                 |                   |   |               |           |         |
| OldEC.3.O_2 | CHS       | 5.31                 | 0.19              | 3 | VSMOW.O       | Qi et al. (2011) | HTC-IRMS with new GC column; calibration to water standards sealed in silver tubes (optimal method) |
|             | CFS       | 5.24                 | 0.02              | 3 |               |           |         |
| EC.O_9*     | KHS       | 20.3                 |                   |   | EC.O_9        | Wassenaar and Hobson (2010) | HTC-IRMS with new GC column; calibration to IAEA.O_1; no drying method reported |
|             | CBS       | 3.8                  |                   |   |               |           |         |
| EC.O_10     | KHS       | 21.46                |                   |   | EC.O_10       | Qi et al. (2011) | HTC-IRMS with new GC column, peak detected on mass 28; calibration to water standards sealed in silver tubes |
|             | CBS       | 2.5                  |                   |   |               |           |         |
| EC.O_11     | KHS       | 21.21                | 0.17              | 4 | VSMOW.O       | Qi et al. (2011) | HTC-IRMS with new GC column; calibration to water standards sealed in silver tubes (optimal method) |
|             | CBS       | 2.39                 | 0.13              | 4 |               |           |         |
| OldUT.O_1   | FH        | 14.85                |                   |   | OldUT.O_1     | Bowen, Chesson, et al. (2005), L.A. Chesson and J.D. Howa (pers. comm.) | TCEA-IRMS; calibration to non-keratin organic standards (cellulose and PEF-1) |
|             | UH        | 5.67                 |                   |   |               |           |         |
| OldUT.O_2   | FH        | 13.22                | 0.21              | 6 | VSMOW.O       | Coplen and Qi (2012) | TCEA-IRMS; calibration to US.O_1 |
|             | UH        | 3.49                 | 0.16              | 6 |               |           |         |
| OldUT.O_4   | FH        | 14.67                | 0.27              | 48| IAEA.O_1      | Table S4 | TCEA-IRMS; calibration to IAEA.O_1 |
|             | UH        | 5.35                 | 0.18              | 48|               |           |         |
| UT.O_2      | ORX       | 25.09                | 0.3               | 6 | IAEA.O_1      | Table S4 | TCEA-IRMS; calibration to IAEA.O_1; calibrated at IsoForensics Inc. |
|             | DS        | 6.02                 | 0.24              | 50|               |           |         |
| UT.O_4      | ORX       | 22.82                |                   |   | UT.O_4        | J.D. Howa (pers. comm.) | TCEA-IRMS; calibration to IAEA.O_1; calibrated at UC Davis stable isotope facility |
|             | DS        | 4.66                 |                   |   |               |           |         |
| UT.O_5      | ORX       | 23.45                |                   |   | UT.O_5        | J.D. Howa (pers. comm.) | TCEA-IRMS; calibration to EC.O_7; no uncertainty reported |
|             | DS        | 4.93                 |                   |   |               |           |         |
| CAN.O_6     | AND       | 12.26                |                   |   | CAN.O_6       | Qi et al. (2011) | HTC-IRMS with new GC column, peak detected on mass 28; calibration to water standards sealed in silver tubes |
|             | CAL-SAL   | 5.95                 |                   |   |               |           |         |
| CAN.O_7     | AND       | 12.22                | 0.12              | 3 | VSMOW.O       | Qi et al. (2011) | HTC-IRMS with new GC column; calibration to water standards sealed in silver tubes (optimal method) |
|             | CAL-SAL   | 5.97                 | 0.08              | 3 |               |           |         |
| CAN.O_5     | AND       | 23.45                |                   |   | VSMOW.O       | Coplen and Qi (2012) | TCEA-IRMS; calibration to US.O_1 |
|             | CAL-SAL   | 5.49                 |                   |   |               |           |         |
| US.O_1*     | USGS43    | 14.11                | 0.10              | 18| VSMOW.O       | Qi et al. (2011), Coplen and Qi (2012) | TCEA-IRMS with new GC column; calibration to water standards sealed in silver tubes (optimal method) |
|             | USGS42    | 8.56                 | 0.10              | 18|               |           |         |
| US.O_5      | USGS43    | 15.96                | 0.41              | 44| IAEA.O_1      | Table S4 | TCEA-IRMS; calibrated to UT.O_2 |
|             | USGS42    | 9.94                 | 0.36              | 56|               |           |         |
| US.O_8      | USGS43    | 14.4                 |                   |   | US.O_8        | Qi et al. (2011) | HTC-IRMS with new GC column, peak detected on mass 28; calibration to water standards sealed in silver tubes |
|             | USGS42    | 8.85                 |                   |   |               |           |         |
| IAEA.O_1    | IAEA601   | 23.3                 |                   |   | IAEA.O_1      | Schimmelmann (2002), Brand et al. (2009) | Online (Max Planck Institute for Biogeochemistry) and offline (Indiana University stable isotope research facility) methods; average values |
|             | IAEA602   | 71.4                 |                   |   |               |           |         |
TABLE 2 (Continued)

