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Validation of dried blood spot sampling for determining trophic positions of Arctic char using nitrogen stable isotope analyses of amino acids

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Abstract

Rationale: Dried blood spots (DBSs) are gaining popularity for biomarker analyses in ecological research due to their advantages for use in field-based research and in remote settings, however, many DBS biomarkers remain unvalidated. We validated the application of compound-specific stable nitrogen isotope analyses of amino acids (CSIA-AAs) to field-prepared DBSs for determining trophic positions of wild-caught Arctic char (*Salvelinus alpinus*).

Methods: Whole blood and muscle from Arctic char were collected and DBSs were created in the field. We measured the stable nitrogen isotope ratios (expressed as $\delta^{15}\text{N}$ values) of the amino acids glutamic acid (Glu) and phenylalanine (Phe) isolated from Arctic char samples using CSIA-AAs. We then compared amino acid $\delta^{15}\text{N}$ values from DBSs and the other sample types (whole blood and muscle) from the same specimens. We calculated and compared trophic position estimates generated from whole blood, DBSs, and muscle.

Results: The $\delta^{15}\text{N}$ values of Glu and Phe, as well as trophic position estimates from DBSs were highly correlated with $\delta^{15}\text{N}$ values and estimates from both whole blood and muscle. DBS amino acid $\delta^{15}\text{N}$ values and trophic position estimates agreed well with those from whole blood. Although, mean differences between amino acid $\delta^{15}\text{N}$ values from DBSs and muscle were noted, the offsets were small and resulted in a 0.2 mean difference between trophic position estimates for DBSs and muscle.

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Conclusions: We demonstrate that the application of CSIA-AAs to field-prepared DBSs of Arctic char generates similar trophic position estimates compared to whole blood and muscle. We suggest that DBSs could be developed as a minimally invasive sampling technique to study feeding ecology of wild fish and perhaps other organisms of interest.

1 INTRODUCTION

There is considerable interest in characterizing the structure and dynamics of aquatic food webs through the use of naturally occurring stable isotope ratios of carbon (expressed as $\delta^{13}\text{C}$ values) and nitrogen (expressed as $\delta^{15}\text{N}$ values)^{1,2}. Ecologists typically apply stable isotope analyses to bulk (total organic carbon and total nitrogen) tissues to infer basal carbon sources and trophic positions of organisms of interest¹⁻³. In particular, the accurate estimation of an organism's trophic level, or position in a food web, is critical for assessing biomass pathways, trophic linkages, and competition in aquatic ecosystems^{2,4}. The use of bulk $\delta^{15}\text{N}$ values to infer an organism's trophic position is based on the predictable stepwise increase in $\delta^{15}\text{N}$ values with increasing trophic level².

Despite the practicality and extensive use of bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in ecological research, a variety of factors can complicate interpretations of bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data, including the influences of multiple primary production sources, trophic transfer, microbial degradation, and spatial variation in isotopic baselines^{2,5,6}. The latter poses a particular challenge as bulk $\delta^{15}\text{N}$ values of aquatic consumers (e.g., fish) alone are not typically sufficient for accurately estimating trophic positions without appropriate baseline $\delta^{15}\text{N}$ values². Defining the appropriate isotopic baseline has been identified as one of the greatest challenges for applying bulk stable nitrogen isotope analyses to multiple food webs because baseline $\delta^{15}\text{N}$ values can vary considerably within and among aquatic ecosystems².

Compound-specific isotope analyses of individual amino acids (CSIA-AAs) is a relatively new approach that helps to sidestep some of the complications related to bulk stable carbon and nitrogen isotope analyses^{4,5-7}. CSIA applied to $\delta^{13}\text{C}$ values of essential amino acids (EAAs), termed "isotope fingerprinting", can be used to quantify and discriminate a wider range of basal carbon sources compared with traditional bulk $\delta^{13}\text{C}$ values^{6,7}. Additionally, the differential fractionation of nitrogen stable isotopes of individual amino acids can be used to determine both the baseline $\delta^{15}\text{N}$ value of an organism's food web, as well as its relative trophic position^{5,8,9}. Specifically, the $\delta^{15}\text{N}$ value of phenylalanine (Phe) does not increase significantly (<1‰) relative to diet during trophic transfer providing an indicator of the $\delta^{15}\text{N}$ value of the base of the food web^{5,10}. In contrast, the $\delta^{15}\text{N}$ value of glutamic acid (Glu) increases significantly (~8‰) relative to diet during trophic transfer⁵. Thus, the difference between the $\delta^{15}\text{N}$ values of Glu and Phe can be used to estimate an organism's trophic position relative to an internally indexed baseline value^{5,10,11}. Therefore, the collection and analysis of potential baseline candidates may not be required for nitrogen CSIA-AA analyses and trophic position estimation because the isotopic baseline is archived within the consumer of interest⁵. Although CSIA-AA has the potential to reduce difficulties associated with bulk nitrogen stable isotope analyses, the interpretation of CSIA-AA results needs to take into account the findings from some of the more recent studies indicating that trophic enrichment factors can vary substantially among taxa and ecosystems for both source

