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PRIMARY RESEARCH PAPER

Differences in the contributions of dietary water to the hydrogen stable isotope ratios of cultured Atlantic salmon and Arctic charr tissues

C. T. Graham · S. S. C. Harrison · C. Harrod

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Abstract Hydrogen stable isotopes of animal tissues are well established tracers of migration ecology in terrestrial ecosystems. Recent research has highlighted δ^2H as a potential tool in studies of aquatic ecosystems, particularly as a robust tracer for quantifying the importance of allochthonous subsidies. Although the use of δ^2H has clear potential, some uncertainties remain, in particular with regard to the contribution of dietary water to consumer δ^2H. Here, we quantify the contribution of dietary water to δ^2H in two salmonid fishes, Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.), reared on diets of known isotopic composition. Furthermore, we examined the capacity of fins (adipose and caudal) to provide a non-lethal means of estimating consumer δ^2H. The proportion of deuterium derived from environmental water of all tissue was substantial in both Atlantic salmon (mean = 0.43 ± 0.1 SD) and Arctic charr (mean = 0.48 ± 0.15 SD) but varied considerably between both individuals and tissue type. White muscle proved to be the least variable of the tissues analysed. Although fins proved to be a possible non-destructive substitute, a degree of caution is recommended with their use, as the proportion of dietary water contributing to the deuterium of fins was considerable more variable.

Keywords Stable isotope · Fish · Environmental water · Deuterium · Hydrogen · Consumers · Fins

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C. T. Graham (✉) · S. S. C. Harrison
School of Biological, Earth and Environmental Sciences, University College Cork, Enterprise Centre, Distillery Fields, North Mall, Cork, Ireland
e-mail: grahamconor@gmail.com

C. Harrod
School of Biological Sciences, Medical Biology Centre, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK

C. Harrod
Instituto de Investigaciones Oceanológicas, Universidad de Antofagasta, Avenida Angamos 601, Antofagasta, Chile

Introduction

Consumers in freshwater food webs typically rely on two principal sources of energy and nutrients: (a) autochthonous materials derived from primary production (e.g. phytoplankton, algae, macrophytes) within the water body itself, which are often subsidised by (b) allochthonous matter originating from outside of the water body, e.g. terrestrial-based production, which in temperate systems are typically in the form of leaf litter, detritus and insect inputs. These sources of energy can differ greatly in their quality (Thorp & Delong, 2002) and availability (Artmann et al., 2003). These terrestrial subsidies

can support distinct groups of secondary producers and can vary in their relative importance both within and between waterbodies (Vannote et al., 1980). The capacity to reliably differentiate between allochthonous and autochthonous inputs and calculate their relative importance to aquatic biota is fundamental to our understanding of the functioning of lotic and lenthic food webs. For example, the supply of terrestrial invertebrates from riparian vegetation to streams can constitute a major component of fish diets (Nakano et al., 1999a, c; Dineen et al., 2007). These terrestrial subsidies are directly available to fish and can provide a considerable energy resource (Nakano et al., 1999c; Kawaguchi & Nakano, 2001; Dineen et al., 2007) of a magnitude that exceeds total in situ aquatic secondary production (Cloe & Garman, 1996). This subsidy can facilitate resource partitioning, allow coexistence of sympatric species and increase the abundance of predatory fish (Nakano et al., 1999b, c), resulting in enhanced fish production, particularly in nutrient limited systems (Nakano et al., 1999a, c; Dineen et al., 2007). As the potential importance of allochthonous inputs to river function is increasingly recognised, there is increasing interest in quantifying terrestrial subsidies to consumers (Doucett et al., 1996b; Grey & Jones, 2001; Cole et al., 2011).

