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Inter-tissue variability in the stable isotope values of European perch (*Perca fluviatilis*) and pumpkinseed (*Lepomis gibbosus*)

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Abstract – Ecological studies on native and invasive populations of European perch *Perca fluviatilis* and pumpkinseed *Lepomis gibbosus* are often based on stable isotope (SI) analysis based on dorsal muscle, where samples are usually taken from sacrificed fishes. However, other tissues, such as scale and fin tissue, can be used as non-lethal alternatives, where their SI values can be standardised to dorsal muscle values for comparative purposes. In both perch and pumpkinseed, there was a pattern of $\delta^{13}C$ enrichment and $\delta^{15}N$ depletion from muscle to fin and scale. As comparative studies must account for these inter-tissue differences prior to analyses, conversion equations for SI data from scale and fin tissue to standardised muscle values are provided.

Keywords: Trophic ecology / diet / fish / non-lethal sampling / $\delta^{13}C$ / $\delta^{15}N$

Dietary analyses provide the basis for understanding the trophic ecology of fishes but are often reliant on destructive sampling, especially where stomach content analyses are used (Sandlund *et al.*, 2016). Stable isotope analysis (SIA) is an alternative method for reconstructing trophic relationships that provides temporally integrated dietary perspectives (Trueman *et al.*, 2012). Dorsal muscle is generally the main tissue analysed in fish SI studies, with its sampling also tending to be destructive, especially in smaller fishes (Hette-Tronquart *et al.*, 2012; Maitland and Rahel, 2021; Boardman *et al.*, 2022). However, other fish tissues (e.g. scale, fin and mucus) can be used non-destructively for SIA (Boardman *et al.*, 2022). These tissues also vary in their rate of stable isotope turnover, being relatively fast in mucus (so SI data provide a short-term dietary perspective, e.g. 4 weeks) and relatively slow in scales (long-term dietary perspective, e.g. 6 months) (Busst and Britton, 2018; Winter *et al.*, 2021). Moreover, when comparing species-specific SI data between studies (e.g. in meta-analyses), data generated from different tissues can be encountered. Providing knowledge is available on the extent to which the SI values vary between the tissues then correction factors and/ or conversion equations can be applied to standardise all values to dorsal muscle equivalents (Maitland and Rahel, 2021; Roberts *et al.*, 2021).

European perch *Perca fluviatilis* and the pumpkinseed *Lepomis gibbosus* are species with relatively large native ranges and increasing non-native ranges, where invasive populations of both species can potentially have ecological consequences for native communities (e.g. Almeida *et al.*, 2014; Furlan and Gleeson, 2016). Where the species co-occur, there is also potential for dietary overlap and competition (Fobert *et al.*, 2011), with SIA providing a tool that can investigate the extent of their trophic interactions (Copp *et al.*, 2017). While destructive sampling for the collection of tissues for SIA may be permissible in the invasive range of these fishes, the species can have relatively high fishery values in their native ranges, especially European perch where catch-and-release angling is increasingly practised (Czarkowski and Kapusta, 2019), especially as harvesting by angling can have strong deleterious effects on population abundances (Lyach and Remr, 2019).

To promote the application of a range of different tissues in European perch and pumpkinseed SI studies, including tissues that can be collected non-destructively, the aim here was to test differences in $\delta^{13}C$ and $\delta^{15}N$ data between dorsal muscle, fin tissue and scales; where significant differences between the tissues were apparent in either species then tissue conversion equations were generated to allow conversion to a standardised dorsal muscle value (SI_{muscle}). The perch ($n = 10$; mean length ± 95 % CI: 93.6 ± 5.0 mm) and pumpkinseed ($n = 18$; 102.4 ± 9.4 mm) were sampled from a lentic fish community in

