Estimating westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) movements in a river network using strontium isoscapes

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Abstract: We used natural variation in the strontium concentration (Sr:Ca) and isotope composition (^{87}Sr:^{86}Sr) of stream waters and corresponding values recorded in otoliths of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) to examine movements during their life history in a large river network. We found significant spatial differences in Sr:Ca and ^{87}Sr:^{86}Sr values (strontium isoscapes) within and among numerous spawning and rearing streams that remained relatively constant seasonally. Both Sr:Ca and ^{87}Sr:^{86}Sr values in the otoliths of juveniles collected from nine natal streams were highly correlated with those values in the ambient water. Strontium isoscapes measured along the axis of otolith growth revealed that almost half of the juveniles had moved at least some distance from their natal streams. Finally, otolith Sr profiles from three spawning adults confirmed homing to natal streams and use of nonoverlapping habitats over their migratory lifetimes. Our study demonstrates that otolith geochemistry records movements of cutthroat trout through Sr isoscapes and therefore provides a method that complements and extends the utility of conventional tagging techniques in understanding life history strategies and conservation needs of freshwater fishes in river networks.

Résumé : Nous avons utilisé les variations naturelles de la concentration (Sr:Ca) et de la composition isotopique (^{87}Sr:^{86}Sr) du strontium dans les eaux de cours d'eau, ainsi que les valeurs correspondantes enregistrées dans des otolites de truites fardées versant de l'ouest (*Oncorhynchus clarkii lewisi*) pour examiner les déplacements de ces poissons au cours de leur cycle biologique dans un grand réseau de rivières. Des différences spatiales significatives ont été observées dans les valeurs de Sr:Ca et de ^{87}Sr:^{86}Sr (le paysage isotopique du strontium) au sein de nombreux cours d'eau de frai et d'alevinage et entre ceux-ci, ces différences demeurant relativement constantes à l'échelle saisonnière. Les valeurs de Sr:Ca et de ^{87}Sr:^{86}Sr d'otolites de juvéniles prélevés dans neuf cours d'eau natals étaient très fortement corrélées aux valeurs correspondantes de l'eau ambiante. Les paysages de strontium mesurés le long de l'axe de croissance des otolites ont révélé que près de la moitié des juvéniles s'étaient éloignés de leurs cours d'eau natals. Enfin, les profils de Sr d'otolites de trois adultes frayants ont confirmé le retour au cours d'eau natal et l'utilisation d'habitats non chevauchants au cours de la durée de vie migratoire. L'étude démontre que la géochimie des otolites, par l'entremise des paysages isotopiques du Sr, fournit un registre des déplacements de la truite fardée et constitue donc une méthode qui complémente et élargit l'application des techniques de marquage traditionnelles à la compréhension des stratégies associées au cycle biologique et des besoins en matière de conservation des poissons d'eau douce dans les réseaux de rivières.

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Introduction

Understanding the movements and life history of stream fishes is important for conservation and management of native populations and freshwater ecosystems. Although the migratory behaviors of anadromous fishes are relatively well known, the extent of movement of potamodromous fishes may be equally common and extensive (Lucas and Baras 2001). Potamodromous salmonids, in particular, are known to make extensive migrations among spawning, growth, and refuge habitats within large stream networks (Northcote 1997). However, despite the fundamental importance of understanding life history diversity and its importance to species management, knowledge about the migratory behaviors of stream fishes through multiple life stages is constrained by limitations of conventional tracking techniques (e.g., mark–recapture, telemetry, passive integrated transponder (PIT) tagging) for following individuals over long time periods, particularly during juvenile life stages (Gowan et al. 1994).

Naturally occurring geochemical signatures in otoliths (fish earstones) have emerged as a powerful tool for determining natal origins and life histories of fishes (Campana and Thor-

