Isotopes in Environmental and Health Studies, 2013
http://dx.doi.org/10.1080/10256016.2013.808635

Taylor & Francis
Taylor & Francis Group

Development of non-lethal sampling of carbon and nitrogen stable isotope ratios in salmonids: effects of lipid and inorganic components of fins

Conor T. Graham[a]*, Simon S.C. Harrison[a] and Chris Harrod[b,c]

[a]*Enterprise Centre, School of Biological, Earth and Environmental Sciences, University College Cork, North Mall, Cork, Ireland;* [b]*Medical Biology Centre, School of Biological Sciences, Queen's University Belfast, Belfast, UK;* [c]*Instituto de Investigaciones Oceanológicas, Universidad de Antofagasta, Antofagasta, Chile*

(*Received 21 November 2012; final version received 12 April 2013*)

The preferred tissue for analyses of fish stable isotope ratios for most researchers is muscle, the sampling of which typically requires the specimen to be sacrificed. The use of non-destructive methods in fish isotopic research has been increasing recently, but as yet is not a standard procedure. Previous studies have reported varying levels of success regarding the utility of non-lethally obtained stable isotope materials, e.g. fins, but none have accounted for the potential compounding effects of inorganic components of fin rays or lipids. Comparisons of carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios of muscle with adipose and caudal fin of two salmonids, Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.), revealed that caudal fin can be used as a non-destructive surrogate for muscle in stable isotope analysis, but that adipose fin, where available, is a better proxy. The use of a published model to inexpensively counteract the confounding effect of lipids, which are depleted in ^{13}C, greatly improved the relationship between fish muscle and fins. However, efforts to account for the inorganic components of fin rays were counterproductive and required twice the biomass of fins clipped from each fish. As this experiment was conducted on wild fish, controlled laboratory studies are required to confirm these field observations.

Keywords: carbon-13; decalcification; fins; fish; isotope ecology; lipids; methodology; muscles; nitrogen-15; sampling techniques

1. Introduction

The use of stable isotope analysis (SIA) in ecological research has increased rapidly during the last two decades [1] and has provided ecologists with a powerful tool within a wide range of ecological contexts [2]. For over a decade, fish biologists have recognised white muscle as the preferred tissue for SIA as it shows less variability in δ^{13}C and δ^{15}N compared with other tissues [3]. However, the use of muscle for SIA typically requires the sacrifice of the individual (but see [4]). For many researchers this poses a dilemma, as frequently the rationale of the research is to further understand the ecology of rare or endangered species to improve conservation strategies [5] or if repeated sampling of individuals is desirable [6]. Some recent studies, however, have advocated the use of alternative structures that can be sampled non-destructively, such as feathers

*Corresponding author. Email: grahamconor@gmail.com

and claw clippings [7] and blood in birds [8], nail clippings in newts [9] and hair in humans [10] and wolves [11]. However, it is arguably in the research of fish that alternative, non-lethally sampled tissues have been used most extensively in SIA, including scales [12–14], adipose fins [15–17], various rayed fins [13,18,19], mucus [20] and potentially blood [21].

Fin tissues represent potential non-destructive proxies for muscle and other lethally sampled tissues such as liver or otoliths [3,22]. However, in the majority of studies, the fins used for stable isotope ratios are rayed fins such as caudal and pectoral fins. Fin rays are an integral part of the skeleton of bony fishes [23], containing inorganic components such as apatites [24] that may be metabolically inert and form a non-diet origin. In order to correct for the influence of inorganic carbon, samples for SIA are typically acidified using a hydrochloric acid solution [3,25,26]. However, this treatment can affect δ^{15}N values [3,26]. Therefore, many workers analyse separate HCl-treated samples for δ^{13}C and untreated samples for δ^{15}N, increasing not only the monetary and labour costs but also the amount of fin clipped from each fish. Although not present in all families of fish, the adipose fin does not contain any fin rays [27] and may represent a better proxy for muscle compared with rayed fins.

When conducting studies of trophic interactions between fish consumers and their prey, the lipid composition in the muscle of fish potentially complicates the interpretation of diet consumed, as lipids are known to be depleted in ^{13}C [28–30]. A number of methodologies have been developed to extract lipids using chloroform–methanol or hexane–isopropanol solvent mixtures prior to analysis. Such lipid-corrected values for δ^{13}C are believed to reflect assimilated carbon more accurately [31]. Although it has been suggested that lipid extraction methodologies may affect δ^{15}N values [31,32] and again, many workers analyse two samples, adding to both the financial and labour costs, other research has concluded no or varying effect of lipid extraction methodologies on sample δ^{15}N on a variety of tissue types [33,34]. However, lipid-normalising models have been developed as the lipid content of tissues can be estimated from the ratio of carbon to nitrogen within tissues. Recently, the most widely used model, that of McConnaughey and McRoy [32], has been modified and improved for fish muscle [31], providing researchers with an accurate and cost effective alternative to lipid removal techniques.

Here, we compare δ^{13}C and δ^{15}N values of muscle with adipose fin and acidified and non-acidified caudal fin (to examine the possible influences of inorganic carbon in fin rays) of two widely distributed salmonid species, the Atlantic salmon (*Salmo salar* L.) and the brown trout (*Salmo trutta* L.), to determine which non-lethally sampled fin is the more suitable proxy for muscle in SIA. As the muscle of salmonids can have high lipid content [31], the relationship between the stable isotope ratios of the fins and muscle corrected for lipid content is examined using the model modified by Kiljunen et al. [31]. We conducted this study on wild fish and therefore had no control on the dietary history of the fish. However, in a laboratory-controlled experiment, Suzuki et al. [35] showed the half-life values were similar for Japanese sea bass (*Labeolabrax japonicus*) muscle and fin tissue for both δ^{13}C and δ^{15}N. Our study also focuses on situations were fish are consumers/predators and not when they themselves are prey, as we did not compare the fin tissue isotope values to that of the entire fish (see [4]), although muscle mass comprises approximately 50% of fish total mass [36].

