Received: 3 October 2020 | Revised: 18 February 2021 | Accepted: 31 March 2021

DOI: 10.1002/rcm.9093

RESEARCH ARTICLE

Rapid Communications in Mass Spectrometry WILEY

Evaluation of muscle lipid extraction and non-lethal fin tissue use for carbon, nitrogen, and sulfur isotope analyses in adult salmonids

Sarah M. Larocque[1] | Aaron T. Fisk[1] | Timothy B. Johnson[2]

[1]Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, N9B 3P4, Canada

[2]Glenora Fisheries Station, Ontario Ministry of Natural Resources and Forestry, R.R. #4, Picton, Ontario, K0K 2T0, Canada

Correspondence
S. M. Larocque, Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, N9B 3P4, Canada.
Email: slarocque9@gmail.com

Funding information
Canada-Ontario Agreement on Great Lakes Water Quality and Ecosystem Health; Natural Sciences and Engineering Research Council of Canada; NSERC

Rationale: Chemical lipid extraction or using alternative tissues such as fish fin as opposed to muscle may alter isotopic ratios and influence interpretations of $\delta^{13}C$, $\delta^{15}N$, and previously unassessed $\delta^{34}S$ values in stable isotope analyses (SIA). Our objectives were to determine if lipid extraction alters these isotope ratios in muscle, if lipid normalization models can be used for lipid-rich salmonids, and if fin isotope ratios are comparable with those of muscle in adult salmonids.

Methods: In six adult salmonid species (n = 106) collected from Lake Ontario, we compared three isotope ratios in lipid-extracted (LE) muscle with bulk muscle, and LE muscle with fin tissue, with paired t-tests and linear regressions. We compared differences between $\delta^{13}C$ values in LE and bulk muscle with predicted values from lipid normalization models and the log-linear model of best fit and determined model efficiency.

Results: The $\delta^{15}N$ values in LE muscle increased (<1‰) relative to bulk muscle for most salmonids, with relationships nearing 1:1. There were either no differences or strong 1:1 relationships in $\delta^{34}S$ values between species-specific bulk and LE muscle. One lipid normalization model had greater model efficiency (97%) than the model of best fit (94%). Fin had higher $\delta^{13}C$ values than LE muscle while $\delta^{15}N$ trends varied (<1‰); however, both isotope ratios had either no or weak linear relationships with fin and LE muscle within species. The $\delta^{34}S$ values in fin were similar to those in LE muscle and had strong 1:1 relationships across species.

Conclusions: We recommend using the lipid normalization model to adjust for $\delta^{13}C$ values in lipid-rich muscle (C:N >3.4). LE muscle could be used without $\delta^{15}N$ or $\delta^{34}S$ adjustments, but the minimal increase in $\delta^{15}N$ values may affect SIA interpretation. With high unexplained variability among adult species in fin-muscle $\delta^{13}C$ and $\delta^{15}N$ relationships, species-specific fin-muscle adjustments are warranted. No fin-muscle tissue adjustment would be required for $\delta^{34}S$ values.

1 | INTRODUCTION

Stable isotope analysis (SIA) is commonly used by ecologists to understand trophic interactions, diets, and food webs.[1,2] In aquatic ecology, two commonly used stable isotope ratios are carbon ($\delta^{13}C$ value) and nitrogen ($\delta^{15}N$ value), which can identify an animal's diet source (e.g., more littoral vs offshore in lake ecosystems[3,4]) and trophic position in a food web, respectively.[2,5] A third stable isotope ratio, that of sulfur ($\delta^{34}S$ value), has been used to distinguish between feeding in marine vs freshwater environments,[2,6] and more recently within freshwater systems to differentiate benthic vs pelagic dietary sources.[7,8] When analyzing for stable isotopes, it has been recognized

that white muscle tissue is the preferred tissue for SIA in fish due to its low lipid content and reduced variability in $\delta^{13}C$ and $\delta^{15}N$ values compared with liver, heart, or whole body.[9] However, white muscle tissue can still have high lipid content (C:N ratios >3.4) which should be accounted for by either chemical lipid extraction or mathematical normalization.[10] In addition, sampling for white muscle tissue usually involves sacrificing the fish. Collection of alternative, non-lethal tissues such as fin material may be more appropriate and often desirable, particularly when trying to better understand the ecology of endangered or rare species or if repeatedly sampling, in order to make minimal impact on the population of the sampled individuals when conducting SIA.[11,12]

Bias can be introduced into SIA from $\delta^{13}C$ values in tissues with a high lipid content due to lipids being depleted in ^{13}C.[10] The muscle of some fish, such as salmonids, can have high lipid content[13,14] and the depletion of ^{13}C from lipids need to be accounted for to allow accurate ecological interpretation of diet[10,15] and isotopic niche analyses.[16,17] Although chemical lipid extraction removes biases in $\delta^{13}C$ values in the muscle, the process can also impact other isotope ratios.[18] For example, some studies indicate that the lipid extraction process either has a negligible impact on the $\delta^{15}N$ value[19] or can increase it.[20–22] However, the influence of lipid extraction on $\delta^{34}S$ values remains unknown. As the $\delta^{34}S$ value becomes increasingly used in SIA it is pertinent to understand the effect that lipid extraction has on interpretations of this isotope ratio. As an alternative to lipid extraction, mathematical lipid normalization models have been used to accurately adjust $\delta^{13}C$ values across groups of taxa, such as freshwater fishes (e.g.,[10,13,23]). Determining the effect of lipid extraction on isotope ratios other than those of C and N and whether the changes in $\delta^{13}C$ values from lipid extraction support published lipid normalization models will further our understanding, confidence, and consistency in adjusting for the effects of lipids in tissue samples for SIA.

Non-lethally sampled tissue for SIA, such as fin, is a potential alternative to lethal sampling of muscle; however, similar to the lipid extraction process, it is important to determine the degree to which the isotopic ratios of fin and dorsal muscle are correlated for accurate isotope interpretation.[24,25] Generally, it has been shown that caudal and pectoral fins have a faster isotopic ratio turnover rate than muscle.[22,26–28] These differences in tissue turnover could potentially influence fin-muscle isotope ratio relationships due to differences in temporal integration and the impact of dietary non-equilibrium. Rayed fins such as caudal, anal, and pectoral fins contain skeletal components that may also limit fin-muscle comparability and application.[25] However, various studies comparing fin and muscle $\delta^{13}C$ and $\delta^{15}N$ values indicate negligible isotopic differences between the two tissues. Generally, fin has a higher $\delta^{13}C$ value and lower $\delta^{15}N$ value than muscle with an overall estimated offset of +1.2‰ and −0.8‰, respectively (see review by Willis et al[29]) and typically no tissue adjustment or correction factor has been recommended.[11,30,31] However, no studies to date have assessed the fin and muscle relationship with $\delta^{34}S$ values. The relationship between fin and muscle for $\delta^{15}N$ values can vary among species but may also be influenced by the size of individuals within a species due to greater differences in tissue-specific turnover rates for adults than for juveniles. These differences can be exacerbated if tissues are not in dietary equilibrium (e.g., ontogenetic or seasonal diet shifts).[22,29,30] Thus, the same tissue adjustment used for juveniles may not apply to adults. As such, it is not clear whether a general fin-muscle relationship for stable isotope ratios can be applied to several species or life stages or whether species-specific relationships need to be determined.[24,25,29]

Studies have begun to evaluate using caudal and adipose fin tissue as a surrogate for muscle in the SIA of salmonids.[11,30,32,33] Most fin-muscle comparisons have focused on juvenile or subadult salmonids[11,31,33] which may have a stronger, and closer 1:1 relationship with muscle tissue than adults, due to faster growth rates and more membranous fin composition in younger fish. With the few studies that have assessed the fin-muscle relationship in adult salmonids,[30] some species such as coho salmon (*Oncorhynchus kisutch*) and Chinook salmon (*Oncorhynchus tshawytscha*) have yet to be described, and assessing more adults of different species will further the ability to understand any biases associated with the use of non-lethal sampling of fin as a surrogate for muscle in SIA.

