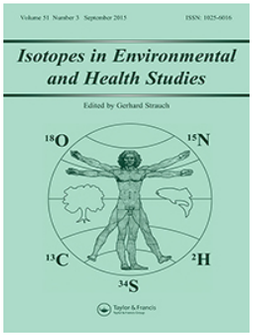

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The efficacy of scale sampling for monitoring trace element concentrations and stable isotopes in commercially harvested walleye (*Sander vitreus*)

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Commercial and sport fishes are subject to rigorous monitoring for concentrations of elements that could pose threats to human health, with numerous advisories issued by authorities annually for those fisheries with high mercury (Hg) concentrations. In Lake Winnipeg, Manitoba, Canada, the commercial walleye fishery is valued at more than $20 million/year, but has historically been subject to Hg advisories. We used an information theoretic approach to evaluate the utility of non-destructive fish-scale sampling to predict As, Mn and Hg concentrations, as well as stable isotope values in walleye muscle by analysing paired samples. Hg concentrations in scales were significantly related to those in muscle ($r^2 = 0.75$), but the relationships were weaker for As and Mn. The δ^{15}N values in scales predicted δ^{15}N in muscle reasonably well ($r^2 = 0.72$), whereas scale δ^{13}C and δ^{34}S had less predictive power for estimating their respective muscle stable isotope values. For all three isotope values, sex was a marginal predictor, with parameter confidence intervals bounding zero. Analytical constraints currently limit the utility of non-destructively analysing scales for Hg, but hindcasting trophic changes using archived walleye scales may be useful in understanding shifts in nutrients and production, particularly in impacted lake systems.

Keywords: arsenic; carbon-13; fish scales; isotope ecology; Lake Winnipeg; manganese; mercury; nitrogen-15; non-destructive analysis; sulphur-34

1. Introduction

Loading of Hg and other metals to freshwater lakes may increase concentrations in the tissues of commercial and sport fishes [1,2], and may have implications for consumer health, as well as the sustainability of recreational and commercial fishing industries. In Canada and the USA, thousands of Hg-related fish consumption advisories have been issued [3,4], including moratoria on the commercial harvest of walleye (*Sander vitreus*) from Lake Erie and Lake Winnipeg in the 1970s [5,6]. Although Lake Winnipeg (53°17′N, 97°58′W) now supports ∼$20 million/year in commercial fisheries, the need for contaminant monitoring continues [7]. Large-scale and stochastic flooding within the lake's watershed may increase the influx rate and bioavailability

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of contaminants entering the lake [8], and subtle food-web changes resulting from eutrophication and the introduction of non-native species may affect the dispersion of contaminants and accumulation of metals in frequently harvested species [7,9].

Current programmes for monitoring concentrations of Hg and other contaminants in fishes are based on direct analysis of muscle [10]. The fillet-based approach allows regulators to monitor the commercial product and set consumption guidelines for local anglers [4]. Unfortunately, fillet-monitoring programmes often require that large numbers of individuals be lethally taken from aquatic systems on an annual basis [11]. The removal of reproductive stock or rare and endangered species may have ecological consequences, and loss of fisheries resources may have implications for the regional economy [12,13]. Perceived fiscal losses associated with lethal fish sampling highlight the need for suitable non-destructive alternatives when monitoring contaminants, or assessing changes in lake trophic structure [14]. Analysis of Hg and other contaminants in scales may provide an attractive proxy for whole-fillet sampling for walleye in Lake Winnipeg, and elsewhere [15]. However, before walleye scales can be used in a regional contaminants monitoring programme, the feasibility and efficacy of this sampling technique require investigation, as elemental concentration or stable isotope relationships between scale or fin tissue and muscle vary among species, or may not be present at all [16,17].

The objective of this study was to determine whether concentrations of trace elements in scales of Lake Winnipeg walleye were accurate predictors of Hg and other trace element concentrations in muscle fillet [18] in its two biologically distinct basins. Since trophic linkages and food sources regulate contaminant transport to apex predators like walleye [7], stable isotope values (δ^{15}N, δ^{13}C and δ^{34}S) in paired tissues were examined. The influence of fish size, sex and maturity on the relative distribution of contaminants to tissues was also considered [2].

2. Methods

2.1. *Field collections*

Beam trawls deployed by Manitoba Fisheries personnel and commercial gillnets were used to collect walleye ($n = 34$) specimens from Lake Winnipeg during the ice-free season (May–October) of 2010. The beam trawl was towed alongside the MV Namao research vessel at a rate of 3.9 km/h for approximately 30 min per trawl [19]. Each station marked in Figure 1 (closed circles) denotes the ship's position at the end of each trawl effort. Gillnets were set overnight at nearshore locations (Figure 1; closed triangles) [20]. Fork lengths (FLs), which were measured to the nearest mm [21], ranged from 232 to 492 mm, and are known to influence the relationship between muscle and fin stable isotope values [16]. Sex and maturity were determined through visual examination of the gonads [22]. One or both fillets, with skin and scales intact, were removed from each fish and stored frozen at -20°C [23,24] until they could be processed at the National Hydrology Research Centre laboratory in Saskatoon, Canada.

