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[Article]

Geochemical Signatures in Scales Record Stream of Origin in Westslope Cutthroat Trout

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Abstract.—We used laser ablation inductively coupled plasma mass spectrometry (ICP-MS) to quantify the elemental composition of Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and Pb:Ca ratios in scales from juvenile westslope cutthroat trout *Oncorhynchus clarkii lewisi* collected from 13 streams in three major drainages (North Fork, South Fork, and Middle Fork) of the upper Flathead River system in Montana during summer 2001 and 2002. We also determined element : Ca levels within natal streams in summer 2001 and 2002. The concentrations of Sr:Ca and Ba:Ca in westslope cutthroat trout scales were highly correlated with those in the water. The multivariate elemental signatures of the scales differed significantly among streams. A forward stepwise discriminant function analysis was used to classify individual fish, first to the drainage of origin and then to the natal stream while considering all 13 streams simultaneously. At the drainage level, cross-validated classification accuracy was 91% in the Middle Fork, 81% in the North Fork, and 78% in the South Fork; overall accuracy was 82%. Of the fish that were correctly classified at the drainage level, 88% were correctly classified to their natal stream at accuracy levels of 100% in the Middle Fork, 88% in the North Fork, and 80% in the South Fork. Finally, the Mn:Ca, Sr:Ca, and Ba:Ca ratios in westslope cutthroat trout scales were significantly correlated with values in the otoliths of individual fish, suggesting that scales may provide a nonlethal alternative to otoliths as natural markers. Our data indicate that elemental signatures in scales may be used as natural tags to identify the natal stream origin of westslope cutthroat trout in the upper Flathead River system and in other similar freshwater environments. However, future work needs to determine whether elemental signatures are sufficiently stable over time to allow for accurate classification of adult fish after emigration from natal streams.

An understanding of the movements and dynamics of fish populations is crucial to fisheries management. Geochemical signatures in the calcified structures of fishes, including otoliths and scales, have been commonly used as natural tags to determine natal origin and understand life history of individual fish (Campana 1999; Gillanders 2001; Kennedy et al. 2002). However, most studies have focused on estuarine and marine environments (Campana et al. 1994; Wells et al. 2000b; Thorrold et al. 2001), while little information exists for freshwater systems (Coutant and Chen 1993; Kennedy et al. 2000; Wells et al. 2003a).

Many studies have shown that geochemical tracers in otoliths provide an accurate and permanent record of the environmental history of individual fish (Campana et al. 1994, 1997; Rieman et al. 1994; Thorrold et al. 1998). Chemical analyses of otoliths, however, require the sacrifice of fish. Scales may provide a nonlethal alternative to otoliths as natural markers in fish populations (Coutant and Chen 1993; Wells et al. 2000b, 2003a; Gillanders 2001). Recent advances in analytical technology, including laser ablation inductively coupled plasma mass spectrometry (ICP-MS), have increased the accuracy and precision of elemental analyses of fish scales (Coutant and Chen 1993; Wells et al. 2000b). Wells et al. (2000b) quantified the elemental composition of the scales of juvenile weakfish *Cynoscion regalis* in natal estuaries along the Atlantic coast of the United States. The authors concluded that scales could be used to determine nursery-specific elemental signatures. Wells et al. (2003a), studying three streams in the Coeur d'Alene River system in Idaho, provided evidence that scale chemistry can be used to describe fish movements in a freshwater

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system, but they recommended that future studies should extend the application to streams within a single freshwater river basin.

Salmonids that rear in natal streams and migrate to rivers and lakes are ideal for examining the potential use of elemental signatures in scales as natural tags of stream origin because fish encounter different water chemistries as they move between trophic, refuge, and reproductive habitats. In the Flathead River system in northwestern Montana, westslope cutthroat trout *Oncorhynchus clarkii lewisi* exhibit both resident (i.e., remaining in natal streams throughout life) and migratory life history strategies (Shepard et al. 1984; Liknes and Graham 1988; Weaver and Fraley 1993). Migratory westslope cutthroat trout rear in natal streams for 1–4 years and then migrate downstream as subadults to Flathead Lake (i.e., adfluvial) or the main-stem rivers (i.e., fluvial). Adult westslope cutthroat trout generally overwinter in the lower river or the lake and then migrate upstream (up to 250 km) during high spring flows. The migrational behaviors between habitats are critical for maintaining genetic diversity and dispersal among populations, and this connectivity is in turn probably essential to the long-term persistence of the species (Allendorf and Leary 1988; Schmetterling 2003).

The Flathead River system is recognized as the regional stronghold for westslope cutthroat trout throughout their historic range (Liknes and Graham 1988; Weaver and Fraley 1993; Hitt et al. 2003). However, the distribution and abundance of westslope cutthroat trout have declined because of hybridization, competition, and predation associated with introduced salmonids; habitat degradation and isolation; and angler exploitation (Liknes and Graham 1988; Behnke 1992; Hitt et al. 2003).

No studies have assessed the use of geochemical tracers in fish scales to determine natal origin in a freshwater river basin. An understanding of fish movements through chemical analyses of scales may provide fisheries managers with the necessary information to identify and manage critical populations and to assess habitat restoration and fish passage programs for recovery of westslope cutthroat trout and other fishes in similar freshwater systems. We were primarily interested in developing a nonlethal technique to determine the stream origin of fish in a freshwater system. Our objectives were to quantify elemental signatures in scales from juvenile westslope cutthroat trout in the upper Flathead River system and to examine whether these signatures were reflective of otolith chemistry in the same fish and water chemistry of the tributary in which the fish were collected.

Methods

Study area.—The Flathead River basin originates in the Rocky Mountains of British Columbia, Canada, and northwestern Montana and includes Flathead Lake and the river system upstream (Figure 1). The drainage area is approximately 18,400 km^2 and comprises the headwaters of the upper Columbia River basin. Our study included fish and water samples from streams in the three major drainages of the upper Flathead River system: the North Fork, Middle Fork, and South Fork of the Flathead River.

Fish collection.—We used a pulsed-DC backpack electrofishing unit to collect 126 fish from five streams in the North Fork drainage (Camas, Hay, Langford, Sage, and Tuchuck creeks) in August and October 2001 (Table 1). In the Middle Fork and South Fork drainages, we used angling (fly-fishing) to collect fish because all sampling sites were located in wilderness areas. We collected 56 trout from four streams in the Middle Fork (Bowl, Cox, Morrison, and Ole creeks) in September 2002 and 109 fish from four streams in the South Fork (White River and Danaher, Little Salmon, and Youngs creeks) in August 2001 (Table 1). One upper site and one lower site were sampled (spaced at least 5 km apart) to obtain a representative sample of elemental signatures in each study stream. Fish were not encountered at four of the upper sampling sites, and we did not sample an upper site in Sage Creek because it was located in British Columbia. All collected fish were placed on ice and were transported to a laboratory or remote field station. We first measured the total length (TL; mm) of each fish and then removed the otoliths and scales for elemental analyses. Scales were sampled directly below the anterior portion of the dorsal fin above the lateral line. Otoliths were not extracted from fish in the Middle Fork, as they were not sacrificed for this study. Otoliths were stored in dry microcentrifuge tubes (0.5 mL), and scales were placed in paper envelopes.