Salmonids are ecologically and economically important top predators in many regions of the world, and developing non-lethal techniques for SIA would assist in conserving and monitoring their population ecology as well as applications in comparative studies. Lake Ontario has six salmonid species, including re-introduced native lake trout (*Salvelinus namaycush*) and Atlantic salmon (*Salmo salar*), and non-native Chinook salmon, coho salmon, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), making it an ideal location to assess multiple salmonid species. Lake Ontario salmonids generally have high lipid contents (C:N ratios >4) due to consumption of the lipid-rich, non-native alewife (*Alosa pseudoharengus*).[34–36] Thus, accounting for lipids in salmonid tissues either through lipid extraction or by mathematical normalization is important for diet analyses. In addition, with the rehabilitation of both lake trout and Atlantic salmon populations in Lake Ontario, non-lethal sampling for SIA would be desirable for their conservation. Determining fin-muscle tissue relationships and adjustments is important if a mix of tissue samples have been collected and will enable comparisons across species for food web analyses and cross study comparisons. By also assessing $\delta^{34}S$ values along with $\delta^{13}C$ and $\delta^{15}N$ values we will improve our understanding of lipid extraction effects as well as the fin-muscle relationship with SIA.

In the present study, we compared isotopic ratios in lipid-extracted (LE) muscle with those of both untreated, "bulk" muscle and caudal fin of the six adult salmonid species from Lake Ontario. We used caudal fin as it is the most commonly sampled fin tissue for SIA.[29] Our objectives were to determine: (1) if lipid extraction of muscle affected $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values; (2) the best lipid normalization model for high lipid content muscle samples as an alternative to lipid extraction; (3) the relationship between the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of fin and muscle in six adult salmonid species, and to provide appropriate tissue adjustment factors where differences exist; and (4) whether the isotopic differences in the

tissues were influenced by fish length. Our results will improve the ability to adjust for high lipid content in muscle samples, to use non-lethal fin samples for more species, particularly larger fish such as adult salmonids, and to provide both lipid extraction and fin-muscle relationships for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values in SIA.

2 | METHODS

2.1 | Sampling

Chinook salmon, coho salmon, brown trout, lake trout, and rainbow trout were angled and captured from Lake Ontario by local anglers at fishing derbies during June–July 2018. Fish harvested by anglers were sampled (n = 20 per species) after the derby had concluded each day. Atlantic salmon were either captured and harvested by local fishing charters, and kept frozen until retrieved for sampling, or captured by the Ontario Ministry of Natural Resources and Forestry (OMNRF) during routine fish community sampling with gill nets and subsequently sampled (n = 6) during June–September 2018. Salmonids were identified and measured for total length (mm). For each fish, a skinless, boneless, muscle sample was taken from the left, dorsal side, posterior to the dorsal fin, and a fin clip was taken from the tip of the upper caudal fin lobe. All equipment was sterilized with 10% betadine solution and rinsed with distilled water between samples. All samples were rinsed with distilled water, placed in 2-mL cryovial tubes, and kept on ice until they could be later frozen. The experimental protocol followed the Canadian Council on Animal Care guidelines (University of Windsor AUPP #16-08).

2.2 | Stable isotope analyses

All samples were freeze dried at −48°C for 48 h under a vacuum pressure of 133×10^3 mbar in preparation for SIA. Muscle tissue was crushed into a fine powder and fin tissue was cut into smaller pieces before being weighed. Due to the high lipid content (C:N >3.4) in the muscle tissues for all species except coho salmon (species-wide mean C:N ± SD = 4.83 ± 2.13; Table 1), SIA was performed for muscle tissue that was both untreated (hereafter called bulk) and lipid-extracted (LE) using the chloroform/methanol lipid extraction method of Bligh and Dyer.[37] Fin tissue had low lipid content for all species (species-wide mean C:N ± SD = 3.20 ± 0.15; Table 1) and did not require lipid extraction.

For SIA, the $\delta^{13}C$ and $\delta^{15}N$ values were determined separately from the $\delta^{34}S$ values. Tissue samples were weighed out (0.4–0.8 mg for $\delta^{13}C$ and $\delta^{15}N$ values, and 5.5–7.0 mg for $\delta^{34}S$ values) and placed into a tin capsule for SIA. Isotope ratios were determined using a Delta V isotope ratio mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech Analytical

TABLE 1 Summary of the fork length (FL), sample size (n), and C:N, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (mean ± SD ‰) of fin, bulk muscle and lipid-extracted (LE) muscle tissue for six salmonid species

Species	FL (mm)	n	Tissue	C:N	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Atlantic salmon	538 ± 88	6	Fin	3.25 ± 0.20	−20.78 ± 0.40	15.31 ± 0.78	5.03 ± 0.10
			Bulk muscle	3.55 ± 0.32	−22.52 ± 0.37	15.35 ± 0.36	5.13 ± 0.19
			LE muscle	3.12 ± 0.06	−21.72 ± 0.43	15.35 ± 0.37	4.99 ± 0.29
Brown trout	554 ± 61	20	Fin	3.27 ± 0.11	−20.59 ± 0.64	15.45 ± 0.44	5.32 ± 0.37
			Bulk muscle	5.41 ± 1.28	−24.19 ± 1.13	15.78 ± 0.29	5.21 ± 0.39
			LE muscle	3.16 ± 0.08	−21.27 ± 0.50	15.95 ± 0.35	5.31 ± 0.39
Chinook salmon	823 ± 112	20	Fin	3.32 ± 0.14	−21.64 ± 0.45	16.12 ± 0.53	5.32 ± 0.24
			Bulk muscle	4.50 ± 1.13	−24.01 ± 1.40	15.21 ± 0.34	5.37 ± 0.29
			LE muscle	3.09 ± 0.10	−21.86 ± 0.29	15.75 ± 0.30	5.41 ± 0.14
Coho salmon	530 ± 57	20	Fin	3.15 ± 0.11	−21.28 ± 0.49	16.67 ± 0.65	5.11 ± 0.43
			Bulk muscle	3.32 ± 0.15	−22.58 ± 0.35	15.17 ± 0.50	5.11 ± 0.27
			LE muscle	3.06 ± 0.04	−22.07 ± 0.12	15.61 ± 0.41	5.06 ± 0.33
Lake trout	725 ± 90	20	Fin	3.17 ± 0.11	−21.08 ± 0.50	17.70 ± 0.59	5.01 ± 0.28
			Bulk muscle	8.02 ± 2.60	−26.00 ± 1.17	16.99 ± 0.41	5.04 ± 0.43
			LE muscle	3.37 ± 0.12	−22.01 ± 0.45	17.42 ± 0.35	4.83 ± 0.41
Rainbow trout	658 ± 55	20	Fin	3.09 ± 0.14	−20.39 ± 0.60	15.55 ± 0.49	5.00 ± 0.52
			Bulk muscle	3.49 ± 0.41	−22.40 ± 0.68	15.46 ± 0.52	4.97 ± 0.63
			LE muscle	3.15 ± 0.08	−21.29 ± 0.26	15.94 ± 0.57	4.80 ± 0.69
Species-wide	651 ± 133	106	Fin	3.20 ± 0.15	−20.98 ± 0.68	16.24 ± 1.00	5.14 ± 0.39
			Bulk muscle	4.87 ± 2.17	−23.76 ± 1.63	15.70 ± 0.77	5.14 ± 0.42
			LE muscle	3.16 ± 0.13	−21.70 ± 0.49	16.09 ± 0.77	5.08 ± 0.49

Technologies Inc., Valencia, CA, USA). Isotopic ratios were reported as: $\delta X = [(R_{sample}/R_{standard}) - 1]$ where X is either ^{13}C, ^{15}N or ^{34}S, R is the ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$, and the standards used were C from Vienna Peedee Belemnite (VPDB), N from atmospheric N, or S from the Canyon Diablo troilite (CDT).

Laboratory and National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) standards were analyzed every 12 samples. The analytical precision (standard deviation (SD)) for NIST standard 1577c (bovine liver), an internal laboratory standard (tilapia muscle), USGS 40 and Urea (n = 86 for all) for $\delta^{13}C$ and $\delta^{15}N$ values was <0.20 and <0.19‰, respectively. The analytical precision for $\delta^{34}S$ values from NIST 1577c, an internal laboratory standard, USGS 42, NIST 8555 and NIST 8529 (n = 118 for all) was <0.25‰. The accuracy was checked monthly using a certified USGS 40 sample (n = 86) and was within 0.02 and 0.06‰ of the mean calculated values for $\delta^{13}C$ and $\delta^{15}N$. For $\delta^{34}S$, the accuracy using USGS 42 (n = 118) was within 0.12‰ of the mean calculated value.

