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BRIEF COMMUNICATION

Acid treatment of Atlantic salmon (*Salmo salar*) scales prior to analysis has negligible effects on $\delta^{13}C$ and $\delta^{15}N$ isotope ratios

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Abstract

There is debate in the literature as to whether scales of fishes require acidification to remove inorganic carbonates prior to stable isotope analysis. Acid-treated and untreated scales from 208 Atlantic salmon from nine locations on both sides of the Atlantic were analysed for $\delta^{13}C$ and $\delta^{15}N$. Linear mixed-effect models determined the effect of acid treatment to be statistically significant. However, the mean difference was small ($\delta^{13}C$ 0.1 ± 0.2‰, $\delta^{15}N$ −0.1 ± 0.2‰) and not of biological relevance. This study concludes that Atlantic salmon scales do not need to be acidified prior to stable isotope analysis.

KEYWORDS

acidification, Atlantic salmon, decalcification, fish scales, *Salmo salar*, stable isotope analysis

Stable isotope analysis (SIA) is a powerful tool in ecology that can be used to determine the prey items of primary importance to consumers (Wieczorek *et al*., 2018), identify the marine feeding location of individual organisms (MacKenzie *et al*., 2011), establish migratory connectivity between populations (Torniainen *et al*., 2013) and investigate the trophic position of consumers (Vander Zanden *et al*., 1997). Dorsal muscle is typically the tissue of choice for SIA of fish (Pinnegar & Polunin, 1999) but alternative tissues have also been analysed, including fins (Graham *et al*., 2013, 2014), mucus (Church *et al*., 2009) and scales (Hutchinson & Trueman, 2006; MacKenzie *et al*., 2011; Perga & Gerdeaux, 2003; Sinnatamby *et al*., 2007; Torniainen *et al*., 2013). Scales are an ideal tissue to analyse as they can be sampled nonlethally, relatively simply and quickly. In addition, data describing age and growth rates can also be obtained from the scales prior to SIA (Einum *et al*., 2002; Hutchinson & Trueman, 2006). Many laboratories around the world hold vast archives of scales from various fish species, from which considerable amounts of invaluable data can be generated. Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are incorporated into the collagen layers of scales as they grow and can be used for analysis of diet, environmental conditions and trophic structure, making SIA of fish scales potentially very informative (Hutchinson & Trueman, 2006). Scale archives may include samples that span up to 100 years, thus providing exciting opportunities to gain unique insights into the lives of not only individual fish but also populations over large timespans. Analyses of archived scales can determine if migration pathways or feeding histories have changed

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over the span of the archive (MacKenzie *et al*., 2011). Combining these analyses with environmental data could illuminate long-term patterns, including climate change effects.

In previous studies that use SIA of fish scales, various methods have been used to prepare the scales for analysis and to deal with the potentially confounding inorganic component of fish scales. In a study on whitefish (*Coregonus lavaretus*, L.) in Lake Geneva, Perga and Gerdeaux (2003) determined that exposing scales to hydrochloric acid (HCl) for 2 min was necessary during preparation for SIA to remove such inorganic carbonates from the scale which can be enriched in ^{13}C (Perga & Gerdeaux, 2003). However, acidification is not a desired step in preparing scales for SIA as it greatly increases the preparation time and has been shown to alter nitrogen isotopic composition, causing enrichment in ^{15}N (Bunn *et al*., 1995; Pinnegar & Polunin, 1999). This would necessitate the analysis of twice as much material, which is very time consuming, costly and highly unfavourable when dealing with archived scales, which may be limited in number. Schlacher and Connolly (2014) recommend that acidification should not be carried out as a general rule and the effects should be determined prior to analysis as acidification can affect both $\delta^{13}C$ and $\delta^{15}N$. Sinnatamby *et al*. (2007) completed a study on Atlantic salmon (*Salmo salar*, L.), yellow perch (*Perca flavescens*, Mitchill) and walleye (*Sander vitreus*, Mitchill) and did not find significant differences between acidified and nonacidified scales. As this contradicts Perga and Gerdeaux (2003), they suggested that the need for acidification of fish scales could vary between species, a possibility that was also proposed by Ventura and Jeppesen (2010), who suggest that varying mineral content in scales of different species could be responsible for disparities between previous studies. Additionally, dissolved inorganic carbon (DIC) in freshwater is usually depleted in inorganic ^{13}C values relative to seawater due to CO_2 input from decomposing terrestrial matter (Boutton, 1991). Therefore, uptake of inorganic carbon in fish scales could be affected by varied availability of DIC between marine and freshwater ecosystems. According to Trueman and Moore (2007), the apatite component of Atlantic salmon scales is less than 30% of the mass of the scale, indicating that acidification is not necessary. However, while the regression models carried out by Sinnatamby *et al*. (2007) showed strong relationships between treated and untreated scale isotope ratios for the two freshwater species yellow perch (R^2 = 0.997 for $\delta^{13}C$, 0.989 for $\delta^{15}N$) and walleye (R^2 = 0.989 for $\delta^{13}C$, 0.980 for $\delta^{15}N$), the relationship for Atlantic salmon was much weaker (R^2 = 0.455 for $\delta^{13}C$, 0.553 for $\delta^{15}N$). The $\delta^{13}C$ values of acidified and nonacidified scales were not significantly different from each other, but the *P* value (0.052) of Atlantic salmon was somewhat inconclusive (Sinnatamby *et al*., 2007).