2.2. *Preparation of scales and muscle samples*

In the laboratory, fillet samples were partially thawed, and 5–10 g of white muscle was removed by making clean cuts into the inner region of each fillet with a clean stainless steel scalpel [23,25]. The muscle sample was then divided into two portions: one was dried at 60°C and homogenised in preparation for isotopic and elemental analyses [26], and a second section was analysed for Hg [12].

Scales were stripped from each fillet and placed in acid-washed polyethylene vials. In order to remove mucus and skin, scales were ultra-sonicated in high-purity acetone for at least 5 min,

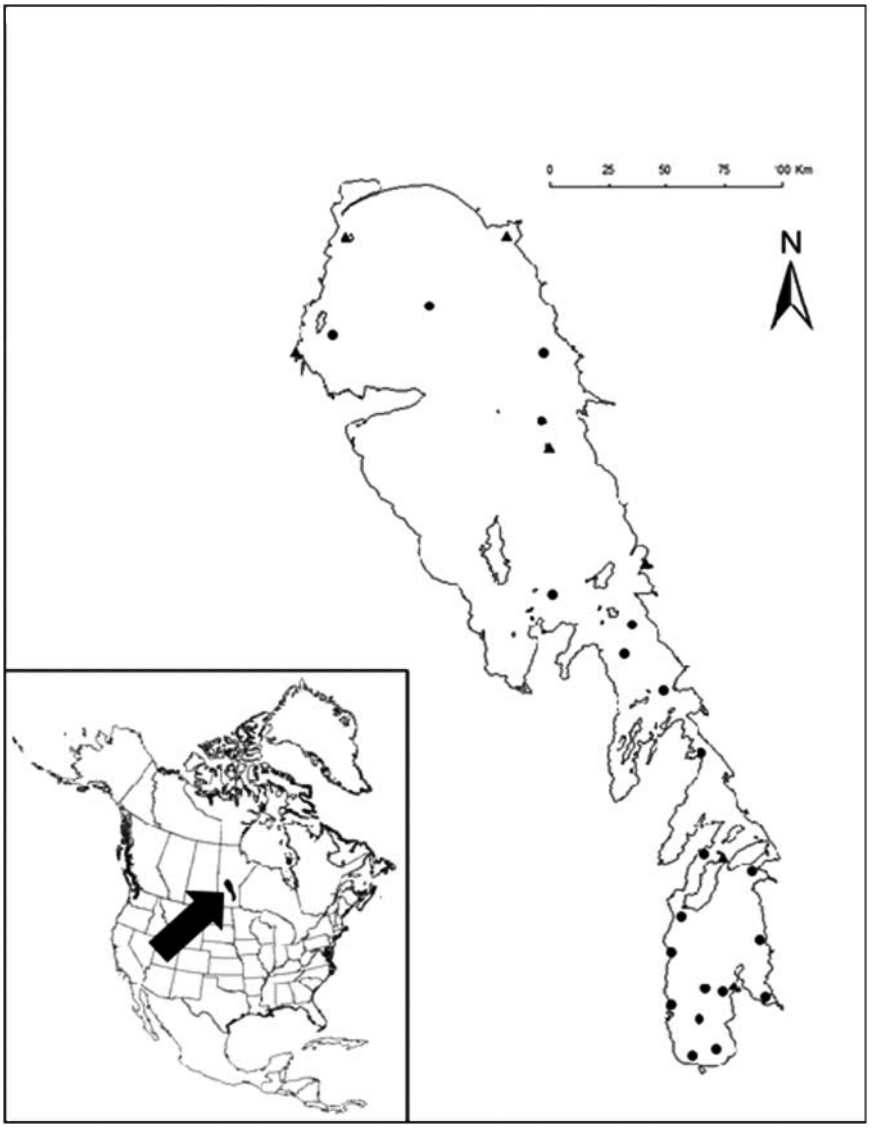

Figure 1. Walleye collection locations on Lake Winnipeg, Canada. Stations where beam trawls and commercial gillnets were used to capture walleye specimens are marked by black circles and black triangles, respectively.

then rinsed multiple times with Milli-Q water [21,27]. Cleaned scale samples were dried at 60°C and ground using a stainless steel ball mill which was cleaned with analytical-grade 5% v/v nitric acid (HNO_3) and Milli-Q water between samples [28,29].