2.3 | Statistical analyses

We used paired t-tests (or paired Wilcoxon tests if residuals were non-normal) to examine differences between LE and bulk muscle in the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values for each species, and all species combined, to determine if the lipid-extraction process affected mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values. Species-specific linear regressions of bulk muscle and LE muscle for each isotope ratio were calculated to provide a model to account for possible isotopic ratio alteration by the lipid extraction process and to be able to account for this effect in future studies that use LE samples with $\delta^{34}S$ values. When relationships between bulk and LE muscle were statistically significant, we used 95% confidence intervals (CIs) of the slope to determine if the slopes differed significantly from 1.0.

A lipid normalization model for $\delta^{13}C$ values in muscle from all species pooled was determined as the log-linear regression of best fit from the difference between the LE muscle and bulk muscle $\delta^{13}C$ values (hereafter indicated by $\Delta\delta^{13}C$) against the log C:N ratio of bulk muscle. Species were combined to increase the range of C:N ratios covered (3.02–14.23). We compared the observed $\Delta\delta^{13}C$ values from the salmonid dataset with predicted values, based on the best fit model and mass balance mathematical lipid normalization models that are commonly used in the literature. The models selected do not require tissue- and species-specific model parameter estimation via lipid extraction from a subset of samples and are thus convenient to use, and have been previously evaluated in salmonid tissues but with lower lipid content (C:N <8.0) by Skinner et al[38] and Abrantes et al.[14] Note that there are many published lipid normalization models derived from taxa not represented in this study[39] or that inaccurately predicted $\delta^{13}C$ values for freshwater fish white muscle tissue (e.g., McConnaughey and McRoy,[40] as tested in Post et al[10]). The lipid normalization models used were: the Fry[23] mass balance model (Fry), the Kiljunen et al[13] normalization model with the McConnaughey and McRoy[40] lipid percent method (KMM), the Kiljunen et al[13] normalization model with the Post[10] lipid percent method (KP), and the Post[10] normalization model (Post). Equations can be found in the respective papers but also in Skinner et al[38] and Abrantes et al.[14] The best fit, Fry, KMM, KP, and Post models for muscle compared the predicted $\Delta\delta^{13}C$ values with the observed values using paired t-tests. Finally, we tested the goodness-of-fit for the lipid normalization models for muscle by calculating the model efficiency (EF): $EF = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}$, where y_i is the observed, $\hat{y}_i$ is the model predicted, and $\bar{y}$ is the observed average value of the respective parameter. Values closer to 1 indicate better model performance.

We also assessed differences between LE muscle and fin tissues in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values using paired t-tests, (or paired Wilcoxon tests if residuals were non-normal) for each species, and with all fish combined. In addition, linear regression models were used to estimate LE muscle $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (dependent variables) from caudal fin $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (independent variables) for each species and with all fish pooled to assess the relationship between isotope ratios in caudal fin and muscle.

Lastly, to help explain variation in the data, we also tested for an effect of fish body length (i.e., total length) on differences in isotope signatures between LE muscle and fin pairs and LE muscle and bulk muscle pairs using linear regression for each species. Isotopic differences between tissues for each individual were also visualized to identify any potential isotopic ratio outliers. No individuals were found to be an outlier across multiple isotope ratios or tissues (Figure S1, supporting information). Location of capture within regions of Lake Ontario also did not drive any of the outliers or trends seen (Figure S1, supporting information) and locations were grouped together for analyses.

All analyses were conducted in R version 4.0.2[41] and significance was assessed at $\alpha = 0.05$. Unless stated otherwise, values are reported in mean ± SD). Assumptions of normality and homoscedasticity were visually assessed using qqplots and fitted versus residual plots.

3 | RESULTS

3.1 | Lipid-extracted vs bulk muscle comparisons

For each of the six species and the species-pooled data, mean $\delta^{13}C$ values of bulk muscle were significantly lower than those of LE muscle (Tables 1 and 2). Linear regression models indicated that bulk muscle $\delta^{13}C$ values were positively related to LE muscle $\delta^{13}C$ values for all species but Atlantic salmon (Figure 1; Table 3). The 95% CI of the slope, however, did not approach 1.0 for any species and the $\delta^{13}C$ values in bulk muscle explained <35% of the variation in $\delta^{13}C$ values of LE muscle, except for Chinook salmon and lake trout (R^2 >60% for both; Figure 1; Table 3). Mean bulk muscle $\delta^{15}N$ values were significantly lower than those of LE muscle for all species except for Atlantic salmon and brown trout which did not differ (Table 2). With all species pooled, mean $\delta^{15}N$ values in bulk muscle were significantly lower than those in LE muscle (mean LE – bulk muscle

TABLE 2 Differences (Δ) in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (mean ± SD ‰) between lipid-extracted (LE) muscle and bulk muscle, and LE muscle and caudal fin. Level of significance from paired t-tests are indicated with asterisks (p <0.05*, p <0.01**, p <0.001***). w = paired Wilcoxon test

	LE muscle – bulk muscle			LE muscle – fin		
Species	**$\Delta\delta^{13}C$**	**$\Delta\delta^{15}N$**	**$\Delta\delta^{34}S$**	**$\Delta\delta^{13}C$**	**$\Delta\delta^{15}N$**	**$\Delta\delta^{34}S$**
Atlantic salmon	0.80 ± 0.39**	0.01 ± 0.27	−0.15 ± 0.18	−0.94 ± 0.55**	0.05 ± 0.96	−0.04 ± 0.32
Brown trout	2.92 ± 0.94***	0.16 ± 0.38	0.09 ± 0.19*	−0.68 ± 0.44***	0.50 ± 0.48***	−0.01 ± 0.22
Chinook salmon	2.15 ± 1.17***	0.54 ± 0.27***	0.05 ± 0.25	−0.22 ± 0.41*	−0.37 ± 0.57*w	0.10 ± 0.23
Coho salmon	0.52 ± 0.31***	0.43 ± 0.21***	−0.05 ± 0.30	−0.79 ± 0.44***	−1.07 ± 0.70***w	−0.05 ± 0.50
Lake trout	3.99 ± 0.91***	0.43 ± 0.32***	−0.21 ± 0.27*w	−0.93 ± 0.45***	−0.28 ± 0.57*w	−0.18 ± 0.33*
Rainbow trout	1.11 ± 0.59***	0.48 ± 0.13***	−0.17 ± 0.24*	−0.90 ± 0.57***	0.39 ± 0.57**	−0.21 ± 0.35*
Species-pooled	2.06 ± 1.49***w	0.39 ± 0.31***w	−0.06 ± 0.27**w	−0.72 ± 0.52***	−0.15 ± 0.81	−0.07 ± 0.35*w