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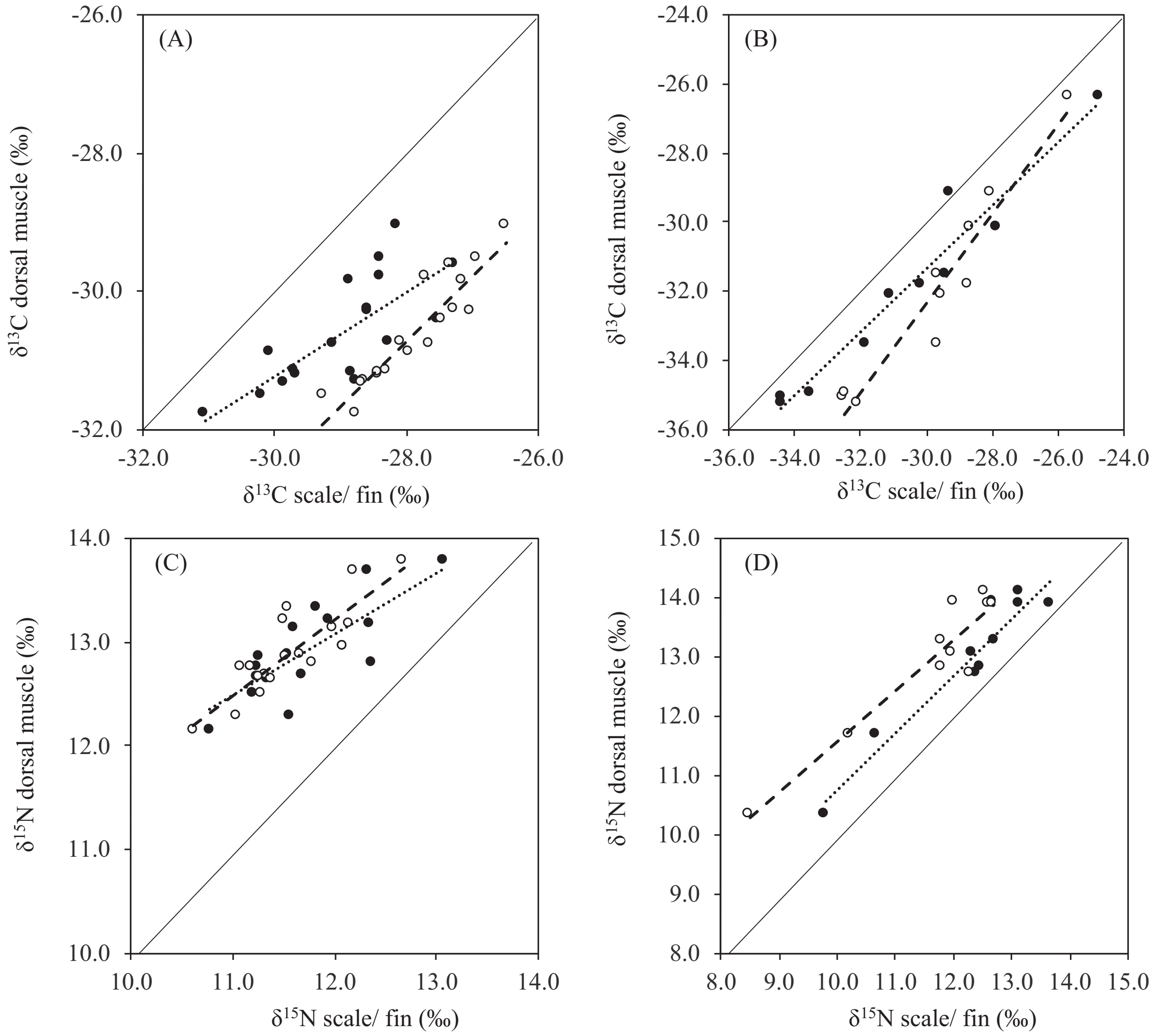

Fig. 1. Comparison of stable isotope data ($\delta^{13}C$, $\delta^{15}N$) of scale (open circle) and fin (filled circle) versus dorsal muscle for pumpkinseed (A, C) and European perch (B, D); solid lines = equality; small dashed line = significant relationship between fin and muscle SI data according to linear regression; long dashed line = significant relationship between fin and muscle SI data according to linear regression.

