**Appendix E −** Calibration and Analytical Uncertainty for Isotopic Measurements

Carbon and nitrogen isotopic and elemental compositions were determined using Thermo Delta V mass spectrometer coupled to a Costech ECS 4010 elemental analyzer in Lab Y (University X). Stable carbon and nitrogen isotope compositions were calibrated relative to VPDB (*δ*13C) and AIR (*δ*15N) using USGS40 and USGS41 (Table S1; Qi*, et al.*, 2003).

**Table S1.** 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 | −26.389 | −4.52 |
| USGS41 | Glutamic Acid | +37.626 | +47.57 |

The following standards were used to monitor analytical uncertainty (Table S2). The isotopic compositions reported here for internal standards represent long term averages calibrated to VPDB and AIR with USGS40 and USGS41.

**Table S3.** Standard reference materials used for to monitor internal accuracy and precision.

|  |  |  |  |
| --- | --- | --- | --- |
| Standard | Material | Mean *δ*13C  (‰, VPDB) | Mean *δ*15N  (‰, AIR) |
| IRM-1 | Deer bone collagen | −19.28±0.07 | +1.79±0.11 |
| IRM-2 | Sea lion bone collagen | −11.59±0.08 | +18.02±0.06 |
| IRM-3 | Cow bone collagen | −15.30±0.07 | +9.62±0.10 |

Table S4 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 ±0.14 ‰ for *δ*13C and ±0.18 ‰ for *δ*15N (*df*=62). Measurement accuracy (bias) was evaluated by comparing the known and measured *δ*13C and *δ*15N values for IRM-1, IRM-2, and IRM-3 and factoring in the long-term uncertainty in these known measurements following (Insert reference here). Measurement bias due to systematic error (accuracy) was determined to be ±0.11 ‰ for *δ*13C and ±0.10 ‰ for *δ*15N.

Table S4. 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 1 | USGS40 | 8 |  | ± | 0.04 |  | ± | 0.04 |
| Session 2 | USGS40 | 8 |  | ± | 0.04 |  | ± | 0.10 |
| Session 1 | USGS41 | 8 |  | ± | 0.05 |  | ± | 0.20 |
| Session 2 | USGS41 | 8 |  | ± | 0.05 |  | ± | 0.20 |
| Session 1 | IRM-1 | 6 | -19.34 | ± | 0.02 | 1.72 | ± | 0.10 |
| Session 2 | IRM-1 | 6 | -19.20 | ± | 0.08 | 1.74 | ± | 0.25 |
| Session 1 | IRM-2 | 6 | -11.63 | ± | 0.05 | 17.94 | ± | 0.17 |
| Session 2 | IRM-2 | 6 | -11.44 | ± | 0.20 | 18.01 | ± | 0.28 |
| Session 1 | IRM-3 | 8 | -15.27 | ± | 0.19 | 9.62 | ± | 0.16 |
| Session 2 | IRM-3 | 8 | -15.24 | ± | 0.05 | 9.65 | ± | 0.30 |

Ten percent of the samples were analyzed in duplicate (18/176), the results of which are presented in Table S5. The measurement precision specific to the samples (the pooled standard deviation of all samples analyzed in duplicate) was ±0.14 ‰ for *δ*13C and *δ*15N (*df*=18).

Table S5. Stable carbon and nitrogen isotopic compositions for all samples analyzed in duplicate. “A” and “B” correspond to Session 1 and Session 2, respectively.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample ID** | ***δ*13CA** | ***δ*13CB** | ***δ*15NA** | ***δ*15NB** |
| 1008 | -19.36 | -19.52 | 4.15 | 4.12 |
| 1019 | -16.98 | -16.76 | 9.10 | 8.87 |
| 1028 | -17.83 | -17.60 | 8.27 | 8.11 |
| 1038 | -16.94 | -16.90 | 8.04 | 7.91 |
| 1048 | -18.03 | -18.08 | 9.90 | 9.74 |
| 1058 | -18.30 | -18.21 | 10.20 | 10.08 |
| 1068 | -17.19 | -17.34 | 10.63 | 10.88 |
| 1078 | -17.38 | -17.62 | 8.40 | 8.25 |
| 1088 | -18.89 | -18.70 | 4.47 | 4.64 |
| 1102 | -18.73 | -18.75 | 9.35 | 9.45 |
| 1104 | -15.65 | -16.00 | 10.11 | 10.45 |
| 1108 | -18.78 | -18.66 | 7.61 | 7.65 |
| 1112 | -16.88 | -17.12 | 8.55 | 8.60 |
| 1116 | -21.03 | -21.36 | 8.20 | 8.12 |
| 1122 | -20.76 | -20.50 | 8.43 | 8.35 |
| 1154 | -18.77 | -19.01 | 11.05 | 10.97 |
| 1188 | -17.86 | -17.93 | 8.53 | 9.03 |
| 1196 | -20.22 | -20.05 | 9.58 | 9.90 |

**Standard Uncertainty**

Standard uncertainty for the *δ*13C and *δ*15N measurements of the samples (*us*) was estimated following (Insert appropriate reference) and was determined to be ±0.20 ‰ for *δ*13C and ±0.24 ‰ for *δ*15N.

**References**

Qi H, Coplen TB, Geilmann H, Brand WA, Böhlke JK. 2003. Two new organic reference materials for δ13C and δ15N measurements and a new value for the δ13C of NBS 22 oil. *Rapid Communications in Mass Spectrometry* **17**: 2483-2487.