2. Methods

In May 2006, 30 wild brown trout (aged between 0+ and 3+; fork length (FL) range 69–232 mm) were collected from the River Awbeg, southwestern Ireland (52.14°N, 8.81°W). Wild Atlantic salmon (aged 1+ and 2+; $n = 25$; FL range 82–135 mm) was collected from the Outeragh River, southern Ireland (52.39°N, 7.92°W) in October 2007. All fish were killed in a solution of 2% clove

oil and transported on ice to the laboratory where they were frozen (−20°C) prior to processing within a week.

After defrosting at room temperature, all fish were measured and weighed to the nearest millimetre and 0.01 g, respectively. Dorsal white muscle tissue was excised from between the dorsal fin and the lateral line, insuring that no skin or bones were included in the sample. The adipose fin was removed, as was a section of the dorsal section of the caudal fin (approximately $<1\,cm^2$). All tissues were oven-dried at a constant temperature of 60°C for 24–48 h. The dried caudal fin sample was divided equally, with one half treated with HCl acid to remove inorganic C, by adding $1\,M\,l^{-1}$ drop-by-drop until effervescence of carbon dioxide gas ceased, following Jacob et al. [26] prior to being re-dried at 60°C for 24–48 h. The remaining caudal fin section was analysed without treatment to determine the $\delta^{15}N$ value unaffected by acid washing. Muscle tissue was ground with a mortar and pestle to a fine homogenous powder prior to being weighed into combustible tin cups. Adipose and caudal fins (both acidified and untreated) were clipped into an appropriate size for analysis using scissors. All samples were weighed to 400 μg. Unfortunately, two of the samples of untreated trout caudal fins were lost. Simultaneous analysis of stable carbon and nitrogen isotope ratios of all samples was determined by continuous flow mass spectrometry at the Max Planck Institute for Limnology, Germany, by combustion in a Eurovector elemental analyser interfaced to a Micromass Isoprime isotope ratio mass spectrometer (see [37]). The reference materials used were secondary standards of known relation to the international standards of Vienna PDB for carbon and atmospheric N_2 for nitrogen. Typical precision for a single analysis was ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Lipid-corrected $\delta^{13}C$ values were calculated based on the model given in Kiljunen et al. [31]. This approach relies on the ratio of elemental C:N in order to estimate lipid content. As such, sample masses must be determined to a high level of precision. The salmon tissue samples in this study were weighed to a precision of 0.001 mg, whereas the trout samples were weighed to a precision of 0.1 mg. Due to this limited resolution, it was not possible to reliably estimate lipid-corrected $\delta^{13}C$ values for trout using the model of Kiljunen et al. [31].

Paired *t*-tests were used to compare $\delta^{13}C$ and $\delta^{15}N$ values between different tissues. The $\delta^{13}C$ and $\delta^{15}N$ values of different fins were regressed on muscle isotope values using least-squares linear regression. These statistical tests were conducted using SPSS 12.1 for Windows. Cochran's test for homogeneity and *t*-tests comparing regression slopes with unity (i.e. to test that the relationship between fin and muscle isotope values was isometric) were conducted in Excel 2003 for Windows XP.

3. Results

The average difference in stable isotope ratios of fins relative to muscle differed between the two species, with all three fin types being ^{13}C and ^{15}N-enriched relative to muscle in trout. Conversely, in salmon, fins were ^{13}C and ^{15}N-depleted relative to muscle. The degree of enrichment differed, however, with generally a greater difference in $\delta^{15}N$ compared with $\delta^{13}C$ (Table 1).

3.1. *$\delta^{15}N$: muscle vs. fins*

There was no difference between the $\delta^{15}N$ values of muscle and adipose fin in trout (average difference = 0.7‰, Table 1). However, there were differences between muscle and both treated and untreated caudal fin $\delta^{15}N$ for both species, and between $\delta^{15}N$ values of muscle and adipose fin in salmon (average differences ranged between −0.9 and 1.3‰, Table 1). While there was no significant difference between the $\delta^{15}N$ values of muscle and adipose fin in trout (Figure 1), there was considerable scatter around the regression line, resulting in a relatively low R^2 value of

Table 1. Results of paired *t*-tests comparing δ^{13}C and δ^{15}N values of muscle and the three fin types examined in salmon and trout.

	Atlantic salmon				Brown trout			
Paired t-tests	*t*	d.f.	Sig.	Av. diff.	*t*	d.f.	Sig.	Av. diff.
δ^{15}N: muscle vs. adipose fin	13.57	24	<0.001	-0.9 ± 0.1	−0.95	29	=0.349	0.7 ± 0.1
δ^{15}N: muscle vs. caudal fin	12.55	24	<0.001	-0.8 ± 0.1	−4.02	27	<0.001	1.3 ± 0.7
δ^{15}N: muscle vs. acidified caudal fin	12.55	24	<0.001	-0.7 ± 0.1	−7.09	29	<0.001	0.6 ± 0.1
δ^{13}C: muscle vs. adipose fin	5.528	24	<0.001	-0.6 ± 0.1	−11.68	29	<0.001	0.7 ± 0.1
δ^{13}C: muscle vs. caudal fin	3.846	24	=0.001	-0.4 ± 0.1	−15.67	27	<0.001	1.2 ± 0.1
δ^{13}C: muscle vs. acidified caudal fin	5.046	24	<0.001	-0.7 ± 0.1	−7.48	29	<0.001	0.7 ± 0.1
δ^{13}C: lipid-corrected muscle vs. lipid-corrected adipose fin	−2.378	24	=0.026	0.2 ± 0.7				
δ^{13}C: lipid-corrected muscle vs. lipid-corrected caudal fin	0.487	24	=0.63	-0.1 ± 0.1				
δ^{13}C: lipid-corrected muscle vs. lipid-corrected acidified caudal fin	−1.112	24	=0.277	0.1 ± 0.1				