Elemental analyses of scales and otoliths.—All scales used for elemental analysis were initially cleaned and decontaminated by means of a protocol similar to that of Wells et al. (2003a, 2003b). Briefly, scales were ultrasonically cleaned for 5 min in ultrapure water to loosen organic debris

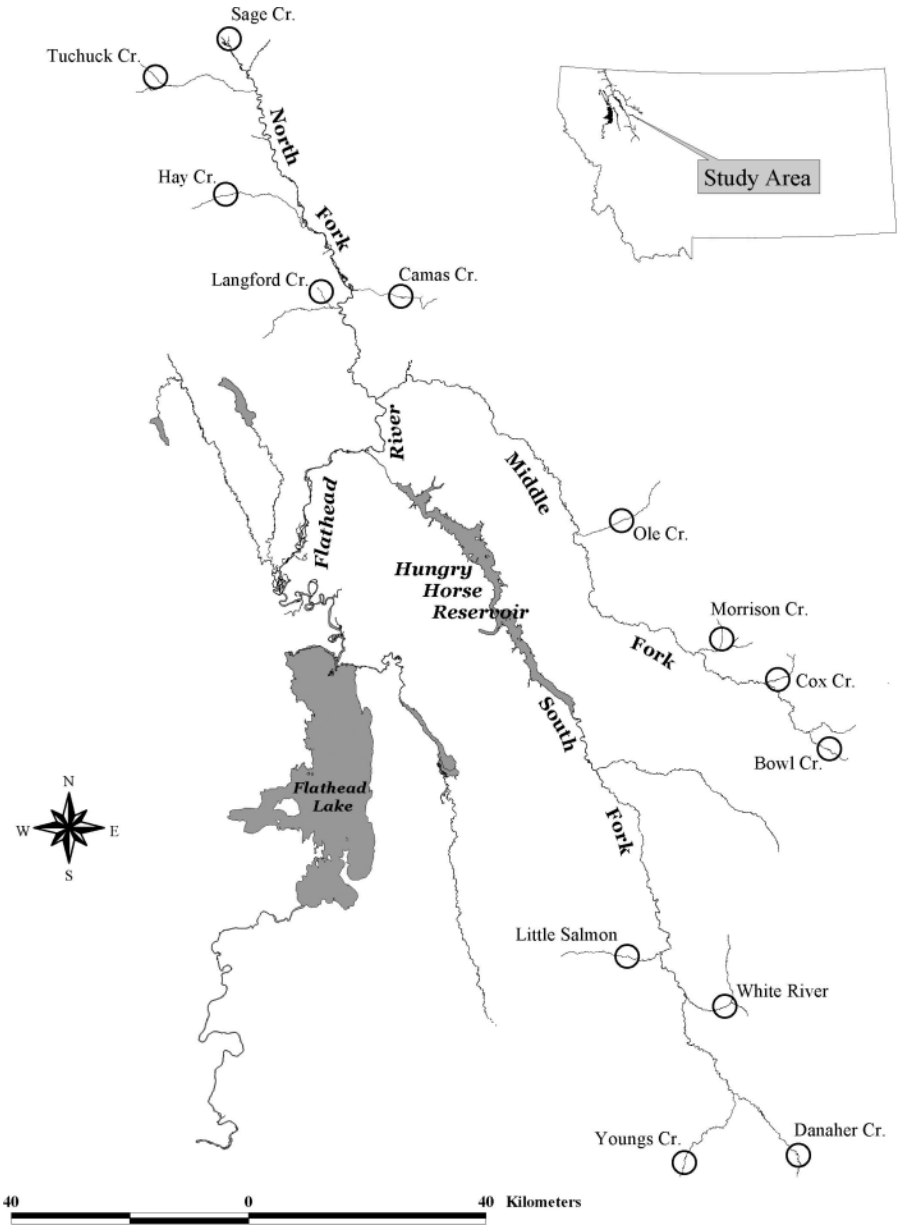

FIGURE 1.—Map showing the locations (circles) of the 13 study streams in the upper Flathead River system, Montana; Cr. = creek.

and then were triple-rinsed with ultrapure water. Scales were dried under a laminar-flow hood and then were mounted to petrographic slides by use of double-sided tape for subsequent analysis by laser ablation ICP-MS.

We used a Thermo Finnigan Element2 sector field ICP mass spectrometer coupled with a 266-nm New Wave Research laser ablation system to analyze the elemental composition of one scale from each juvenile fish (Thorrold and Shuttleworth 2000). Samples were analyzed at two positions on the scale, one close to the center and the second at the outside edge. The laser software was used to scan along a 500-m line at a rate of 5 μm/s; we employed laser settings of 20% power at 10 Hz and a 30-μm spot size. The ablated material was

TABLE 1.—Sample locations, collection dates, sample sizes, mean total lengths (TL), and SDs for juvenile westslope cutthroat trout collected in the upper Flathead River system, Montana, in 2001 and 2002. Letters indicate significant differences in TLs among streams within each drainage. Post hoc comparisons were conducted with Sheffe's test.

Drainage or stream	Date collected	*N*	TL	
			Mean	SD
North Fork				
Camas Creek	10 Oct 2001	28	129 y	22.1
Hay Creek	13 Aug 2001	28	161 z	30.4
Langford Creek	14 Aug 2001	27	138 y	47.9
Sage Creek	3 Oct 2001	14	118 y	23.4
Tuchuck Creek	15 Aug 2001	29	164 z	21.0
Middle Fork				
Bowl Creek	19 Sep 2002	12	216 z	31.8
Cox Creek	19 Sep 2002	26	135 y	29.5
Morrison Creek	16–17 Sep 2002	11	155 y	32.9
Ole Creek	25 Sep 2002	7	198 z	43.7
South Fork				
Danaher Creek	27 Aug 2001	27	179 y	22.9
Little Salmon Creek	21 Aug 2001	26	245 z	79.5
White River	24 Aug 2001	29	174 y	34.4
Youngs Creek	24 Aug 2001	27	202 z	21.8