(e.g., Phe) and trophic (e.g., Glu) AAs^{4,9,12,13}. Additional research is required to account for sources of variation in CSIA-AA studies, including those that compare results from various tissues.

For fish, $\delta^{15}\text{N}$ values are typically analysed from samples of dorsal muscle samples obtained via lethal sampling¹⁴. However, recent studies have explored the use of non-lethal sampling techniques as an alternative method for fish tissue collection for bulk stable isotope analyses. Alternatives include sampling of muscle biopsies¹⁵, fin clips¹⁴⁻¹⁷, scales^{18,19}, and skin mucus^{19,20}. Dried blood spot (DBS) sampling, which involves the collection of a small volume of blood onto a cellulose paper card²¹, also shows great promise as a non-lethal sampling technique for fish^{22,23} and other wildlife^{24,25}. Recently, we demonstrated that CSIA of $\delta^{13}\text{C}$ values from EAAs isolated from DBSs could be used to “fingerprint” dietary carbon sources of landlocked Arctic char (*Salvelinus alpinus*) collected in the Canadian High Arctic²³. As DBS sampling is low-cost and can reduce the logistical and financial challenges of sampling in resource limited settings²¹, the use of this sampling strategy is ideal for the Arctic and other remote regions with limited research infrastructure.

Here we build upon our previous work with CSIA of $\delta^{13}\text{C}$ values from EAAs by describing and validating the use of CSIA of $\delta^{15}\text{N}$ values from field-prepared DBSs for estimating the trophic positions of landlocked Arctic char. Specifically, we measured and compared $\delta^{15}\text{N}$ values of the amino acids Glu and Phe from DBSs, whole blood, and muscle of landlocked Arctic char. We also compared trophic position estimates generated from each of these sample types in order to assess whether DBS sampling may be developed as a non-lethal sampling technique for fish and possibly other wildlife.

2 EXPERIMENTAL

2.1 Arctic char collection and dried blood spot preparation

Landlocked Arctic char collections and DBS preparation have been described in detail elsewhere²³. Briefly, landlocked Arctic char were collected from five lakes (Meretta, Small, Amituk, Resolute, and Char; n=3 fish per lake) in the Canadian High Arctic (Cornwallis Island, Nunavut). Axial muscle samples were collected from each fish. Whole blood was collected from the caudal vein of each fish and immediately transferred to Vacutainers®. DBSs were created by pipetting 40 μL samples of whole blood within the circles of cellulose-based filter paper cards (Whatman 903™ Protein Saver Cards, GE Healthcare Services, Mississauga, ON, Canada). Cards were allowed to air-dry lakeside for ~1 to 2 hr before being placed in individual plastic bags with desiccant packs. Axial muscle and remaining whole blood samples were maintained at -20°C . DBS cards were transported and stored at ambient temperature. The storage time before analyses for tissues and DBS cards was approximately 1 yr. Fish weights and total lengths are provided in the Supporting Information (Table S1). In the laboratory, axial muscle and whole blood samples were freeze-dried in preparation for GC-C-IRMS analyses.

