**Appendix C –** Preparation, Calibration, and Analytical Uncertainty for Isotopic Measurements

To prepare samples for analysis, we crushed bone into 1-2 mm fragments, sonicated with ultrapure water for 30 minutes, and used a modified Longin (1971) method to extract collagen. We soaked bone in 5 ml of 0.5M hydrochloric acid (HCl), refrigerated at 4o C to demineralize, rinsed with ultrapure water, and soaked in 5 ml of sodium hydroxide (NaOH) to remove humic contaminants. We rinsed samples and extracted lipids using a 1:2:.0.8 (v/v) ratio of CHCl3(chloroform), CH3OH (methanol), and ultrapure water. We then refluxed samples in a dry bath with 0.01M HCl for 16 hours. We then froze samples and lyophilized collagen prior to shipment for analysis.

Carbon and nitrogen isotopic and elemental compositions were determined using a Thermo DeltaPlus XP coupled to a Costech 4010 Elemental Analyzer in the Yale Analytical Stable Isotope Center (YASIC) of Yale University. Stable carbon isotopic compositions are expressed as delta values relative to VPDB (Vienna Peedee belemnite) on a scale normalized such that the δ13C values of NBS 19 calcium carbonate and L-SVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively. Stable nitrogen isotopic compositions are expressed relative to atmospheric nitrogen, which is isotopically homogenous. Our in-house glycine standards (glycine low and glycine high) were characterized using USGS40 and USGS41 reference material (Table A1). Glycine low and glycine high were used as scale anchors in every analytical session (Table A1). Stable isotope ratios are reported in per mil () notation, e.g., *δ*13C = (((13C/12C)/(13C/12Cstandard)) – 1) x 1000 (Coplen, 1994).

**Table A1.** Standard reference materials used for calibration of *δ*13C relative to VPDB and *δ*15N relative to AIR.

|  |  |  |  |
| --- | --- | --- | --- |
| Standard | Material | Accepted *δ*13C  (‰, VPDB) | Accepted *δ*15N  (‰, AIR) |
| USGS40 | Glutamic Acid | –26.39±0.04 | –4.52±0.06 |
| USGS41 | Glutamic Acid | +37.63±0.05 | +47.57±0.11 |
| YGA | Yale Glutamic Acid | +31.02±0.22 | +31.80±0.17 |
| CN2 | Glutamic Acid 2 | –28.32±0.16 | –4.52±0.12 |

The following standards were used to monitor analytical uncertainty (Table A2). The isotopic compositions reported here for internal standards represent long term averages calibrated to VPDB and AIR with USGS40 and USGS41.

**Table A2.** Standard reference materials used for to monitor internal accuracy and precision.

|  |  |  |  |
| --- | --- | --- | --- |
| Standard | Material | Mean *δ*13C  (‰, VPDB) | Mean *δ*15N  (‰, AIR) |
| Cocoa | Cocoa | −29.11±0.02 | +5.34±0.20 |
| Trout | Tout | −28.97±0.02 | +15.78±0.10 |

Table A3 presents the means and standard deviations of the *δ*13C and *δ*15N values for the check and calibration standards (standard deviations only) as well as the number of standards included in each analytical session. Of the basis of the check and calibration standards, measurement precision (the pooled standard deviation of the check and calibration standards) was ±0.26 ‰ for *δ*13C and ±0.01 ‰ for *δ*15N (*df*=33). Measurement accuracy (bias) was evaluated by comparing the known and measured *δ*13C and *δ*15N values for trout and cocoa and factoring in the long-term uncertainty in these known measurements following the methods outlined by Szpak et al. (2017). Measurement bias due to systematic error (accuracy) was determined to be ±0.34 ‰ for *δ*13C and ±0.17 ‰ for *δ*15N.

Table A3. Mean and standard deviation of all check and calibration standards for all analytical sessions containing data presented in this paper.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Session ID** | **Standard** | **n** | ***δ*13C (‰, VPDB)** | | | ***δ*15N (‰, AIR)** | | |
| Session 19-125 | CN2 | 6 | −28.32 | ± | 0.10 | −4.52 | ± | 0.12 |
| Session 19-125 | YGA | 6 | +31.02 | ± | 0.04 | +31.80 | ± | 0.12 |
| Session 19-125 | Trout | 7 | −29.14 | ± | 0.15 | +15.77 | ± | 0.03 |
| Session 17-192 | CN2 | 5 | −28.13 | ± | 0.14 | −4.52 | ± | 0.11 |
| Session 17-192 | YGA | 4 | +31.80 | ± | 0.08 | +31.80 | ± | 0.08 |
| Session 17-192 | Cocoa | 6 | −28.96 | ± | 0.13 | +5.34 | ± | 0.08 |
| Session 17-192 | Trout | 6 | −29.11 | ± | 0.15 | +15.77 | ± | 0.03 |

No samples were analyzed as replicates due to the paucity of collagen extracted from each sample. Therefore, standard uncertainty could not be calculated.

**References**

Longin, R., 1971. New method of collagen extraction for radiocarbon dating, Nature 230, 241-242.

Szpak, P., Metcalfe, J.Z., Macdonald, R.A., 2017. Best practices for calibrating and reporting stable isotope measurements in archaeology, Journal of Archaeological Science: Reports 13, 609-616.