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rold 2001; Kennedy et al. 1997; Thorrold et al. 2001). Over the past decade, the use of chemical analysis of otoliths to reconstruct environmental histories of anadromous, catadromous, and marine fishes has expanded rapidly (e.g., Gillanders and Kingsford 1996; Ohji et al. 2006; Zimmerman and Reeves 2002). Yet relatively few studies have used this approach to estimate movement patterns and natal origins of freshwater fishes (Muhlfeld et al. 2005; Munro et al. 2005; Wells et al. 2003). Furthermore, previous studies have primarily used elemental concentrations in otoliths to reconstruct environmental histories that may lack sufficient discriminatory power to identify different ambient waters used by a fish throughout its life history (Rieman et al. 1994; Wells et al. 2003). Isotope systematics of a number of elements, including C, O, and Sr, may in some instances provide a more sensitive method for identifying unique chemical signatures of ambient waters (Walther and Thorrold 2008). In particular, Sr isotopes (^{87}Sr:^{86}Sr) have proved useful in freshwater systems (Barnett-Johnson et al. 2005; Kennedy et al. 2000, 2002). Dissolved Sr in surface waters has an isotopic composition that integrates all upstream sources (Kennedy et al. 2000). Moreover, Sr isotope ratios show no evidence of biological fractionation, and therefore otolith ^{87}Sr:^{86}Sr values are typically very similar to that found in ambient waters (Kennedy et al. 2000, 2002; Walther and Thorrold 2008). Thus, measurement of Sr isotopic composition along the axis of otolith growth may permit identification of natal origin and reconstruction of environmental history of individual fish over their entire lifetime (e.g., Bacon et al. 2004; Barnett-Johnson et al. 2005; Milton and Chenery 2003). However, the use of Sr isotopes to identify life cycle movements of freshwater fishes has had limited application thus far; most applications to date have focused on determining freshwater natal origins of anadromous species (e.g., Bacon et al. 2004; Barnett-Johnson et al. 2005; Kennedy et al. 2002).

Westslope cutthroat trout *Oncorhynchus clarkii lewisi* are typical of many potamodromous salmonids, exhibiting a diversity of life history strategies ranging from a resident life history (remaining in natal streams throughout life) to highly migratory over large distances (Liknes and Graham 1988). Westslope cutthroat trout and other salmonids often exhibit complex movements within large drainage networks for spawning and habitat utilization (Muhlfeld et al. 2009*a*; Schmetterling 2001; Shepard et al. 1984), but such patterns have been difficult to decipher using conventional methods for marking and tracking of individual fish. Thus, the use of natural variation in Sr:Ca and ^{87}Sr:^{86}Sr values, or Sr isoscapes, offer great potential to more accurately reconstruct a fish's environmental history (natal origin, spatial distributions, movement patterns, and spawning locations) within large stream networks. However, Sr isoscapes as a natural tag requires that Sr:Ca and ^{87}Sr:^{86}Sr ratios in ambient waters and fish are sufficiently different at the necessary spatial scales to reconstruct fish movements with the required accuracy.

In this study, we evaluated the use of Sr isoscapes to identify natal origins, reconstruct environmental history, and estimate spawning site fidelity of westslope cutthroat trout. First, we assessed the use of Sr isoscapes to determine natal origin by (*i*) determining the spatial and temporal variation of Sr:Ca and ^{87}Sr:^{86}Sr values in water samples from numerous tributaries used for spawning and early rearing by westslope cutthroat trout and (*ii*) assessing the degree of correlation between Sr:Ca and ^{87}Sr:^{86}Sr ratios in the water and otoliths of juvenile trout collected in nine streams. Finally, we used Sr:Ca and ^{87}Sr:^{86}Sr ratios measured across the axis of otolith growth to reconstruct the environmental history and spawning site fidelity of cutthroat trout.

Materials and methods

The Flathead River drainage originates in the Rocky Mountains of British Columbia, Canada, and northwestern Montana, USA, and includes Flathead Lake and the river system upstream (Fig. 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 headwaters of the Flathead River system: the North Fork, South Fork, Middle Fork, and mainstem Flathead rivers (Fig. 1). Hungry Horse Dam, located on the South Fork 8.5 km upstream of the confluence with the mainstem Flathead River, regulates river discharge, impedes upstream fish migration, and isolates fish populations upstream (Fig. 1). Otherwise, fish movement among tributaries is unconstrained across this large river network.