2.2 Compound-specific stable nitrogen isotope analyses of amino acids

For CSIA-AA analyses, we used about one quarter of a DBS (equivalent to ~10 μL of whole blood sample) and approximately 2 mg of freeze-dried whole blood and muscle from each fish. Amino acids were converted to N-acetyl methyl ester derivatives²⁶ in the Alaska Stable Isotope Facility at the University of Alaska Fairbanks. We described the derivatization procedure of Arctic char samples in greater detail in our previous work²³. Briefly, whole blood, DBSs, and muscle samples were hydrolyzed with 6 N HCl at 100°C for 20 h. Next, 15 μL norleucine (nLeu) (6.6 mg/mL) was added as an internal standard to all samples and a liquid-liquid extraction was performed using a mixture of hexane and dichloromethane. Amino acids were methylated, acetylated, and then washed with a mixture of potassium phosphate buffer and chloroform. Residual amino acids from the organic layer were reconstituted in ethyl acetate and caffeine was added as an internal standard. Samples were analyzed using a TRACE 1310 gas chromatograph coupled via a GC Isolink II Conversion Unit and a ConfloIV universal continuous flow interface to a Delta V Plus IRMS system (Thermo Scientific, Bremen, Germany). An inline cryogenic trap was used to prevent CO_2 delivery to the IRMS system. Samples were injected using a Thermo Scientific Triplus RSH autosampler into a Restek liner (4 $\times$ 6.5 $\times$ 78.5 mm; double gooseneck splitless; hold at 280°C for 2 min) (Thermo Scientific, Bremen, Germany). Amino acids were separated using a VF-35ms column (30m $\times$ 0.32mm $\times$ 1 μm ; Agilent Technologies, Santa Clara, California). The GC temperature program consisted of: 80 °C (hold 2 min), increase to 135 °C at a rate of 20 °C/min, increase to 160 °C at a rate of 5 °C/min (hold 3 min), and increase to 300 °C at a rate of 8 °C/min (hold 3 min). The fish samples used in this study were derivatized in six separate batches. Fish were randomized across batches, however whole blood, DBSs, and muscle from an individual fish were derivatized and analyzed in a single batch to minimize any batch potential effect. A mixed amino acid standard also containing Glu, Phe, leucine (Leu), isoleucine (Ile), proline (Pro), and caffeine (all with known $\delta^{15}\text{N}$ values) was derivatized and analyzed with each batch. Acid blanks (n=3) and DBS blanks (n=3; portions of filter paper without blood from cards used in the field) were also derivatized and analyzed. All derivatized samples were analyzed by GC-C-IRMS in triplicate. Stable nitrogen isotope values of amino acids are expressed in delta ($\delta^{15}\text{N}$) notation relative to the isotopic ratio of an international standard (atmospheric air) in parts per thousand. Delta values are defined as $\delta X = (\text{R sample}/\text{R standard} - 1)$, where X is ^{15}N and R is the ratio of heavy to light isotope ($^{15}\text{N}/^{14}\text{N}$).

2.3 Data handling and statistical analyses

Initially, we used data from the triplicate injections to calculate means and standard deviations of $\delta^{15}\text{N}$ values for Glu, Phe, Leu, Ile, Pro, and caffeine from the mixed amino acid standard. For the standards, we specifically targeted Glu and Phe as these are the amino acids most commonly used for trophic position estimations⁵. We also report $\delta^{15}\text{N}$ values for Leu, Ile, and Pro from the standard as these amino acids represented a larger range of known $\delta^{15}\text{N}$ values (8.5, -3.0, and -6.5 ‰, respectively) relative to Glu (-3.1 ‰), Phe (3.8 ‰), or caffeine (-3.3 ‰), thus allowing us to judge precision and accuracy across a larger range of $\delta^{15}\text{N}$ values. We then used the deviation between the measured and expected $\delta^{15}\text{N}$ value of the internal standard caffeine (expected $\delta^{15}\text{N}$ value of -3.3 ‰) to correct the measured $\delta^{15}\text{N}$ values of targeted amino acids for each injection. We then recalculated caffeine-corrected

mean and standard deviation $\delta^{15}\text{N}$ values for Glu, Phe, Leu, Ile, and Pro from the mixed amino acid standards. We compared both mean uncorrected and caffeine-corrected $\delta^{15}\text{N}$ values to known $\delta^{15}\text{N}$ values for each of the compounds and report the differences () in per mil. . We also plotted mean measured versus expected $\delta^{15}\text{N}$ values and generated ordinary least-squares equations for both uncorrected and corrected $\delta^{15}\text{N}$ values for the standards. For the Arctic char samples, we generated caffeine-corrected mean and standard deviation $\delta^{15}\text{N}$ values of triplicate injections for Glu, Phe, Leu, Ile, and Pro. However, we present and discuss only Glu and Phe in the main text, whereas Leu, Ile, and Pro are presented in the Supporting Information.