Providing reliable estimates of the relative contribution of allochthonous material in aquatic food webs has long provided a challenge to biologists. The application of carbon and nitrogen stable isotope analyses has proved valuable in quantifying the role of terrestrial-derived matter in aquatic systems (Doucett et al., 1996b; Jones et al., 1998; Grey & Jones, 2001). This approach relies on distinct and measurable differences in the isotopic values of the two basal resources (Rounick et al., 1982; Rounick & Hicks, 1985; Grey & Jones, 2001). In temperate regions, the carbon fixed by terrestrial C3 plants during photosynthesis typically has a $\delta^{13}C$ value of ca. −27 ‰ (Doucett et al., 1996b; Finlay et al., 2002). The $\delta^{13}C$ of aquatic plants can range much more widely than terrestrial plants (−50 ‰ to −10 ‰) (Keeley & Sandquist, 1992; Doucett et al., 1996b). This variation reflects taxonomic differences, but also importantly, site-specific physicochemistry and biogeochemistry, particularly with regards to concentrations of dissolved inorganic carbon and flow rates (Doucett et al., 1996a, b; Finlay et al., 2002). However, $\delta^{13}C$ values of allochthonous- and autochthonous-derived materials are frequently too similar to discriminate between the two carbon sources, rendering stable isotope analysis invalid when calculating the contribution of allochthonous resources to aquatic consumers (France, 1994; Doucett et al., 2007; Solomon et al., 2009).

Recent studies have shown the potential of hydrogen stable isotope ratios (deuterium (D): hydrogen, or $\delta^{2}H$) as a tool for quantifying allochthony in aquatic systems (Doucett et al., 2007; Finlay et al., 2010). Aquatic primary producers are typically markedly depleted in D relative to allochthonous organic matter from the same location by up to 100 ‰ (Doucett et al., 2007; Finlay et al., 2010). The use of hydrogen stable isotope ratios may therefore provide a universally applicable technique for calculating the contribution of allochthonous energy in aquatic systems, but also, due to the very large differences between the $\delta^{2}H$ values of the two basal resources, afford greater accuracy in these calculations (Doucett et al., 2007). Furthermore, factors known to influence autochthonous $\delta^{13}C$ of aquatic basal resources such as water velocity, stream productivity and seasonal variation appear to have little influence on algal $\delta^{2}H$ (Finlay et al., 2010).

The use of any stable isotope tracer in a foodweb context requires knowledge on how isotope ratios of the food sources are reflected in consumer tissues (McCutchan et al., 2003; Solomon et al., 2009). Differences between consumer $\delta^{2}H$ and that of their prey have been attributed to isotopic fractionation of D during biosynthesis (Estep & Dabrowski, 1980; Malej et al., 1993; Birchall et al., 2005). However, as early as 1981, DeNiro and Epstein showed that a significant portion of the H in animal tissues is determined by the $\delta^{2}H$ of drinking water as well as the food consumed. In controlled laboratory experiments with isotopically distinct sources of both water and food, water accounted for between 12 and 20 % of tissue H of birds reared in the laboratory (Hobson et al., 1999; Wolf et al., 2011). Recently, using multiple approaches, it was revealed that the proportion of tissue H derived from environmental water (often called "dietary water") of aquatic consumers was substantial yet highly variable, ranging from 0 to 40 % (Solomon et al., 2009). No evidence for fractionation between consumers and food sources has been identified (Hobson et al., 1999; Solomon et al., 2009).

Current uncertainty regarding the influence of dietary water contributions to consumer $\delta^{2}H$ restricts

the accuracy of calculations of allochthonous subsidies. In this study, we estimate the contribution of dietary water to consumer δ^2H in cultured two salmonid fishes of considerable conservation and economic importance, Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.).

As biologists increasingly attempt to minimise the impacts of field studies on wild populations, there is a need to develop non-destructive sampling protocols. The preferred tissue for stable isotope analysis of large organisms such as fish is typically white muscle, as it shows less variability in $\delta^{13}C$ and $\delta^{15}N$ compared to other tissues (Pinnegar & Polunin, 1999; Suzuki et al., 2005). For ethical reasons, this typically involves the killing of the animal. However, an increasing number of studies have advocated the use of alternative structures that can be sampled non-destructively that are suitable for stable isotope analyses (Jardine et al., 2005; Church et al., 2009; Grey et al., 2009). Such approaches have great potential in the study of species of conservation or economic concern, e.g. salmonids. Here, we also investigate the suitability of fins as a non-lethal method of obtaining stable isotope samples of hydrogen.

Methods

Sampling

Juvenile Arctic charr and Atlantic salmon were reared at Stofinfiskur aquaculture facility, Co. Galway, Ireland on a diet of known isotopic composition. Twenty-five salmon (mean ± SD wet mass = 19 ± 7.4 g, range 5.4–34.5 g) were selected for isotopic analyses from a single tank of 1+ (fish aged more than 1 but <2 years of age) salmon parr. Arctic charr were selected for isotopic analyses from two separate tanks, eight 1+ charr (mean ± SD wet mass = 15.4 ± 6.5 g, range 12.1–20.9 g) from the first and a further 17 age 2+ (more than 2 but <3 years of age) individuals (114 ± 14.9 g, range 28.3–226 g) were collected from the second. Charr were sampled from two separate age cohorts as the contribution of dietary water to consumer tissue δ^2H can vary with size (Soto et al., 2011).