2.3. *Trace element analysis*

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is used for the successful recovery of Hg and other trace elements from fish scales [30]. Here, trace elements in scales were analysed via inductively-coupled plasma mass spectrometry (ICP-MS); however, the scales were liquefied, rather than ablated, prior to analysis [31]. Aliquots of dried muscle and scale samples were digested with high-purity nitric acid and 30% v/v analytical-grade hydrogen

peroxide (H_2O_2) [32]. Blanks, reference materials and duplicates were included in each digestion batch at a ratio of one blank, reference and duplicate per 10 field samples. The concentrations of Al, As, Cd, Cu, Fe, Mn and Se in paired muscle and scale samples were measured via ICP-MS [31,33]. Acceptable elemental recoveries from DORM-2 (National Research Council Canada; Ottawa, ON) and coefficients of variation (CVs) on duplicate samples were $\pm 20\%$ and $\pm 15\%$, respectively [1]. For the scale samples, only Al, As and Mn met these criteria. Therefore, the ICP-MS data presented here were limited to these three elements. Limits of detection (LoD) for Al, As and Mn were 0.76 µg/g, 5.2×10^{-4} µg/g and 1.3×10^{-4} µg/g dry weight (dw), respectively.

2.4. *Mercury*

Microwave digestion (US EPA, 1996) and total Hg determinations via cold-vapour atomic fluorescence spectrometry (CVAFS) [12] were conducted at the Saskatchewan Research Council (SRC) Environmental Analytical Laboratory in Saskatoon, Canada. Blanks, reference materials and duplicate samples were included in each analytical batch. The LoD for walleye muscle was 0.01 µg/g fresh weight (fw), and recoveries of DORM-2 were within 85–115%. The LoD for scale samples was 0.05 µg/g dw, and recoveries were 72%. The CV on duplicate samples was within 13% for both tissue types. In order to standardise the units of measurement across the study, per cent moisture data for each of the muscle samples (unpublished data, Environment Canada) was used to convert the fw Hg data to units of µg/g dw.

2.5. *Isotopic analyses*

Of the dried sample aliquots reserved for stable isotope analyses, only those muscle samples used for $\delta^{13}C_m$ determination had lipids extracted using a 2:1 chloroform:methanol solution [9,34]. Lipid removal was not required for the walleye scales, which consisted of collagen and an upper, calcified layer [35]. One milligram (± 0.01 mg) each of un-extracted muscle ($\delta^{15}N_m$), lipid-extracted muscle ($\delta^{13}C_m$) and homogenised scales ($\delta^{15}N_s$ and $\delta^{13}C_s$) were weighed into tin capsules [36]. Sulphur isotope ratios were determined for 3.50 ± 0.02 and 10.00 ± 0.10 mg of scale ($\delta^{34}S_s$) and muscle tissue ($\delta^{34}S_m$), respectively. All $^{15}N/^{14}N$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$ ratios were measured via continuous-flow isotope-ratio mass spectrometry [36]. In-house calibration standards BWB ($\delta^{15}N = 14.4$ ‰, $\delta^{13}C = -18.5$ ‰, $\delta^{34}S = 17.5$ ‰), PUGEL ($\delta^{15}N = 5.6$ ‰, $\delta^{13}C = -12.6$ ‰) and CFS ($\delta^{34}S = -3.8$ ‰) were analysed along with the samples for data normalisation. Analytical precision on control samples was better than ± 0.3 ‰ for all stable isotopes.

All results for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ ($n = 34$) are expressed in per mil (‰) relative to the isotopic reference materials atmospheric air, Vienna Peedee Belemnite (VPDB) and Vienna Cañon Diablo Troilite (VCDT), respectively:

$$\delta X = \left(\frac{R_A}{R_S} - 1\right),$$

where X was one of ^{15}N, ^{13}C or ^{34}S, R_A was the ratio of the heavy to light isotope (e.g. $^{13}C/^{12}C$) in the sample and R_S was the ratio of the heavy to light isotope in one of the isotopic reference materials [37].

2.6. *Statistical analyses*

All statistical analyses were conducted in R 3.0.2 [38]. A Shapiro–Wilk goodness-of-fit test and Levene's test were used to assess the normality and heterogeneity of variance, respectively, for

the walleye data [21,39,40]. Where data transformation was required, $\log(x)$ performed the best [26]. Although the muscle/scale isotopic offset ($\delta X_m/\delta X_s$) appeared to be species specific, we considered whether isotopic differences between the basins' nutrient pools imparted an effect on this relationship [9,21]. A Wilcoxon rank sum test was used to determine if all $\delta X_m/\delta X_s$ for north and south basin walleye came from the same distribution [41].