Trophic position estimates and statistical analyses were performed using mean caffeine-corrected $\delta^{15}\text{N}$ values of Glu and Phe. We calculated individual Arctic char trophic positions using a previously reported equation⁵ :

$$[\text{Trophic Position}]_{\text{aquatic}} = [(\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}} - 3.4) / 7.6] + 1$$

The distributions of mean $\delta^{15}\text{N}$ values of Glu and Phe and trophic position estimates from whole blood, DBSs, and muscle were initially observed in bivariate scatterplots and tested by simple correlation (Pearson's r). If this indicated a possible relationship, regressions were tested with ordinary least-squares equations when the required assumptions (normality and homoscedasticity of residuals) were satisfied. Normality of residuals was tested with the Shapiro-Wilks test and constant variance was assessed by visual inspection of residual/predicted plots. To better understand differences in agreement, Bland-Altman plots were generated to compare amino acid $\delta^{15}\text{N}$ values and trophic position estimates between DBSs and the other sample types. The Bland-Altman approach involves plotting the difference of the two paired measures (DBS - whole blood or DBS - muscle) against the mean of the two measurements²⁷. A mean difference (or bias), a 95 % confidence interval around the mean difference, and a 95 % confidence interval for the differences were calculated to help assess the significance of any potential disagreements between measurements.

RESULTS AND DISCUSSION

3.1 Quality control

In the amino acid standard, measured $\delta^{15}\text{N}$ values of Glu, Phe, Leu, Ile, Pro, and caffeine averaged $-3.5\text{‰} \pm 0.6$, $3.2\text{‰} \pm 0.5$, $8.0\text{‰} \pm 0.8$, $-3.5\text{‰} \pm 0.5$, $-7.2\text{‰} \pm 0.5$, and $-3.6\text{‰} \pm 0.5$, respectively. The associated differences () from expected $\delta^{15}\text{N}$ values for these same compounds were 0.4, 0.6, 0.5, 0.5, 0.7, and 0.3 ‰, respectively. For the same standard, caffeine-corrected Glu, Phe, Leu, Ile, Pro $\delta^{15}\text{N}$ values averaged $-3.3\text{‰} \pm 0.8$, $3.5\text{‰} \pm 0.7$, $8.3\text{‰} \pm 1.1$, $-3.2\text{‰} \pm 0.7$, and $-6.9\text{‰} \pm 0.7$, respectively. The differences () between caffeine-corrected and expected $\delta^{15}\text{N}$ values of Glu, Phe, Leu, Ile, and Pro in the amino acid standard, were 0.2, 0.3, 0.2, 0.2, and 0.4 ‰, respectively. The measured and expected $\delta^{15}\text{N}$ values were highly correlated for both uncorrected ($r^2=1.0$, slope=0.99) and corrected values ($r^2=1.0$, slope=1.0) (Figure S1). Collectively these results indicate acceptable accuracy and precision of measured $\delta^{15}\text{N}$ values for both amino acids and the caffeine standard. As the differences from expected $\delta^{15}\text{N}$ values were slightly lower for caffeine-corrected values for

the five amino acids, we chose to also apply the correction to the various Arctic char samples prior to further statistical analysis. Note that because it is taken through the entire derivatization procedure, nLeu may be a more suitable candidate for performing the type of correction we conducted herein. However, nLeu was added to samples but inadvertently not the amino acid standards. Therefore, we chose to use caffeine for correcting $\delta^{15}\text{N}$ values. Future work could also use scale-normalization to improve the accuracy and precision of amino acid $\delta^{15}\text{N}$ values, which has been shown to decrease the analytical error associated with CSIA-AA²⁸. However, despite these future opportunities to decrease analytical error we are still able to illustrate very strong relationships between $\delta^{15}\text{N}$ values of amino acids from DBSs and whole blood and the main findings from our study remain valid.

For all Arctic char samples, the uncorrected and caffeine-corrected $\delta^{15}\text{N}$ values for the internal standard nLeu were similar ($18.6\text{‰} \pm 0.6$ and $18.8\text{‰} \pm 0.8$, respectively), and both values were similar ($< 1\text{‰}$ difference) to the expected value (19.3‰). The precision associated with measuring the $\delta^{15}\text{N}$ values of Glu and Phe were similar across Arctic char sample types. The averages of the standard deviations for Glu were 0.6, 0.7, and 0.5 ‰ for all triplicate measures of Arctic char whole blood, DBSs, and muscle, respectively. For Phe, the average standard deviation was 0.6 ‰ for all types of Arctic char samples. The relatively low variability in replicate measures indicates excellent analytical precision across sample types. No amino acid peaks were detected in either the blank filter paper or acid blank samples, indicating that there was no amino acid contamination during either field preparation of DBSs or during the hydrolysis and derivatization procedure. Moreover, these results indicate that the cellulose-based filter paper cards did not contribute amino acids during the amino acid isolation procedure.