swept by an Ar carrier gas into Scott's double-pass spray chamber through a port in the perfluoroalkoxy (PFA) endcap of the spray chamber. The Ar stream was then mixed with a wet aerosol (1% HNO_3) from a 50-μL/min PFA self-aspirating nebulizer. A total of six isotopes were quantified (^{25}Mg, ^{48}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba, and ^{208}Pb). Blanks (1% HNO_3) and a laboratory standard solution were introduced at regular intervals throughout an analysis session to account for instrument drift and changes in mass bias, thus alleviating the need for matrix matching with solid reference standards. The laboratory standard was introduced by moving the autosampler probe from the solution containing the 1% HNO_3 to the standard solution while maintaining the carrier gas flow through the ablation cell. Elemental concentrations in the laboratory standard were as follows: 10 ng of Mg per gram of standard, 51.1 mg Ca/g, 4.1 ng Mn/g, 100.4 ng Sr/g, 2.0 ng Ba/g, and 0.02 ng Pb/g. Isotopic counts were converted to elemental intensities by multiplying the percent natural occurrence of the isotopes. All data were standardized to Ca to account for variability in laser energy and weight of ablated material and were converted to molar ratios. Limits of detection (LODs) were calculated as 3 times the SD of the 1% HNO_3 sample blanks ($n = 97$) that were run throughout the analyses. These limits were 0.9% of the average sample intensity for ^{25}Mg, 11% for ^{48}Ca, 40% for ^{55}Mn, 23% for ^{88}Sr, 1% for ^{138}Ba, and 27% for ^{208}Pb. The precision (relative SD) of elemental ratios in the laboratory standard, uncorrected for mass bias, were as follows: 5.4% for Mg:Ca, 1.1% for Mn:Ca, 4.4% for Sr:Ca, 3.8% for Ba:Ca, and 3.6% for Pb:Ca. Because laser samples were corrected for changes in mass bias by means of the laboratory standard, the precision of the technique was probably similar to that of the uncorrected solution estimate.

Otoliths used for elemental analyses were mounted on petrographic slides and were then ground to the plane of the nucleus using 30- and 3-μm Al_2O_3 lapping film. The sections were then rinsed in ultrapure water, scrubbed with a nylon brush in a solution of ultrapure water, sonified for 2 min in ultrapure water, and finally triple-rinsed again with ultrapure water. The section was dried under a positive-flow hood for 24 h and was stored in a polyethylene bag.

Elemental assays of otoliths were conducted with the Element2 ICP mass spectrometer coupled with a 213-nm New Wave Research laser ablation system. Thirty fish were randomly selected for otolith analysis, and one otolith was analyzed per fish. A laser beam (20 Hz, 100% power) with a nominal diameter of 20 μm traced a line of 720 μm at 5 μm/s at a site just inside of the initial annual check visible in the otoliths and at the otolith edge. The ablated material was swept by a He carrier gas into

a dual-inlet quartz cyclonic spray chamber. The He carrier gas was mixed with a wet aerosol (2% HNO_3) from a 20-μL/min PFA self-aspirating nebulizer. We monitored the same set of six isotopes as was used in the scale analyses. Limits of detection were calculated as 3 times the SD of the 2% HNO_3 sample blanks ($n = 8$) that were run after every 10 samples. The limits were 0.15% of the average sample intensity for ^{25}Mg, 0.02% for ^{48}Ca, 3.5% for ^{55}Mn, 0.12% for ^{88}Sr, 0.22% for ^{138}Ba, and 22% for ^{208}Pb. Quality control was maintained by assays of an otolith reference material (Yoshinaga et al. 1999, 2000), dissolved and diluted to 40 μg per gram of Ca in solution, after every blank sample. Precision (relative SD) of elemental ratios in the otolith reference were as follows: 0.2% for Mg:Ca, 3.8% for Mn:Ca, 0.5% for Sr:Ca, 1.1% for Ba:Ca, and 2.2% for Pb:Ca. Quantification followed the approach outlined by Rosenthal et al. (1999) for obtaining precise element : Ca ratios with sector field ICP-MS (Thorrold et al. 2001). Elemental mass bias was calculated by reference to certified values of the otolith reference, and a correction factor was then interpolated and applied to the laser samples bracketed between adjacent measurements of the reference material.

Water collection.—Water samples were collected to quantify the dissolved Mg, Ca, Mn, Sr, Ba, and Pb concentrations in the North Fork and South Fork by means of the ultra-clean procedures described by Horowitz et al. (1994). Water samples were collected on the same date and location as the fish. At each sampling location, duplicate water samples were collected in the thalweg by integrating a 60-mL syringe vertically and horizontally in the water column. We vacuum-filtered 50 mL of the water through a 0.45-μm sterile filter and then preserved each sample with two drops (~60 μL) of trace-metal-grade, concentrated HCl. All bottles were pre-cleaned by washing in a 6-N HCl bath for 2 h and a 1% trace-metal-grade HNO_3 bath for 24 h; multiple ultrapure water rinses were used before and after each cleaning stage.

Water samples were assayed with the Element2 ICP mass spectrometer equipped with a dual-inlet quartz cyclonic spray chamber and a 50-μL/min PFA self-aspirating nebulizer. We initially diluted water samples and a certified riverine water standard (National Research Council Canada SLRS-4) 20× with 2% HNO_3. Preliminary analyses of the river water found that Pb concentrations in the samples were considerably lower than concentrations of the other elements, and we therefore quantified ^{43}Ca and ^{208}Pb in low resolution (300) by use of the ICP mass spectrometer. All other isotopes (^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba) were monitored in medium resolution (4,000) to reduce the possibility of molecular interferences on the isotopes of interest. Limits of detection were calculated as 3 times the SD of the 2% HNO_3 sample blanks ($n = 9$) that were run after every seven samples. The LODs were 0.01% of the average sample intensity for ^{24}Mg, 0.02% for ^{43}Ca, 0.1% for ^{55}Mn, 0.04% for ^{88}Sr, 0.05% for ^{138}Ba, and 19% for ^{208}Pb. Quantification of elemental ratios in the water samples followed the same approach as outlined above for scales and otoliths. Elemental fractionation of the ICP mass spectrometer was quantified from assays of the SLRS-4 river water standard, and a correction factor was then interpolated and applied to the samples between adjacent measurements of the standard. Precision (relative SD) of elemental ratios in a laboratory river water standard were as follows: 3.2% for Mg:Ca, 1.0% for Mn:Ca, 1.3% for Sr:Ca, 1.3% for Ba:Ca, and 3.9% for Pb:Ca.