The current study furthered the research of Sinnatamby *et al*. (2007) on Atlantic salmon scales. We focused mainly on marine feeding salmon as they are under-represented in other similar studies that focus on freshwater habitats (Perga & Gerdeaux, 2003; Ventura & Jeppesen, 2010). The aim was to examine a much larger sample size from many regions across the range of the fish, including Canada, Ireland and the UK, to determine if acidification to remove inorganic carbonates is necessary prior to SIA of Atlantic salmon scales. Our study included scales from varying life histories including ranched, farmed and wild fish. This research is particularly important for Atlantic salmon as an increasing number of studies are carrying out SIA of both modern and archived salmon scales to better understand marine migrations and long-term trends.

Scale samples were obtained from Atlantic salmon in nine locations in Canada, Ireland, Northern Ireland and Wales in 2018. Our samples included adult Atlantic salmon from freshwater tanks, marine aquaculture pens, ranched adults returning to Ireland and wild adult salmon returning to rivers in Eastern Canada and Europe. Scales were obtained from adult captive broodstock from the Tobique River, hereafter Tobique (n = 30), in north-western New Brunswick, Canada during hatchery spawning. Samples from the Tobique population represent growth exclusively in a freshwater hatchery environment. The marine aquaculture fish were reared in the Bay of Fundy, New Brunswick, Canada in two separate aquaculture facilities: fish hereafter known as Aqua 1 (n = 30) were sampled during routine health screening while in sea cages; wild origin smolts, grown to maturity in modified net pens at the world's first Marine Conservation Farm (Aqua 2, n = 25) were sampled during the autumn tagging period and subsequently released back to their natal rivers. Adult salmon returning to Canadian rivers were sampled at the Big Salmon River (BSR, n = 24), Upper Salmon River (USR, n = 7) and Gaspereau River (Gaspereau, n = 8) in the Bay of Fundy, New Brunswick. Adult salmon returning to European rivers were sampled at fish traps in the Bush River, Northern Ireland (hereafter Bush, n = 26), the River Dee, Wales (hereafter Dee, n = 48) and the Burrishoole River on the west coast of Ireland (hereafter Burrishoole, n = 10). Scales collected at the Burrishoole River were from ranched fish, reared until the smolt stage in a hatchery at the Marine Institute's Newport Research Facility in County Mayo, then released into Lough Furnace to begin their sea migration and sampled on their return as grilse.

Prior to analysis, all scales were soaked in distilled water for a minimum of 2 min and then scraped gently with a scalpel on both sides to remove any mucus. Suitable scales from each fish were chosen for imaging. Burrishoole scales were imaged using an Olympus BX51 compound microscope and ImagePro Plus software Version 6.3.1.542. All other scales were imaged using a Leica MZ16 A microscope with Auto-Montage Pro software. The scales were allowed to air dry following imaging. Scale material corresponding to the last summer at sea was excised under a dissecting microscope or magnifier to obtain a temporally distinct sample (MacKenzie *et al*., 2011). Between 1 and 1.2 mg of the scale cuttings were weighed into tin capsules (elemental microanalysis pressed tin capsules, 5 × 3.5 mm) and folded for analysis. The remainder of the cut scales were submerged in 1 M HCl for 2 min, then rinsed with distilled water and placed in an oven at 60°C for approximately 24 h. Acidified scale sections were then weighed into tin capsules as above. All analyses were carried out at the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick, Fredericton, NB, Canada. A combination of CE NC2500 and Costech 4010 elemental analysers connected to either a Delta-Plus/Conflo II or a Delta^{Plus} XP/Conflo III continuous-flow isotope ratio mass spectrometer (*CF*-IRMS) were used for analysis of carbon and nitrogen isotopes. Stable isotope