Linear regression models for predicting $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ from $\delta^{15}N_s$, $\delta^{13}C_s$, $\delta^{34}S_s$ and other morphological parameters were evaluated with Akaike's Information Criterion [42] corrected for small sample sizes (AIC_c) [43,44]. Concentrations of Al, As, Mn and Hg in walleye muscle were modelled from scale concentrations, isotopic parameters, fork length, sex and maturity. A null model was also included in the analyses [45]. The *AICcmodavg* package was used to identify the models that had the best fit given the data available [44,46]. Since analysing all possible models can lead to illusory results, especially when the number of parameters (K) is large [43], a parsimonious approach to model fitting was taken. The number of models considered for each response variable never exceeded the sample size (n), and, where possible, the number of parameters per model was maintained at roughly $n/10$ [43]. The top model ($\Delta AIC_c = 0.00$) and models with $\Delta AIC_c \leq 2.00$ were considered to have similar support [44]. Whenever the top-ranking model did not have an Akaike weight (w_i) ≥ 0.90, model averaging across a 95% confidence set of models ($\Sigma w_i \geq 0.95$) was used to identify those parameters which had a strong effect [44,47]. A given parameter was identified as having a strong effect on $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$, or Al, As, Mn and Hg in muscle if the 95% unconditional confidence interval around the model-averaged estimate excluded zero. The results of the model-selection procedures were evaluated graphically by plotting the observed versus the predicted values [48].

3. Results

We collected scale and muscle samples from 34 walleye (10 females, 23 males, and 1 individual of unknown sex) with FLs ranging from 232 to 664 mm.

3.1. *Trace elements*

Concentrations of Mn and Hg were significantly different between the paired muscle and scale samples; however, no difference was found for As concentrations in the two tissues (Wilcoxon rank sum test; $p \leq 0.05$; Table 1). Al concentrations in both tissues were consistently below the LoD. Hg was the only element for which the top-ranking model had an Akaike weight ≥ 0.90 ($w_i = 0.99$; Table 2). The null model was ranked first for As (Table 3), although $\log(As_s)$ and $\delta^{13}C_s$ appeared in models with $\Delta AIC_c \leq 2.00$. Model averaging revealed $\log(As_s)$ did not have a strong influence on the prediction of $\log(As_m)$ (95% unconditional confidence

Table 1. Mean ± SD concentrations of Al, As, Hg and Mn in paired walleye scales and muscle. Values are expressed in µg/g dw.

	Al	As	Mn	Hg
	$n = 25$	$n = 16$	$n = 18$	$n = 17$
Scales	< LoD	0.36 ± 0.22	7.22 ± 2.3	0.06 ± 0.04[a]
Muscle	< LoD	0.34 ± 0.09	0.43 ± 0.18	1.05 ± 1.26

[a]$n = 9$ of the scale Hg concentrations fell below the LoD; these were assigned a value of LoD/2 = 0.025 prior to calculation of the mean ± SD.

Table 2. Scale-based models which best explain the variation in As_m, Hg_m and Mn_m for Lake Winnipeg walleye.

Model	K	AIC_c	ΔAIC	w_i
As_m				
Null model	1	6.50	0.00	0.34
$Log(As_s) + \delta^{13}C_s$	3	7.37	0.88	0.22
$Log(As_s)$	2	7.77	1.28	0.18
$Log(As_s) + \delta^{15}N_s$	3	9.13	2.63	0.09
$Log(As_s) + \delta^{34}S_s$	3	9.71	3.21	0.07
$Log(As_s)$ + fork length	3	10.46	3.96	0.05
$Log(As_s)$ + maturity	3	11.16	4.66	0.03
Hg_m				
$Log(Hg_s)$ + fork length	3	21.74	0.00	0.99
Mn_m				
$Log(Mn_s)$ + maturity	3	19.20	0.00	0.67
Null model	1	22.31	3.12	0.14
$Log(Mn_s)$	2	24.18	4.98	0.06
$Log(Mn_s)$ + fork length	3	24.46	5.27	0.05
$Log(mn_s)$ + sex	3	24.66	5.46	0.04

Notes: The number of parameters (K), AIC_c, ΔAIC_c and Akaike weight (w_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the 'best' model ($\Delta AIC_c = 0.00$) did not have $w_i \geq 0.90$, a model-averaging approach was used.

Table 3. Parameter estimates for predicting As_m, Hg_m and Mn_m in walleye muscle based on concentrations in scales (X_s) and morphological parameters.

Model	Intercept (SE)	$Log(As_s)$ (SE)	$\delta^{13}C_s$ (SE)
$Log(As_s) + \delta^{13}C_s$	0.85 (1.09)	− 0.04 (0.10)[a]	0.08 (0.04)
$Log(As_s)$	− 1.24 (0.13)	− 0.12 (0.10)[a]	NA
Model-averaged estimate	− 0.28 (1.03)	− 0.08 (0.11)[a]	0.08 (0.04)
Model	Intercept (SE)	$Log(Hg_s)$ (SE)	Fork length (SE)
$Log(Hg_s)$ + fork length	− 2.22 (0.62)	0.93 (0.15)	0.01 (0.00)
Model	Intercept (SE)	$Log(Mn_s)$ (SE)	Maturity, M (SE)
$Log(Mn_s)$ + maturity	− 1.02 (0.50)	0.17 (0.24)[a]	− 0.47 (0.16)
Model-averaged estimate	− 0.98 (0.48)	0.18 (0.25)[a]	− 0.47 (0.16)

Notes: Parameters were estimated based on simple linear models and MAEs were determined from models with Akaike weights (w_i) adding to ≥ 0.95.
[a]Parameter did not have a strong effect.

interval = −2.29 to 1.73; Figure 2), and that $\delta^{13}C_s$ was a weak predictor of $log(As_m)$ (95% unconditional confidence interval = 0.00 to 0.16; Table 3). Similarly, calculation of the model-averaged estimate for log-transformed Mn data in scales revealed that $log(Mn_s)$ was a poorly supported parameter, based on its large unconditional standard error (0.25) and wide confidence intervals which overlapped zero (95% unconditional confidence interval = −0.32 to 0.68; Figure 2).