| Calibration | Standards | $\delta^{18}$O (mean) | $\delta^{18}$O (SD) | $n$ | Reference scale | Citation | Methods |
|-------------|-----------|-----------------------|---------------------|-----|----------------|----------|---------|
| IAEA_O_2    | IAEA601   | 23.14                 |                     | 71.28 |                     | Brand et al. (2009, 2014) | HTC-IRMS methods; calibration to water and barium sulphates standards |
|             | IAEA602   |                       |                     |       |                 |          |         |
| IAEA_O_3    | IAEA601   | 23.02                 |                     | 71.26 |                     | Brand et al. (2009, 2014) | TCEA-IRMS; calibration to water and inorganic standards; calibrated at USGS Reston stable isotope laboratory |
|             | IAEA602   |                       |                     |       |                 |          |         |
| IAEA_O_4    | IAEA601   | 23.14                 |                     | 71.24 |                     | Brand et al. (2009, 2014) | HTC-IRMS; calibration to water and inorganic standards; calibrated at Eldgenoessische Technische Hochschule Zurich stable isotope laboratory |
|             | IAEA602   |                       |                     |       |                 |          |         |
| IAEA_O_5    | IAEA601   | 23.03                 | 1.1                 | 10    | IAEA_O_5        | Brand et al. (2009, 2014) | HTC-IRMS; calibration to water and inorganic standards; calibrated at University of Groningen Centre for Isotope Research |
|             | IAEA602   |                       | 0.8                 |       |                 |          |         |
| IAEA_O_6    | IAEA601   | 22.95                 |                     | 71.01 |                     | Brand et al. (2009, 2014) | HTC-IRMS; calibration to water and inorganic standards; calibrated at Max Planck Institute for Biogeochemistry |
|             | IAEA602   |                       |                     |       |                 |          |         |
| IAEA_O_7    | IAEA601   | 23.43                 |                     | 71.01 |                     | Brand et al. (2009, 2014) | HTC-IRMS; calibration to water and inorganic standards; calibrated at Helmholtz Centre for Environmental Research isotope laboratory |
|             | IAEA602   |                       |                     |       |                 |          |         |
| IAEA_O_8    | IAEA601   | 23.48                 |                     | 71.31 |                     | Brand et al. (2009, 2014) | HTC-IRMS; calibration to water and inorganic standards; calibrated at Australian National University stable isotope laboratory |
|             | IAEA602   |                       |                     |       |                 |          |         |
| VSMOW_O     | VSMOW     | 0                     | VSMOW               | 0     | VSMOW_O         | https://nucleus.iaea.org/rpst/documents/VSMOW_SLAP.pdf |         |
|             | SLAP      |                       | SLAP                | 0     |                 |          |         |

Note: CRM calibrations are noted by *.

2.2 | Secondary standard database

We compiled summary data and methodological information for each secondary standard calibration (Tables 1 and 2), a description of all unpublished calibration data (Table S1; Table 2), and, when available, the raw unpublished calibration data themselves (Tables S3 and S4).

A summary of the calibrations was added as a new object stds within the ASSIGNR r-package (Ma et al., 2020). This list object contains two data frames (hstds and ostds) that record assigned secondary standard values, uncertainty and calibration methods for each calibration. A Ref_scale field records the reference scale to which the assigned values are calibrated (either another scale, or, for a floating calibration, the calibration itself). Two adjacency matrices (ham and oam) record which scales are linked, either because they share a common reference scale or because they represent different assigned values for the same physical materials.