Southern England, where the waterbody is used for recreational angling. Due to the non-native status of pumpkinseed in the fishery, the exact location cannot be provided to protect business confidentiality. Fish samples were collected through a combination of rod-and-line angling and baited fish traps in August 2022 (mid-summer in the study area, when water temperatures tend to be in the range of 18–22 °C). Following capture, fish were identified to species, euthanised (anaesthetic overdose, MS-222), with individual fish placed into plastic sample bags and taken to the laboratory, where they were measured (total length (TL), nearest mm) before a sample of dorsal muscle, and fin tissue was excised from all individuals, and up to 3 scales removed. Mucus samples could not be taken as the amounts able to be collected from both species were below the threshold required for SIA.

All muscle, fin and scale samples were then dried to constant weight (60°C for 48 h), before being bulk analysed for $\delta^{13}C$ and $\delta^{15}N$ in a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Analytical precision of the $\delta^{13}C$ and $\delta^{15}N$ sample runs was estimated against an internal standard sample of animal (deer) material every 10 samples, with the overall standard deviation estimated at 0.08 and 0.04‰ respectively. The C:N ratios of the samples were low (mean ± 95% CI: perch 3.25 ± 0.09 (2.91–3.72); pumpkinseed: 3.16 ± 0.06 (2.78–3.71)) and so were not mathematically corrected for lipid content (Post *et al.*, 2007).

In perch, there was a pattern of $\delta^{13}C$ enrichment from muscle (mean ± 95% CI: −31.99 ± 0.91‰) to fin: (−30.68 ± 0.97‰) to scale (−29.71 ± 0.68‰), with a pattern of $\delta^{15}N$ depletion across these tissues (muscle: 12.98 ± 0.38‰, fin: 12.29 ± 0.37‰. scales: 11.64 ± 0.42‰) (Fig. 1). These patterns were also apparent for pumpkinseed ($\delta^{13}C$: muscle −30.60 ± 0.36‰; fin: −28.96 ± 0.45‰; scale: −27.87 ± 0.35‰; $\delta^{15}N$: muscle: 12.90 ± 0.20‰; fin: 11.70 ± 0.26‰; scales: 11.57 ± 0.23‰) (Fig. 1). Testing for differences in the SI data by tissue and species, using Wilcoxon tests (where data were not normally distributed) and *t*-tests (data normally distributed), revealed that in both

Table 1. (A) Significance of differences in the stable isotope data of different tissues per species, according to Wilcoxon tests and *t*-tests; (B) results of linear regression of the relationships of dorsal muscle versus fin and scales SI data per species, and the associated regression coefficients of *a* and *b* for use in equation (1). *P*-values are Bonferroni corrected at α 0.05/2 = 0.025 because two sets of tests were derived from the same dataset (or subsets thereof).

(A)

Species	Isotope	Relationship	Test type	Test value	*P*-value
European perch	$\delta^{13}C$	Muscle/Fin	*t*-test	5.71	<0.001
		Muscle/Scales	*t*-test	−7.15	<0.001
	$\delta^{15}N$	Muscle/Fin	Wilcoxon	0.00	0.002
		Muscle/Scales	Wilcoxon	55.00	0.002
Pumpkinseed	$\delta^{13}C$	Muscle/Fin	*t*-test	10.81	<0.001
		Muscle/Scales	*t*-test	−37.15	<0.001
	$\delta^{15}N$	Muscle/Fin	*t*-test	−14.43	<0.001
		Muscle/Scales	*t*-test	21.73	<0.001

(B)