3.2. *Stable isotopes*

Results of the isotopic analyses indicated that walleye scales were generally enriched in ^{13}C and ^{34}S, but depleted in ^{15}N, relative to their corresponding muscle sample (Table 4). The $\delta X_m/\delta X_s$ offset for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ did not vary significantly with basin (Wilcoxon rank sum test; $\delta^{13}C$: $W = 123$, $p = 0.66$; $\delta^{15}N$: $W = 138$, $p = 0.72$; $\delta^{34}S$: $W = 99$, $p = 0.12$), and

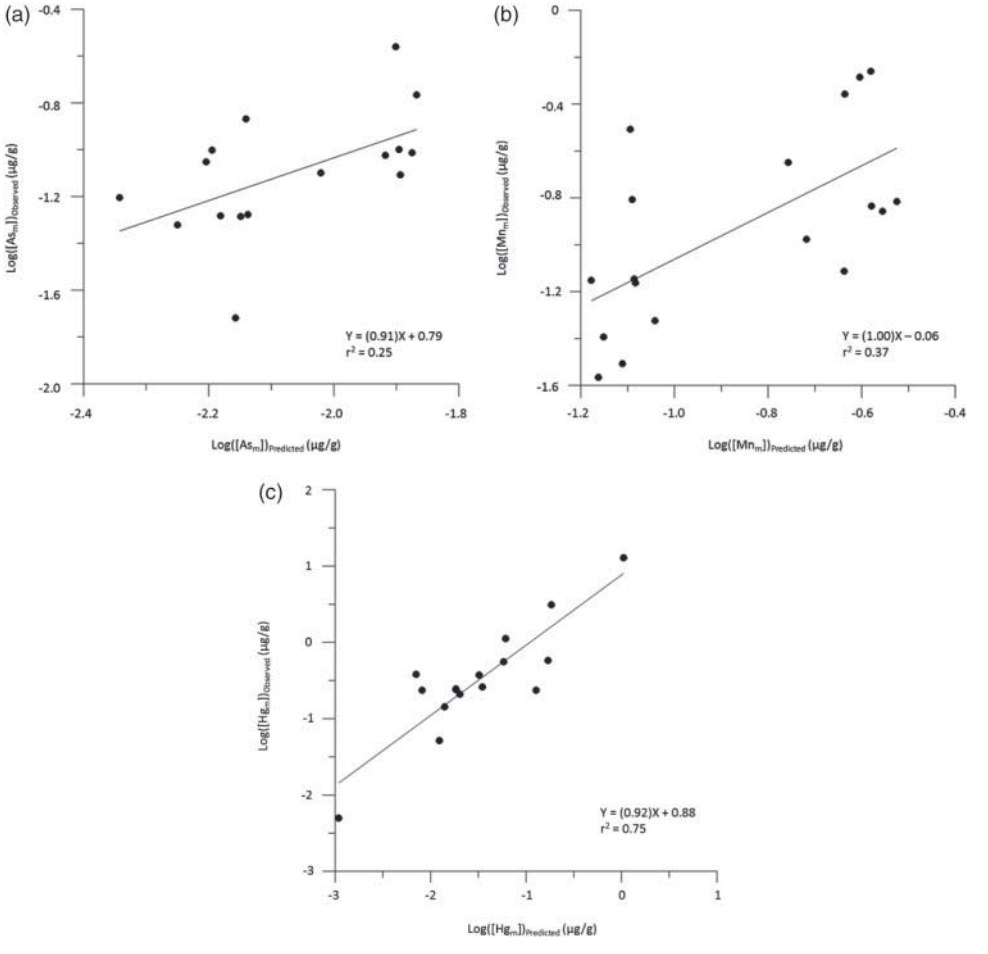

Figure 2. Observed versus predicted values for (a) As, (b) Mn and (c) Hg in the walleye muscle. Predicted values are based trace element and/or isotopic data for fish scales, as well as morphological parameters (see Table 3). The line associated with the data points in each graph represents the linear fit of the model. Note that Hg is the only element for which all parameters in the predictive model had a strong effect.

Table 4. Mean ± SD isotope data (in ‰) for scales and muscle of Lake Winnipeg walleye.