3.3 $\delta^{15}\text{N}$ values of amino acids and trophic position estimates from whole blood, DBSs, and muscle of Arctic char

Mean $\delta^{15}\text{N}$ values of Glu, Phe, Leu, Ile, and Pro from all of the Arctic char samples are provided in the Supporting Information (Table S2). A representative chromatogram for an Arctic char sample is provided in the Supporting Information (Figure S2). For all Arctic char, we noted similar ranges in Glu $\delta^{15}\text{N}$ values for whole blood (19.7 to 26.7‰), DBSs (19.5 to 26.7‰), and muscle (20.0 to 28.7‰). The ranges of Phe $\delta^{15}\text{N}$ values were also similar for Arctic char whole blood (-1.0 to 3.2‰), DBSs (-0.9 to 3.4‰), and muscle (-1.4 to 3.3‰). We present comparisons between amino acid $\delta^{15}\text{N}$ values from DBSs and the other sample types in Figures 1 and 2. The $\delta^{15}\text{N}$ values of Glu from DBSs were highly correlated with Glu $\delta^{15}\text{N}$ values from both whole blood ($r^2 = 0.93$, $p < 0.0001$) and muscle ($r^2 = 0.94$, $p < 0.0001$). Similarly, the $\delta^{15}\text{N}$ values of Phe from DBSs were highly correlated with Phe $\delta^{15}\text{N}$ values from both whole blood ($r^2 = 0.91$, $p < 0.0001$) and muscle ($r^2 = 0.82$, $p < 0.0001$). The slopes of these lines varied from 0.89 to 1.1, indicating near 1:1 relationships between DBS $\delta^{15}\text{N}$ values and those of whole blood and muscle.

We further assessed the agreement between DBS amino acid $\delta^{15}\text{N}$ values and those from whole blood and muscle by constructing Bland-Altman plots, which reveal the differences in two measures relative to their averages²⁷. There was negligible bias between Glu $\delta^{15}\text{N}$ values from DBSs and whole blood (mean difference = -0.005‰). The 95 % confidence

interval for this bias includes 0, indicating that there was no significant difference between the two measurements. Moreover, almost all of the data points fell within the 95 % confidence interval and their variability was consistent across their average values (Figure 1B). In comparison, the bias between Phe $\delta^{15}\text{N}$ values from DBSs and whole blood was slightly greater (mean difference = -0.2‰), though this difference was still quite low especially when compared to analytical precision. Most of the differences in DBS and whole blood Phe $\delta^{15}\text{N}$ values were within the 95% confidence interval and there was no relation between these differences and their average values (Figure 1D). We observed modest biases between amino acid $\delta^{15}\text{N}$ values from DBSs and muscle for both Glu and Phe. On average, Glu $\delta^{15}\text{N}$ values were 0.7‰ higher and Phe $\delta^{15}\text{N}$ values were 0.8‰ lower in muscle than in DBSs. Similar to other comparisons, most of the differences in amino acid $\delta^{15}\text{N}$ values were within the 95 % confidence intervals and these differences were consistent across average values (Figures 2B and 2D).

The individual Arctic char trophic position estimates produced from the various sample types are provided in the Supporting Information (Table S3). For all Arctic char, the ranges in trophic position estimates were similar for whole blood (3.1 to 3.7), DBSs (3.0 to 3.9), and muscle (3.2 to 4.2). We present comparisons of trophic position estimates from DBSs with whole blood and muscle in Figure 3. The DBS trophic position estimates were highly correlated with estimates from both whole blood ($r^2 = 0.81$, $p < 0.0001$) and muscle ($r^2 = 0.90$, $p < 0.0001$) (Figure 3). We observed a small bias between trophic position estimates produced from DBSs and whole blood, however the value was relatively low (0.03) and the 95 % confidence interval for this value also captured 0 indicating that the two measures have a high level of agreement (Figure 3B). Conversely, trophic position estimates from muscle were on average 0.2 higher than estimates from DBSs and the 95 % confidence interval around this bias did not include 0. This indicates that there is likely a mean difference in muscle trophic position estimates compared to those generated from DBSs (Figure 3D). Similar to the amino acid $\delta^{15}\text{N}$ values, most of the differences in trophic positions estimates fell between the 95 % confidence interval and the level of dispersion was not a function of the absolute isotopic composition.