Notes: The mean (±SD) difference between tissues reflects enrichment/depletion relative to muscle. Due to limited resolution in the weighing of trout samples, it was not possible to estimate lipid-corrected δ^{13}C values for these fish.

0.42 (Table 2). Although there were significant differences between muscle and both treated and untreated caudal fin δ^{15}N values for both species, and between δ^{15}N values of muscle and adipose fin in salmon (Table 1), regressions between muscle δ^{15}N and all three fin types were highly significant for both species (Table 2, all $p < 0.01$). In salmon, the relationships were strongest in adipose fin, intermediate in caudal fin and less strong in decalcified caudal fin. Conversely, in trout they were strongest in caudal fin, then adipose fin and finally decalcified caudal fin (Table 2).

3.2. δ^{13}C: muscle vs. fins

There were significant differences between the δ^{13}C of muscle and each of the three fin types in both salmon and trout (Table 1). However, the average difference between δ^{13}C of muscle and each of the three fin types in both species was small (0.4–1.2‰, Table 1), resulting in relationships between muscle and fin δ^{13} carbon values with R^2 values between 0.53 and 0.82 (Figure 1 and Table 2). In both species, relationships were strongest for adipose, then caudal and finally decalcified caudal fin (Table 2). The regression between δ^{13}C of muscle and adipose fin was strong in salmon ($R^2 = 0.66$) and particularly so in trout ($R^2 = 0.82$).

3.3. δ^{13}C: lipid-corrected muscle vs. lipid-corrected fins

When the lipid correction model of Kiljunen et al. [31] was applied to the Atlantic salmon tissue types, the mean difference between lipid-normalised muscle and fins decreased substantially to <0.2‰ for all three fin types (Table 1). Furthermore, arithmetic lipid correction also increased predictive power (as estimated by R^2). Following lipid correction, adipose fin once again showed the highest predictive power ($R^2 = 0.85$), followed in turn by caudal fin ($R^2 = 0.7$) and decalcified caudal fin ($R^2 = 0.62$). There were no significant differences between lipid-corrected δ^{13}C values for muscle and lipid-corrected caudal fin and decalcified caudal fin tissues (Table 1). Although lipid-corrected adipose fin was enriched by on average less than 0.2‰ relative to lipid-corrected