$\delta^{13}C$

Species	Tissue sampled	Tissue predicted	Test result	*a* (±95% CI)	*b*
Perch	Fin	Muscle	$F_{1,8}$ = 133.5; R^2 = 0.94; P < 0.001	0.92 (±0.08)	−3.80
	Scale	Muscle	$F_{1,8}$ = 94.67; R^2 = 0.91; P < 0.001	1.28 (±0.13)	6.08
Pumpkinseed	Fin	Muscle	$F_{1,16}$ = 21.05; R^2 = 0.54; P < 0.003	0.60 (±0.13)	−13.15
	Scale	Muscle	$F_{1,16}$ = 86.32; R^2 = 0.83; P < 0.001	0.94 (±0.10)	−4.36

$\delta^{15}N$

Species	Tissue sampled	Tissue predicted	Test result	*a* (±95 % CI)	*b*
Perch	Fin	Muscle	$F_{1,8}$ = 93.61; R^2 = 0.91; P < 0.001	0.97 (±0.01)	1.09
	Scale	Muscle	$F_{1,8}$ = 66.89; R^2 = 0.88; P < 0.001	0.86 (±0.10)	3.00
Pumpkinseed	Fin	Muscle	$F_{1,16}$ = 26.22; R^2 = 0.60; P < 0.001	0.59 (±0.12)	5.96
	Scale	Muscle	$F_{1,16}$ = 45.12; R^2 = 0.72; P < 0.001	0.73 (±0.11)	4.44

species, there were significant differences in values of muscle SI data versus fin and scales (Tab. 1A). Consequently, linear regression was applied (independent variable: scale or fin tissue SI data; dependent variable: muscle SI data) to generate the regression coefficients *a* and *b* for application in equation (1) that enables the standardised dorsal muscle (SI_{muscle}) values to be predicted from scale and fin SI data (SI_{other}) (Tab. 1B; Fig. 1):

$$SI_{muscle} = (SI_{other} \times a) + b \quad (1).$$

To compare inter-species differences in trophic niche sizes between adjusted scale SI data (long isotopic turnover rate) and dorsal muscle data (relatively short isotopic turnover rate) standard ellipse areas (SEA) were calculated that provides an indication of the isotopic niche size, using the SIBER package in R (Jackson *et al.*, 2011, 2012). As the ellipses enclose the core 40% of SI data, they represent the typical resource use of the analysed population (Jackson *et al.*, 2011). A Bayesian estimate of SEA (SEA_b) was used due to the small sample sizes; this utilises a Markov chain Monte Carlo simulation (10^4 iterations per group) and provides 95% confidence limits for the isotopic niche size (Jackson *et al.*, 2011). To quantify trophic niche overlap, the bivariate area shared by both species in isotopic space and percentage of overlap was also calculated using SEA_c (subscript '$_c$' indicates a small sample size correction was used) (Jackson *et al.*, 2011, 2012). Values of SEA_b were similar for each species and tissue (perch muscle: 7.79 ± 4.12‰^{2}, scale: 7.81 ± 3.99‰^{2}, fin: 9.43 ± 4.77‰^{2}, pumpkinseed muscle: 1.10 ± 0.47‰^{2}, scale: 1.25 ± 0.42‰^{2}, fin: 1.84 ± 0.56‰^{2}). SEA_c revealed very similar patterns for all tissues (Supplementary material: Tab. S1 and Fig. S1). In entirety, these results suggest that both the isotopic niche sizes and the resource use of the species (and the extent of inter-specific interactions) were consistent temporally.

The results thus demonstrated that where the trophic relationships of both perch and pumpkinseed are assessed using SIA, the tissue analysed will affect their SI values. Correspondingly, meta-analyses that use SI data from different tissues to assess the spatial and/ or temporal patterns in the trophic relationships of these species can use the information here to generate standardised SI data. The data presented here also enables the collection of fin and/ or scale material from both species using non-destructive methods in their native ranges, promoting the use of sustainable sampling methods.

The SI conversion data for perch were, however, collected from a relatively limited sample size (n = 10), with no fish used above 138 mm. As European perch can attain lengths exceeding 350 mm, with ontogenetic dietary shifts towards piscivory with increasing fish length, then some caution is needed in applying these conversion values beyond the size

Table 2. Comparison of non-lethal tissue – muscle conversion factors for perch and pumpkinseed from across the literature (*combined data for three sunfish species, including pumpkinseed).