measurements are reported in the standard delta (δ) notation in parts per thousand (‰) relative to the international standards: Vienna Pee Dee Belemnite (VPDB) for carbon (Craig, 1957) and atmospheric air (AIR) for nitrogen (Mariotti, 1983). Isotope values were normalized using in-house secondary standards [USGS61, BLS (Bovine Liver Standard) and MLS (Muskellunge Muscle Standard)], which were all calibrated against International Atomic Energy Agency (IAEA) standards. To assess analytical accuracy, the following check standards were analysed: nicotinamide, N_2 and CH_7. Repeated analysis of internal standards shows that the analytical precision was better than ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$. Approximately 7% of samples were run in replicate to monitor instrument drift over time. Following acidification, some samples achieved low weights of 0.6 mg and below. These samples were run separately and the *CF*-IRMS was amplified for low-weight samples to achieve accurate results. All statistical analyses were carried out using R Version 3.5.2 in RStudio Version 1.2.5019.

Across locations, mean $\delta^{13}C$ values ranged from −16.7‰ to −14.9‰ in untreated scales and from −16.7‰ to −14.8‰ in acid-treated scales. Mean values for $\delta^{15}N$ ranged from 8.1‰ to 15.0‰ in untreated scales and 8.00‰ to 15.1‰ in treated scales. The difference between means at each location was small (from 0.0 ± 0.1 S.D. to 0.3 ± 0.2 S.D.; Table 1). Linear mixed-effect models were used to examine the effect of acid treatment on $\delta^{13}C$ and $\delta^{15}N$ values in the scale. Three models were tested for each of $\delta^{13}C$ and $\delta^{15}N$ (models and AIC values displayed in Supporting Information Table 1). For both $\delta^{13}C$ and $\delta^{15}N$, the best-fitting model (based on AIC values: for $\delta^{13}C$, the best-fitting model had an AIC value of 115.5, while the other two models had AIC values of 529.4 and 540.5; for $\delta^{15}N$, the best-fitting model had an AIC value of 414.3, while the other two models had AIC values of 869.5 and 886.2) included treatment as a fixed effect with location and fish ID as random effects. The total explained variance indicated a good model fit (conditional R^2 = 0.96 for $\delta^{13}C$, 0.99 for $\delta^{15}N$), with a very small proportion of that variance attributed to the acid treatment (marginal R^2 = 0.002 for $\delta^{13}C$, 0.0005 for $\delta^{15}N$). The fixed-effect model estimate indicated that $\delta^{13}C$ values of untreated scales were 0.07‰ (±0.02‰ S.E.) higher than $\delta^{13}C$ values of treated scales. $\delta^{15}N$ values of untreated scales were 0.08‰ (±0.02‰ S.E.) lower than $\delta^{15}N$ values of treated scales. Treatment had a significant effect on $\delta^{13}C$ ($P < 0.001$) and $\delta^{15}N$ ($P < 0.001$) values but the differences between acid-treated and untreated scales were negligible considering that analytical precision was estimated at ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$. A linear mixed-effect model was used to model the relationships between $\delta^{13}C$ in acid-treated and untreated scales, with location included as a random effect (models and AIC values can be viewed in Supporting Information Table S2). The marginal R^2 (0.899) and conditional R^2 (0.934) show that the majority of the variability in $\delta^{13}C$ of acid-treated scales is due to variation in $\delta^{13}C$ before treatment, with less than 4% of variability accounted for by location. A similar linear mixed model was run for nitrogen (models and AIC values can be viewed in Supporting Information Table S2), where marginal R^2 (0.983) and conditional R^2 (0.989) showed that less than 0.01% of variability in $\delta^{15}N$ of acid-treated scales was due to location.