Data analysis.—Pearsons' product-moment correlation analysis was used to assess the relations between (1) the trace element ratio concentrations at the core and rim of all individual fish scales, (2) scale and water chemistries (mean values) of the 13 study streams, and (3) scale and otolith trace element concentrations. Multivariate analysis of variance (MANOVA) was used to test for differences in elemental signatures among streams in each drainage (Statistica 1995). It was necessary to $\log_{10}$ transform all element : Ca ratios to meet normality and homogeneity of variance assumptions. Post-hoc comparisons were conducted by means of Hotelling's *t*-tests. Differences in the multivariate scale signatures among streams were visualized with a canonical discriminant analysis. A forward stepwise discriminant function analysis was used to estimate the accuracy of fish classification, first to the natal drainage and then to the natal stream, based on the multivariate elemental signatures of the scales (Statistica 1995). Drainage and stream classification accuracies were considered simultaneously (all 13 streams) to maintain the covariance structure of the data in one model. Accuracy of the discriminant functions in assigning an unknown fish to a particular stream was assessed by means of a ''leave-one-out'' cross-validation strategy in which each sample was removed sequentially from the data matrix, the discriminant function was estimated from the remaining samples, and then this function was used

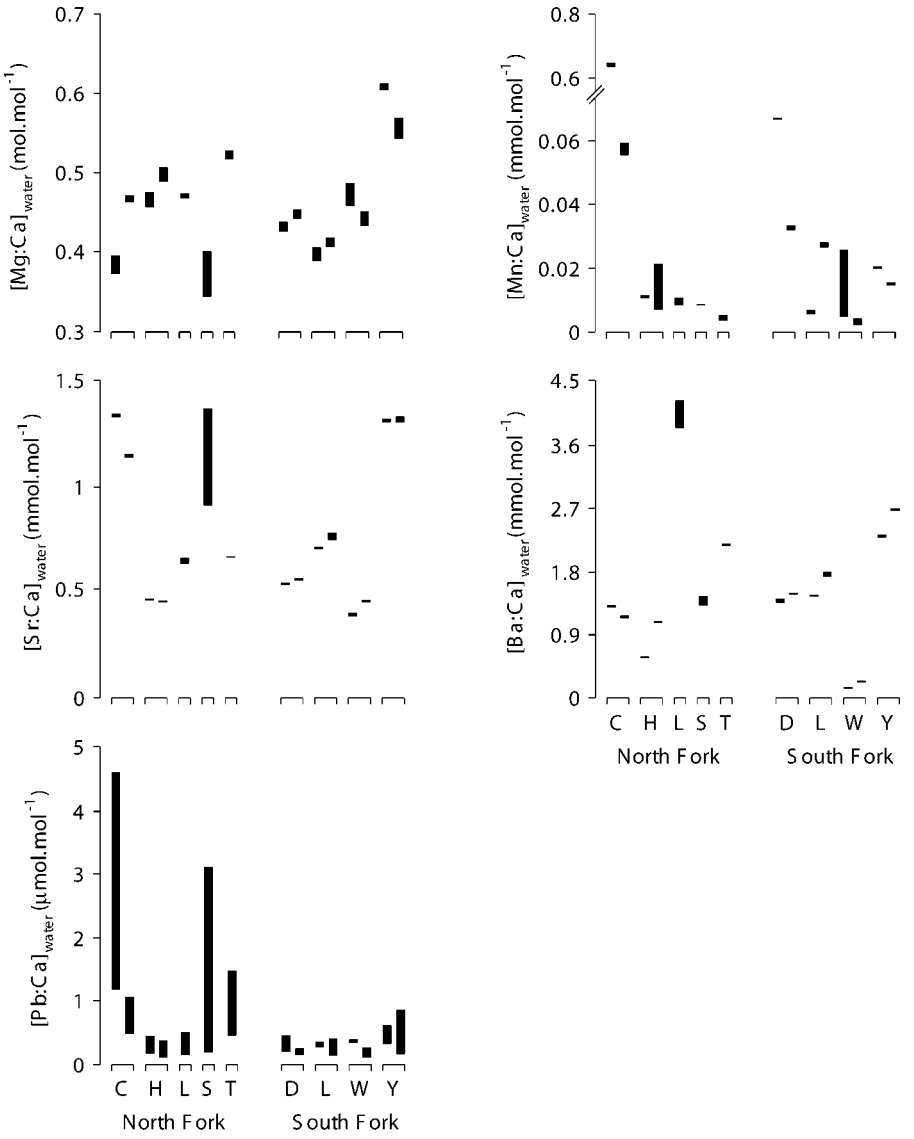

FIGURE 2.—Water chemistry in the North Fork (Camas [C], Hay [H], Langford [L], Sage [S], and Tuchuck [T] creeks) and South Fork (Danaher Creek [D], Little Salmon Creek [L], White River [W], and Youngs Creek [Y]) of the upper Flathead River, Montana. Bars show minimum and maximum values recorded from duplicate samples collected from one or two locations within each stream in summer 2001 and 2002.

to classify the sample that was removed (Thorrold et al. 1998).

Results

We quantified Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and Pb:Ca ratios in the scales of 291 juvenile westslope cutthroat trout from 13 streams in the upper Flathead River system in 2001 and 2002. There were significant differences in mean TLs among streams in the three drainages (Table 1). We also quantified Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and Pb:Ca ratios in water samples from a total of nine streams in the North Fork and the South Fork of the upper Flathead River (Figure 2). These data revealed consistent differences in water chemistry among streams for five elements, suggesting that these elements could be useful discriminators of natal stream if water chemistry is accurately recorded

by the elemental composition of scales. Further, elemental concentrations at the scale center and edge were significantly correlated ($P < 0.05$) for Mg:Ca (Pearson's product-moment correlation coefficient, $r = 0.58$), Mn:Ca ($r = 0.91$), Sr:Ca ($r = 0.91$), Ba:Ca ($r = 0.90$), and Pb:Ca ($r = 0.72$) ratios.

The water chemistry of individual natal streams was significantly correlated with the Mn:Ca ($r = 0.76$; $P < 0.05$), Sr:Ca ($r = 0.95$; $P < 0.0001$), and Ba:Ca ($r = 0.90$; $P < 0.0001$) ratios in westslope cutthroat trout scales (Figure 3). However, the significant correlation between water and scale chemistry for Mn:Ca appeared to be driven by high values from Camas Creek, and when this point was removed the correlation was no longer significant ($r = 0.45$; $P > 0.05$). Scale values for both Mg:Ca ($r = -0.01$; $P > 0.05$) and Pb:Ca ($r = -0.09$; $P > 0.05$) ratios were not significantly correlated with dissolved levels in streams. Plots of scale elemental composition by stream confirmed patterns predicted by the strength of correlations between scale and water chemistry. We found relatively subtle among-stream differences in Mg:Ca, Mn:Ca, and Pb:Ca ratios in scales, whereas Sr:Ca and Ba:Ca ratios showed greater differences (Figure 4).