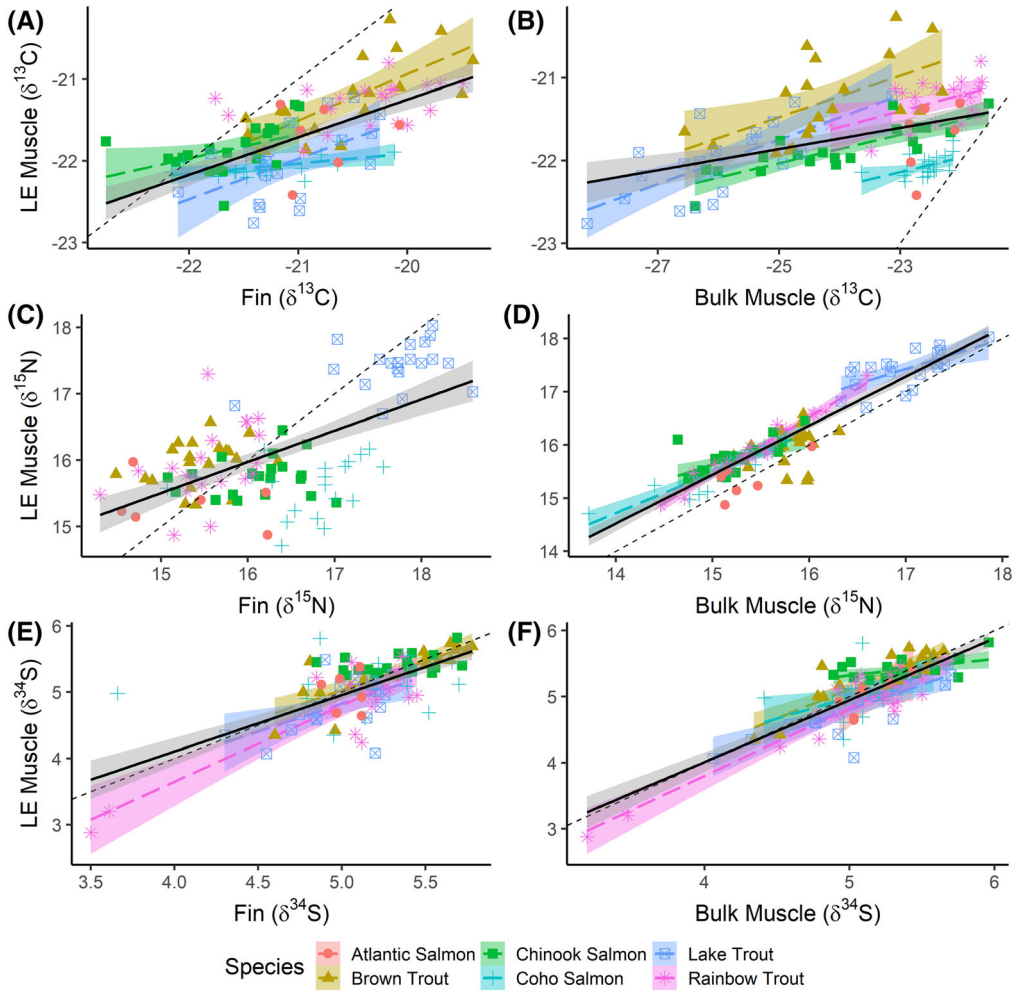

FIGURE 1 Relationships between $\delta^{13}C$ (top panels), $\delta^{15}N$ (middle panels) and $\delta^{34}S$ (‰) (lower panels) values of lipid-extracted (LE) muscle and fin (left panels) or LE muscle and bulk muscle (right panels) of six salmonid species from Lake Ontario. Long-dashed coloured lines indicate a significant species relationship, solid black lines indicate significant species-combined relationships, and short black dashed lines indicates the 1:1 relationship for reference

difference = 0.39 ± 0.34‰; p <0.001; Table 2). Linear regression models indicated that for all species except Atlantic salmon and brown trout, there were positive relationships between bulk muscle and LE muscle for $\delta^{15}N$ values, with $\delta^{15}N$ values in bulk muscle explaining 40–95% of the variation in $\delta^{15}N$ values of LE muscle (Figure 1; Table 3). The 95% CIs of the slopes included 1.0 for rainbow trout and approached 1.0 for all the remaining species (Figure 1; Table 3). Mean $\delta^{34}S$ values in bulk muscle were not

TABLE 3 Linear regression equations and 95% confidence intervals (CIs) of slopes from stable isotope ratios of muscle (M) or caudal fin (F) and lipid-extracted (LE) muscle for six salmonid species. (p <0.05*, p <0.01**, p <0.001***, NS: nonsignificant relationship)

Species	Stable isotope	Bulk muscle – LE muscle	R^2	95% CI of slope	Fin – LE muscle	R^2	95% CI of slope
Atlantic salmon	C	NS			NS		
	N	NS			NS		
	S	LE = 1.264·M – 1.499*	0.58	0.012–2.515	NS		
Brown trout	C	LE = 0.2508·M – 15.205**	0.29	0.071–0.431	LE = 0.5703·F – 9.5294***	0.51	0.310–0.831
	N	NS			NS		
	S	LE = 0.8920·M + 0.6553***	0.76	0.650–1.134	LE = 0.8802·F + 0.6272***	0.67	0.588–1.172
Chinook salmon	C	LE = 0.1728·M – 17.712***	0.67	0.116–0.230	LE = 0.2962·F – 15.452*	0.16	0.007–0.586
	N	LE = 0.5746·M + 7.0103**	0.40	0.248–0.901	NS		
	S	LE = 0.2511·M + 4.0652*	0.23	0.045–0.457	NS		
Coho salmon	C	LE = 0.1744·M – 18.128*	0.20	0.021–0.328	LE = 0.1242·F – 19.423*	0.20	0.015–0.234
	N	LE = 0.7559·M + 4.1378***	0.83	0.590–0.922	NS		
	S	LE = 0.6434·M + 1.7718*	0.23	0.114–1.172	NS		
Lake trout	C	LE = 0.2680·M – 15.045***	0.45	0.131–0.405	LE = 0.4944·F – 11.5904*	0.26	0.119–0.870
	N	LE = 0.5663·M + 7.7986**	0.40	0.245–0.888	NS		
	S	LE = 0.7552·M + 1.0266***	0.62	0.475–1.036	LE = 0.8382·F + 0.6332**	0.31	0.262–1.414
Rainbow trout	C	LE = 0.1918·M – 16.992*	0.21	0.027–0.357	NS		
	N	LE = 1.0728·M – 0.6452***	0.95	0.952–1.194	NS		
	S	LE = 1.0258·M – 0.3016***	0.88	0.841–1.211	LE = 1.146·F – 0.936***	0.74	0.821–1.471
Species-pooled	C	LE = 0.1228·M – 18.793***	0.16	0.069–0.177	LE = 0.4600·F – 12.057***	0.40	0.352–0.568
	N	LE = 0.9133·M + 1.7540***	0.85	0.839–0.988	LE = 0.4698·F + 8.4644***	0.37	0.352–0.588
	S	LE = 0.0.9360·M + 0.2659***	0.69	0.814–1.058	LE = 0.8506·M + 0.7012***	0.48	0.679–1.023

different from those in LE muscle for Atlantic salmon, Chinook salmon and coho salmon but were significantly higher for lake trout and rainbow trout, and lower for brown trout (Tables 1 and 2). With all species pooled, results suggested that mean $\delta^{34}S$ values were significantly higher in bulk muscle than in LE muscle; however, the absolute differences were quite small (mean bulk – LE muscle difference = 0.06 ± 0.27‰; p = 0.007; Table 2). Linear regression models indicated that $\delta^{34}S$ values in bulk muscle were positively related to those in LE muscle for all species (Figure 1; Table 3). A range of variance in $\delta^{34}S$ values of LE muscle was explained by $\delta^{34}S$ values of bulk muscle across species (R^2 = 23–88%), and the 95% CIs of the slopes included 1.0 in all cases except for Chinook salmon (Figure 1; Table 3). With all species pooled, regression models indicated that bulk muscle was significantly related to LE muscle for the three isotope ratios but only had a 95% CI of the slope that included 1.0 for $\delta^{15}N$ and $\delta^{34}S$ values.

Lipid normalization models (Fry, KP, KMM, Post) and the log-linear best fit model predicted $\Delta\delta^{13}C$ values from the bulk muscle C:N ratios (Figure 2). Only the predicted $\Delta\delta^{13}C$ values from the log-linear best fit model were not different from the observed values (t = 0.44, df = 98, p = 0.951), while the predicted $\Delta\delta^{13}C$ values from the Fry, KP, KMM, and Post normalization models were significantly different from observed values (KMM: p = 0.038; remaining models: p <0.001; Figure 2). However, model efficiency, or goodness-of-fit, was highest with the KMM model (0.97), followed by the log-linear best fit model (0.94), and lower model fits were seen with the KP (0.77), Post (0.40), and Fry (0.24) models.