The model-estimated differences between acid-treated and untreated scales are too small to be biologically relevant. Kennedy *et al.* (2005) examined over 200 salmon fry stocked in 11 tributaries of the Connecticut River in the eastern United States. Using stable isotopes of carbon and nitrogen, they were able to distinguish 7 out of 11 sites between 40 and 104 days after being stocked in the river with the difference in $\delta^{13}C$ between sites ranging from 0.25‰ to 6.1‰. As those fish were released from the same hatchery just 4 months prior to recapture, this confirms that a difference of 0.07 ± 0.02‰ for $\delta^{13}C$ in adult fish with a wide-ranging migration is not likely to be biologically relevant. The mean difference of −0.08 ± 0.02‰ for $\delta^{15}N$ is also not likely to be biologically relevant. $\delta^{15}N$ is most commonly used to estimate trophic position, and trophic fractionation of $\delta^{15}N$ is widely accepted to be ~3‰ on average (McCutchan *et al.*, 2003; Post, 2002) and can vary from 1.3‰ to

TABLE 1 The mean (±S.D.) of $\delta^{13}C$ and $\delta^{15}N$ isotope signatures and the difference between untreated and acid-treated Atlantic salmon scales for each location and the combined data

				Carbon			Nitrogen		
				Mean $\delta^{13}C$ ± S.D. (‰)			Mean $\delta^{15}N$ ± S.D. (‰)		
Location	*n*	Region	Life history	Untreated	Acidified	Difference	Untreated	Acidified	Difference
Aqua 1	30	Canada	Aquaculture	−15.2 ± 0.2	−15.5 ± 0.3	0.2 ± 0.2	8.1 ± 0.2	8.0 ± 0.2	0.0 ± 0.1
Aqua 2	25	Canada	Aquaculture	−14.9 ± 0.2	−15.0 ± 0.2	0.2 ± 0.2	15.0 ± 0.3	15.1 ± 0.3	−0.1 ± 0.2
BSR	24	Canada	Wild	−14.9 ± 0.2	−14.8 ± 0.3	−0.0 ± 0.2	10.3 ± 0.4	10.3 ± 0.5	0.0 ± 0.2
Burrishoole	10	Ireland	Ranched	−16.4 ± 0.3	−16.6 ± 0.3	0.2 ± 0.2	10.6 ± 0.8	11.0 ± 0.8	−0.3 ± 0.2
Bush	26	N. Ireland	Wild	−16.7 ± 0.5	−16.7 ± 0.3	0.1 ± 0.3	11.6 ± 0.7	11.7 ± 0.7	−0.1 ± 0.2
Dee	48	Wales	Wild	−16.7 ± 0.3	−16.7 ± 0.3	0.0 ± 0.2	11.3 ± 0.6	11.5 ± 0.6	−0.3 ± 0.2
Gaspereau	8	Canada	Wild	−16.5 ± 0.3	−16.3 ± 0.6	−0.2 ± 0.4	11.6 ± 0.6	11.5 ± 0.6	0.2 ± 0.2
Tobique	30	Canada	Freshwater	−15.0 ± 0.2	−15.1 ± 0.2	0.1 ± 0.2	10.0 ± 0.2	10.0 ± 0.2	0.0 ± 0.2
USR	7	Canada	Wild	−15.2 ± 0.4	−15.1 ± 0.5	−0.0 ± 0.2	11.1 ± 1.9	11.2 ± 2.1	−0.2 ± 0.3
Combined	208			−15.7 ± 0.9	−15.8 ± 0.9	0.1 ± 0.2	11.0 ± 1.9	11.1 ± 2.0	−0.1 ± 0.2

Abbreviations: BSR, Big Salmon River; USR, Upper Salmon River.