Sample collections

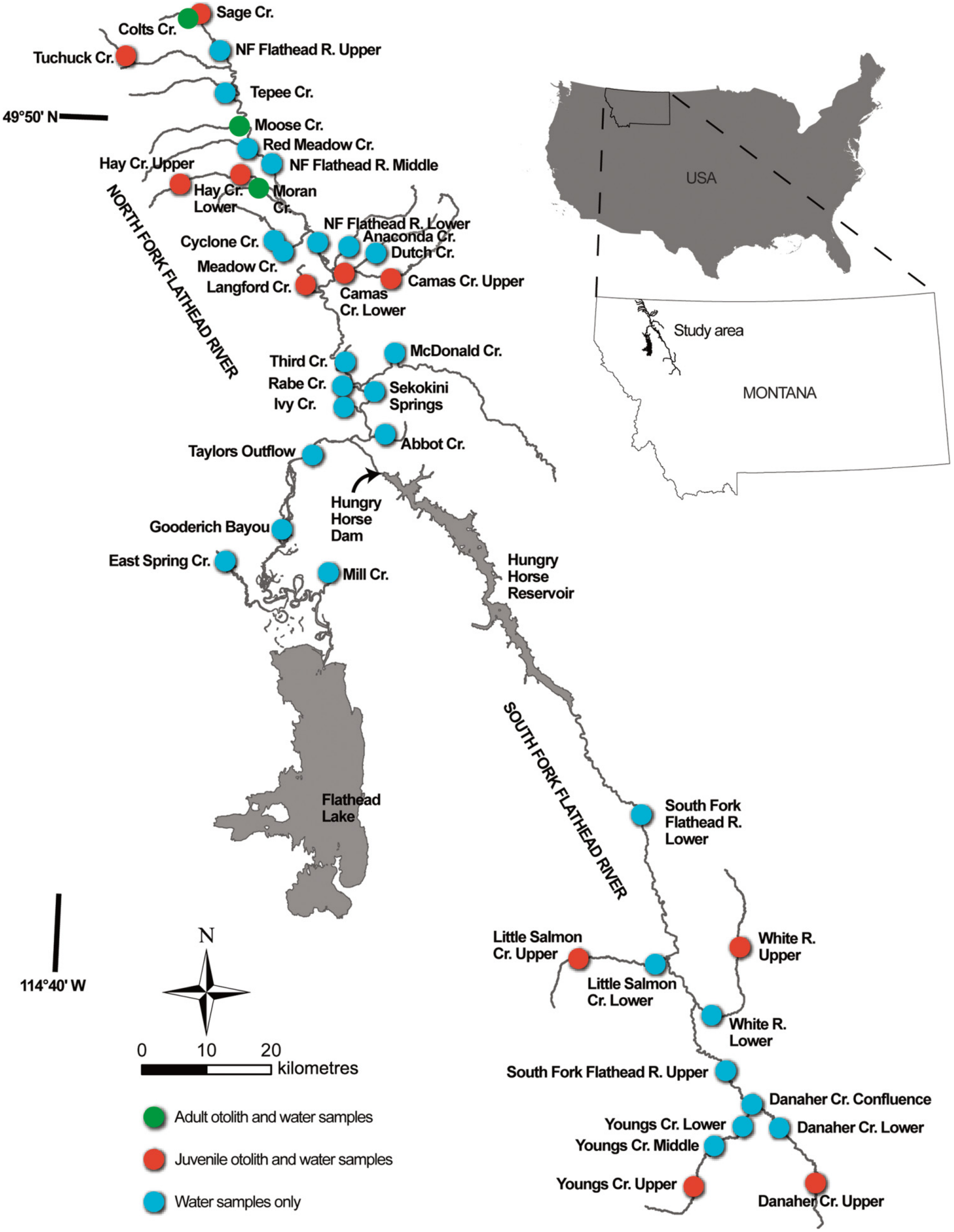
Water samples

The spatial variation of ^{87}Sr:^{86}Sr isotopes and Sr:Ca ratios in the ambient water was assessed among 41 sites used for spawning and early rearing by westslope cutthroat trout and westslope cutthroat trout × rainbow trout (*Oncorhynchus mykiss*) hybrids in the basin (Fig. 1). Water samples were collected during summer base flow conditions during August 2001 and 2006 following ultra-clean procedures described by Horowitz et al. (1994). Additionally, water samples were collected in five streams on 21 August 2006, 30 November 2006, 6 February 2007, and 8 May 2007 to assess seasonal variation in water chemistry. 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 precleaned by washing in a 6 mol·L^{-1} HCl bath for 2 h and a 1% trace metal grade HNO_3 bath for 24 h, with multiple ultrapure water rinses before and after each cleaning stage.