2.3 | Known-origin database

We updated ASSIGNR’s database of known-origin tissue samples by adding additional keratin data and information on sample preparation and analysis. The new knownOrig database consists of three objects.

knownOrig$sources includes attribution and methodological information, where available, for the compiled datasets (see Table S5). Documentation includes (a) sample type (e.g. feather); (b) sampling method (e.g. vane vs. rachis); (c) whether the samples were powdered (Y/N); (d) whether lipids (i.e. surface oils) were extracted (Y/N) and (e) lipid extraction method (e.g. 2:1 chloroform:methanol); (f) whether H exchange was corrected for (Y/N) and (g) the H exchange correction method (multiple waters/comparative equilibration); (h) equilibration temperature (high/ambient); (i) the reference scale originally used for data calibration (e.g. OldEC.1_H_1); (j) whether the standards used for calibration were powdered (Y/N); (k) whether procedures that limit the contribution of adsorbed water to the analyses, either through dedicated sample preparation devices (Soto et al., 2017; Wassenaar et al., 2015) or careful drying and rapid handing of dried samples (e.g. Bowen, Chesson, et al., 2005), were used (Y/N); (l) the analysis method (TCEA/H device, Cr vs. glassy carbon reduction) and (m) the analysis type (H/HO).

knownOrig$sites is a spatial object that records location information for all sample collection sites, including geographical coordinates, site name, state and/or country.

knownOrig$samples is a data frame containing information about taxonomy, age class, material type (e.g. hair) and matrix (e.g. keratin),
and δ²H and/or δ¹⁸O values and uncertainty for each known-origin sample. This object can be linked to the knownOrig$Sources object through the Dataset_ID field and to the knownOrig$Sites object through the Site_ID field. If available, analytical uncertainty is calculated from replicate analyses of the sample itself (we do not distinguish between replicates from a single or multiple analytical runs, as this is often not reported). In most cases, information on sample replicates was not available and we record a generic estimate of reproducibility based on replicate analyses of a quality control material.

2.4 | Data analysis

2.4.1 | Recalibration functions

A new ASSGNR function refTrans identifies and conducts transformations among reference scales based on the above-compiled calibration data. Briefly, a breadth-first algorithm is applied to the H or O adjacency matrix to identify the shortest chain linking the starting and target reference scales. This chain is composed of a sequence of calibrations, each linked to the next through a shared reference scale (i.e. two sets of secondary standards, one calibrated to the other) or a shared set of standards (i.e. two calibrations for the same physical materials). Links involving a single set of standards imply a reassignment of values and addition of uncertainty associated with the new calibration. For each of such links, the algorithm calculates a linear scale transformation relating δ values on the new and old reference scales (Equations 1–3):

\[
d_\text{new} = d_\text{old} \times m_{\text{new-old}} + b_{\text{new-old}}
\]

\[
m_{\text{new-old}} = \frac{SH_{\text{new}} - SL_{\text{new}}}{SH_{\text{old}} - SL_{\text{old}}}, \quad \text{and}
\]

\[
b_{\text{new-old}} = SH_{\text{new}} - SH_{\text{old}} \times m_{\text{new-old}}
\]

where \( m \) and \( b \) refer to the slope and intercept of the transformation and \( SH \) and \( SL \) new and old are the high and low values assigned to the standards in the new and old calibrations, respectively. Uncertainty is propagated by randomly sampling (default = 5,000 times) from normal distributions defined by the means and standard errors of (a) the reported sample values; (b) the standard values for the original reference scale, if they have associated uncertainty and (c) each calibrated standard value in the chain with associated uncertainty; and refitting the transformation equations. Because floating calibrations are self-referenced, they have no associated uncertainty; calibrations that are linked to a second reference scale add uncertainty associated with the underlying cross-calibration.

These functions allow transformations between most reference scales compiled here. We used calibration chains (Figures S3 and S4) to transform all compiled datasets to the VSMOW reference scale for analysis.

2.4.2 | Tissue–water relationships

We compared within-species site-average keratin values for known-origin samples with local precipitation amount-weighted annual average δ²H and δ¹⁸O values extracted from precipitation isoscapes (http://www.waterisotopes.org; Bowen & Revenaugh, 2003; Bowen, Wassenaar, et al., 2005). These values offer a standardized, first-order estimate of spatial variation in local environmental water isotope ratios that can be used to characterize tissue–water relationships across different groups of taxonomically and/or ecologically related species. We derived such relationships using both original and recalibrated keratin data.