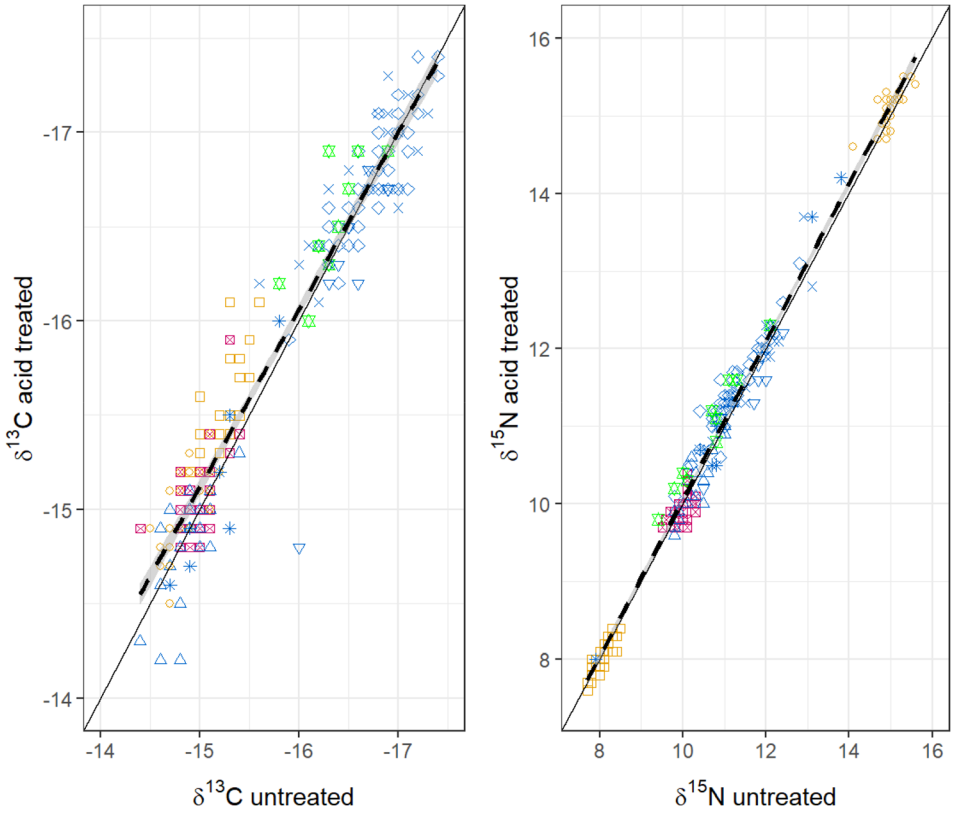

FIGURE 1 Relationships between acid-treated and untreated $\delta^{13}C$ and $\delta^{15}N$ of Atlantic salmon scales. The continuous line is the 1:1 line and the dashed line is the line of best fit for the data, included for illustrative purposes. Colours represent the life history environment of the fish, where Freshwater represents hatchery rearing and Ranched represents fish that were hatchery reared until the smolt stage when they were released to migrate to sea: (●) Aquaculture; (●) Freshwater; (●) Ranched; (●) Wild; (□) Aqua 1; (○) Aqua 2; (△) Big Salmon River, BSR; (✡) Burrishoole; (×) Bush; (◇) Dee; (▽) Gaspereau; (⊠) Tobique; (✳) Upper Salmon River, USR