The tight correlations and the negligible biases between amino acid $\delta^{15}\text{N}$ values from DBSs and whole blood demonstrate that DBS sampling is an appropriate means for collecting fish blood for amino acid-specific stable nitrogen isotope analyses. The drying, transport, and storage processes did not alter the isotopic composition of the amino acids or change their ecological interpretation, as evidenced by the similar $\delta^{15}\text{N}$ values and trophic position estimates from DBSs and whole blood. Moreover, the cellulose filter paper cards did not alter or interfere with these analyses. An important assumption of DBS sampling is that blood and biomarkers are stable on the filter paper cards once dried and that this holds true at ambient temperature. Importantly, previous work reports that concentrations of both Glu and Phe in human DBSs decrease by ~ 4 to 5% per year^{29,30}. Our research demonstrates that the relatively short storage time (~ 1 year) had little effect on the amino acid $\delta^{15}\text{N}$ values in Arctic char DBSs, although it is possible that the concentrations and isotopic compositions of Glu and Phe could feasibly change over a longer time period. We suggest that future research could assess the stability of amino acid $\delta^{15}\text{N}$ values over longer time periods, which would in turn pave the way for significant retrospective analyses.

Although DBS amino acid $\delta^{15}\text{N}$ values and trophic position estimates were tightly correlated with those generated from muscle, we did note slight mean differences between the two sample types. Specifically, on average Glu $\delta^{15}\text{N}$ values from muscle were higher than those from DBSs, while Phe $\delta^{15}\text{N}$ values were lower from muscle than those from DBSs. However both mean differences were modest ($<1\text{ ‰}$) and resulted in only a slightly higher average trophic position estimate (mean difference = 0.2) for muscle tissue. While this mean difference is apparent based on our statistical approach, the ecological significance of such a minor difference in trophic position estimate is unlikely. Ultimately, researchers can decide if this offset is important for their own studies, and if so correct DBS-based trophic position estimates accordingly. The noted mean differences (or biases) revealed from our statistical approach may reflect differences in trophic enrichment factors, in isotope turnover rates, or isotopic routing^{9,12}. Differences in isotopic turnover rates between tissues could lead to differences in isotopic composition and trophic enrichment factors for blood and muscle. Moreover, isotopic routing could also contribute to differences in trophic enrichment factors between tissues¹². In turn, these differences can affect trophic position estimates, which are highly dependent on the trophic enrichment factor that is selected and applied^{12,31}. Notably, a previous study with four species of penguin chicks demonstrated that trophic enrichment factors for blood were consistently lower than the widely accepted universal $\sim 7\text{ ‰}$ trophic enrichment factor³¹. As blood transports AAs derived from dietary proteins, the $\delta^{15}\text{N}$ value of Glu in blood may be influenced by the isotopic composition of the fish itself, as well as its food sources. Additionally, there are known differences in isotopic turnover rates between muscle and blood³². Trophic positions of fish are typically inferred from bulk $\delta^{15}\text{N}$ values from analyses of muscle¹⁴, because this tissue has low isotopic variability and an intermediate isotopic turnover rate^{14,33}. In comparison, fish blood has a quicker isotopic turnover rate³².

Although in the present study we applied the “universal” trophic enrichment factor proposed by Chikaraishi and colleagues¹⁰, we acknowledge that trophic enrichment factors can vary significantly among tissues and taxa^{9,4,12}. Therefore, future studies could develop both tissue-specific and a species-specific trophic enrichment factors for Arctic char. Although, a comparison of AA-specific isotope ratios and trophic position estimates between tissues does not indicate whether trophic position estimates are indeed accurate, the Arctic char trophic position estimates (~ 3 to 4) produced from CSIA-AA in the present study are reasonable and consistent with previous studies using bulk stable isotope analyses to generate trophic positions for Arctic char from several of the same study lakes³⁴ and other high-latitude lakes³⁵.

