BRIEF COMMUNICATION

Non-lethal sampling for stable isotope analysis of pike *Esox lucius*: how mucus, scale and fin tissue compare to muscle

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ABSTRACT

Stable isotope analysis (SIA) was used to examine the isotopic relationships between dorsal muscle and fin, scale and epidermal mucus in pike *Esox lucius*. $\delta^{13}C$ and $\delta^{15}N$ varied predictably within each tissue pairing, with conversion factors calculated for the surrogate tissues, enabling their application to the non-lethal sampling of *E. lucius* for SIA.

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KEYWORDS

conversion factor, Esocidae, non-invasive sampling, stable isotope isotopic signature

Carbon and nitrogen stable isotopes are widely used to determine aspects of organismal trophic ecology, including for fishes (Boecklen *et al.*, 2011). Dorsal white muscle (muscle hereafter) is the most common fish tissue used for stable-isotope analysis (SIA), in-part due to its relative isotopic stability (Pinnegar & Polunin, 1999). However, it usually requires lethal sampling of the fish, unless the individuals are sufficiently large to enable muscle biopsies (Hanisch *et al.*, 2010). Where lethal sampling is not desirable, such as in studies involving telemetry or investigating threatened species, then alternative tissues are required for SIA that can be collected non-lethally (Speed *et al.*, 2012). These surrogate tissues include fin, blood, scales and mucus (Church *et al.*, 2009; Suring and Wing, 2009; Busst *et al.*, 2015; Winter *et al.*, 2019). The SI data from these surrogate tissues can be converted to muscle values, for example, for use in Bayesian mixing models that predict diet composition from putative prey (Nolan & Britton, 2018). Without conversion, researchers risk misinterpretation of ecological findings, such as resource use (Hayden *et al.*, 2017), arising from the wide variation in isotopic data that would result from diverse rates of isotopic turnover and differences in fractionation between body tissues (Busst *et al.*, 2015; Winter *et al.*, 2019).

Since *c.* 2010, the isotopic relationships of different fish tissues have been investigated across various taxonomic families, including Salmonidae, Cyprinidae and Centrarchidae (Hanisch *et al.*, 2010; Jardine *et al.*, 2011; Tronquart *et al.*, 2012). There is, however, a paucity of tissue conversion data for the family Esocidae, despite studies on these fishes using fin tissue as a non-lethal muscle surrogate (Nyqvist *et al.*, 2018). Moreover, predatory species such as pike *Esox lucius* L. 1758 are frequently used within freshwater SI

studies, particularly as they can have top-down effects on prey communities (Søndergaard *et al.*, 1997). Previously, *E. lucius* dietary studies have been based on SIA (Pedreschi *et al.*, 2015). This can be preferable to the use of stomach contents analyses, which usually result in considerable numbers of *E. lucius* being sacrificed, but with a high proportion of stomachs often found empty (Sandlund *et al.*, 2016). To overcome this knowledge gap in isotopic relationships between muscle and surrogate tissues in the Esocidae, *E. lucius* was used as the model species to test the relationships of $\delta^{13}C$ and $\delta^{15}N$ between muscle, pelvic fin, scale and mucus, and to generate conversion factors for $\delta^{13}C$ and $\delta^{15}N$ of non-lethally sampled tissues.

The SI relationships were tested using juvenile fish (n = 15) collected from a side channel of the River Frome, Dorset, England (50° 412092 N, 2° 112092 W) in June 2010. Most of the fish were sampled using a hand-net and then held in controlled aquaria conditions in the laboratory for up to 100 days, with daily feeding of earthworms *Dendrobaena veneta* (n = 13, 78–123 mm length, 3.36–14.31 g; for full details, see Nyqvist *et al.*, 2013). These fish were then euthanised with an overdose of MS-222 and frozen. Two additional wild fish were collected by electric fishing; one within the length and mass range of the laboratory fish and another of 260 mm fork length (L_F) 139.40 g live mass (M). These fish were also euthanised and frozen, but without feeding. Following defrosting, the tissues were sampled from each fish. Mucus was lightly scraped from the dorsal surface of each fish using a sterile cover slip (Winter *et al.*, 2019), muscle was excised from the dorsal region of each fish (above the lateral line, below the dorsal fin) and scales were removed from above the lateral line (due to their small size, whole scales were used, one per fish). Fin tissue was removed from the outer edge of the pelvic fin, where the ratio of fin membrane to ray is at its highest.

This minimises any variation in isotope ratios that may be attributed to a difference in isotopic turnover rates between fin membrane and ray (Hayden *et al.*, 2015). The tissues were then rinsed and cleaned in distilled water, then dried for 48 h at 60°C. Samples were bulk analysed for $\delta^{13}C$ and $\delta^{15}N$ at the Cornell University Stable Isotope Laboratory, New York, USA. Analytical precision of the $\delta^{13}C$ and $\delta^{15}N$ sample runs was estimated at 0.15 and 0.42‰, respectively. All fish sampling and experimental procedures were completed under UK Home Office licence PPL30/2626 and after ethical review.