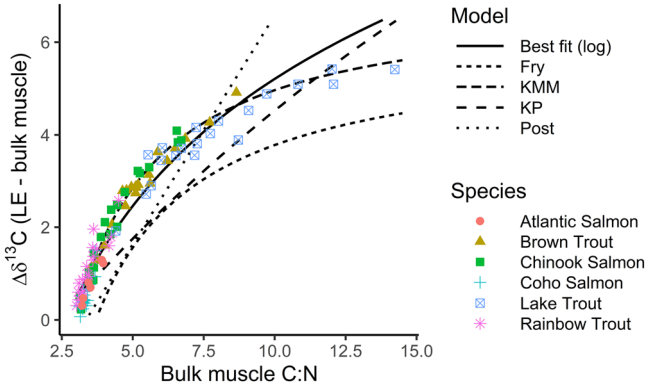

FIGURE 2 Relationship between bulk muscle C:N ratios and the difference in lipid-extracted (LE) muscle and bulk muscle in $\delta^{13}C$ ($\Delta\delta^{13}C$) values for salmonid species with the species-combined log-linear best fit regression (solid line) and previously published lipid normalization models. Model names refer to Fry[23] mass balance model (Fry), Kiljunen et al[13] normalization model with the McConnaughey and McRoy[40] lipid percent method (KMM), Kiljunen et al[13] normalization model with the Post[10] lipid percent method (KP), and the Post[10] normalization model (Post)

3.2 | Tissue comparisons

Mean fin $\delta^{13}C$ values were higher than those of LE muscle for all six species and of all species pooled (Tables 1 and 2). Fin $\delta^{13}C$ values were positively related to LE muscle $\delta^{13}C$ values for brown trout, Chinook salmon, coho salmon and lake trout; however, the fin $\delta^{13}C$ values explained <52% of the variation in LE muscle $\delta^{13}C$ values and none of the 95% CIs of the slopes included 1.0 (Figure 1; Table 3). The mean $\delta^{15}N$ value in fin was significantly different from the value in LE muscle for all species except Atlantic salmon, in which brown trout and rainbow trout fin were depleted in ^{15}N relative to LE muscle, and Chinook salmon, coho salmon, and lake trout fin were enriched in ^{15}N relative to LE muscle (Tables 1 and 2). With all species pooled, the mean $\delta^{15}N$ value did not differ between fin and LE muscle. Although paired tests indicated differences, there was no relationship between fin and LE muscle $\delta^{15}N$ values for all six species (Figure 1; Table 3). The mean $\delta^{34}S$ values in fin were not different from those in LE muscle for Atlantic salmon, brown trout, Chinook salmon, and coho salmon but were significantly higher for lake trout and rainbow trout (Tables 1 and 2). The mean $\delta^{34}S$ value in fin was significantly higher than in LE muscle when all species were pooled; however, the absolute differences were small (mean fin – LE muscle difference = 0.07 ± 0.35‰; p = 0.021; Tables 1 and 2). The fin $\delta^{34}S$ values were positively related to the LE muscle values for brown trout, lake trout, and rainbow trout in which the $\delta^{34}S$ values in fin explained >65% of the variation of $\delta^{34}S$ values in LE muscle for brown trout and rainbow trout, but only 31% of the variation in LE muscle for lake trout. For all three species, the 95% CIs of the slopes included 1.0 for the fin – LE muscle $\delta^{34}S$ linear regressions (Figure 1; Table 3). The fin $\delta^{34}S$ values were not related to the LE muscle values for Atlantic salmon, Chinook salmon and coho salmon (Figure 1; Table 3). With all species pooled, regression models for fin were related to LE muscle for the three isotope ratios (R^2 = 0.32–0.48) but the 95% CIs of the slopes only included 1.0 for $\delta^{34}S$ values.

3.3 | Effect of body length

Fish body length rarely had significant effects (p >0.05) on the difference in isotope ratios between LE muscle and bulk muscle or between LE muscle and fin (Figure 3). Atlantic salmon, Chinook salmon and rainbow trout showed no body length effect for any isotope difference with both tissue comparisons (p >0.05; Figure 3). The difference in LE muscle and bulk muscle $\delta^{13}C$ values was positively related to fork length for brown trout (y = 0.008x – 1.522; $F_{1,18}$ = 6.567; p = 0.020; R^2 = 0.23) and coho salmon (y = 0.003x – 1.18; $F_{1,17}$ = 8.645; p = 0.009; R^2 = 0.30; Figure 3B). The coho salmon and lake trout LE muscle-fin difference for $\delta^{15}N$ had a positive (y = 0.009x – 6.179; $F_{1,17}$ = 27.39; p <0.001; R^2 = 0.59) and negative relationship (y = –0.003x + 1.928; $F_{1,18}$ = 5.447; p = 0.031; R^2 = 0.19) with fork length, respectively (Figure 3C).

4 | DISCUSSION

We compared $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values from LE muscle with those from both bulk muscle and fin tissue to assess the need for lipid and tissue adjustments, respectively, for six common salmonid species. Although mathematical lipid normalization models have been used to adjust $\delta^{13}C$ values for lipid-rich samples (C:N >3.4), the use of normalization models with untreated vs LE comparisons and expanding tissue comparisons over more species[19] are recommended to confirm this, as we do here. Fin and LE muscle comparisons were quite variable between species for $\delta^{13}C$ and $\delta^{15}N$ values but were relatively consistent with $\delta^{34}S$ values, and species-specific fin-muscle adjustments would be warranted at least for $\delta^{13}C$ and $\delta^{15}N$ values as the literature suggests.[24,25,29] However, the low R^2 values, particularly for $\delta^{13}C$ values, indicate high variability within a species, and accurate fin-muscle adjustments may not be possible. As such, caudal fin may not be a highly reliable alternative to muscle for SIA in large salmonids, and other fins such as adipose fins may be worth investigating. Bulk and LE muscle isotope ratios were quite similar between species except $\delta^{13}C$ values, as was expected. The highly related trends with $\Delta\delta^{13}C$ (LE muscle – bulk muscle) values to bulk muscle C:N ratios allow for the use of a general lipid normalization model across species and comparisons with other lipid normalization models could be made. Determining whether the lipid extraction process affects other isotope ratios is important, and, in this case, the $\delta^{15}N$ value was generally lower in bulk muscle than in LE muscle, by 0.39 ± 0.310‰, but these differences were small, and the $\delta^{34}S$ value was generally unaffected, with a 1:1 relationship between bulk and LE muscle; we thus suggest that no $\delta^{15}N$ or $\delta^{34}S$ adjustment is required for lipids.

Our study further supports the use of the mass balance KMM lipid normalization model for fish muscle tissue.[13,38] The KMM model fits the data better than any other normalization model, including the log-linear best fit regression. Studies by Skinner et al[38] also found the KMM model to be the best fit model for lipid normalization in fish muscle tissue. However, we found that the lipid extraction process used here had increased the $\delta^{15}N$ values relative to that in bulk muscle for all species except Atlantic salmon and brown trout. Previous studies have found that lipid extraction increased $\delta^{15}N$ values in muscle and liver tissues and this is related to the chemical extraction process,[18,20,38] which could be further influenced by different solvents used with different chemical extraction methods. The amount of enrichment of ^{15}N from lipid extraction can vary across species and fauna which can have implications in population or community level isotopic niche analyses[16,17] or diet reconstruction by mixing models.[15] In our study, differences between LE and bulk muscle in $\delta^{15}N$ values were <1‰, nearing a 1:1 relationship, suggesting that a $\delta^{15}N$ adjustment is probably unnecessary, particularly if all samples in a study were lipid-extraxted. No previous study has determined if the chemical extraction process impacts $\delta^{34}S$ values, and we found that the $\delta^{34}S$ value was generally unaffected, with a 1:1 relationship between bulk and LE muscle, and requires no adjustment. Thus, our data support the use of the KMM model for