2.4.3 | Validation

We validated the quality of the calibration chain transformations using several datasets in which data for the same or related samples were originally reported on different reference scales. These include modern human hair δ²H data reported on the OldUT_H_1 (Ehleringer et al., 2008; Thompson et al., 2010) and CAN_H_1 scales (Bataille et al., 2020; C.P. Bataille, pers. comm.), a collection of Lesser scaup (Aythya affinis) feathers calibrated to the OldEC_H_1 (Hobson et al., 2009) and OldUT_H_1 (G.J. Bowen, pers. comm.) scales, and data from taxonomically and ecologically similar birds reported on several different scales. Our expectation was that values for the scaup samples would be more similar after transformation to a common scale, and that the tissue–water relationship for other ecologically related samples would be more uniform following transformation. For two sample groups (modern humans and ground-foraging non-passerine birds), enough data were available to allow statistical testing. Levene’s test was used to assess whether residual variance from tissue–water regressions was reduced using different regressions for data originally calibrated to different reference scales; the test was repeated for pre- and post-transformation data.

3 | RESULTS

3.1 | Recalibration

3.1.1 | Hydrogen

The compiled known-origin dataset includes 935 human hair and 3,075 bird feather samples analysed for δ²H values. Hair data were originally reported on the OldUT_H_1 or CAN_H_1 reference scales. The majority of the feather data were referenced to the OldEC_H_1 scale, although five other scales were represented (Figure 1). None of the data were reported on scales that were directly traceable to the VSMOW reference scale based on the criteria used here (e.g. EC_H_9 and US_H_6), so all values were shifted during transformation. Most sample values are higher after transformation, but the magnitude of
change varies greatly among scales (Figure 1a). For most datasets, the range of recalibrated values is compressed compared to that of the original data, with the exception of data calibrated to the CAN_H_1 scale. The largest contributors to scale compression are generally the calibrations of the keratins to the VSMOW scale based on optimal methods. The estimated uncertainty of transformed sample values
reflects a combination of the originally reported analytical uncertainty, contraction (or expansion) of the δ²H scale during transformation and uncertainty added within the calibration chain. Reported uncertainties varied widely among datasets, and uncertainties converge somewhat following transformation: δ¹⁸O scale compression drives a reduction in estimated uncertainty for many samples with high reported uncertainty, and the addition of uncertainty from transformation dominates for those with low reported uncertainty (Figure 1b).

3.2 | Oxygen

The compilation contains 358 human hair and 337 bird feather samples analysed for δ¹⁸O values. All hair δ¹⁸O data used the OldUT_O_1 reference scale. Feather δ¹⁸O data were referenced to four different scales, including one dataset using the benzoic-acid-based IAEA_O_1 scale (Figure 2). As for δ²H values, all δ¹⁸O datasets required transformation to be linked to the VSMOW reference scale. Transformed values are somewhat lower than values calibrated to the original scales, but scale compression/expansion during the transformations is more modest and less uniform than for δ²H values (Figure 2a). Estimated uncertainty is slightly increased for most recalibrated datasets, with the exception of those originally reported on the IAEA_O_1 and UT_O_2 scales where scale compression drives small reductions in uncertainty (Figure 2b).

4 | DISCUSSION

4.1 | Validation

Calibration chain transformations leverage cross-calibration of keratin standards to develop scale transformations that are intended to improve data comparability. Examples in which known or presumed relationships exist between data originally calibrated to different scales allow us to test for improved comparability of transformed data and evaluate that improvement relative to other sources of variability among datasets.

We used the method to transform two sets of modern human hair δ²H data originally calibrated to the OldUT_H_1 (Ehleringer et al., 2008; Thompson et al., 2010) and CAN_H_1 (Bataille et al., 2020; C.P. Bataille, pers. comm.) reference scales. Although we expect some regional variation in hair isotope ratios due to dietary differences (Bowen et al., 2009), hair δ²H values are known to correlate strongly with local environmental water values (Ehleringer et al., 2008). Before recalibration, values in the CAN_H_1 dataset, consisting of samples from Canadian residents, were ~8‰ higher, on average, than those calibrated to OldUT_H_1, which included samples from the United States and east Asia (Figure 1a). This pattern is opposite to that expected based on water δ²H values for these regions (Bowen, Wassenaar, et al., 2005), and regression relationships between the two groups of data and local precipitation δ²H values were statistically distinct (Levene’s test p value = 0.05; Figure 3a,b).