Cutthroat trout otoliths

To assess the correlation between Sr isoscapes of water and otoliths from known-origin trout, we collected juvenile westslope cutthroat trout (mean total length (TL) range, 118 to 245 mm) using a pulsed-DC backpack electrofishing unit or by angling from nine different streams in the North and South forks of the Flathead River drainage (Muhlfeld et al. 2005; Fig. 1). In the Flathead system, migratory westslope cutthroat trout juveniles generally rear in their natal stream for 1 to 4 years and then migrate downstream as subadults to large rivers and lakes (Liknes and Graham 1988; Shepard et al. 1984). All study fish were sampled from populations containing nonhybridized westslope cutthroat trout (Boyer et al.

Fig. 1. Map showing the study streams and sampling locations in the upper Flathead River system, Montana, USA (Cr. = creek; R. = river).



2008); however, hybridized individuals have been detected in two of the study streams (Langford and Camas creeks).

We analyzed Sr:Ca and ^{87}Sr:^{86}Sr ratios within the sagittal otoliths of two westslope cutthroat trout in nine streams (Fig. 1). All collected fish were placed on ice and transported to a laboratory or remote field station. We first measured the TL (mm) of each fish and then removed the otoliths for elemental and isotopic analyses. Otoliths were stored in dry microcentrifuge tubes (0.5 mL). Duplicate water samples were collected at the same time and location as the otoliths. Finally, we sampled otoliths from a single postspawning westslope cutthroat trout from three additional streams (Colts, Moose, and Moran creeks) that contain nonhybridized westslope cutthroat trout (Boyer et al. 2008; Muhlfeld et al. 2009*b*).

Geochemical analyses

Water

Water samples were diluted 10-fold with 2% HNO_3 and analyzed using solution-based inductively coupled plasma mass spectrometry (ICP–MS) to measure ^{43}Ca and ^{88}Sr on a Thermo Finnigan Element2 single collector ICP–MS. Liquid standards and instrument blanks of 2% HNO_3 were run every six samples. Instrument mass bias was corrected for using certified values from a river water standard (SLRS-4, NRC), and an internal laboratory river water standard was used to assess measurement precision. External precision (relative standard deviation) of Sr:Ca ratios for the laboratory standard ($n = 20$) was 1.3%.

Strontium isotopes in water samples were quantified using solution-based multiple collector ICP–MS. Prior to analysis of ^{87}Sr:^{86}Sr ratios in stream water, a 20 mL aliquot of each sample was evaporated to dryness, redissolved in 50% HNO_3, and eluted through a Sr-specific cation exchange resin. This procedure separated Sr from all other constituents in the water samples. The sample was again evaporated to dryness and then redissolved in 1 mL of 5% HNO_3 for Sr isotope analysis using a Thermo Finnigan Neptune multiple collector ICP–MS. The isotope ratio ^{87}Sr:^{86}Sr was calculated by correcting for interferences of ^{87}Rb on ^{87}Sr and ^{86}Kr on ^{86}Sr intensities and mass bias corrections were applied using the method outlined by (Barnett-Johnson et al. 2005; Jackson and Hart 2006). All data were normalized to NIST SRM987 ^{87}Sr:^{86}Sr value of 0.71024 based on mean ^{87}Sr:^{86}Sr values measured in SRM987. The mean (±1 standard deviation, SD) value of ^{87}Sr:^{86}Sr values in SRM 987 ($n = 6$) run throughout the analyses was 0.71026 ± 0.00002.

Otoliths

We assayed Sr:Ca and ^{87}Sr:^{86}Sr ratios of juvenile cutthroat otoliths during the same sample run using laser ablation multiple collector ICP–MS (e.g., Walther et al. 2008). One sagittal otolith from each pair was randomly chosen for laser ablation analysis, mounted on petrographic slides, and then ground to just above the plane of the nucleus using 30 and 3 μm Al_2O_3 lapping film. The sections were then rinsed and sonified in ultrapure water and then dried under a class 100 laminar flow hood. We sampled Sr:Ca and ^{87}Sr:^{86}Sr profiles across the otoliths of two juvenile cutthroat trout collected from nine streams in the South Fork and North Fork Flathead River (Fig. 1). Juvenile otoliths were sampled along a transect from the otolith core to the edge that represented time intervals of approximately 3–4 weeks between adjacent measurements based on the typical age distribution of westslope cutthroat juveniles in natal streams (Liknes and Graham 1988; Shepard et al. 1984). All profiles began with a 140 μm × 200 μm raster centered on the otolith core followed by concentric lines 900 μm in length tracing the contour of individual growth increments and spaced approximately 100 μm apart from the raster to the otolith edge (e.g., Fitzgerald et al. 2004). Laser parameters for the raster and the lines were as follows: diameter 90 μm, repetition rate 20 Hz, scan speed 5 $\mu m \cdot s^{-1}$. We also sampled Sr:Ca and ^{87}Sr:^{86}Sr profiles of otoliths from three adult westslope cutthroat trout using a similar sampling strategy as for juvenile otoliths but with a slightly larger beam diameter (120 μm), core raster (250 μm × 250 μm), and distance between adjacent lines (150 μm). Decreased growth rates towards the edge of the otolith along with the larger distance between adjacent lines meant that the sampling resolution increased to 2–3 months in the adult profiles.