	δ^{15}N	δ^{13}C	δ^{34}S
	$n = 34$	$n = 34$	$n = 34$
Scales	13.7 ± 1.8 (17.8 ± 2.0)	− 23.5 ± 1.4 (− 20.6 ± 1.6)	− 6.0 ± 1.5 (NA)
Muscle	14.4 ± 1.7 (17.7 ± 1.4)	− 25.2 ± 1.3 (− 23.3 ± 1.6)	− 6.9 ± 2.5 (NA)
Whole pelvic fin	(17.3 ± 1.7)	(− 23.1 ± 1.9)	NA

Notes: Mean ± SD values for walleye samples collected from North Dakota [23] are included in parentheses for comparison.

therefore there were no regional differences in the available nitrate, dissolved inorganic carbon and sulphate pools. Consequently, basin was not considered as a potential parameter in the model-selection procedures.

Since the elemental isotope discrimination processes governing the δ^{15}N, δ^{13}C and δ^{34}S values in tissues are not the same, models for $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ were developed separately

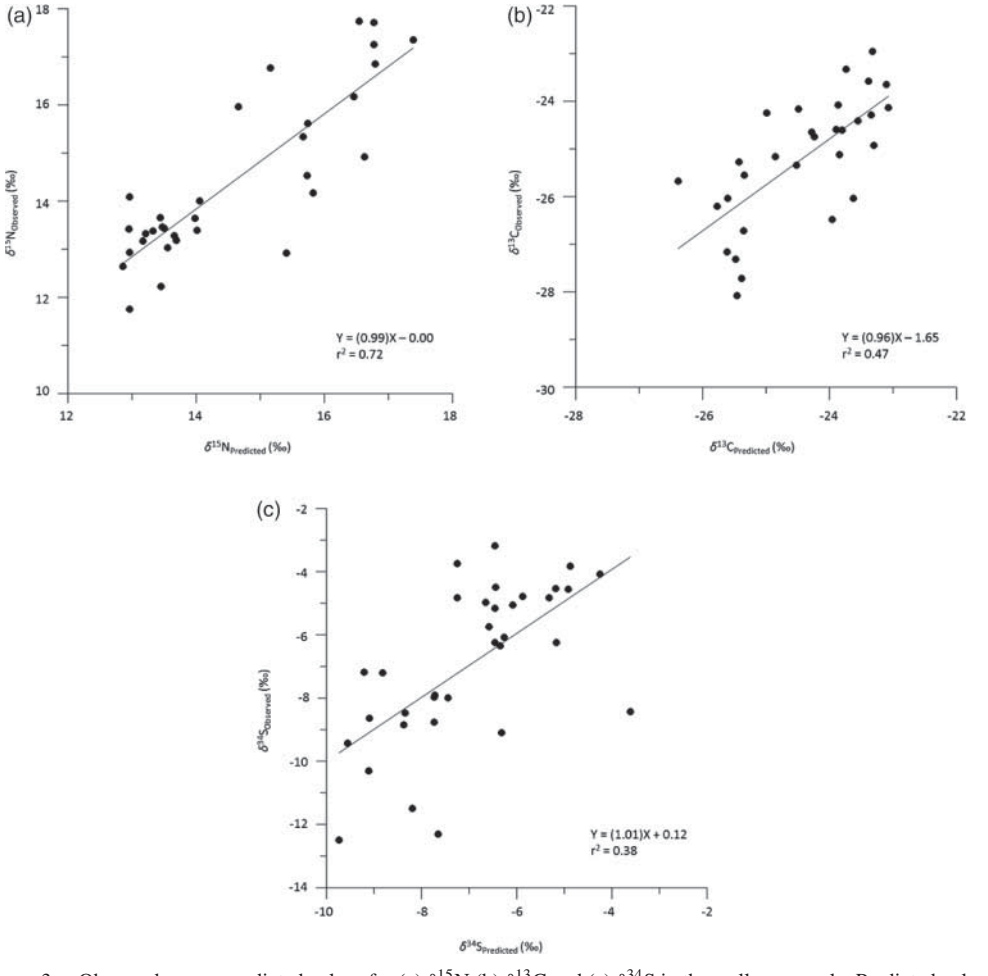

Figure 3. Observed versus predicted values for (a) δ^{15}N (b) δ^{13}C and (c) δ^{34}S in the walleye muscle. Predicted values are based on stable isotope ratios in walleye scales and sex. The line associated with the data points in each graph represents the linear fit of the model. When individuals' sex is not considered, the relationships are $0.617x - 10.751$ ($r^2 = 0.42$) for δ^{13}C, $0.817x + 3.183$ ($r^2 = 0.73$) for δ^{15}N and $1.048x - 0.851$ ($r^2 = 0.40$) for δ^{34}S.