After transformation to the VSMOW reference scale, values in the Canadian dataset are lower than those for USA/Asia, as expected (Figure 1a), and the hair–water relationships are no longer distinct (Levene’s test p value = 0.96; Figure 3a,b). This strongly suggests that the originally calibrated data were not comparable, and that the calibration chain transformation eliminates (or greatly reduces) the disparity between these datasets.

We also evaluated transformation effects on multi-species data for two broadly defined ecological guilds of birds. The vast majority of passerine δ²H values in the database were originally calibrated to the OldEC.1_H_1 and EC_H_5 reference scales. However, the compilation contains a dataset for a population of spotted towhees (Pipilo maculatus) from a single site in Utah (Magozzi et al., 2020) that was calibrated to UT_H_2. Both pre- and post-transformation, the towhee site-average value clusters well with other passerine data from environments with similar water δ²H values, but the post-transformation value falls closer to the mean tissue–water relationship for the composite dataset (Figure 3c,d). The same is true for pre- and post-transformation δ¹⁸O data for passerine feathers originally calibrated to IAEA_O_1, UT_O_1 and EC_O_10 scales (Figure 4c,d). These are relatively weak tests in that we lack a firm basis for predicting expected differences between species, but the results are consistent with the idea that calibration chain transformation increases comparability among datasets.

Values of δ²H for ground-foraging non-passerine birds originally calibrated to DEN_H_1 (Wunder et al., 2005; M.B. Wunder, pers. comm.) and OldEC.1_H_1 (Hobson et al., 2004) defined two discrete clusters when plotted against local water values (Figure 3e). Following transformation, the offset between these groups is reduced but not eliminated (Levene’s test p value pre-transformation = 5e⁻⁵; post-transformation = 4e⁻³; Figure 3f). In this case, the residual offset may represent real, ecologically driven differences among taxa, rather than an analytical artefact. The DEN_H_1-calibrated data represent a single species (Charadrius montanus) that occupies dry grasslands with sparse vegetation cover, in which evaporative isotope effects might lead to higher food web δ²H values (e.g. Magozzi et al., 2019) than for the other species represented in the database.

The database includes only one case in which the same samples were calibrated to two different scales. Lesser scap feathers analysed and calibrated to OldEC.1_H_1 (Hobson et al., 2009) were subsequently reanalysed at Purdue University relative to OldUT_H_1 (G.J. Bowen, pers. comm.; the data show a small but consistent (mean = 6.7‰) offset. In this case, the transformation results in almost no relative shift in values (Figure 1a). The BWB secondary standard was analysed alongside the feathers at Purdue, and both the originally calibrated and transformed data show good agreement with its value on the OldEC.1_H_1 scale. This suggests that the original reference scales are themselves closely comparable, and that the small offset in the scap sample data might result from other methodological effects. One possibility may be inaccurate correction for H exchange due to differences in the physical condition (powdered vs. cut) of the samples and standards (Coplen & Qi, 2012) or difference in their amino acid composition. Thus, these data highlight the potential importance of
minor deviations from PIT, which remain common in analytical work with complex organic materials, as an unresolved source of uncertainty in data compilations. Standardization of protocols across laboratories will be needed to reduce or eliminate this uncertainty.

4.2 | Tissue–water relationships

Differences in tissue–water isotope relationships may reflect environmentally or biologically controlled isotope effects during
the routing of elements from precipitation through hydrological and ecological systems to consumer tissues (Hobson et al., 2012; Magozzi et al., 2019; Meehan et al., 2003). Questions about the comparability of data from different studies have previously limited the extent to which cross-group comparisons could be made. Here we explore such comparisons based on the VSMOW-transformed keratin data. We caution that the compiled data are a non-random sample, and comparisons may be complicated by differences in geographical range, body size and other ecophysiological characteristics. Regardless, several patterns consistent with first-order expectations emerge, supporting the idea that differences among groups may be meaningful and, to some degree, predictable.