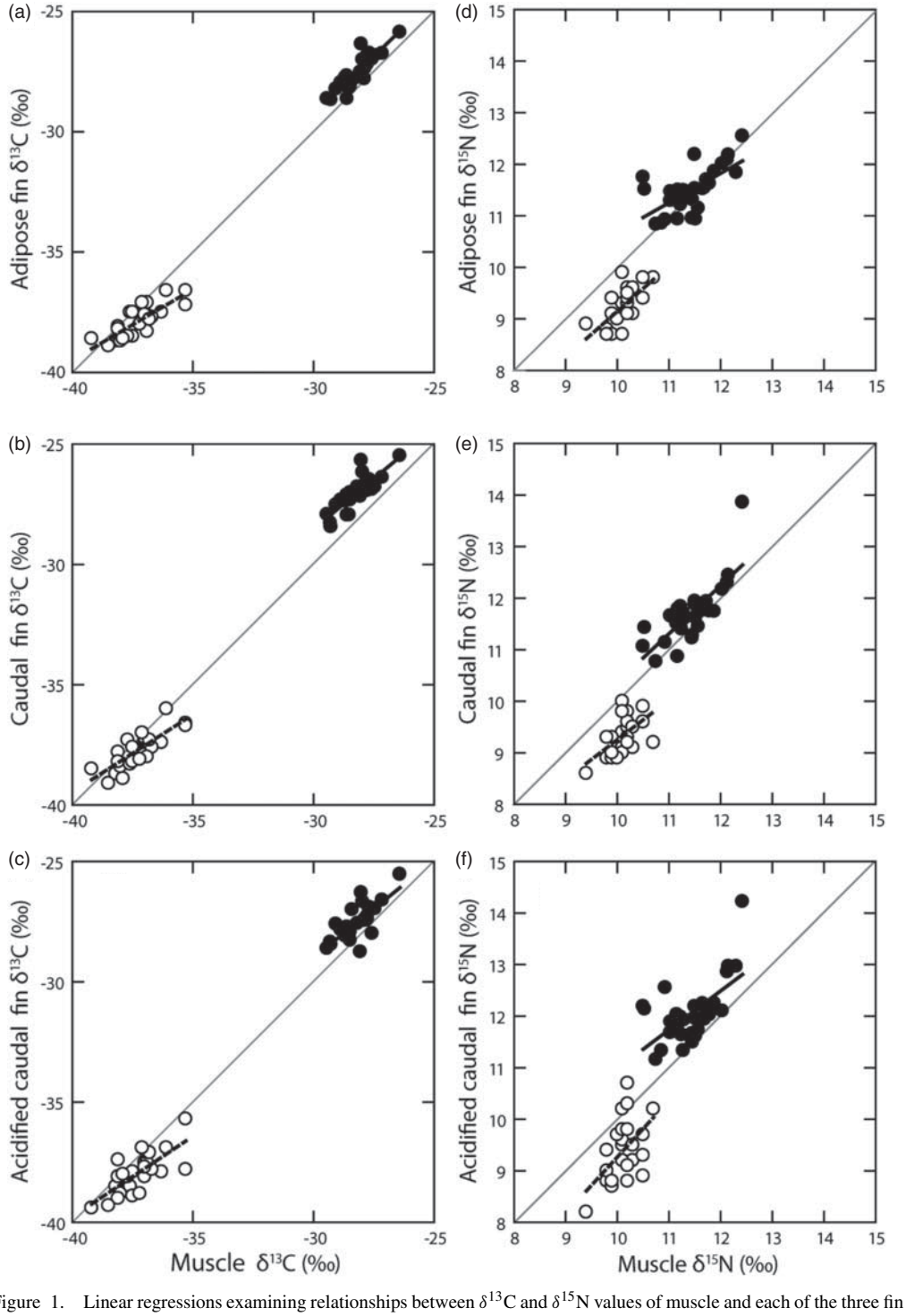

Figure 1. Linear regressions examining relationships between $\delta^{13}C$ and $\delta^{15}N$ values of muscle and each of the three fin types: adipose fin, caudal fin and decalcified caudal fin for salmon (open markers) and trout (filled markers). A 1:1 line is shown for clarity.

Table 2. Parameters of least-square regressions of $\delta^{13}C$ and $\delta^{15}N$ of muscle and three different fin types of salmon and trout (±standard error).

	Atlantic salmon					Brown trout				
Linear regression	n	R^2	Slope (b)	Intercept (a)	p	n	R^2	Slope (b)	Intercept (a)	p
$\delta^{15}N$: muscle vs. adipose fin	25	0.37	0.88 (±0.24)	2.41 (±2.4)	=0.001	30	0.42	0.57 (±0.13)	4.979(±1.5)	<0.001
$\delta^{15}N$: muscle vs. caudal fin	25	0.36	0.77 (±0.23)	1.59 (±2.3)	=0.002	28	0.58	0.938(±0.15)	0.996(±1.8)	<0.001
$\delta^{15}N$: muscle vs. acidified caudal fin	25	0.26	1.1 (±0.38)	−1.7 (±3.9)	=0.009	30	0.38	0.754(±0.18)	3.438(±2.1)	<0.001
$\delta^{13}C$: muscle vs. adipose fin	25	0.66	0.59 (±0.9)	−15.97 (±3.3)	<0.001	30	0.82	0.969(±0.09)	−0.215(±2.5)	<0.001
$\delta^{13}C$: muscle vs. caudal fin	25	0.64	0.64 (±0.1)	−13.73 (±3.8)	<0.001	28	0.69	0.838(±0.11)	−3.389(±3.1)	<0.001
$\delta^{13}C$: muscle vs. acidified caudal fin	25	0.53	0.67 (±0.13)	−12.93 (±4.9)	<0.001	30	0.57	0.812(±0.13)	−4.630(±3.8)	<0.001
$\delta^{13}C$: lipid-corrected muscle vs. lipid-corrected adipose fin	25	0.85	0.71 (±0.64)	−10.23 (±2.3)	<0.001					
$\delta^{13}C$: lipid-corrected muscle vs. lipid-corrected caudal fin	25	0.7	0.62 (±0.09)	−13.71 (±3.1)	<0.001					
$\delta^{13}C$: lipid-corrected muscle vs. lipid-corrected acidified caudal fin	25	0.62	0.72 (±0.12)	−9.94 (±4.3)	<0.001					

Table 3. Average (±SD), minimum and maximum C:N values of Atlantic salmon muscle and adipose, caudal and acid-treated caudal fin tissue.

	Muscle	Adipose fin	Caudal fin	Acidified caudal fin
Average	3.46 (±0.02)	3.97 (±0.03)	3.69 (±0.02)	3.99 (±0.05)
Minimum	3.41	3.65	3.49	3.49
Maximum	3.88	4.28	4.08	4.49

muscle, they were still statistically significantly different, despite the strong significant relationship between the two (Table 1 and Figure 2). The average, minimum and maximum of the Atlantic salmon muscle, adipose, caudal and HCl-treated caudal fin C:N ratios are shown in Table 3.

Table 4. Results of t-tests examining whether the slopes of regressions between different tissues types were significantly different from unity (i.e. the relationship was isometric).

	Salmon			Trout		
Comparison of regression slope with unity	t	d.f.	Sig. diff.	t	d.f.	Sig.
$\delta^{15}N$: muscle vs. adipose fin	0.38	46	$p > 0.05$	3.39	56	$p < 0.01$
$\delta^{15}N$: muscle vs. caudal fin	1.04	46	$p > 0.05$	0.4	52	$p > 0.05$
$\delta^{15}N$: muscle vs. decalcified caudal fin	−0.25	46	$p > 0.05$	1.36	56	$p < 0.01$
$\delta^{13}C$: muscle vs. adipose fin	4.63	46	$p < 0.01$	0.36	56	$p > 0.05$
$\delta^{13}C$: muscle vs. caudal fin	3.53	46	$p < 0.01$	1.47	52	$p > 0.05$
$\delta^{13}C$: muscle vs. decalcified caudal fin	2.49	46	$p < 0.01$	1.41	56	$p > 0.05$
$\delta^{13}C$: estimated defatted muscle vs. estimated defatted adipose fin	0.45	46	$p > 0.05$			
$\delta^{13}C$: estimated defatted muscle vs. estimated defatted caudal fin	4.37	46	$p < 0.01$			
$\delta^{13}C$: estimated defatted muscle vs. estimated defatted decalcified caudal fin	2.35	46	$p < 0.01$			