We found significant variation in the multivariate elemental signatures among streams. The MANOVA found significant differences in elemental signatures among streams within the North Fork (Wilk's lambda = 0.0266; $P < 0.0001$), Middle Fork (Wilk's lambda = 0.0221; $P < 0.0001$), and South Fork (Wilk's lambda = 0.1914; $P < 0.0001$). Further, all element : Ca ratios contributed significantly ($P < 0.05$) to stream separation (main effect) except the Mg:Ca ratios for the Middle Fork and South Fork (Table 2). Multiple comparisons revealed that there were significant differences in the multivariate elemental signatures among all streams in each drainage ($P < 0.05$). Differences in the multivariate elemental signatures among streams were visualized in a canonical discriminant analysis, and examination of plots of the first and second canonical variates revealed that streams were clearly separated in discriminant space (Figure 5). For the North Fork drainage, Camas, Sage, and Hay creeks formed separate groups in discriminant space, but Tuchuck and Langford creeks slightly overlapped. In the South Fork drainage, the White River and Youngs and Danaher creeks separated from each other, while Little Salmon Creek overlapped with the other three streams. Streams in the Middle Fork were clearly separated from each other in discriminant space.

Forward stepwise discriminant function analysis was used to classify juvenile westslope cutthroat trout to their natal tributaries based on the multivariate elemental signatures of the scales; classification considered all 13 streams in the basin simultaneously (Table 3). Classification of scale elemental signatures indicated that all trace element ratios contributed significantly to stream separation (Table 4; Wilk's lambda = 0.1690, $P < 0.0001$). The cross-validated classification accuracy of assigning fish to their respective natal drainages was 91% in the Middle Fork, 81% in the North Fork, and 78% in the South Fork; overall accuracy was 82%. Of the fish that were correctly classified at the drainage level, 88% were correctly classified to their natal streams, and cross-validated accuracies were 100% for the Middle Fork, 88% for the North Fork, and 80% for the South Fork. In general, Sr:Ca and Ba:Ca ratios were the most important variables in the first and second discriminant functions. Overall cross-validated classification accuracy was 73% when all 13 streams were considered simultaneously, and accuracy ranged from 29% for fish from Sage Creek to 96% for individuals from Cox Creek (Table 4).

Finally, we compared the elemental composition of scales with that of otoliths taken from the same fish. Absolute levels of the elemental ratios varied considerably between scales and otoliths, often by several orders of magnitude (Figure 6). Levels of all elements except Sr were higher in scales than in otoliths. Despite these differences, we detected significant correlations between otolith ratios and ratios at the scale center for Mg:Ca ($r = 0.41$, $P < 0.05$), the scale edge for Mn:Ca ($r = 0.53$, $P < 0.05$), and the center and edge for Sr:Ca (center: $r = 0.85$, $P < 0.05$; edge: $r = 0.79$, $P < 0.05$) and Ba:Ca (center: $r = 0.78$, $P < 0.05$; edge: $r = 0.72$, $P < 0.05$). We found no significant correlation in Pb:Ca ratios between otoliths and scales.

Discussion

We found that the elemental signatures in juvenile westslope cutthroat trout scales could be used to accurately assign individual fish to the drainage and stream of capture in the upper Flathead River system. Further, we found that scale and otolith chemistries were highly correlated. Together, these data suggest that elemental signatures in scales offer a useful and nonlethal alternative to elemental analyses of otoliths as natural tags of

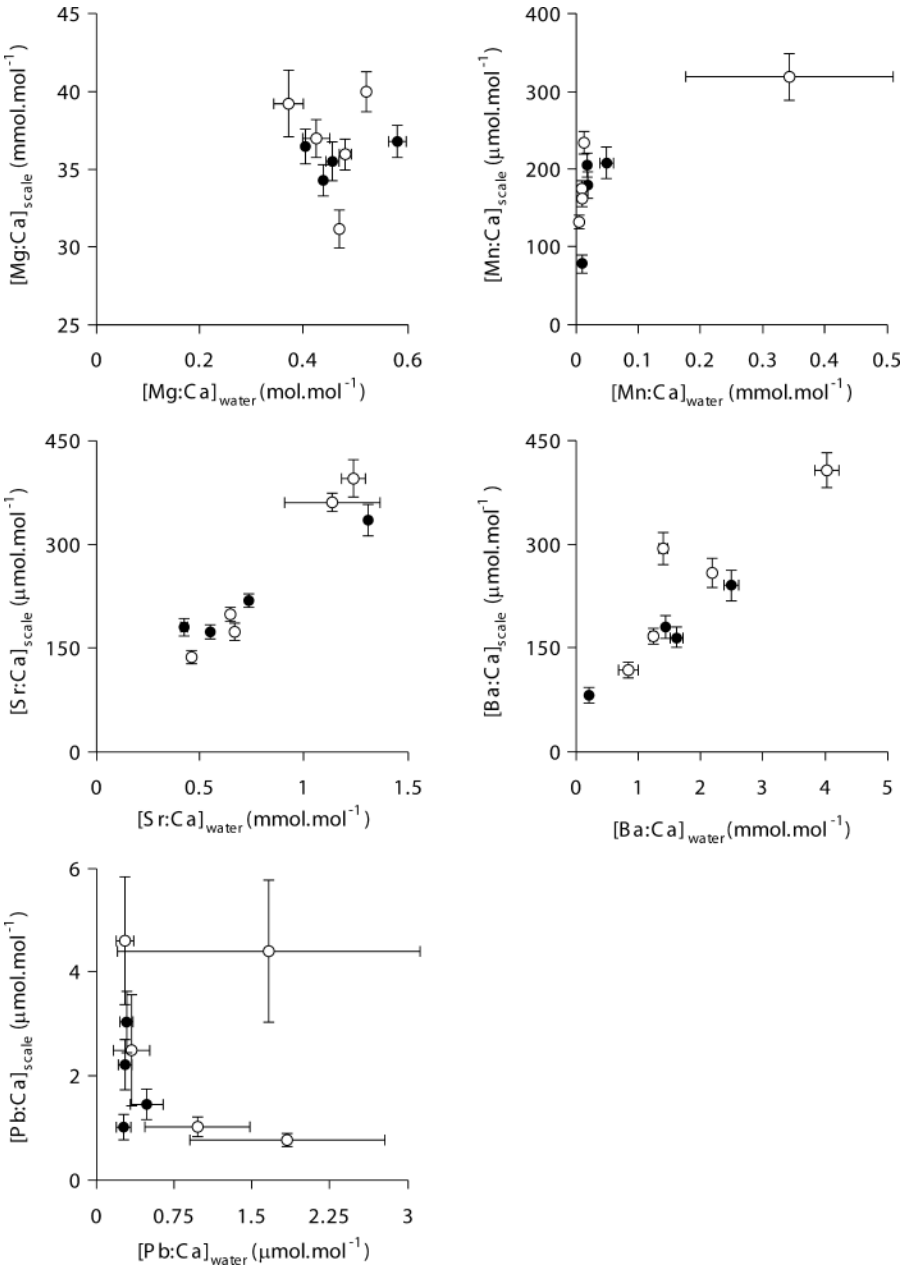

FIGURE 3.—Scatterplots of the relationships between the mean element: Ca concentrations in juvenile westslope cutthroat trout scales and the concentrations in the water from nine streams in the North Fork (open circles; N = 5 streams) and South Fork (solid circles; N = 4 streams) of the upper Flathead River, Montana. Scales and stream samples were collected in summer 2001 and 2002. Barred lines show minimum and maximum values.