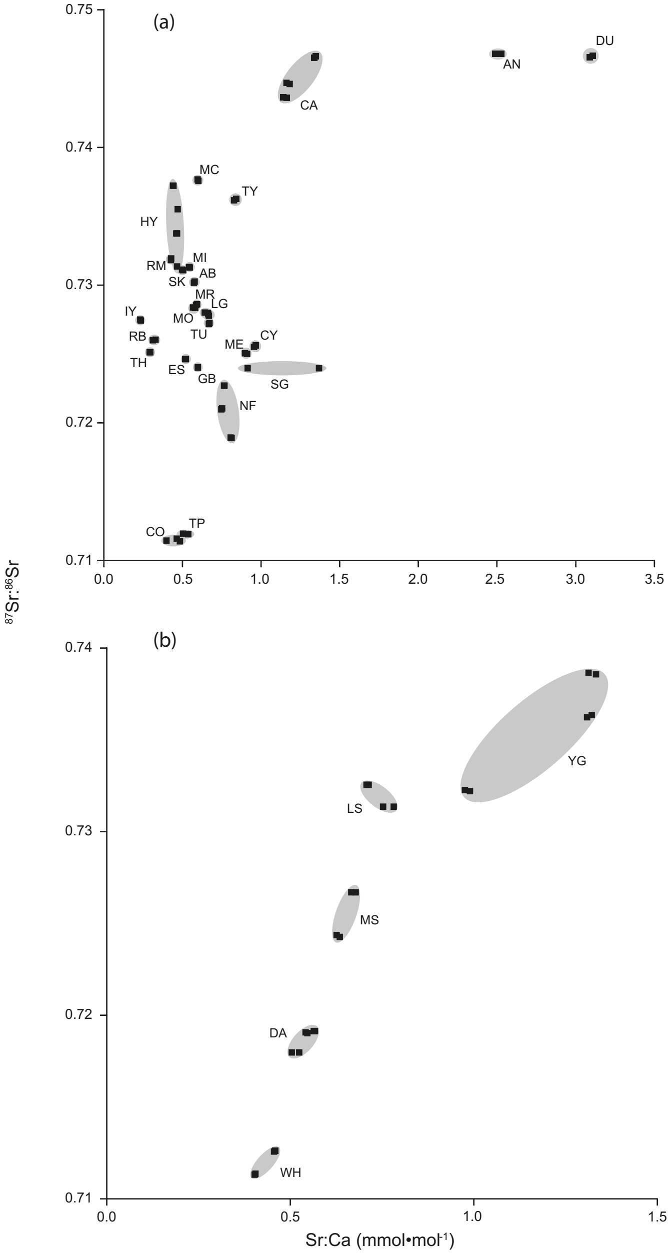
Assay methods including blank and mass bias corrections were similar to those outlined in Walther et al. (2008). During each ablation pass, the instrument cycled between monitoring two sets of monitored isotopes: (*i*) ^{83}Kr, ^{84}Sr, ^{85}Rb, ^{86}Sr, ^{87}Sr, and ^{88}Sr were monitored simultaneously for 3 s; and (*ii*) ^{48}Ca was monitored for 1 s. By cycling through the sets of monitored isotopes, we quantified Sr:Ca, and ^{87}Sr:^{86}Sr with a single ablated raster on the core of each otolith. External precisions (relative SDs) for Sr:Ca values based on repeated measurements of a dissolved otolith certified reference material (CRM; Sturgeon et al. 2005) was 0.4% ($n = 96$). Data were corrected for isotopic interferences as described above for the water analyses. The mean (±1 SD) value of ^{87}Sr:^{86}Sr values in the SRM987 ($n = 37$) run throughout the analyses was 0.71026 ± 0.00002, which is within 1 SD of the accepted value of SRM987 (0.71024). Additional validation included periodic measurements of an aragonitic marine sclerosponge sample ($n = 26$), which yielded a mean (±1 SD) value of 0.70917 ± 0.00002, which was very close to the global marine ^{87}Sr:^{86}Sr value of 0.70918 for this material.