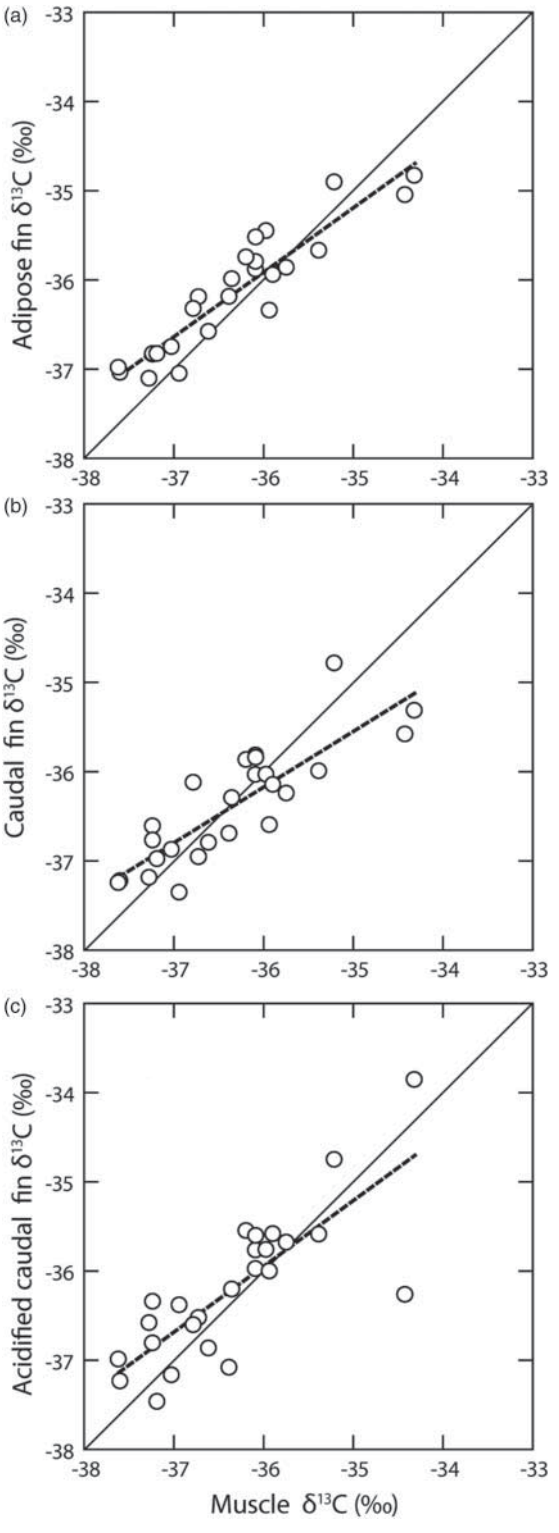

Figure 2. Scatterplots showing the relationship between estimated lipid-free muscle $\delta^{13}C$ values for salmon and three different fin types: (A) adipose fin, (B) caudal fin and (C) acidified caudal fin. 1:1 lines are shown for clarity.

3.4. *Correction factor to account for enrichment/depletion of fins relative to muscle*

The regression of salmon adipose, caudal and decalcified caudal fin δ^{15}N values on muscle δ^{15}N showed that these tissues were predictably ^{15}N-depleted relative to muscle (Table 4), allowing a simple arithmetic correction to estimate muscle δ^{15}N from fin tissues. In trout, adipose fin δ^{15}N values were not statistically distinguishable from muscle values. The relationship between trout muscle δ^{15}N and that of acid-treated and non-treated caudal fins differed. Whereas a simple correction factor could be applied to non-treated caudal fins, the slope of the regression between muscle and acid-treated caudal fin was significantly different from unity and therefore a simple correction factor was not possible (Table 4).

4. Discussion

Comparison of δ^{13}C and δ^{15}N values of muscle, caudal and adipose fin tissues taken from the same individuals has revealed the existence of significant relationships between muscle tissue and each of the fin types. However, there was a considerable difference between the capacities of each of the three fin types in predicting muscle δ^{13}C or δ^{15}N values, with adipose fin proving to be the most suitable proxy.

Brown trout adipose fins were a suitable proxy for δ^{15}N values of muscle as the δ^{15}N values of the two tissues were statistically indistinguishable. The isometric relationship between all three Atlantic salmon fin tissues and brown trout untreated caudal fin with muscle δ^{15}N values means that a simple correction factor could be applied in order to predict muscle δ^{15}N values from the fins of these salmon. However, this was not the case with brown trout decalcified caudal fins, which were an unsuitable surrogate for muscle δ^{15}N values. Despite significant relationships between muscle δ^{15}N and each of the fin types, there was considerable variation in the strength of these relationships, with a maximal R^2 values of 0.58. This compares to values generally ranging from 0.5 to 0.98 in the literature [19,38,39], although in some tropical fish this can be as low as 0.1 [19]. Previous studies have shown that relationships between fin and muscle δ^{15}N are typically less robust compared with fin–muscle δ^{13}C relationships [13,18,19].