natal origin in westslope cutthroat trout and other freshwater fishes.

Our work is the first comprehensive evaluation of the potential use of geochemical signatures in fish scales to determine natal stream origin by means of a hierarchical approach within a single freshwater system (basin → drainage → stream). Several researchers have used elemental analyses of scales to determine natal origin (Wells et al. 2000b, 2003a) and to reconstruct environmental

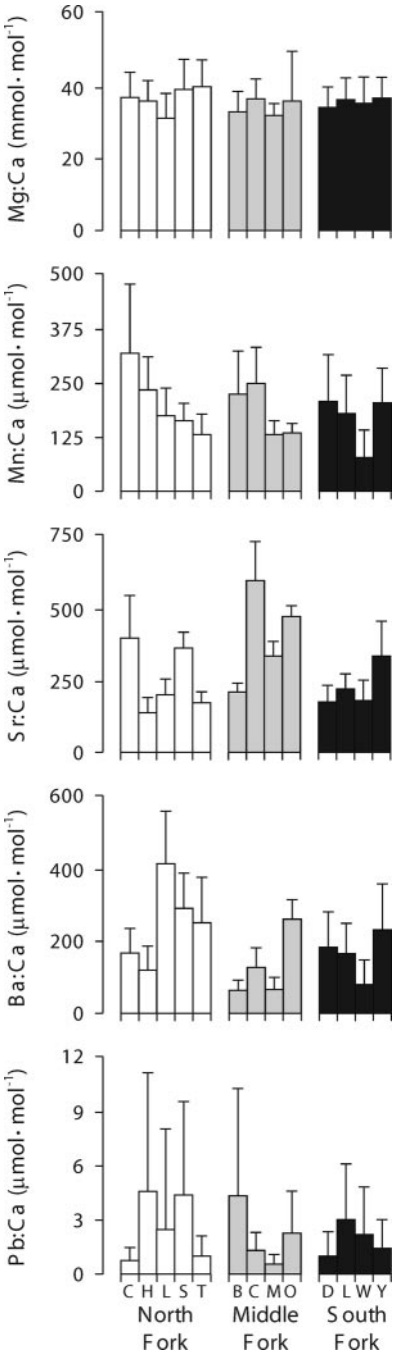

FIGURE 4.—Mean concentrations (±SD) of Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and Pb:Ca in scales from streams of the North Fork (white bars; Camas [C], Hay [H], Langford [L], Sage [S], and Tuchuck [T] creeks), Middle Fork (gray bars; Bowl [B], Cox [C], Morrison [M], and Ole [O] creeks), and South Fork (black bars; Danaher Creek [D], Little Salmon Creek [L], White River [W], and Youngs Creek [Y]) of the Flathead River, Montana.

TABLE 2.—Multivariate analysis of variance main-effect tables for trace element ratios quantified in juvenile westslope cutthroat trout scales from streams in the North Fork, Middle Fork, and South Fork Flathead River drainages, Montana. Log-transformed values of each element ratio (dependent variables) were analyzed. Units for Mg:Ca are mmol/mol; units for all other ratios are µmol/mol.

Element ratio	Mean square effect	Mean square error	F[a]	P
North Fork				
Mg:Ca	0.0492	0.0063	7.8571	0.0000
Mn:Ca	0.5473	0.0219	24.9717	0.0000
Sr:Ca	0.9848	0.0104	94.6743	0.0000
Ba:Ca	1.2672	0.0261	48.5331	0.0000
Pb:Ca	2.1620	0.2278	9.4917	0.0000
Middle Fork				
Mg:Ca	0.0107	0.0049	2.1845	0.1009
Mn:Ca	0.2482	0.0175	14.1476	0.0000
Sr:Ca	0.5876	0.0043	135.2806	0.0000
Ba:Ca	0.8120	0.0195	41.5905	0.0000
Pb:Ca	0.8219	0.1532	5.3634	0.0027
South Fork				
Mg:Ca	0.0055	0.0048	1.1300	0.3404
Mn:Ca	1.6098	0.0586	27.4713	0.0000
Sr:Ca	0.4802	0.0127	37.8722	0.0000
Ba:Ca	1.5357	0.0561	27.3899	0.0000
Pb:Ca	1.0209	0.2784	3.6672	0.0147

[a] North Fork df (1, 2) = 4, 121; Middle Fork = 3, 52; South Fork = 3, 105.

histories of fish (Van Coillie and Rousseau 1974; Coutant and Chen 1993; Pender and Griffin 1996; Wells et al. 2003b). However, most of these studies have been conducted in marine and estuarine systems. Moreover, some researchers have used one or two elements, such as Sr and Ba, to discriminate nursery-specific signatures, which often resulted in a high degree of overlap in elemental concentrations among groups. Our results suggest that multiple elements can improve the accuracy of nursery-specific elemental signatures in freshwater systems.