Fish were fed on two different single batches of feed for the 4 months prior to sampling. The 1+ salmon and charr (tanks 1 and 2) were fed Skretting (Stavanger, Norway) Nutra Salmon Fry 02 (herein called feed 1) while the older charr (tank 3) were fed solely Skretting Nutra Rainbow trout feed 45 (herein called feed 2). δ^2H was determined from multiple replicates ($n = 4$) for each food type and water samples ($n = 2$) collected in 50 ml polypropylene vials directly from the inlet header tank on the farm. Replicate water samples were not collected throughout the experiment as both seasonal and interannual variation in water δ^2H is very low (Bowen et al., 2007; Doucett et al., 2007).

Laboratory analyses

Fish were killed by blunt trauma and transported to the laboratory on ice, where they were frozen (−20°C) prior to processing. After defrosting at room temperature, all fish were measured (fork length) and weighed (blotted wet mass) to the nearest 1 mm and 0.01 g, respectively. Dorsal muscle tissue was excised from between the dorsal fin and the lateral line, ensuring that no skin or bones were included in the sample. The adipose fin was removed, as was a piece of the caudal fin. All tissues were oven dried at a constant temperature of 60°C for 24–48 h. Muscle tissue was ground with a mortar and pestle to a fine homogenous powder prior to being weighed to ca. 350 μg into combustible silver cups for δ^2H analysis. Adipose and caudal fins were clipped to appropriate size for analysis using scissors.

Analysis of stable isotope ratios of hydrogen of all samples was conducted at the Colorado Plateau Stable Isotope Laboratory, Arizona, USA. Prior to analysis, all organic samples were equilibrated using the bench-top procedure of Wassenaar & Hobson (2003) to correct for exchange of H atoms between samples and ambient water vapour (DeNiro & Epstein, 1981). In this procedure, both samples and standards with known δ^2H values for non-exchangeable H are exposed to water vapour in laboratory air for a period of at least 2 weeks to allow all exchangeable H to obtain a constant δ^2H value.

Organic samples were pyrolyzed at 1,400°C to H_2 gas using a Thermo-Electron (Bremen, Germany) thermal-chemical elemental analyser (TC/EA). Chromatographically purified H_2 gas was then introduced to a Thermo-Electron Delta Plus XL gas isotope-ratio mass spectrometer for measurement of δ^2H using an open-split interface (CONFLOII). δ^2H values were normalised on the Vienna standard mean ocean water

(VSMOW) scale using known δ^2H values for three standards: chicken feather ($\delta^2H = -147$ ‰), cow hoof ($\delta^2H = -187$ ‰) and bowhead whale baleen ($\delta^2H = -108$ ‰). Repeated analysis of these standards showed that organic δ^2H values were precise (SD) to within ±1.5 ‰. Water samples were analysed for δ^2H via cavity-ring-down laser spectrometry on a Los Gatos Research DLT-100 liquid-water analyser. Water δ^2H values were normalised on the VSMOW scale using internal water standards that had been previously calibrated against VSMOW. Repeated analysis of several internal water standards showed that water δ^2H values were precise (SD) to within ±0.2 ‰ on average. All δ^2H data are expressed relative to VSMOW, using the standard δ notation expressed as units per mil, according to the following equation:

$$\delta^2H\ (‰) = 1000\left[\left(R_{sample}/R^{-1}_{standards}\right) - 1\right]$$

The reference materials ($R_{standard}$) were secondary samples of known isotope composition to the international standards of VSMOW. The percentage contribution to fish tissues from water (ω) was calculated using the following equation:

$$\omega = \left(\delta^2H\ \text{tissue} - \delta^2H\ \text{food}\right)/\left(\delta^2H\ \text{water} - \delta^2H\ \text{food}\right)$$