In our study, salmon fins were ^{15}N-depleted compared with muscle, similar to previous findings [17,18]. However, the trout we sampled had fins that were ^{15}N-enriched relative to muscle which is in contrast with the results of McCarthy and Waldron [40], who reported that adipose fin tissues of brown trout were ^{15}N-enriched in relation to muscle. The existence of such differences among not only a family of fish but between populations of the same species is notable. This may reflect a seasonal effect or variation in the turnover times between different tissues following a recent dietary switch, despite evidence that ^{15}N turnover time in fish fins and muscle is similar [35]. Regardless, it does suggest that study or population-specific conversion factors may be required. Similar to the recommendations of Jardine et al. [19] we suggest that δ^{15}N values of fin tissues can be used in the place of muscle but that there will be a small degree of error associated with this substitution.

Comparison between salmon and trout muscle δ^{13}C values with the different fin tissues has revealed the clear potential for adipose fins to be used as a reliable proxy for muscle tissue. In trout, all fin tissues were slightly ^{13}C-enriched but closely related to muscle δ^{13}C. This reflects the results of other studies on a range of fish species from both tropical and temperate regions, including other salmonids [18,19,41]. Although the relationships between salmon fin and muscle tissue δ^{13}C were also strong, in contrast to trout, fin tissues were ^{13}C-depleted relative to muscle. However, this difference may reflect inter-species differences in the lipid content of the different tissues, which would be nullified by the lipid correction model, such as was the case with the Atlantic salmon here.

The coefficients of determination estimated in our regressions of fin δ^{13}C values on muscle δ^{13}C varied between 0.53 and 0.82. These results compare favourably with other studies, e.g. of tropical fishes (R^2 between 0.59 and 0.96) [19], salmonids (R^2 between 0.33 and 0.97) [18,38, 41] and other temperate fishes (R^2 between 0.92 and 0.97) [13,39,42]. However, although our regressions were significant, there were differences between all fin and muscle δ^{13}C values with considerable scatter around the regression line. However, this phenomenon is not uncommon and has been reported for lake trout (*Salvelinus namaycush*) [38], steelhead trout (*Oncorhynchus mykiss*) [41] and barramundi (*Lates calcarifer*) [19]. It is possible that individual variation and weak relationship between muscle and fin tissue may reflect variation in the level of influence of lipid-derived C at both the level of the individual and between tissues.

The use of stable isotopes to infer consumer diet requires an understanding of how the isotope ratio of the food source is reflected in the tissues of the consumer [43], especially when utilising mixing models to calculate the contribution of different sources to the tissues of an animal [44,45]. It is well established that lipids are ^{13}C-depleted [28–30] and that lipid-corrected values for δ^{13}C are believed to reflect assimilated carbon more accurately. Therefore, the comparison of interest for δ^{13}C should be if the lipid-corrected fin values accurately reflect the lipid-corrected muscle values and hence, diet of the study fish. With the application of the lipid correction model [31], the average difference of δ^{13}C between muscle and fin was reduced considerably to less than 0.2‰ for all fin types, which is very similar to the typical analytical precision for a single analysis of δ^{13}C ($\pm$0.1‰ based on the standard samples analysed). Despite this minor discrepancy, there was a difference between the lipid-corrected δ^{13}C values of salmon muscle and adipose fin. The isometric relationship of the regression between lipid-corrected adipose fin and lipid-corrected muscle was strong, and a simple correction factor of -0.2‰ applied to the lipid-corrected δ^{13}C values of adipose fin would provide reliable and accurate estimates of the lipid-corrected δ^{13}C values of salmon muscle. However, as the difference between lipid-corrected δ^{13}C values of salmon muscle and each fin type was similar to the level of precision for a single analysis of δ^{13}C, there is most likely no correction factor required.

The use of this lipid correction model [31] undoubtedly greatly improved the ability of fins to predict the δ^{13}C isotopic ratio of muscle. This is the first study that we know which has used the model of Kiljunen et al. [31] when testing the suitability of any tissue as a non-destructive method of sampling fish. Dempson and Power [17] lipid-corrected the δ^{13}C values of muscle using the model of McConnaughey and McRoy [32] prior to their analysis. In contradiction to the results here, the lipid-corrected δ^{13}C values of the adipose fins were enriched in ^{13}C by 0.5‰ relative to muscle, which they attributed to the larger multi sea-winter salmon having more lipids in their adipose fins. However, the lipid-normalisation model used by McConnaughey and McRoy's [32] assumed a C:N ratio of 4 to be normal for animal tissue. Therefore, the discrepancy between the lipid-corrected δ^{13}C values of muscle and adipose fins in the salmon tested by Dempson and Power [17] is most probably caused by an underestimation of muscle C:N values in adult salmon, which can be quite high and variable (mean C : N = 6.78 $\pm$2.67 SD) compared with other fish species as reported by Kiljunen et al. [31]. Other researchers investigating the use of other tissues as a non-lethal form of obtaining stable isotope samples from fish have indicated that lipid correction was not necessary when C:N ratios are below 4 [19,38,41]. However, lipids can result in considerable depletion in ^{13}C at C:N ratios between 3.2 and 4 of up to 1.3‰ [31]. Jardine et al. [19] reported an increase of up to 0.9 in the C:N ratio of muscle compared with caudal fin of tropical fish, which is higher than the 0.7 discrepancy between tissues of any of the salmon individuals in this study. It is likely that the correction of these tissues would have greatly enhanced the relationship between these δ^{13}C values tissues. However, we must highlight that this lipid correction model of Kiljunen et al. [31] was developed and validated for correcting the lipid content of fish muscle and no other tissue such as fins, particularly those containing inorganic constituents such as caudal fins, as we have applied it here. However, it is clear that

lipids complicate the interpretation of mixing model outputs [31] and hence lipid-corrected $\delta^{13}C$ data are ideally what is desired when applying such mixing models.