We found significant variability in elemental signatures of scales among streams within the upper Flathead River basin. At least some of these differences presumably reflect concomitant variability in geology and stream chemistry in the Flathead River system, whereas significant overlap occurred among streams with similar geology and water chemistry. Differences in elemental signatures allowed us to classify fish to their natal streams with an overall accuracy of 82% at the drainage level and 88% at the stream level for 13 streams in three major drainages of the upper Flathead River system. Schmetterling and Dawson

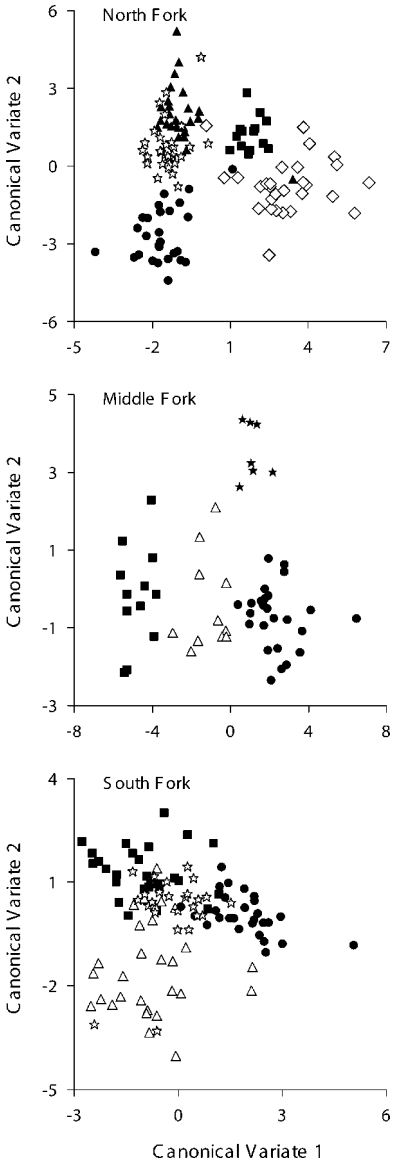

FIGURE 5.—Plots of the first and second canonical variates, demonstrating differences in the scale elemental signatures of juvenile westslope cutthroat trout in streams of the North Fork, Middle Fork, and South Fork Flathead River drainages, Montana, 2001–2002. The variables used in the canonical analysis were the Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and Pb:Ca ratios. North Fork streams include Camas (black circles), Hay (diamonds), Langford (black squares), Sage (black triangles), and Tuchuck (stars) creeks. Middle Fork streams include Bowl (black squares), Cox (black stars), Morrison (triangles), and Ole (black circles) creeks. South Fork streams include Danaher Creek (triangles), Little Salmon Creek (stars), the White River (black circles), and Youngs Creek (black squares).

(2002) were able to discriminate between the Blackfoot and Clark Fork rivers, Montana, and 8 of 12 study streams based on C and N isotope analyses of scales from juvenile westslope cutthroat trout. However, their technique required collection of scale material in an amount (a minimum of 1 mg) that is often lethal to juvenile salmonids (Schmetterling and Dawson 2002). Because multiple scales had to be pooled for analysis, any temporal information from the chemistry of specific growth increments was lost. Wells et al. (2003a) reported that Mn:Ca, Sr:Ca, and Ba:Ca ratios in scales usefully distinguished among a small sample of juvenile westslope cutthroat trout from three streams in the Coeur d'Alene River system. Thus, our results suggest that elemental signatures in scales show considerable promise for assigning natal origin, tracing movements, and delineating stock structure in freshwater systems.

The Sr:Ca and Ba:Ca ratios in otoliths were significantly correlated with the ratios determined for scale centers and edges, while the Mg:Ca, Mn:Ca, and Pb:Ca ratios were less consistent between the two structures. Prior to our work, few studies had compared scale and otolith chemistries (Wells et al. 2000b, 2003a; Gillanders 2001). Wells et al. (2003a) found a high correlation between these ratios in scales and otoliths of westslope cutthroat trout in the Coeur d'Alene River basin. Scale chemistries were, however, more variable and less accurate than otoliths in determining natal origin in their study. Fish otoliths are a useful environmental archive because they are chemically stable and thus provide a permanent record of the geochemical history of individual fish (Campana 1999). However, elemental signatures in fish scales may reset over time because of metabolic resorption of Ca as the fish matures or because of subsequent mineralization (e.g., crystallization of the central regions of the scale) as the scale grows (Yoshitomi et al. 1997). Wells et al. (2003b) found that elemental signatures in scales of adult weakfish degraded after the juvenile period and after maturation, and the authors concluded that scales are unstable for use as natural tags of natal origin. Additional research is clearly needed to assess the persistence of elemental signatures in fish scales, because stability is required if scales are to be a reliable alternative to otoliths as natural tags of natal origin in freshwater fish populations. The use of scales as a nonlethal alternative may still be of value if the elemental signatures persist for sufficient time after fish emigrate from their natal tributaries. Annuli in trout scales record rapid growth

TABLE 3.—Main effects of a forward stepwise linear discriminant function analysis for classifying juvenile westslope cutthroat trout to their natal tributaries based on the elemental signatures of scales collected from 13 streams in the upper Flathead River system, Montana. Element ratios were the dependent variables. The tolerance value is equal to 1 minus the R^2 value.

Element ratio	Wilk's lambda	Partial lambda	*F*-remove (df = 12, 274)	*P*	Tolerance value	R^2
Sr:Ca	0.1066	0.1584	121.3003	0.0000	0.6383	0.3617
Ba:Ca	0.0625	0.2703	61.6508	0.0000	0.6024	0.3976
Mn:Ca	0.0354	0.4779	24.9438	0.0000	0.7107	0.2893
Pb:Ca	0.0228	0.7419	7.9422	0.0000	0.8794	0.1206
Mg:Ca	0.0216	0.7806	6.4177	0.0000	0.8747	0.1253

immediately after fish emigrate from their natal tributaries; these annuli can subsequently be used to identify age of emigration. Thus, it may be possible to identify the natal origin of individuals collected after emigration based on elemental signatures in scales if recently migrant age-classes are selected for analysis.

Our results suggest that it is important to consider spatial scale when elemental signatures in scales are used as natural tags of natal origin in freshwater systems. Classification accuracy at both the drainage and stream levels was probably sufficient to estimate stock composition, but was substantially higher when fewer streams were considered within the same river drainage. Using the same data, we found that classification accuracy was higher when we ran a discriminant model separately for each drainage; cross-validated classification accuracy was 92% for the North Fork, 98% for the Middle Fork, and 78% for the South Fork (C.C. Muhlfeld, unpublished data). Therefore, it may be prudent for sampling to occur in mainstem river sites immediately downstream of natal rearing streams, such as one of the three major forks in the upper Flathead River system.

We found that scale chemistry in juvenile westslope cutthroat trout scales was strongly related to the Sr:Ca and Ba:Ca ratios in the water. Similarly, Wells et al. (2000a) showed that Sr:Ca, Cd:Ca, and Ba:Ca concentrations in the scales of juvenile spot *Leiostomus xanthurus* reflected differences in elemental concentrations of the ambient water. It may be possible, therefore, to determine stream origin of individual fish based on a comparison of water and scale chemistries within a freshwater river basin. These data also suggest that water chemistry analyses should precede the scale chemistry work for future applications of this technique. We did not assess temporal differences in scale and water chemistries. However, Wells et al.