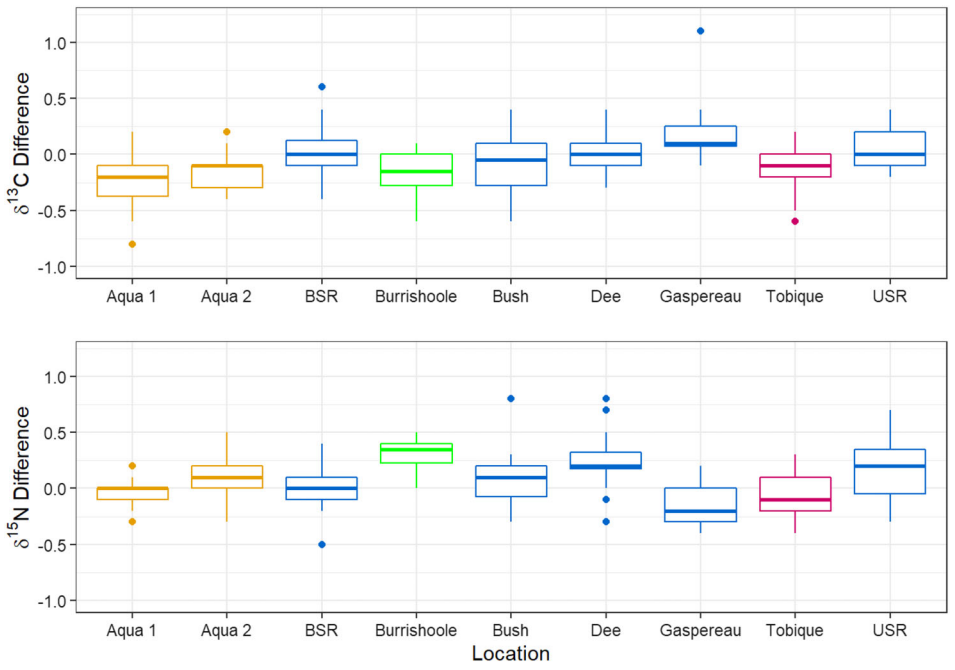

FIGURE 2 Boxplots of the difference in $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios between untreated and acidified Atlantic salmon scales, organized by sampling location. The bold line in each box represents the median for each location. Colours depict life history environment as in Figure 1, where orange represents aquaculture, red represents freshwater, green represents ranched, and blue represents wild

5.3‰ depending on factors including diet, physiological stress and tissue type analysed (McCutchan *et al.*, 2003; McMahon *et al.*, 2015; Minagawa & Wada, 1984). These values are considerably higher than the mean differences in $\delta^{15}N$ reflected in our analyses. Figure 1 outlines the relationship between acid-treated and untreated scale data for both $\delta^{13}C$ and $\delta^{15}N$. The slope of the relationship was significantly different from 1 for $\delta^{13}C$ ($P < 0.05$) but not for $\delta^{15}N$ ($P = 0.05$). The line of best fit deviates slightly from the 1:1 line for $\delta^{13}C$ where the freshwater samples are clustered. This suggests that further research is needed on the effect of acidification on scales of Atlantic salmon residing in freshwater environments. However, the difference between acid-treated and untreated scales was very small for the freshwater samples (0.1 ± 0.2‰ for $\delta^{13}C$). Boxplots of the difference in isotopic composition between untreated and acidified Atlantic

salmon scales are displayed in Figure 2 and show that differences are relatively low and constant across locations.

The data from this study show that treatment has a statistically significant effect on Atlantic salmon scales, contrary to the results of Sinnatamby *et al*. (2007), but the difference is very small and not likely to be of biological relevance. Therefore, this study finds that acidification has a negligible effect on Atlantic salmon scales and is not necessary prior to carbon SIA, confirming the findings of Sinnatamby *et al*. (2007). The mean difference in this study is much smaller than that recorded by Perga and Gerdeaux (2003), where acidifying whitefish scales increased the mean δ^{15}N by 1.3 ± 0.3‰. It is possible that scales are affected differently by acidification depending on the species or the habitat occupied by the fish, that is freshwater *versus* marine. The majority of fish in this study had spent at least 1 year in the marine environment, but the Tobique fish were reared exclusively in freshwater. Tobique data showed very small differences between acid-treated and untreated scales (0.1 ± 0.2‰ for δ^{13}C, 0.0 ± 0.2‰ for δ^{15}N), much smaller than that reported by Perga and Gerdeaux (2003), who also examined fish that exclusively inhabited freshwater. This is consistent with the findings of Ventura and Jeppesen (2010), who suggest that the effect of acid treatment on scales may vary between species. This study has answered an important contradiction in the literature by investigating acid-treated and untreated scales from 208 Atlantic salmon. These fish were from nine different locations on both sides of the Atlantic and were from ranched, farmed and wild life histories. Using a large sample size and a variety of locations, this study agrees with Sinnatamby *et al*. (2007) and concludes that acidification prior to SIA is not necessary for Atlantic salmon.

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REFERENCES

Boutton, T. W. (1991). *Stable carbon isotope ratios of natural materials: 2 atmospheric, terrestrial, marine, and freshwater environments*, San Diego, California: Academic Press, Inc.

Bunn, S. E., Loneragan, N. R., & Kempster, M. A. (1995). Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: Implications for food-web studies using multiple stable isotopes. *Limnology and Oceanography*, *40*(3), 622–625.

Church, M. R., Ebersole, J. L., Rensmeyer, K. M., Couture, R. B., Barrows, F. T., & Noakes, D. L. G. (2009). Mucus: A new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*(1), 1–5.

Craig, H. (1957). Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta*, *12*(1), 133–149.

Einum, S., Thorstad, E. B., & Næsje, T. F. (2002). Growth rate correlations across life-stages in female Atlantic salmon. *Journal of Fish Biology*, *60*(3), 780–784.