[49]. Overall, models that included sex along with the isotopic data for scales were ranked first (Table 5). Although models containing FL and maturity performed poorly relative to those which included sex data, none of the top models had $w_i \geq 0.90$. Model averaging revealed that $\delta^{15}N_s$, $\delta^{13}C_s$ and $\delta^{34}S_s$ were strongly supported parameters (Table 6); however, sex-based differences were found to be fairly weak, based on the 95% unconditional confidence intervals for each model-averaged estimate. The unconditional standard errors (denoted as SE in Table 6) around model-averaged estimates were the smallest for nitrogen, and the plotted observed versus predicted $\delta^{15}N_m$ values revealed a nearly 1:1 relationship (Figure 3(a); $r^2 = 0.72$). The predicted $\delta^{13}C_m$ and $\delta^{34}S_m$ values explained much less variation in the observed $\delta^{13}C_m$ and $\delta^{34}S_m$ values (Figure 2(b) and 2(c); $r^2 = 0.47$ and 0.38, respectively) relative to δ^{15}N. This may have been the result of greater model uncertainty (lower precision), as indicated by the larger SE values for the sulphur and carbon models in Table 6. When the effect of sex is removed, the relationships are similar.

Table 5. Scale-based models which best explain the variation in $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ for Lake Winnipeg walleye.

Model	K	AIC_c	ΔAIC	w_i
$\delta^{15}N_m$				
$\delta^{15}N_s$ + sex	3	89.55	0.00	0.43
$\delta^{15}N_s$	2	89.93	0.38	0.36
$\delta^{15}N_s$ + fork length	3	92.13	2.58	0.12
$\delta^{15}N_s$ + maturity	3	92.55	3.00	0.10
$\delta^{13}C_m$				
$\delta^{13}C_s$ + sex	3	87.46	0.00	0.67
$\delta^{13}C_s$ + fork length	3	89.83	2.37	0.20
$\delta^{13}C_s$	2	91.23	3.77	0.10
$\delta^{34}S_m$				
$\delta^{34}S_s$ + sex	3	146.86	0.00	0.44
$\delta^{34}S_s$	2	147.45	0.59	0.33
$\delta^{34}S_s$ + maturity	3	149.23	2.37	0.14
$\delta^{34}S_s$ + fork length	3	150.03	3.17	0.09

Notes: The top model did not have $w_i \geq 0.90$, a model-averaging approach was used. The number of parameters (K), AIC_c, ΔAIC_c and Akaike weight (w_i) are given for each of the models used to estimate parameters.

Table 6. Parameter estimates for predicting $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ in walleye muscle based on $\delta^{15}N_s$, $\delta^{13}Cs$ and $\delta^{34}S_s$, respectively, in scales.

Model	Intercept (SE)	$\delta^{15}N_s$ (SE)	Sex, male (SE)
$\delta^{15}N_s$ + sex	3.01 (1.30)	0.83 (0.10)	0.12 (0.37)[a]
$\delta^{15}N_s$	3.18 (1.27)	0.82 (0.09)	NA
Model-averaged estimate	3.22 (1.37)	0.82 (0.09)	0.12 (0.37)[a]
Model	Intercept (SE)	$\delta^{13}C_s$ (SE)	Sex, male (SE)
$\delta^{13}C_s$ + sex	− 8.67 (3.23)	0.73 (0.14)	0.77 (0.41)[a]
Model-averaged estimate	− 7.87 (3.67)	0.73 (0.15)	0.77 (0.41)[a]
Model	Intercept (SE)	$\delta^{34}S_s$ (SE)	Sex, male (SE)
$\delta^{34}S_s$ + sex	− 0.90 (1.42)	1.04 (0.25)	0.05 (0.80)[a]
$\delta^{34}S_s$	− 0.85 (1.36)	1.05 (0.23)	NA
Model-averaged estimate	− 0.97 (1.52)	1.04 (0.24)	0.05 (0.80)[a]

Notes: Parameters were estimated based on simple linear models. Model-averaged parameter estimates were estimated from models with Akaike weights (w_i) adding to ≥ 0.95.
[a]Parameter did not have a strong effect.

4. Discussion

Consideration of observed versus predicted values for all of the isotopic (Figure 2(a)–(c)) and trace element (Figure 3(a)–(c)) models revealed that the strongest correlations between scale and muscle parameters existed for $\delta^{15}N$ ($r^2 = 0.72$) and Hg ($r^2 = 0.75$). The ability to predict muscle-$\delta^{15}N$ from non-lethal walleye scale collection may therefore be useful in monitoring temporal and spatial variability in the species' trophic position and potential changes to nutrient cycling [50]. The inclusion of sex in all top-ranking isotopic models would suggest that the processes governing the relative distribution of nutrients to muscle, scales, and gonads were different for male and female walleye [24]. Sex has also been identified as a predictor of Hg accumulation for other Canadian walleye populations [2]; however, no such phenomenon was observed in Lake Winnipeg, though this could be the result of our small sample size. Sex, however, had a

generally weak effect on predicted isotope values, and in all cases, 95% confidence intervals of parameter estimates bounded zero (Table 6).