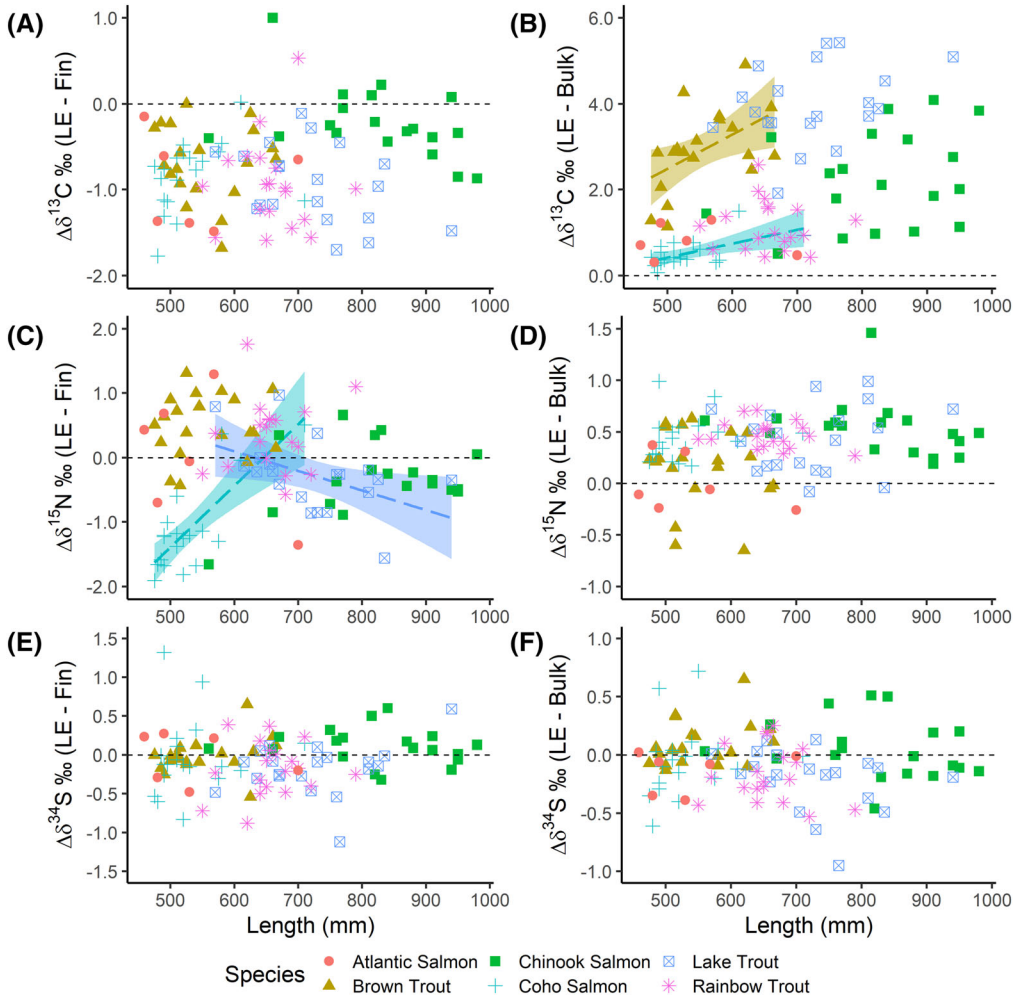

FIGURE 3 Regressions of the differences (Δ) in $\delta^{13}C$ (top panels), $\delta^{15}N$ (middle panels) and $\delta^{34}S$ (‰) (lower panels) values between lipid-extracted (LE) muscle and fin (left panels) or LE muscle and bulk muscle (right panels) against fork length (mm) of six salmonid species from Lake Ontario. Long-dashed coloured lines indicate a significant species relationship, and the short black dashed lines indicate zero difference for reference

lipid normalization of $\delta^{13}C$ values in bulk muscle samples of fish. LE muscle isotopic ratios can still be used, although we caution that increases of <1‰ in $\delta^{15}N$ values may occur and have implications on fine-scale SIA interpretations.

Many studies have shown that SIA using tissue from different types of fin, including caudal, is a suitable non-lethal alternative to the use of muscle.[11,29,30,42,43] Fin-muscle tissue comparisons previously found that no tissue adjustments were warranted when isotopic ratio differences were <1‰ and had a strong correlation.[11,30,31] In our study, we found that species-specific LE muscle-fin regressions for both $\delta^{13}C$ and $\delta^{15}N$ values were not always related and, when they were related, fin isotope ratios did not explain a lot of the variance for the isotope ratios of LE muscle within or among species. In addition, although the differences were sometimes <1‰, a 1:1 relationship did not exist for either isotope ratio. Thus, a tissue adjustment is warranted given the large differences between tissues (species-pooled $\delta^{13}C_{LE\text{-}Fin}$ = −0.72 ± 0.52‰; $\delta^{15}N_{LE\text{-}Fin}$ = −0.15 ± 0.81‰). However, the weak relationships (with some species not having any significant linear relationship) and high variability within and among species makes developing a species-specific or species-pooled adjustment difficult. Larger sample sizes, particularly for Atlantic salmon (n = 6), may have created stronger relationships. The linear regressions here can provide a fin-muscle tissue adjustment for $\delta^{13}C$ and $\delta^{15}N$ values for six species of adult salmonids; however, if applied to other species, a subset of samples should compare fin and muscle tissues to create a species-specific tissue adjustment for $\delta^{13}C$ and $\delta^{15}N$ values, particularly for adults or slower growing species. Notably, differences in $\delta^{15}N$ values between fin and muscle for some species here were <1‰ (e.g., brown trout, Chinook salmon, and rainbow trout), and may not be biologically significant nor require an adjustment for $\delta^{15}N$. A tissue adjustment is not warranted for $\delta^{34}S$ values because tissues did not differ between species or had a strong, positive relationship around 1:1, and all species-specific differences were <1‰.

A key difference between our study and others previously published on similar species is that we assessed large adult salmonids

instead of juveniles.[11,30,31,33] Length was not responsible for the isotopic differences between fin and LE muscle found in our study, and the effect of fish length on tissue differences had been minor in other studies.[11,30] Thus, a change in diet between smaller and larger adult salmonids in our study was not responsible for the large variation between LE muscle and fin within a species. Fin tissue composition may be responsible for the differences among adult species in our study, and differences with other studies using juveniles or individuals <500 mm length. Although fin generally has a higher δ^{13}C value than muscle ([29,30]; this study), Hayden et al[25] found that juvenile and subadult caudal fin rays had lower δ^{13}C values (by ~1‰) than fin membrane, and thus fish species with less membranous caudal fins or more fin ray in the fin tissue sample would have a relationship more similar to muscle. Hayden et al[25] found that fin membrane had higher δ^{15}N values (by <0.5‰) than fin ray yet this was not considered biologically significant. However, Sholto-Douglas et al[44] found δ^{15}N values to be >2‰ higher in muscle than in bone collagen and so greater discrepancies may occur in δ^{15}N values depending on the composition of the fin tissue analyzed. Relative to juveniles, large adults with larger caudal fins can have more bone elements in the fins and this may explain why our study found weak relationships between fin and muscle. Some species in our study could have had more bone elements in the caudal fins and this could explain differences among species; however, sample variability in fin tissue composition may also explain individual differences within a species. There is little reason to suspect that different rayed fins (e.g., pectoral, dorsal, anal) would impact results[42]; however, adipose fin which lacks fin rays (and only certain fish species have fin rays) may be a better proxy of muscle than caudal fin in SIA and should be further investigated across species.[33] Comparing isotopic differences between fin ray and membrane in larger adults may give insight into whether isotope ratios in muscle and fin vary due to tissue composition and this would assist in determining a more precise fin-muscle adjustment.

The differences seen between fin and muscle tissue could also be related to tissue turnover rates and seasonal changes in diet, and fish growth. There was no shift in isotope composition with length; however, the salmonids may be at a size in which the tissue turnover of muscle is slower than that of fin, and less dictated by growth rates. McIntyre and Flecker[26] found that N turnover in catfish muscle decreased with increasing fish size, and fin N turnover was faster than muscle turnover (albeit it was not significant). The fish in their study were very small (max of 2.1 g), but the trends may be accentuated in larger fish such as in this study. Furthermore, other studies have indicated that muscle tissue turnover is slower than that of fin,[27,28] including a study on a slow-growing adult marine fish species.[22] If muscle tissue turnover is slower than that of fin, the two tissues may reflect seasonal differences in diet and the isotope ratios in the two tissues would not be in dietary equilibrium at the time of the study. Species with a more variable diet in the summer (as more prey items generally become available) may have this reflected in the fin, while the longer-term diet would be reflected in the muscle tissue, and this would thus increase the variability in the isotope ratios of fin and reduce the potential for strong 1:1 relationships relative to muscle. In addition, the differences between fin and muscle tissue relationships among species may be from species growing at different rates. Some species such as Chinook salmon and coho salmon are fast growing and their tissue turnover rates may be faster than those of slower growing species like lake trout. Improving our understanding of tissue turnover in larger organisms is generally difficult logistically but perhaps important to determine as they may not respond similarly to smaller organisms.