Graham, C. T., Harrison, S. S. C., & Harrod, C. (2013). Development of non-lethal sampling of carbon and nitrogen stable isotope ratios in salmonids: Effects of lipid and inorganic components of fins. *Isotopes in Environmental and Health Studies*, *49*(4), 555–566.

Graham, C. T., Harrison, S. S. C., & Harrod, C. (2014). Differences in the contributions of dietary water to the hydrogen stable isotope ratios of cultured Atlantic salmon and Arctic charr tissues. *Hydrobiologia*, *721*(1), 45–55.

Hutchinson, J. J., & Trueman, C. N. (2006). Stable isotope analyses of collagen in fish scales: Limitations set by scale architecture. *Journal of Fish Biology*, *69*(6), 1874–1880.

Kennedy, B. P., Chamberlain, C. P., Blum, J. D., Nislow, K. H., & Folt, C. L. (2005). Comparing naturally occurring stable isotopes of nitrogen, carbon, and strontium as markers for the rearing locations of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(1), 48–57.

MacKenzie, K. M., Palmer, M. R., Moore, A., Ibbotson, A. T., Beaumont, W. R. C., Poulter, D. J. S., & Trueman, C. N. (2011). Locations of marine animals revealed by carbon isotopes. *Scientific Reports*, *1*(21), 1–6.

Mariotti, A. (1983). Atmospheric nitrogen is a reliable standard for natural 15N abundance measurements. *Nature*, *303*(5919), 685–687.

McCutchan, J. H., Lewis, W. M., Jr., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102*(2), 378–390.

McMahon, K. W., Thorrold, S. R., Elsdon, T. S., & McCarthy, M. D. (2015). Trophic discrimination of nitrogen stable isotopes in amino acids varies with diet quality in a marine fish. *Limnology and Oceanography*, *60*(3), 1076–1087.

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. *Geochimica et Cosmochimica Acta*, *48*(5), 1135–1140.

Perga, M. E., & Gerdeaux, D. (2003). Using the δ13C and δ15N of whitefish scales for retrospective ecological studies: Changes in isotope signatures during the restoration of Lake Geneva, 1980–2001. *Journal of Fish Biology*, *63*(5), 1197–1207.

Pinnegar, J. K., & Polunin, N. V. C. (1999). Differential fractionation of δ13C and δ15N among fish tissues: Implications for the study of trophic interactions. *Functional Ecology*, *13*(2), 225–231.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703–718.

Schlacher, T. A., & Connolly, R. M. (2014). Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: A review and synthesis. *Methods in Ecology and Evolution*, *5*(6), 541–550.

Sinnatamby, R. N., Bowman, J. E., Dempson, J. B., & Power, M. (2007). An assessment of de-calcification procedures for delta C-13 and delta N-15 analysis of yellow perch, walleye and Atlantic salmon scales. *Journal of Fish Biology*, *70*(5), 1630–1635.

Torniainen, J., Vuorinen, P. J., Jones, R. I., Keinanen, M., Palm, S., Vuori, K. A. M., & Kiljunen, M. (2013). Migratory connectivity of two Baltic Sea salmon populations: retrospective analysis using stable isotopes of scales. *Ices Journal of Marine Science*, *71*(2), 336–344.

Trueman, C. N., & Moore, A. (2007). Use of the stable isotope composition of fish scales for monitoring aquatic ecosystems. In T. E. Dawson & R. T. W. Siegwolf (Eds.), *Stable isotopes as indicators of ecological change* (pp. 145–161). London, England: Elsevier.

Vander Zanden, M. J., Cabana, G., & Rasmussen, J. B. (1997). Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ15N) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(5), 1142–1158.

Ventura, M., & Jeppesen, E. (2010). Evaluating the need for acid treatment prior to δ13C and δ15N analysis of freshwater fish scales: Effects of varying scale mineral content, lake productivity and CO2 concentration. *Hydrobiologia*, *644*(1), 245–259.

Wieczorek, A. M., Power, A. M., Browne, P., & Graham, C. T. (2018). Stable-isotope analysis reveals the importance of soft-bodied prey in the diet of lesser spotted dogfish *Scyliorhinus canicula*. *Journal of Fish Biology*, *93*(4), 685–693.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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