Despite the utility of the Hg data presented here, the application of fish-scale sampling in contaminant monitoring still suffers from a number of methodological and analytical constraints. Isotopic studies or elemental analysis by ICP-MS currently require that as much as 1 g of scale material be removed from a single fish [23]. Mercury analyses by CVAFS, although extremely reliable [51], require approximately 2 g of scale material to achieve a detection limit of 0.05 µg/g (B. Stanek, SRC, personal communication; 2010). This equated to approximately half of the scale biomass available from the specimens studied. Removing such large volumes of scales and overlying mucus from live fish is very likely to result in infection and possibly death [52]. Other analytical techniques, however, require much less material for element-specific assays, and can measure smaller concentrations [53]. ICP-MS is advantageous in that it can measure several elemental concentrations simultaneously, so the choice of analytical technique will depend on the particular question of interest (e.g. a particular element such as Hg or a broader survey of elemental concentrations). An alternative to sampling live walleye may involve removal of scales from the carcasses processed for commercial sale. The risk of harming live fishes would be removed, yet the prized walleye fillets would remain unaffected. However, removal of scales during commercial processing, rather than in a clean laboratory, may heighten the risk of sample contamination. Although scales are often cleaned with acetone, water or detergents prior to isotopic and contaminant analyses, no standardised, reliable method has yet been developed. A combination of acetone and ultra-sonication was effective in cleaning the walleye scales analysed here, whereas deionised water and hydrogen peroxide are often ineffective or destructive to the sample [27,54]. Finally, the biochemical structure of fish scales was likely to have interfered with the ICP-MS elemental scan conducted here. As much as 60% (dw) of a scale's biomass may be composed of mineralised calcium phosphate and carbonates, depending on the species [35,55]. Scales from Lake Winnipeg walleye were well calcified (AFAO, personal observation), and may have had a greater calcium content than what could be effectively managed by the ICP-MS instrumentation under normal operating conditions. Even after complete digestion of tissues, high-calcium sample matrices may have suppressed the measurable signal of other elements, or potentially fouled the inner components of the ICP-MS instrument [56]. Heavy calcification can also affect scale $\delta^{13}C$ values [36], though this may not always be the case [57]. We did not acid treat scales to remove carbonates, but the relationship between treated and untreated scale samples is strongly linear, suggesting that it would have little effect on our interpretations here [36].

Fish scales, including archived samples, can also be used to examine trophic and contaminant changes over time, which is especially important as introduced species change the productivity of different parts of freshwater lakes [15,57]. The introduction of dreissenid mussels in the Great Lakes, for example, has resulted in significant shifts in $\delta^{13}C$ and $\delta^{15}N$ in lake whitefish (*Coregonus clupeaformis*) as offshore areas become less productive [50,58]. And the ability to access archival scale material for hindcasting can be a powerful tool in understanding changes in contaminant burden and trophic structure [59].

$\delta^{34}S$ values can be used to distinguish benthic and pelagic nutrient origins in freshwater systems, which is useful in interpreting food-web structure and contaminant dynamics [60–62]. To our knowledge, ours is the first study to investigate the relationship in $\delta^{34}S$ between fish scales and muscle, and while the relationship between $\delta^{34}S_s$ and $\delta^{34}S_m$ ($r^2 = 0.38$, Figure 3(c)) was not as strong as $\delta^{13}C$ and $\delta^{15}N$ ($r^2 = 0.47$ and 0.72, respectively; Figure 2(a) and 2(b)), the regression slope was essentially unity ($\beta = 1.01$; Figure 3(c)).

Relationships between elemental concentrations or stable isotopes in paired scale and muscle samples vary among species, and may not always be reliable [16,17]. For example, the slopes predicting muscle $\delta^{13}C$ ($\beta = 1.00$) using scale isotope values in South Dakota [23] were similar to

that in Lake Winnipeg ($\beta = 0.96$), that for δ^{15}N differed considerably (South Dakota $\beta = 0.65$, Lake Winnipeg $\beta = 0.99$; Figure 3), suggesting that the relationship is not universal, and is subject to local conditions.

In addition, different tissues typically represent different periods of dietary integration and metabolic pathways, and so direct comparisons between tissues of wild-caught fish require that these factors be taken into account. Adult fish typically have relatively slow isotopic and elemental turnover rates in muscle [63], and fish scales presumably represent similarly long periods of dietary and spatial integration [25]. So, muscle and scale samples may indeed provide similar information on dietary and contaminant history of individuals. However, non-calcified samples, such as muscle biopsies and fin web clips, may be more viable options for the non-destructive monitoring of trace elements in Lake Winnipeg walleye populations [23], but the analysis of Hg in walleye scales showed promise as a suitable proxy for monitoring Hg concentrations in fillets. Further studies that contrast other tissues for trace metals and stable isotopes relative to muscle are now encouraged in order to inform contaminant monitoring programmes in freshwater ecosystems.

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