Results

Water chemistry

We found substantial variations in Sr:Ca and ^{87}Sr:^{86}Sr among streams within both the South Fork and North Fork basins of the Flathead River (Fig. 2). In the South Fork basin, Sr:Ca values ranged from 0.40 to 1.32 $mmol \cdot mol^{-1}$ while ^{87}Sr:^{86}Sr ratios ranged 0.71131 to 0.73862. Samples from all four creeks and mainstem locations were clearly separated in the bivariate Sr isoscape plot. The Sr isoscape data from the North Fork basin was considerably more complicated because of the total number of creeks sampled. Values ranged from 0.23 to 3.1 $mmol \cdot mol^{-1}$ and 0.71145 to 0.74679 for Sr:Ca and ^{87}Sr:^{86}Sr ratios, respectively. Nonetheless, almost all of the individual creek and mainstem samples were clearly separated on the Sr isoscape plot. Creeks in close geographic proximity were often, although not always, grouped together. For instance, Ivy, Rabe, and Third creeks from the lower section

Fig. 2. Surface water Sr:Ca and ^{87}Sr:^{86}Sr values for streams in the (*a*) North Fork and (*b*) South Fork and mainstem Flathead rivers. Squares represent individual samples, and ellipses are the range of values around each stream. Stream codes for the South Fork are as follows: WH, White River; DA, Danaher Creek; LS, Little Salmon Creek; MS, mainstem South Fork Flathead River; and YG, Youngs Creek. Stream codes for the North Fork and mainstem are as follows: AB, Abbott Creek; AN, Anaconda Creek; CA, Camas Creek; CO, Colts Creek; CY, Cyclone Creek; DU, Dutch Creek; ES, East Spring Creek; GB, Gooderich Bayou; HY, Hay Creek; IY, Ivy Creek; LG, Langford Creek; MC, McDonald Creek; ME, Meadow Creek; MI, Mill Creek; MR, Moran Creek; MO, Moose Creek; NF, North Fork Flathead River; RB, Rabe Creek; RM, Red Meadow Creek; SG, Sage Creek; SK, Sekokini Springs Creek; TY, Taylor Creek; TP, Tepee Creek; TH, Third Creek; and TU, Tuchuck Creek. McDonald Creek (MC) is a tributary to the Middle Fork Flathead River.



and Anaconda, Dutch, and Camas creeks from the middle section were each clustered together in Sr isoscape space. However, Sr:Ca and ^{87}Sr:^{86}Sr ratios from Colts and Sage creeks were quite distinct despite being directly adjacent to one another in the upper reaches of the North Fork basin. Additionally, the longitudinal variation in Sr isoscapes within six streams (Camas, Hay, Youngs, Danaher, Whites, and Little Salmon creeks) and within the mainstems of the North Fork and South Fork rivers showed that samples within all six creeks and mainstem locations were clearly separated in the bivariate Sr isoscape plots. Mean distance between stream sampling locations was 12.1 km and ranged from 8.0 to 15.2 km.

We also assessed the seasonal variation in Sr:Ca and ^{87}Sr:^{86}Sr ratios in five streams (Fig. 3). While variability in both Sr:Ca and ^{87}Sr:^{86}Sr ratios among streams was clearly the dominant source of variability, we also found minimal temporal variability within streams. The average differences (range) between duplicate samples were 2% (0.1%–7.1%) and 0.062‰ (0.005‰–0.115‰) for Sr:Ca and ^{87}Sr:^{86}Sr ratios, respectively. Mean differences (range) among sampling periods within a stream were 5.7% (0.7%–18%) and 0.94‰ (0.005‰–4.0‰) for Sr:Ca and ^{87}Sr:^{86}Sr ratios, respectively. Finally, mean differences (range) among streams, averaged over time, were 46% (12.8%–97.1%) and 7‰ (1.8‰–15‰) for Sr:Ca and ^{87}Sr:^{86}Sr ratios, respectively. Expressed in another way, temporal variation in Sr:Ca was on average approximately three times higher than between duplicate samples, while spatial variability was approximately eight times greater than differences among sampling periods. Similarly, temporal variation in ^{87}Sr:^{86}Sr ratios was on average almost 16 times greater than between duplicate samples, while spatial variability was approximately seven times higher than differences among sampling periods.