Statistical analyses

Least squares linear regression was used to examine whether the contribution of H from environmental water to consumer tissue H varied with fish mass. The proportional contribution of dietary water was arcsine transformed prior to analysis. The suitability of fish fins as an alternative tissue to white muscle to obtain samples for determining δ^2H values was performed by comparison with the white muscle and also by directly estimating the contribution of dietary water to the δ^2H of the fin tissue. Differences between δ^2H of muscle tissue and adipose and caudal fins were compared using paired *t*-tests or Wilson signed rank test when assumptions of normality and/or homogeneity of variance could not be met. The relationship between fin and muscle δ^2H was assessed using linear regression and regression slopes compared to unity using *t*-tests. Homogeneity of variance was tested using the Cochran's test and normality was tested using a Kolmogorov–Smirnov test. All statistical tests were conducted using SPSS 12.1 for windows, with Cochran's test for homogeneity and t-tests conducted in Excel 2003 for Windows XP.

Results

The δ^2H of the three fish tissues for all three groups of fish was intermediate between food and water δ^2H (Fig. 1). The proportion of muscle D derived from dietary water was substantial and varied between the three groups of fish, with estimates ranging from 0.32 (±0.03 SD) for the larger charr, to 0.36 (±0.05 SD) and 0.41 (±0.04 SD) for the smaller charr and salmon, respectively. The proportion of fin tissue D derived from dietary water was generally higher and more variable, ranging between 0.4 and 0.73 (Table 1).

There was very little effect of fish size on the proportion of dietary water D incorporated into the tissues of any of the three tanks of fish (Table 1). However, when the size range of the fish was increased by pooling the two tanks of charr, there was a significant negative relationship ($r^2 = 0.43$, $P < 0.001$) between the mass of charr and the proportion of D of muscle derived from environmental water (Table 1).

There were significant differences between the δ^2H of muscle and each of the two fin types in both salmon and charr (Table 2). These differences

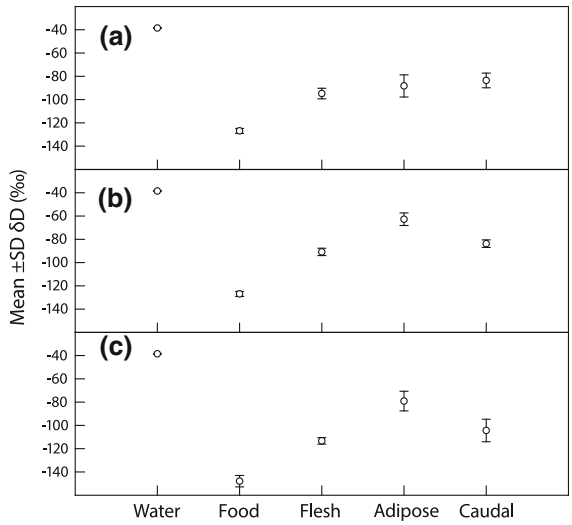

Fig. 1 δ^2H (±SD) of water, fish feed, muscle tissue, adipose and caudal fin of the (*a*) Atlantic salmon (*Salmo salar*) and (*b*) small and (*c*) large Arctic charr (*Salvelinus alpinus*). Note that in some cases the error bars are concealed by the marker

Table 1 The proportion of D derived from dietary water in the three tissue types, and regressions on the effect of fish mass on the proportion of D derived from dietary water in the salmon and charr

	Salmon ($n = 25$)	Charr tank 1 ($n = 9$)	Charr tank 2 ($n = 17$)	All charr ($n = 25$)
Muscle				
Mean proportion	0.36	0.41	0.32	0.35
Standard deviation	0.05	0.04	0.03	0.05
Standard error	0.01	0.01	0.01	0.01
Range	0.21–0.44	0.35–0.46	0.29–0.37	0.29–0.46
Linear regression	$r^2 = 0.03$, $P = 0.44$	$r^2 = 0.14$, $P = 0.36$	$r^2 = 0.06$, $P = 0.33$	**$r^2 = 0.43$, $P < 0.001$**
Adipose fin				
Mean proportion	0.44	0.73	0.63	0.66
Standard deviation	0.11	0.06	0.08	0.08
Standard error	0.02	0.02	0.02	0.02
Range	0.25–0.65	0.65–0.83	0.48–0.74	0.48–0.83
Linear regression	**$r^2 = 0.17$, $P = 0.041$**	$r^2 = 0.3$, $P = 0.16$	$r^2 = 0.05$, $P = 0.4$	$r^2 = 0.06$, $P = 0.23$
Caudal fin				
Mean proportion	0.49	0.49	0.40	0.43
Standard deviation	0.07	0.04	0.09	0.09
Standard error	0.01	0.01	0.02	0.02
Range	0.36–0.66	0.43–0.53	0.24–0.52	0.24–0.53
Linear regression	**$r^2 = 0.16$, $P = 0.046$**	$r^2 = 0.03$, $P = 0.67$	$r^2 = 0.01$, $P = 0.68$	**$r^2 = 0.16$, $P = 0.047$**