The utilization of δ^{34}S values in freshwater food web ecology has been increasing[8,45,46]; however, it is unknown how it varies with the lipid extraction process or between tissues. In this study, we determined that due to the strong relationship and minimal (<0.5‰) variation in δ^{34}S values between both bulk and LE muscle, and fin and muscle tissue, no lipid or tissue adjustments of δ^{34}S values are required. The lipid extraction process did not affect δ^{34}S values which may in part be due to the very low amount of sulfolipids present in the lipids within fish muscle tissue.[47] In tissues with more sulfolipids present, lipid extraction could cause a loss of isotopically depleted sulfur and enrich tissues with ^{34}S.[48] Furthermore, understanding the tissue turnover rate of the δ^{34}S value and how it is incorporated in the body, relative to the δ^{13}C and δ^{15}N values, could determine why such low variation in δ^{34}S values was seen between fin and muscle tissue compared with the other isotope ratios. However, it has been found that isotope ratio specific turnover estimates for sulfur tend not to differ notably from those of other elements, although this has yet to be tested in fin tissue.[49] The strong relationship between fin and muscle δ^{34}S values may be more related to a changing diet relatively consistent in δ^{34}S compared with its δ^{13}C and δ^{15}N values. The faster tissue turnover rates in fin than in muscle may reflect the temporal variation in the δ^{13}C and δ^{15}N values of the fishes' diet, adding "noise" to the relationships for those isotope ratios, but not in δ^{34}S values, by not being in dietary equilibrium isotopically. It is important to know the effects on alternative tissues or sampling processes prior to analyses, and in this case the δ^{34}S value was not affected.

Quantifying how stable isotope ratios are affected by chemical processes such as lipid extraction and how they change among different tissues with species-specific examples is important for increasing our knowledge base and potentially determining adjustments applicable to a broad range of taxa, such as freshwater fishes. We determined that the δ^{34}S value would require no adjustments for either lipid extraction or the use of fin tissue. However, this is one of the first studies to find that fin tissue did not strongly relate to muscle for δ^{13}C and δ^{15}N values. Differences in fin and muscle isotope ratios can probably be attributed to either different tissue turnover rates or different fin composition between the adult, large-bodied fish used here and the juvenile fish used in other studies. Further investigations on tissue turnover rates and the effects of caudal fin ray bone vs membrane are recommended. This study does provide fin-muscle adjustments for six common salmonid adults (>500 mm) of similar size. We recommend using a small sample subset to compare fin vs muscle for study-specific verification with SIA of large fish fin tissue if the species has not been assessed. As

studies increase and more data become available, a general fin-muscle isotope ratio adjustment can potentially be determined and used across freshwater fish species. Our study further supports the use of the KMM lipid normalization model across freshwater fish species due to the high correlation in salmonids that ranged widely in lipid content in this study and the use of the normalization model as opposed to lipid extraction is recommended due to the potential for an increase in δ^{15}N value arising from the lipid extraction process. Using a lipid normalization model will also reduce sample processing costs by negating the lipid extraction step in SIA. As the use of SIA increases in ecological studies, it is important to understand the effects that lipid extraction and using alternative tissues can have on isotope ratios and minimize potential analytical biases in the process.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the logistical assistance from NYSDEC (M. Connerton, J. Lantry, C. Legard), OMNRF (M. Yuille), N. Foxcroft, and J. Felluca. They would also like to recognize the Lake Ontario fishing derbies (specifically Niagara County Lake Ontario Pro-Am, Oak Orchard Open Tournament, Oswego Pro-Am) and thank the organizers and participants for providing samples for them. They would like to thank everyone who assisted in the field (K. Hudelson, C. Huevel, N. Klinard) and in the lab (M. Charrone, M. Lucas, K. Johnson). This project was funded by NSERC DG to ATF, NSERC scholarship to SML, and the Canada-Ontario Agreement on Great Lakes Water Quality and Ecosystem Health through the OMNRF.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/rcm.9093.

ORCID

Sarah M. Larocque https://orcid.org/0000-0002-2088-4988

REFERENCES

1. Boecklen WJ, Yarnes CT, Cook BA, James AC. On the use of stable isotopes in trophic ecology. *Annu Rev Ecol Evol Syst*. 2011;42(1):411-440. https://doi.org/10.1146/annurev-ecolsys-102209-144726
2. Peterson BJ, Fry B. Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst*. 1987;18(1):293-320.
3. Post DM. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*. 2002;83(3):703-718.
4. France RL. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol Oceanogr*. 1995;40(7):1310-1313. https://doi.org/10.4319/lo.1995.40.7.1310
5. DeNiro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta*. 1981;45(3):341-351. https://doi.org/10.1016/0016-7037(81)90244-1
6. Doucett RR, Hooper W, Power G. Identification of anadromous and nonanadromous adult brook trout and their progeny in the Tabusintac River, New Brunswick, by means of multiple-stable-isotope analysis. *Trans Am Fish Soc*. 1999;128(2):278-288. https://doi.org/10.1577/1548-8659(1999)128<0278:ioaana>2.0.co;2
7. Croisetière L, Hare L, Tessier A, Cabana G. Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. *Freshwater Biol*. 2009;54(5):1006-1015. https://doi.org/10.1111/j.1365-2427.2008.02147.x
8. Colborne SF, Rush SA, Paterson G, Johnson TB, Lantry BF, Fisk AT. Estimates of lake trout (*Salvelinus namaycush*) diet in Lake Ontario using two and three isotope mixing models. *J Great Lakes Res*. 2016;42(3):695-702. https://doi.org/10.1016/j.jglr.2016.03.010
9. Pinnegar JK, Polunin NVC. Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: Implications for the study of trophic interactions. *Funct Ecol*. 1999;13(2):225-231. https://doi.org/10.1046/j.1365-2435.1999.00301.x
10. Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG. Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*. 2007;152(1):179-189. https://doi.org/10.1007/s00442-006-0630-x
11. Sanderson BL, Tran CD, Coe HJ, Pelekis V, Steel EA, Reichert WL. Nonlethal sampling of fish caudal fins yields valuable stable isotope data for threatened and endangered fishes. *Trans Am Fish Soc*. 2009;138(5):1166-1177. https://doi.org/10.1577/T08-086.1
12. Jardine TD, Hunt RJ, Pusey BJ, Bunn SE. A non-lethal sampling method for stable isotope studies of tropical fishes. *Mar Freshwater Res*. 2011;62(1):83-90. https://doi.org/10.1071/MF10211
13. Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H, Jones RI. A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *J Appl Ecol*. 2006;43(6):1213-1222. https://doi.org/10.1111/j.1365-2664.2006.01224.x
14. Abrantes KG, Semmens JM, Lyle JM, Nichols PD. Normalisation models for accounting for fat content in stable isotope measurements in salmonid muscle tissue. *Mar Biol*. 2012;159(1):57-64. https://doi.org/10.1007/s00227-011-1789-1
15. Tarroux A, Ehrich D, Lecomte N, Jardine TD, Bêty J, Berteaux D. Sensitivity of stable isotope mixing models to variation in isotopic ratios: Evaluating consequences of lipid extraction. *Methods Ecol Evol*. 2010;1(3):231-241. https://doi.org/10.1111/j.2041-210X.2010.00033.x
16. Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL. A niche for isotopic ecology. *Front Ecol Environ*. 2007;5(8):429-436. https://doi.org/10.1890/060150.1
17. Choy ES, Roth JD, Loseto LL. Lipid removal and acidification affect nitrogen and carbon stable isotope ratios of beluga whales (*Delphinapterus leucas*) and their potential prey species in the Beaufort Sea ecosystem. *Mar Biol*. 2016;163(10):1-13. https://doi.org/10.1007/s00227-016-2992-x
18. Yurkowski DJ, Hussey NE, Semeniuk C, Ferguson SH, Fisk AT. Effects of lipid extraction and the utility of lipid normalization models on δ^{13}C and δ^{15}N values in Arctic marine mammal tissues. *Polar Biol*. 2015;38(2):131-143. https://doi.org/10.1007/s00300-014-1571-1
19. Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME. Lipid corrections in carbon and nitrogen stable isotope analyses: Comparison of chemical extraction and modelling methods. *J Anim Ecol*. 2008;77(4):838-846. https://doi.org/10.1111/j.1365-2656.2008.01394.x
20. Murry BA, Farrell JM, Teece MA, Smyntek PM. Effect of lipid extraction on the interpretation of fish community trophic relationships determined by stable carbon and nitrogen isotopes. *Can J Fish Aquat Sci*. 2006;63(10):2167-2172. https://doi.org/10.1139/F06-116
21. Hoffman JC, Sutton TT. Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep Res Part I Oceanogr Res Pap*. 2010;57(8):956-964. https://doi.org/10.1016/j.dsr.2010.05.003
22. Matley JK, Fisk AT, Tobin AJ, Heupel MR, Simpfendorfer CA. Diet-tissue discrimination factors and turnover of carbon and nitrogen stable isotopes in tissues of an adult predatory coral reef fish, *Plectropomus leopardus*. *Rapid Commun Mass Spectrom*. 2016;30(1):29-44. https://doi.org/10.1002/rcm.7406