Mean C:N ratios (± 95% CI) of the tissues ranged between 2.96 ± 0.05 (scale) to 3.66 ± 0.16 (mucus), indicating relatively low lipid content and therefore no requirement for lipid normalisation of $\delta^{13}C$. As the distribution of the SI data was non-normal (Shapiro-Wilk test, $P < 0.05$), differences in $\delta^{13}C$ and $\delta^{15}N$ between muscle and the corresponding values for fin, scale and mucus (surrogate tissues hereafter) were tested using paired Wilcoxon tests. SI conversion factors (CF) were generated from mean isotopic differences between muscle and the surrogate tissues. These revealed that muscle $\delta^{13}C$ was significantly depleted *v.* fin (CF = −0.76; $P < 0.01$) and scale (CF = −1.39; $P < 0.001$), but not mucus ($P > 0.05$; Figure 1). Conversely, muscle $\delta^{15}N$ was significantly enriched versus fin (CF = 1.26; $P < 0.001$) and mucus (CF = 2.41; $P < 0.001$), but not with scales ($P > 0.05$; Figure 1).

The two electric-fished *E. lucius* were clear outliers due to their difference in diet compared with the laboratory fish (Figure 1). Therefore, to examine the effects of these two fish on the paired tissue relationships, they were removed from the analysis prior to it being repeated. In this adjusted dataset, muscle $\delta^{13}C$ was significantly depleted versus fin (CF = −0.66; $P < 0.01$) and scale (CF = −1.32; $P < 0.001$), but not mucus ($P > 0.05$). Muscle $\delta^{15}N$

was significantly enriched versus fin (CF = 1.27; $P < 0.001$) and mucus (CF = 2.39; $P < 0.001$), but not scales ($P > 0.05$).

Where significant differences in isotope ratios were evident, converted surrogate tissue data were generated using CFs calculated both before and after outlier removal. To test the effects of the outliers on the converted data, the residual errors of each method were compared using paired Wilcoxon tests. The results indicated errors were not reduced following outlier removal (pre-removal = 0.29−0.58 ‰; post-removal = 0.30−0.58 ‰; $P > 0.05$ in all cases).

These results provide the first comparisons and conversion factors between muscle and surrogate tissues for $\delta^{13}C$ and $\delta^{15}N$ of a species of the Esocidae. Values of $\delta^{13}C$ and $\delta^{15}N$ were strongly correlated between muscle and fin, scale and epidermal mucus (Figure S1). Isotope signatures did not differ between muscle and mucus $\delta^{13}C$, nor between muscle and scale $\delta^{15}N$, thus conversion factors were not necessary for these pairings and their SI data can be used interchangeably. Where significant differences in isotope ratios were apparent, the calculated conversion factors were robust to the effects of outliers, indicating consistency in isotopic relationships across the range of recorded $\delta^{13}C$ and $\delta^{15}N$ values.

The pattern here of increasing $\delta^{13}C$ enrichment from muscle to scale was consistent with studies on a wide range of other fishes, including salmonids and cyprinids (Hanisch *et al.*, 2010; Jardine *et al.*, 2011; Busst *et al.*, 2015). It is explained by the relative abundance of ^{13}C-depleted lipids in different tissues, with muscle generally containing more lipids than fin or scale (Pinnegar & Polunin, 1999). Mucus primarily consists of glycoproteins, therefore depleted ^{13}C is expected and, indeed, was evident here and elsewhere (Maruyama *et al.*,

2017; Shigeta *et al.*, 2017; Nolan and Britton, 2018). Across studies, δ^{15}N isotopic relationships between muscle and surrogate tissues are more varied (Hanisch *et al.*, 2010). Here, muscle was more enriched in δ^{15}N than fin tissue, but did not differ to scale. However contrasting relationships have been recorded for other species, e.g. *Lepomis* spp. (Kelly *et al.*, 2006). Mucus was depleted in δ^{15}N *v.* muscle and fin, as was also apparent in *Pseudorasbora parva* (Temminck & Schlegel 1846) (Shigeta *et al.*, 2017) and *Sander lucioperca* (L. 1758) (Nolan and Britton, 2018).

In summary, non-lethal and non-invasive tissue sampling on *E. lucius* can be used reliably in place of dorsal white muscle for SIA in studies on their trophic ecology. The species-specific correction factors derived here should be used, therefore, in future stable-isotope studies on juvenile *E. lucius*. These results also deliver the first step towards a more robust understanding of the isotopic relationships between tissues of this species and for the Esocidae family.

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FIGURE 1 Mean (solid symbols; ± 95% CI) stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ for *Esox lucius* dorsal muscle (O), fin (□), scale (△) and epidermal mucus (◇).values are in bold and error bars represent 95% CIs.