Slopes of the tissue–water relationships reflect the degree to which geographical differences in water isotope ratios are transferred to local consumers as opposed to being damped by H or O from sources not tied to the local water signature (e.g. atmospheric O2 and stored or transported non-local resources). For δ2H values, the slopes for all avian groups except ground-foraging non-passerine birds (for which weak correlation lowers the OLS regression slope) are similar, and slopes for all bird groups are substantially higher than that for humans (Figure 3). This is consistent with the idea that widespread consumption of non-local food resources dampens the isotopic variability that would otherwise be expected in human hair due to geography (Bowen et al., 2009; Ehleringer et al., 2008). Slope comparisons for δ18O values are complicated by the smaller sample sizes and weaker tissue–water correlations for avian groups, but at minimum suggest that human–avian differences are less apparent than for δ2H values (Figure 4). Values of δ2H for waterbirds are lowest, and for raptors highest, across most of the sampled range (Figure 3). The close association of waterbirds with aquatic systems may reduce evaporation-driven 2H-enrichment in the food webs on which they depend (Hobson et al., 2012 and references therein). In contrast, the longer food chain of raptors involves additional potential for evaporation and dilution of 2H-depleted organic H by relatively 2H-enriched environmental water (Magozzi et al., 2019). Enrichment of 2H in feather keratin in ground-foraging relative to shrub- and canopy-feeding birds, reported by Hobson et al. (2012), is apparent here for mountain plovers but not for other ground-foraging non-passerine species (Figure 3). This may reflect the dry, high elevation habitat occupied by the plovers, characterized by high levels of evapotranspiration. Human hair δ2H values are low to intermediate, and δ18O values quite low, relative to all avian groups (Figures 3 and 4). Dietary effects (dominantly non-aquatic food resources, low to intermediate trophic level) might be expected to give intermediate values for human hair δ2H, whereas the large body size and relatively low rate of evaporative water loss relative to other body water fluxes, plus high drinking water consumption, could contribute to reducing both δ2H and δ18O values of human keratin samples (Kohn, 1996; Magozzi et al., 2019).

5 | CONCLUSIONS

Although keratin δ2H and δ18O data from known-origin biological samples are important in movement ecology research, they are difficult to compare among studies and reuse due to heterogeneity in analytical methods. The calibration chain method introduced here attempts to resolve one major source of heterogeneity by reducing or eliminating differences related to the use of different
secondary standards in data calibration. Comparisons of pre- and post-transformation data show that the method improves comparability, and suggests systematic isotopic differences between groups of organisms that likely reflect differences in isotope routing and fractionation within food webs. The method is implemented in the ASSIGNR r-package, and provides a basis for improved reuse of the database of >4,000 samples included therein and for transformation of user-generated data. The approach developed here could be extended to other matrices and isotope systems in future work.

We emphasize, however, that post-hoc correction such as that introduced here is not ideal, and encourage continued efforts within the research community to increase the availability of suitable standards and adoption of optimal analytical methods. Standard development has thus far been conducted largely by self-organized groups, leading to the diversity of heterogeneity of materials evidenced here. In addition, other methodological factors continue to contribute uncertainty to comparisons among studies. Some have been more thoroughly discussed in other papers (e.g. Soto et al., 2017), and are documented in the ASSIGNR database but not accounted for in calibration chain transformation. To leverage the method developed here and continue to support improvements in the standardization and comparability of organic H and O isotope data, we suggest the following three priorities for the community:

1. Organize and support coordinated efforts to develop, characterize (including regular round-robin comparisons among laboratories) and distribute large amounts of standard materials for keratin and other commonly studied biological materials (e.g. chitin).
2. Conduct analyses in compliance with PIT principles to the maximum extent possible, and, where identical treatment is not possible, adopt technologies and methodologies that are demonstrated to eliminate analytical effects known to impart bias in non-PIT analyses (e.g. Hunsinger & Stern, 2012; Wassenaar et al., 2015).
3. Ensure that all new data reports include essential quality control and methodological information, such as measured weight % H and O, the identity and accepted values of the standards used, and details of sample preparation, handling and drying, needed to assess and conduct post-hoc re-evaluation of the results.

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AUTHORS’ CONTRIBUTIONS
S.M. and G.J.B. conceived the ideas and designed the methodology; S.M. compiled and the data; S.M. and G.J.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/2041-210X.13556.

DATA AVAILABILITY STATEMENT
The ASSIGNR package version 2.0.0 is available on the CRAN repository: https://cran.r-project.org/web/packages/assignR/index.html. The release (Bowen & Ma, 2021) is archived on Zenodo: https://doi.org/10.5281/zenodo.4430639. Previously unpublished non-aggregated δ2H and δ18O data (Tables S3 and S4) are archived on Figshare: https://doi.org/10.6084/m9.figshare.12769928.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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