23. Fry B. Stable isotopic indicators of habitat use by Mississippi River fish. *J North Am Benthol Soc*. 2002;21(4):676-685.
24. Kelly MH, Hagar WG, Jardine TD, Cunjak RA. Nonlethal sampling of sunfish and slimy sculpin for stable isotope analysis: How scale and fin tissue compare with muscle tissue. *North Am J Fish Manag*. 2006; 26(4):921-925. https://doi.org/10.1577/m05-084.1
25. Hayden B, Soto DX, Jardine TD, et al. Small tails tell tall tales – intra-individual variation in the stable isotope values of fish fin. *PLoS One*. 2015;10(12):1-18. https://doi.org/10.1371/journal.pone.0145154
26. McIntyre PB, Flecker AS. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. *Oecologia*. 2006;148(1):12-21. https://doi.org/10.1007/s00442-005-0354-3
27. Charest MA. Turnover rates and trophic discrimination factors of $\delta^{34}S$ in brook trout, *Salvelinus fontinalis*. BSc thesis. University of New Brunswick. Published Online 2016.
28. Heady WN, Moore JW. Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecologia*. 2013;172(1):21-34. https://doi.org/10.1007/s00442-012-2483-9
29. Willis TJ, Sweeting CJ, Bury SJ, et al. Matching and mismatching stable isotope (δ ^{13}C and $\delta^{15}N$) ratios in fin and muscle tissue among fish species: A critical review. *Mar Biol*. 2013;160(7):1633-1644. https://doi.org/10.1007/s00227-013-2216-6
30. Hanisch JR, Tonn WM, Paszkowski CA, Scrimgeour GJ. $\delta^{13}C$ and $\delta^{15}N$ signatures in muscle and fin tissues: Nonlethal sampling methods for stable isotope analysis of salmonids. *North Am J Fish Manag*. 2010;30(1):1-11. https://doi.org/10.1577/m09-048.1
31. Jardine TD, Gray MA, McWilliam SM, Cunjak RA. Stable isotope variability in tissues of temperate stream fishes. *Trans Am Fish Soc*. 2005;134(5):1103-1110. https://doi.org/10.1577/T04-124.1
32. Curry RA, Gautreau MD, Culp JM. Fin tissues as surrogates of white muscle when assessing carbon and nitrogen stable isotope levels for Arctic and brook char. *Environ Biol Fishes*. 2014;97(6):627-633. https://doi.org/10.1007/s10641-013-0165-z
33. Graham CT, Harrison SSC, Harrod C. Development of non-lethal sampling of carbon and nitrogen stable isotope ratios in salmonids: Effects of lipid and inorganic components of fins. *Isot Environ Health Stud*. 2013;49(4):555-566. https://doi.org/10.1080/10256016.2013.808635
34. Mumby JA, Larocque SM, Johnson TB, et al. Diet and trophic niche space and overlap of Lake Ontario salmonid species using stable isotopes and stomach contents. *J Great Lakes Res*. 2018;44(6): 1383-1392. https://doi.org/10.1016/j.jglr.2018.08.009
35. Brandt SB. Food of trout and Salmon in Lake Ontario. *J Great Lakes Res*. 1986;12(3):200-205. https://doi.org/10.1016/S0380-1330(86)71719-X
36. Rand PS, Stewart DJ. Dynamics of salmonine diets and foraging in Lake Ontario, 1983–1993: A test of a bioenergetic model prediction. *Can J Fish Aquat Sci*. 1998;55(2):307-317.
37. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*. 1959;37(8):911-917.
38. Skinner MM, Martin AA, Moore BC. Is lipid correction necessary in the stable isotope analysis of fish tissues? *Rapid Commun Mass Spectrom*. 2016;30(7):881-889. https://doi.org/10.1002/rcm.7480
39. Bodin N, Le Loc'h F, Hily C. Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *J Exp Mar Biol Ecol*. 2007;341(2):168-175. https://doi.org/10.1016/j.jembe.2006.09.008
40. McConnaughey T, McRoy CP. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar Biol*. 1979; 53(3):257-262. https://doi.org/10.1007/BF00952434
41. R Core Team. R: A Language and Environment for Statistical Computing. 2020. https://www.r-project.org/
42. Hette-Tronquart N, Mazeas L, Reuilly-Manenti L, Zahm A, Belliard J. Fish fins as non-lethal surrogates for muscle tissues in freshwater food web studies using stable isotopes. *Rapid Commun Mass Spectrom*. 2012;26(14):1603-1608. https://doi.org/10.1002/rcm.6265
43. Wang SK, Xu C, Wang Y, et al. Nonlethal sampling for stable isotope analysis of juvenile Chinese sturgeon (*Acipenser sinensis*): Comparing $\delta^{13}C$ and $\delta^{15}N$ signatures in muscle and fin tissues. *J Appl Ichthyol*. 2017;33(5):877-884. https://doi.org/10.1111/jai.13433
44. Sholto-Douglas AD, Field JG, James AG, van der Merwe NJ. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios in the southern Benguela ecosystem: Indicators of food web relationships among different size- classes of plankton and pelagic fish; differences between fish muscle and bone collagen tissues. *Mar Ecol Prog Ser*. 1991;78(1):23-31. https://doi.org/10.3354/meps078023
45. Ofukany AFA, Wassenaar LI, Bond AL, Hobson KA. Defining fish community structure in Lake Winnipeg using stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$): Implications for monitoring ecological responses and trophodynamics of mercury & other trace elements. *Sci Total Environ*. 2014;497–498:239-249. https://doi.org/10.1016/j.scitotenv.2014.07.125
46. Heuvel CE, Haffner GD, Zhao Y, Colborne SF, Despenic A, Fisk AT. The influence of body size and season on the feeding ecology of three freshwater fishes with different diets in Lake Erie. *J Great Lakes Res*. 2019;45(4):795-804. https://doi.org/10.1016/j.jglr.2019.05.001
47. Moradi Y, Bakar J, Motalebi AA, Syed Muhamad SH, Che Y. A review on fish lipid: Composition and changes during cooking methods. *J Aquat Food Prod Technol*. 2011;20(4):379-390. https://doi.org/10.1080/10498850.2011.576449
48. Oppel S, Federer RN, O'Brien DM, Powell AN, Hollmén TE. Effects of lipid extraction on stable isotope ratios in avian egg yolk: Is arithmetic correction a reliable alternative? *Auk*. 2010;127(1):72-78. https://doi.org/10.1525/auk.2009.09153
49. Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC. Stable isotope turnover and half-life in animal tissues: A literature synthesis. *PLoS One*. 2015;10(1):1-16. https://doi.org/10.1371/journal.pone.0116182

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Larocque SM, Fisk AT, Johnson TB. Evaluation of muscle lipid extraction and non-lethal fin tissue use for carbon, nitrogen, and sulfur isotope analyses in adult salmonids. *Rapid Commun Mass Spectrom*. 2021;35:e9093. https://doi.org/10.1002/rcm.9093