Otolith geochemistry

We found Sr:Ca and ^{87}Sr:^{86}Sr ratios at the edge of juvenile westslope cutthroat trout otoliths were highly correlated with values in the ambient water from which the fish were collected (Fig. 4). Least squares regression described a linear relation between otolith Sr:Ca ($(\mathrm{Sr{:}Ca})_{\mathrm{otolith}}$) and water Sr:Ca ($(\mathrm{Sr{:}Ca})_{\mathrm{water}}$) with the following equation:

$$(\mathrm{Sr} : \mathrm{Ca})_{\mathrm{otolith}} = 0.32(\mathrm{Sr} : \mathrm{Ca})_{\mathrm{water}} - 0.025, \quad r^2 = 0.86$$

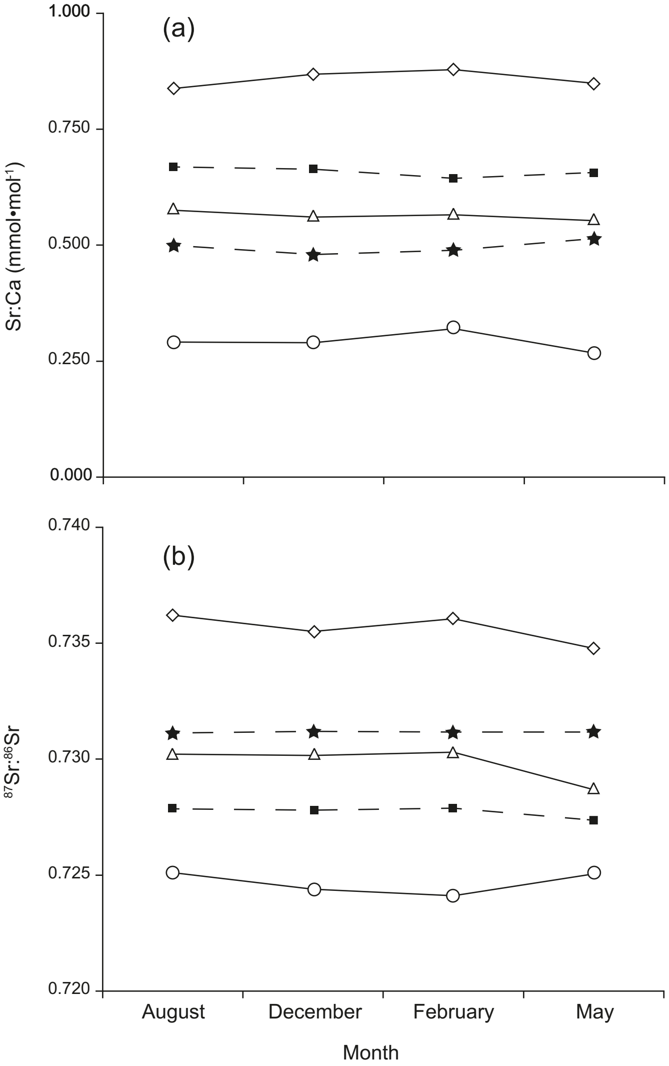
after removing data from a single stream where juveniles had only recently moved to the stream in which they were captured (Fig. 4*a*; see Otolith profiles section below). Values of Sr:Ca in otoliths were clearly lower than corresponding water values, so we calculated a Sr:Ca partition coefficient ($D_{\mathrm{Sr:Ca}}$) for westslope cutthroat trout using the following equation:

$$D_{\mathrm{Sr:Ca}} = (\mathrm{Sr} : \mathrm{Ca})_{\mathrm{otolith}}/(\mathrm{Sr} : \mathrm{Ca})_{\mathrm{water}}$$

The mean (± 1 SD; n = 10) partition coefficient was 0.285 ± 0.047. This value was subsequently used to facilitate comparison between water and otolith Sr:Ca values in the Sr isoscapes developed for the South Fork and North Fork drainages.