We hypothesised that the inorganic content of the caudal fin would reduce the effectiveness of this tissue to accurately predict the stable isotope ratios of carbon of the fish muscle. However, the decalcification process actually impaired rather than improved the ability of caudal fin to act as a proxy for flesh. HCl treatment can affect the C:N ratio, even on samples that are presumably calcium carbonate free [26] and therefore may have influenced the comparison of lipid-normalised decalcified caudal fin with muscle. This procedure also doubles the number of samples required for analyses as the $\delta^{15}N$ values were affected by the decalcification process as demonstrated elsewhere [3,26], doubling not only the price but the biomass of fin clip required. We therefore conclude that there is no benefit in the decalcification of inorganic fin rays when using rayed fins as a non-lethal form of obtaining stable isotope ratios of fish.

The clipping of adipose fin as a standard marking technique is widely used on millions of salmonids every year that are reared for stocking fisheries. Traditionally, the adipose fin has been considered as vestigial without clear function. Recently, however, the detection of an extensive neural network throughout the adipose fin suggests that it is operating as a mechanosensory organ nervous tissue [27]. Laboratory experiments have indicated reduced swimming proficiency after the removal of the adipose fin of rainbow trout (*O. mykiss*), suggesting that it may act as a precaudal flow sensor, particularly in turbulent water [46]. The clipping of any fin also exposes the fish to potential of infection. Nevertheless, experimental evidence indicates that the removal of the adipose fin has less impact than the removal of other fins [47] and its removal may be preferential over the killing of the fish to obtain a sample for SIA.

The destructive sampling of fish is undesirable and the results of this study support the use of non-lethally obtained fin samples of fish for SIA of $\delta^{13}C$ and $\delta^{15}N$. Although it appears from the data here that adipose fin is the most suitable for predicting $\delta^{13}C$ and $\delta^{15}N$ values of muscle, many species of fish do not possess an adipose fin. However, the use of rayed fins also accurately predicts the $\delta^{13}C$ and $\delta^{15}N$ values of muscle, without the requirement to decalcify this tissue to account for inorganic carbon components of the fin rays. However, samples ought to be corrected for lipid content when estimating the $\delta^{13}C$ values of fins. This is not solely because of the difference in the lipid content of various tissues but rather due to the fact that lipid-corrected values reflect the assimilated carbon from the diet more accurately.

There are numerous advantages of non-lethal sampling of fish fins, e.g. it permits the repeated collection of values from the same individual over time, which can be very useful in tracking individual migration patterns or ontogenetic shifts in feeding ecology. Most importantly, it allows researchers to use this powerful tool to study fish that are threatened or endangered without the need to sacrifice every individual they sample. However, difference in fractionation recorded in $\delta^{15}N$ among not only a family of fish but between populations of the same species is notable and may restrict the general application of these results and therefore may require the sacrifice of some individuals to determine any conversion factor required.

Acknowledgements

This work was funded through a grant awarded by Science Foundation Ireland (05/RFP/EEB0055) under the Research Frontier Programme. We would like to thank R. Macklin for assistance in the field. The authors would like to thank the two anonymous reviewers for their constructive comments and suggestions on an earlier draft that improved the quality of the paper.

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;42:411–440.