		δ^{13}N		δ^{13}C		Reference
Species	Conversion	*a*	*b*	*a*	*b*	
Perch	Fin-muscle	0.97	1.09	0.92	−3.80	This study
	Fin-muscle	1.02	0.44	0.96	−1.86	Hette-Tronquart *et al.* (2012)
Pumpkinseed	Fin-muscle	0.59	5.96	0.60	−13.15	This study
	Fin-muscle	1.17	−1.72	0.84	−5.71	Hette-Tronquart *et al.* (2012)
	Fin-muscle	1.26	−3.46	1.03	0.50	Cano-Rocabayera *et al.* (2015)
	Scale-muscle	0.73	4.44	1.28	6.08	This study
	Scale-muscle	1.05	0.88	0.93	−3.89	Cano-Rocabayera *et al.* (2015)
	Scale-muscle	0.85	2.67	1.17	1.05	Kelly *et al.* (2006)*

range analysed. There are other non-lethal tissues to muscle conversion factors available for both species (Tab. 2), with Vollaire *et al.* (2007) also indicating that the fractionation between perch muscle and a formulated diet was greater for muscle than scales for δ^{15}N (2.88 ± 0.42‰ versus 1.26 ± 0.39‰) but was greater for scales than muscle for δ^{13}C (4.02 ± 0.13‰ versus 5.98 ± 0.20‰). Thus, when researchers apply fin and/ or scales from these and require conversion to standardised muscle values species, they must decide on the conversion factors to use. We argue that our conversion equations provide robust estimates of standardised muscle values. For example, the mean standardised residuals of the predicted SI_{muscle} data versus the observed SI muscle data of both species were significantly smaller using *a* and *b* values in Table 1 in equation (1) versus the conversion equations of Hette-Tronquart *et al.* (2012) (HT) (mean standardised residuals ± 95% CI: δ^{13}C: HT: 1.00 ± 0.37, Tab. 1: 0.41 ± 0.37, ANOVA: $F_{1,54} = 4.93$, $P = 0.03$; δ^{15}N: HT: −0.99 ± 0.37, Tab. 1: 0.00 ± 0.37, ANOVA: $F_{1,54} = 13.89$, $P < 0.001$). Note, however, that the predicted data here were generated from our data that produced the regression coefficients (i.e. it was a circular analysis), so some caution is needed in interpretation. Accordingly, future studies could consider creating their own regression coefficients from a small number of lethally sampled individuals (*e.g.* $n = 10–12$ per species) and apply these over their larger dataset generated from non-lethally sampled fish. Finally, we suggest that the provision here of scale to muscle SI values is important, as it provides the opportunity to use historical scale samples of European perch that are more likely to be available for SIA than fin samples across their native range, given their collection in long-term datasets (*e.g.* Kankaala *et al.*, 2019), where scales are routinely collected for age and growth analyses.

Ethical statement

The study was completed following the gaining of all relevant ethical and legislative approvals (UK Home Office Project Licence P47216841).

CRediT authorship contribution statement

Irmak Kurtul: Conceptualization, Writing – review & editing, Formal analysis, Writing – original draft, Visualization. Ali Serhan Tarkan: Conceptualization, Formal analysis, Writing – original draft. J. Robert Britton: Conceptualization, Writing – original draft, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available upon reasonable request to the corresponding author.

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Supplementary Material

The Supplementary Material is available at https://www.alr-journal.org/10.1051/kmae/2023018/olm.

Table 1. Standard ellipse area (SEAb) and corrected standard ellipse area (SEAc) of different tissues in perch and pumpkinseed.

Figure S1. Trophic niche sizes (as the isotopic niches, standard ellipse areas, SEAc) of perch and pumpkinseed for corrected fin and dorsal muscle, and scale.

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