TABLE 4.—Cross-validated classification results of a forward stepwise linear discriminant function analysis for classifying juvenile westslope cutthroat trout to their natal tributaries based on the elemental signatures of scales collected from 13 streams in the upper Flathead River system, Montana. Values indicate predicted classifications based on Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and Pb:Ca ratios as dependent variables.

			Stream												
Sample stream	Sample size	Percent correct	Bowl	Cox	Morrison	Ole	Camas	Hay	Langford	Sage	Tuchuck	Danaher	Little Salmon	White	Youngs
Bowl	12	83	10										2		
Cox	26	96		25			1								
Morrison	11	91			10										1
Ole	7	86				6				1					
Camas	28	61	1	6			17		1				2		1
Hay	28	89						25				2			1
Langford	27	85					1		23		3				
Sage	14	29				1				4					9
Tuchuck	29	83							3		24	1	1		
Danaher	27	48						4	2	1	3	13	4		
Little Salmon	26	62	1							1	1	5	16	2	
White	29	66	1		1						3	1	3	19	1
Youngs	27	78				1	2			1	1		1		21
Total	291	73	13	31	11	8	21	29	29	8	35	22	29	21	34

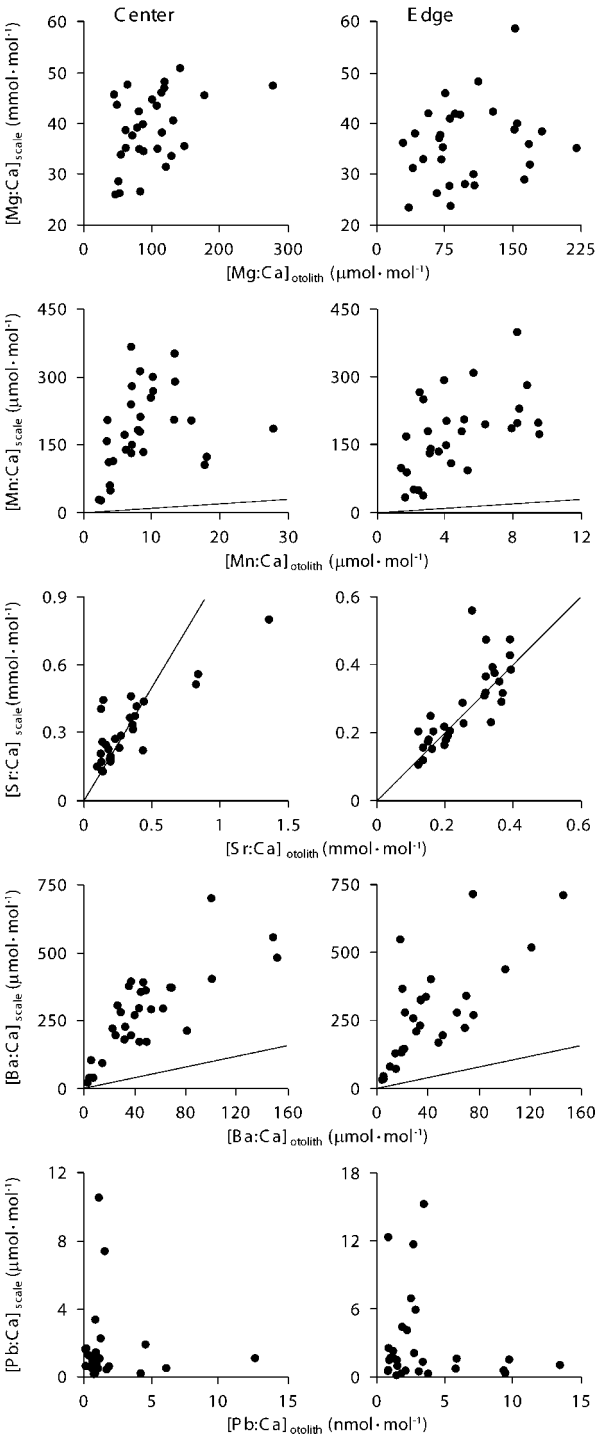

FIGURE 6.—Pearson's product-moment correlations for mean element : Ca concentrations in otoliths and at scale centers (left panels) and scale edges (right panels) for juvenile westslope cutthroat trout from the Flathead River basin, Montana, 2001–2002. See Results for a summary of significant correlations, including *r*- and *P*-values. The lines represent 1:1 ratios.

(2003a) found that scale and water chemistries were temporally stable in a similar freshwater stream system with heterogeneous lithology. Kennedy et al. (2000) reported little seasonal and annual variation in Sr isotope ratios of stream water in tributaries of the Connecticut River. As recommended by Kennedy et al. (2000), we sampled during base flow conditions, as these represent the average conditions for stream hydrology and represent the optimal season for salmonid growth.

Our results indicate that juvenile fish were probably rearing in natal streams, as elemental concentrations at the core and rim of individual fish scales were significantly correlated. These results corroborate previous studies, which have shown that migratory westslope cutthroat trout rear in their natal streams for 1–4 years and then migrate downstream as subadults to large rivers and lakes, whereas resident forms spend their entire lives in natal streams (Shepard et al. 1984; Liknes and Graham 1988). The significant differences in TLs among fish from our study streams may therefore reflect differences in life history or population characteristics (i.e., age or length at emigration).

Elemental analysis of fish scales offers several advantages over traditional marking techniques. Information gathered from physical tag studies (i.e., floy tags, coded wire tags, or passive integrated transponder tags) is often limited and questionable because of sample size concerns (few fish are typically recaptured in such studies), tag retention and detection problems, and logistical constraints. Additionally, juvenile fish may be too small to receive tags or other physical markers without experiencing significant behavioral alterations or mortality. Recent technology has enabled researchers to track individual fish by use of radiotelemetry or sonic telemetry, which allows quantification of movements and habitat use. However, telemetry is labor intensive and expensive, and usually too few animals are monitored to permit accurate conclusions to be drawn. Genetic markers have proven to be useful for stock identification, especially for cutthroat trout *O. clarkii*. Allendorf and Leary (1988) found large variation in DNA from westslope cutthroat trout among streams. However, the interpretation of population genetic data is difficult when a population experiences significant hybridization with nonnative fish (Hitt et al. 2003).

Our work suggests that elemental analysis of juvenile westslope cutthroat trout scales can be used to determine natal stream origin in a single freshwater river basin. Additionally, if scale elemental signatures persist for a sufficient duration after fish emigrate from natal tributaries, scales may offer a nonlethal alternative to retrospectively describing the environmental history of individual fish. Future applications of this technique may allow researchers to estimate the relative contribution of stocks, verify the effectiveness of habitat restoration activities, and quantify life histories. Future work needs to determine whether elemental signatures are sufficiently stable over time to allow for accurate classification of adult fish after emigration from natal streams.

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