[2] Fry B. Stable isotope ecology. New York: Springer; 2006.
[3] Pinnegar JK, Polunin VC. Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. Funct Ecol. 1999;13:225–231.
[4] Schielke EG, Post DM. Size matters: comparing stable isotope ratios of tissue plugs and whole organisms. Limnol Oceanogr Methods. 2010;8:348–351.
[5] Andvik RT, VanDeHey JA, Fincel MJ, French WE, Bertrand KN, Chipps SR, Klumb RA, Graeb BDS. Application of non-lethal stable isotope analysis to assess feeding patterns of juvenile pallid sturgeon *Scaphirhynchus albus*: a comparison of tissue types and sample preservation methods. J Appl Ichthyol. 2010;26:831–835.
[6] Cunjak RA, Roussel J-M, Gray MA, Dietrich JP, Cartwright DF, Munkittrick KR, Jardine TD. Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. Oecologia. 2005;144: 636–646.
[7] Bearhop S, Furness RW, Hilton GM, Votier SC, Waldron S. A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. Funct Ecol. 2003;17:270–275.
[8] Hobson KA, Clark RG. Turnover of ^{13}C in celluar and plasma fractions of blood: implications for non-destructive sampling in avian dietary studies. Auk. 1993;110:638–641.
[9] Finlay JC, Khandwala S, Power MG. Spatial scales of carbon flow in a river food web. Ecology. 2002;83:1845–1859.
[10] Macko SA, Engel MH, Andrusevich V, Lubea G, O'Connell TC, Hedges REM. Documenting the diet in ancient human populations through stable isotope analysis of hair. Philos Trans R Soc B. 1999;354:65–76.
[11] Dairmont CT, Reimchen TE. Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet. Can J Zool. 2002;80:1638–1642.
[12] Grey J, Graham CT, Britton JR, Harrod C. Stable isotope analysis of archived roach (*Rutilis rutilis*) scales for retrospective study of shallow lake responses to nutrient reduction. Freshwater Biol. 2009;54:1663–1670.
[13] 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 Manage. 2006;26:921–925.
[14] Syväranta J, Vesala S, Rask M, Ruuhijärvi J, Jones RI. Evaluating the utility of stable isotope analyses of archived freshwater sample materials. Hydrobiologia. 2008;600:121–130.
[15] McCarthy ID, Fraser D, Waldron S, Adams CE. A stable isotope analysis of trophic polymorphism among Arctic charr from Loch Ericht, Scotland. J Fish Biol. 2004;65:1435–1440.
[16] Doucett R, 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;128:278–288.
[17] Dempson JB, Power M. Use of stable isotopes to distinguish farmed from wild Atlantic salmon, *Salmo salar*. Ecol Freshwater Fish. 2004;13:176–184.
[18] Jardine TD, Gray MA, McWilliam SM, Cunjak RA. Stable isotope variability in tissues of temperate stream fishes. Trans Am Fish Soc. 2005;134:1103–1110.
[19] Jardine TD, Hunt RJ, Pusey BJ, Bunn SE. A non-lethal sampling method for stable carbon and nitrogen isotope studies of tropical fishes. Mar Freshwater Res. 2011;62:83–90.
[20] Church MR, Ebersole JL, Rensmeyer KM, Couture RB, Barrows FT, Noakes DLG. Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. Can J Fish Aquat Sci. 2009;66:1–5.
[21] German DP, Miles RD. Stable carbon and nitrogen incorporation in blood and fin tissue of the catfish *Pterygoplichthys disjunctivus* (Siluriformes, Loricariidae). Environ Biol Fishes. 2010;89:117–133.
[22] Radtke RL, Lenz P, Showers W, Moksness E. Environmental information stored in otoliths: insights from stable isotopes. Mar Biol. 1996;127:161–170.
[23] Lagler KF, Bardach JE, Miller RR. Ichthyology. New York: Wiley; 1962.
[24] Cameron JN. The bone compartment in a teleost fish, *Ictalurus punctatus*: size, composition and acid based response to hypercapnia. J Exp Biol. 1985;117:307–318.
[25] Angradi TR. Trophic linkages in the lower Colarado River: multiple stable isotope evidence. J North Am Benthol Soc. 1994;13:479–495.
[26] Jacob U, Mintenbeck K, Brey T, Knust R, Beyer K. Stable isotope food web studies: a case for standardized sample treatment. Mar Ecol Prog Ser. 2005;287:251–253.
[27] Buckland-Nicks JA, Gillis M, Reimchen TE. Neural network detected in a presumed vestigial trait: ultrastructure of the salmonid adipose fin. Proc R Soc B Biol Sci. 2011;297:553–563.
[28] Peterson BJ, Fry B. Stable isotopes in ecosystem studies. Annu Rev Ecol Syst. 1987;18:293–320.
[29] DeNiro MJ, Epstein S. Influence of diet in the distribution of carbon isotopes in animals. Geochim Cosmochim Acta. 1978;42:495–506.
[30] Focken U, Becker K. Metabolic fractionation of stable carbon isotopes: implications of different proximate compositions for studies of the aquatic food webs using δ^{13}C data. Oecologia. 1998;115:337–343.
[31] Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H, Jones RI. A revised model for lipid-normalisation of carbon stable isotope values from aquatic organisms, with implications for the use of isotope mixing models to evaluate diets of consumers. J Appl Ecol. 2006;43:1213–1222.
[32] McConnaughey T, McRoy CP. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar Biol. 1979;53:257–262.
[33] Ingram T, Matthews B, Harrod C, Stephens T, Grey J, Mazumder A. Little effect of lipid extraction on δ^{15}N of aquatic consumers. Limnol Oceanogr Methods. 2007;5:338–343.
[34] 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;77:838–846.

[35] Suzuki KW, Kasai A, Nakayama K, Tanaka M. Differential isotope enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. Can J Fish Aquat Sci. 2005;62:671–678.
[36] Webb PW. Fast-start performance and body form in seven species of teleost fish. J Exp Biol. 1978;74:211–226.
[37] Harrod C, Grey J. Isotopic variation complicates analysis of trophic relations within the fish community of Plußsee: a small, deep, stratifying lake. Arch Hydrobiol. 2006;167:281–299.
[38] Hanisch JR, Tonn WM, Paszkowski CA, Scrimgeour GJ. δ^{13}C and δ^{15}N signatures in muscle and fin tissues: nonlethal sampling methods for stable isotope analysis of salmonids. North Am J Fish Manage. 2010;30:1–11.
[39] Syväranta J, Cucherousset J, Kopp D, Crivelli A, Céréghino R, Santoul F. Dietary breadth and trophic position of introduced European catfish *Silurus glanis* in the River Tarn (Garonne River basin), southwest France. Aquat Biol. 2010;8:137–144.
[40] McCarthy ID, Waldron S. Identifying migratory *Salmo trutta* using carbon and nitrogen stable isotope ratios. Rapid Commun Mass Spectrom. 2000;14:1325–1331.
[41] 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;138:1166–1177.
[42] Fincel MJ, VanDeHey JA, Chipps SR. Non-lethal sampling of walleye for stable isotope analysis: a comparison of three tissues. Fish Manage Ecol. 2011;19:283–292.
[43] Solomon C, Doucett RR, Pace M, Preston N, Smith L, Weidel B. The influence of dietary water on the hydrogen stable isotope ratio of aquatic consumers. Oecologia. 2009;161:313–324.
[44] Phillips DL, Gregg JW. Uncertainity in source partitioning using stable isotopes. Oecologia. 2001;127:171–179.
[45] Phillips DL, Gregg JW. Source partitioning using stable isotopes: coping with too many sources. Oecologia. 2003;136:261–269.
[46] Reimchen TE, Temple NF. Hydrodynamic and phylogenetic aspects of the adipose fin in fishes. Can J Zool. 2004;82:910–916.
[47] Vander Haegen GE, Blankenship HL, Hoffmann A, Thompson DA. The effects of adipose fin clipping and coded wire tagging on the survival and growth of spring Chinook salmon. North Am J Fish Manage. 2005;25:1161–1170.