The food webs supporting landlocked Arctic char from the various study lakes are relatively simple, consisting mainly of landlocked Arctic char, non-biting midges (Diptera, Chironomidae), and basal resources³⁶⁻³⁸. Research shows that there are seasonal differences in chironomid availability, feeding activity, and bulk $\delta^{15}\text{N}$ values of Arctic char^{39,40}, which in concert with the differing isotopic turnover rates, isotopic routing, and trophic enrichment factors for blood and muscle could explain the slight offset in DBS and muscle values observed here. For Arctic char or other species of fish sampled from more dynamic and/or complex food webs the $\delta^{15}\text{N}$ values of amino acids from blood and muscle may differ to a greater degree than what we observed in the present study. If this is the case, DBS-based

trophic position estimates of these fish may better reflect recent feeding ecology. The potential differences in trophic enrichment factors could be most important for researchers interested in using DBSs to generate trophic position estimates for migratory species or lake-dwelling species that exhibit seasonal or ontogenetic shifts in habitat use.

4 CONCLUSIONS

In the present study, we validated the application of CSIA-AA to field-prepared DBSs of landlocked Arctic char collected from lakes in the Canadian High Arctic. Our work demonstrates that DBS sampling, which is ideal for use in resource-limited settings like the Arctic, provides an excellent approximation of trophic position. In our previous work, we demonstrated that the application of CSIA-AA to generate $\delta^{13}\text{C}$ values of EAAs isolated from DBSs could be used to “fingerprint” dietary carbon sources of landlocked Arctic char from many of the same arctic lakes²³. By coupling CSIA of stable carbon and nitrogen isotope ratios of amino acids isolated from DBSs a higher resolution understanding of food web relationships can be obtained (relative to bulk stable isotope analyses) from a small volume of dried blood (~10 μL). Notably, the same digestion and amino acid derivatization procedure for DBSs can be used for CSIA of stable carbon and nitrogen isotopes, which reduces the difficulty of coupling these techniques in future studies. Furthermore, as CSIA-AA requires only a small volume of blood, DBS sampling could be developed as a non-lethal sampling technique for fish and perhaps other wildlife.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Comparisons of glutamic acid $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{Glu}}$; Panels A and B) and phenylalanine $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{Phe}}$; Panels C and D) values from dried blood spots (DBSs) and whole blood (WB) of landlocked Arctic char (*Salvelinus alpinus*). Panels A and C present linear regressions where the gray shaded regions indicate the 95 % confidence regions about the fitted lines. Panels B and D present Bland-Altman plots showing relationships between the average of the $\delta^{15}\text{N}$ values for the two sample types and the difference between $\delta^{15}\text{N}$ values for the two sample types. The blue solid line represents the bias or mean difference between the two measurements, the dashed blue lines represents the 95 % confidence interval for the bias, and the red dashed lines represent the 95 % confidence interval for all of the measurements. In all panels, the different symbols represent Arctic char from different lakes.

Figure 2.

Comparisons of glutamic acid $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{Glu}}$; Panels A and B) and phenylalanine $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{Phe}}$; Panels C and D) values from dried blood spots (DBSs) and muscle of landlocked Arctic char (*Salvelinus alpinus*). Panels A and C present linear regressions where the gray shaded regions indicate the 95 % confidence regions about the fitted lines. Panels B and D present Bland-Altman plots showing relationships between the average of the amino acid $\delta^{15}\text{N}$ values for the two sample types and the difference between amino acid $\delta^{15}\text{N}$ values for the two sample types. The blue solid line represents the bias or mean difference between the two measurements, the dashed blue lines represents the 95 % confidence interval for the bias, and the red dashed lines represent the 95 % confidence interval for all of the measurements. In all panels, the different symbols represent Arctic char from different lakes.

Figure 3.

Comparisons of dried blood spot (DBS) trophic position estimates (TP) with estimates from whole blood (WB; Panels A and B) and muscle (Panels C and D) of landlocked Arctic char (*Salvelinus alpinus*). Panels A and C present linear regressions where the gray shaded regions indicate the 95 % confidence regions about the fitted lines. Panels B and D present Bland-Altman plots showing relationships between the average of the estimates for the two sample types and the difference between the estimates for the two sample types. The blue solid line represents the bias or mean difference between the two estimates, the dashed blue lines represents the 95 % confidence interval for the bias, and the red dashed lines represent the 95 % confidence interval for all of the estimates. In all panels, the different symbols represent Arctic char from different lakes.