Significant regressions are highlighted in bold

Table 2 Summary of the statistical tests used to compare the difference of D/H ratios between muscle and the two fin types in both salmon and charr

	Salmon			Charr		
	t/*Z*	df	Sig.	*t*	df	Sig.
δ^2H: muscle vs. adipose fin	−2.79 (Z^a)	24	<0.01	−20.17	24	<0.01
δ^2H: muscle vs. caudal fin	−3.88 (Z^a)	24	<0.01	−5.24	24	<0.01
δ^2H: adipose fin vs. caudal fin	−2.65	24	0.014	13.4	24	<0.01

[a] Non parametric test, Wilcoxon signed rank test used and therefore these values are *Z* values

reflected enrichment of D in adipose and caudal fins relative to muscle in all fish treatments (Fig. 2). However, the degree of enrichment in D of the fin tissue relative to muscle tissue varied between the two fish species (Table 3; Fig. 1). The caudal fins of salmon were more enriched in D relative to muscle tissue and adipose fins, with the opposite case in the charr (Table 3; Fig. 1). The enrichment of charr adipose fins relative to muscle tissue was significantly higher than that in the salmon. However, the enrichment of caudal fin relative to muscle was marginally higher in salmon than in charr (Table 3). Furthermore, charr adipose fin was enriched relative to muscle D in all 25 individuals sampled, but was depleted relative to muscle in some of the 25 salmon individuals tested (Fig. 2). Both salmon and charr had individuals that were either D enriched or depleted in caudal fin tissues relative to muscle tissue, with a larger range in δ^2H relative to muscle δ^2H in the salmon than in the charr for both fin types (Fig. 2; Table 3).

Despite significant differences between muscle and both caudal and adipose fin in charr δ^2H (Table 2), the regressions between charr muscle and adipose and caudal fin δ^2H were highly significant (Table 4). The slope ($b = 0.71 \pm 0.14$) of the regression between charr adipose fin and muscle δ^2H was significantly

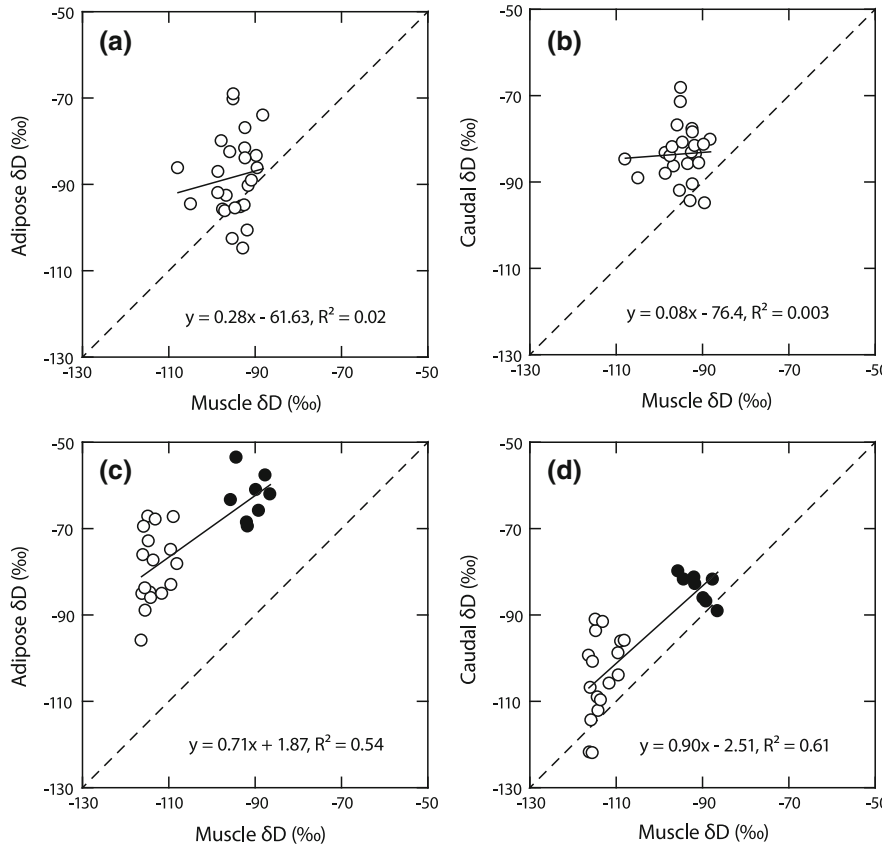

Fig. 2 The relationship between δ^2H of muscle and Atlantic salmon **a** adipose and **b** caudal fin, and Arctic char **c** adipose and **d** caudal fin. For Arctic char: *filled circle* = small and *empty circle* = large individuals. The *dashed line* in each graph running through the origin is the 1:1 line

Table 3 Mean differences in δ^2H values in fin tissues relative to muscle tissue

	Salmon ($n = 25$)	Charr tank 1 ($n = 9$)	Charr tank 2 ($n = 17$)	All charr ($n = 25$)
Muscle → adipose fin				
Average enrichment (‰)	+6.6	+28.1	+34.2	+31.9
Standard error	±2	±2.22	±1.97	±1.6
Standard deviation	±10.02	±6.29	±8.12	±8
Range (‰)	−12.1 → 25.8	+22.2 → 40.8	+20.4 → 47.5	+20.4 → 47.5
Muscle → caudal fin				
Average enrichment (‰)	+11.1	+7.1	+9	+8.5
Standard error	±1.45	±2.11	±2.15	±1.59
Standard deviation	±7.28	±5.99	±8.88	±7.94
Range (‰)	−11.1 → 26.7	−2.6 → 15.7	−6.6 → 23.6	−6.6 → 23.6

different to a slope of 1 ($t = 2.08$, df $= 46$, $P < 0.05$) as was the slope of salmon caudal fin and muscle δ^2H ($b = 0.07$: $t = 3.19$, df $= 46$, $P < 0.05$). However, the slope ($b = 0.89$) of the regression between charr caudal fin was statistically indistinguishable from unity ($t = 0.68$, df $= 46$, $P > 0.05$). There was a noticeable amount of scatter ($r^2 = 0.02$) around the slope ($b = 0.28$, SE $= 0.44$) of salmon adipose fin and muscle δ^2H and this likely influenced its overlap with unity ($t = 1.65$, df $= 46$, $P > 0.1$).

The mean, standard deviation and range of δ^2H of all fish samples are shown in Table 5. The δ^2H of the

Table 4 The relationship between stable isotope ratios of hydrogen between muscle and the two fin types in both salmon and charr, using linear regressions

Linear regression	df	r^2	Slope (±SE)	Intercept	P
Atlantic salmon					
δ^2H muscle vs. adipose fin	23	0.02	0.28 (0.44)	−62.1	0.53
δ^2H muscle vs. caudal fin	23	0.02	0.07 (0.29)	−76.6	0.8
Arctic charr					
δ^2H muscle vs. adipose fin	23	0.54	0.71 (0.14)	1.82	<0.001
δ^2H muscle vs. caudal fin	23	0.61	0.89 (0.15)	−2.45	<0.001

Table 5 Mean, standard deviation and range of δ^2H of Atlantic salmon and Arctic charr muscle, adipose and caudal fins

	Salmon (n = 25)	Charr tank 1 (n = 9)	Charr tank 2 (n = 17)
Muscle			
Mean	−94.8	−90.8	−113.3
Standard deviation	4.53	3.17	2.79
Range	−107.9 → −88.1	−95.6 → −86.5	−116.3 → −108
Adipose fin			
Mean	−88.3	−62.7	−79.1
Standard deviation	9.56	5.39	8.42
Range	−104.9 → −69.1	−53.6 → −69.5	−95.9 → −67.2
Caudal fin			
Mean	−83.5	−83.7	−104.3
Standard deviation	6.3	3.24	9.66
Range	−94.9 → −68.2	−89.1 → −79.9	−122 → 91.1

two water samples were −38.65 and −38.29, of feed 1 were −127.9 and −126 and of feed 2 were −144.7 and 151.2.

Discussion

The fish in this study derived large proportion of their D from dietary water, ranging between 32 and 73 %. Early studies on the use of D isotope in ecology concluded that environmental water did not have a significant effect on the δ^2H of a consumer (Estep & Dabrowski, 1980; Macko & Estep, 1983). However, in both these experiments, consumer δ^2H was intermediate between the δ^2H of water and that of their diet and therefore some contribution of dietary water to consumer D was probable. It has been shown that approximately 20 % of hydrogen in birds was derived from dietary water (Hobson et al., 1999), 14 % in *Chaoborus*, 20 % in zooplankton, 39 % in (Culicidae) mosquitoes (Solomon et al., 2009), 31 % (Wang et al., 2009) and 47 % (Soto et al., 2013) in Chironomidae, 30 % in microbes (Kreuzer-Martin et al., 2003) and between 27 and 35 % in humans (Sharp et al., 2003; O'Brien & Wooler, 2007; Ehleringer et al., 2008). Prior to this study, only two estimates have been made for the contribution of environmental water to muscle D in fish: Solomon et al. (2009) calculated the contribution of dietary water at 12 % (±2 SD), considerably lower than that seen in other taxa, while Soto et al. (2013) estimated that 33 % of tissue D was from dietary water. However, their estimates for four groups of cultured salmonids were based on a more limited number of replicates (n = 3). In our study, using much larger sample sizes, the range of contributions of dietary water to muscle D was 21–44 % in salmon and 29–46 % in charr. This may indicate that determinations of dietary water contributions are sensitive to sample size.

The disparity between the results of the two studies of fish dietary water (i.e. Solomon et al., 2009 and the current study) may reflect other biological factors, e.g. the growth rate of the fish. The strong negative relationship between the proportion of muscle D

derived from environmental water and individual charr size suggests the contribution of dietary water to consumer δ^2H may be higher in faster growing fish, as growth is generally faster in younger fish. Although the water content of fish muscle varies inversely with specific growth rate (Pelletier et al., 1995), the proportion of dietary water incorporated into non-exchangeable H of consumers, i.e. that portion of H that provides information on dietary sources (Hobson et al., 1999), may increase with growth. Clearly, there is a need for investigation into the effect of growth rate on the contribution of dietary water to consumers and any other factors that affect this process.

The significant depletion in the D of muscle relative to fins in both the salmon and the charr, and the variation in the proportion of D derived from dietary water possibly reflects variation in lipid content. Salmonid muscle tissues can have a high lipid content (Kiljunen et al., 2006) and lipids are typically depleted in H compared with proteins (Smith & Epstein, 1970; Estep & Hoering, 1981; Hobson et al., 1999; Post et al., 2007). The higher degree of enrichment in H of charr adipose fin relative to muscle may simply reflect a lower concentration of lipids in the adipose fin of Arctic charr relative to the salmon sampled. Differences in lipid concentrations may also contribute to the disparity between the results presented here and Solomon et al. (2009) proportion of D derived from dietary water. Interestingly, Jardine et al. (2009) and Soto et al. (2013) both noted a considerable enrichment in D of brook trout (*Salvelinus fontinalis*) and water striders (*Aquarius remigis*) after lipid extraction. Indeed, the very recent work of Soto et al. (2013) highlights the importance of measuring lipid δ^2H of consumers when using stable isotopes of hydrogen as a trophic tracer in aquatic food webs.

Hydrogen in carbohydrates can potentially exchange with drinking water hydrogen and can be fixed into non-exchangeable hydrogen during conversion from carbohydrates to lipids (Hobson et al., 1999). This incorporation of drinking water elements into lipids in animal tissue is related to diet and nutritional status of the animal and therefore physiological status may have an important influence on the interpretation of δ^2H values (Hobson et al., 1999). Alternatively, or in combination with the effects of D depletion in lipids, the drinking water effect could simply be more pronounced in the adipose and caudal fins of the fish sampled than in the muscle tissue. Hobson et al. (1999) noted that the effect of drinking water varied between different tissues of Japanese quail (*Coturnix coturnik japonica*), even though lipids were removed prior to analysis. They concluded that this would suggest more opportunity for hydrogen exchange with consumed water during the synthesis of these tissues in comparison with muscle tissue (Hobson et al., 1999).

In many studies examining the potential use of alternative tissues to muscle for stable isotope analysis, the surrogate structure is compared to muscle tissue using paired *t*-tests and/or regression analysis to see how the substitute tissue relates to muscle (Kelly et al., 2006; Grey et al., 2009; Jardine et al., 2011). Where the isotope ratio of the alternative tissue is significantly different to that of muscle, but a significantly strong isometric relationship exists between the two, a simple correction factor of the average difference between the two sets of values can be applied (Hanisch et al., 2010). Both the adipose and caudal fin δ^2H in the salmon and the charr examined here were statistically different from the muscle δ^2H. Regressions showed that while charr muscle δ^2H was significantly related to the δ^2H of both fin types, these relationships were only moderately strong (muscle—adipose fin $r^2 = 0.52$; muscle–caudal fin $r^2 = 0.61$) and the slope of the line (δ^2H muscle vs. δ^2H fins) was either isometric (caudal fin) or non-isometric (adipose fin). The linear regressions between salmon muscle δ^2H and δ^2H of both fin types were non-significant. The slope of the line between δ^2H of salmon muscle and adipose fin was not significantly different to a slope of 1 reflecting a large amount of scatter around the regression line for δ^2H of salmon muscle and adipose fin. However, despite apparent isometric relationship between δ^2H muscle and caudal fins of char, this relationship is almost certainly a result of pooling of the Arctic char from the two separate tanks into a single regression and is therefore a function of increasing the range in D values used in the regression analyses. Therefore, based on this statistical approach one could not use charr caudal fin as an alternative tissue to muscle in D stable isotope analyses.

The use of stable isotopes to infer diet of a consumer requires an understanding of how the isotope ratio of the food source is reflected in the tissues of the consumer (Solomon et al., 2009), but also the accuracy of this relationship, especially when utilising mixing models to calculate the contribution

of different sources to the tissues of an animal (Phillips & Gregg, 2001, 2003). For example, the use of charr caudal fin δ^2H as a proxy for muscle δ^2H as outlined above (the impact of pooling of the two tanks of char aside), has a degree of error associated with it. The regression between the two is highly significant but the strength of the association is only moderately strong ($r^2 = 0.61$). Added to this uncertainty is the error associated with the calculation of the proportion of δ^2H derived from dietary water to the flesh δ^2H (Table 1).

In previous studies where the use of alternative tissues to muscle for stable isotope analysis of carbon and nitrogen is examined e.g. (Jardine et al., 2005; Church et al., 2009; Grey et al., 2009), the substitute tissue is compared to muscle, as muscle is the tissue of choice in isotope analysis (Pinnegar & Polunin, 1999). Ultimately, however, the most important factor is how well the fins reflect the diet of the consumer, regardless of whether a correction factor is used or not. As the δ^2H of the diet was controlled in this experiment, it was possible to calculate directly how well the fins reflect the diet of the salmon and charr and any error associated with it. As there is negligible trophic fractionation associated with trophic transfer of hydrogen (Hobson et al., 1999; Solomon et al., 2009; Soto et al., 2011), once the δ^2H of the environmental water and all relevant putative prey is known within a system, the proportion of D derived from dietary water in the specific fin (Table 1) can be corrected for, to infer the diet of the consumer. However, a degree of caution is recommended with their use, as the proportion of dietary water contributing to the deuterium of fins was considerably more variable than the muscle tissue.

Conclusions

The proportion of tissue H derived from environmental water of aquatic consumers is highly variable depending on the taxa and tissue analysed. The nutritional status, growth rate and lipid content of the consumers' tissue may exert an influence on the dietary water effect on the δ^2H of the consumer. If any of these factors, alone or in conjunction with one another, affect the calculation of dietary water effect on the δ^2H of the consumer, it could reduce the accuracy in the use of D as a tracer of diet, such as the calculation of allochthony in streams. Further work addressing these issues is required in order to reduce error in calculations when natural abundance of hydrogen isotopes is used in mixing models in tracing energy flow in food webs. Until such data is available, results utilising δ^2H from aquatic ecosystems should be interpreted with caution.

Of the tissues investigated, muscle showed the least isotopic variability and of those analysed, appears to be the most suitable as a tracer of energy. In situations where a non-lethal alternative is required, fin tissue may be utilised, despite being significantly different in its δ^2H ratio compared to muscle. However, from our results it is clear that knowing the specific proportion of tissue H derived from environmental water of the alternative structure, is required. The proportion of dietary water contributing to the D of fins was considerable more variable in fins than flesh and a degree of caution is recommended if using this approach.

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