Otolith ^{87}Sr:^{86}Sr ratios ($(^{87}\mathrm{Sr}{:}^{86}\mathrm{Sr})_{\mathrm{otolith}}$) were also highly correlated with ambient water values (Fig. 4*b*). Least squares regression described a linear relation between $(^{87}\mathrm{Sr}{:}^{86}\mathrm{Sr})_{\mathrm{otolith}}$ and ambient water ^{87}Sr:^{86}Sr values ($(^{87}\mathrm{Sr}{:}^{86}\mathrm{Sr})_{\mathrm{water}}$):

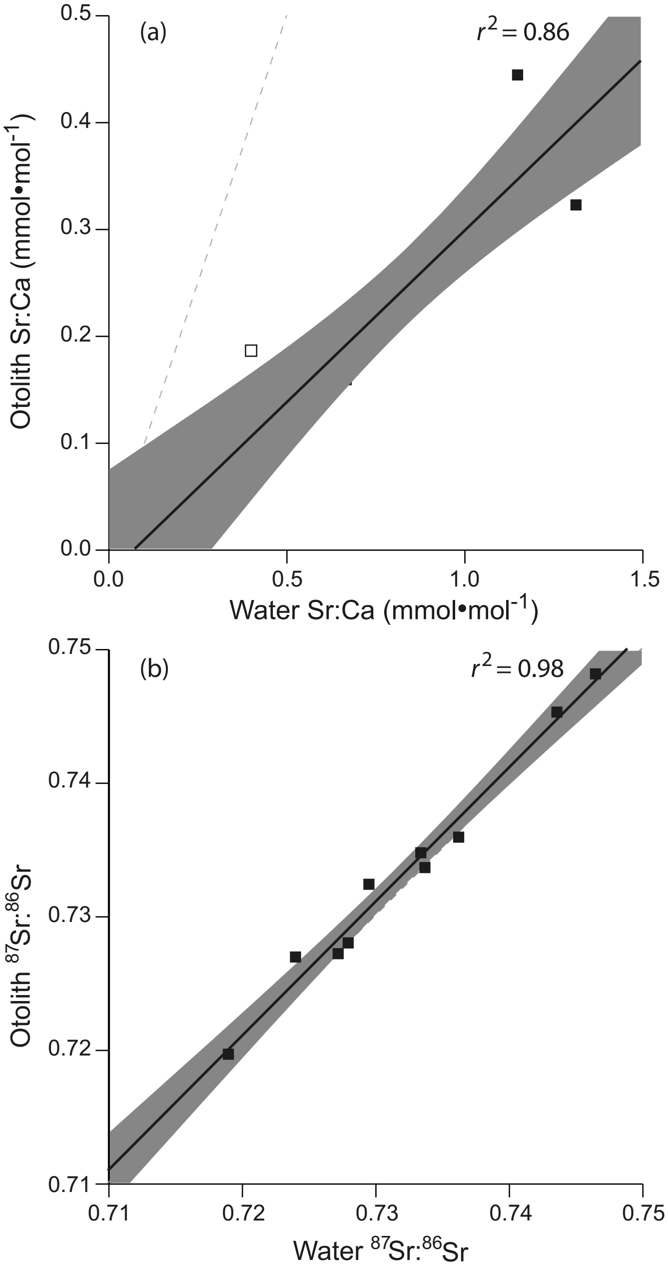
Fig. 3. Temporal variability in Sr:Ca (*a*) and ^{87}Sr:^{86}Sr (*b*) values for five streams sampled over 261 days. Streams are as follows: Taylor Creek (diamonds); Sekokini Springs Creek (stars); Abbott Creek (triangles); Langford Creek (squares); and Rabe Creek (circles).



$$(^{87}\mathrm{Sr}{:}^{86}\mathrm{Sr})_{\mathrm{otolith}} = 1.004(\mathrm{Sr}{:}^{86}\mathrm{Sr})_{\mathrm{water}} - 0.002,\quad r^2 = 0.98$$

Unlike the Sr:Ca values, the slope of the fitted regression line was not significantly different to 1 (Fig. 4*b*), confirming that no measureable fractionation between ambient water and otoliths occurred.

Fig. 4. Mean (n = 2) Sr:Ca values (*a*) and ^{87}Sr:^{86}Sr ratios (*b*) in paired otolith edge and water samples from juvenile cutthroat trout collected in five streams in the North Fork and four streams in the South Fork Flathead River, Montana. Fish from one site (open square) had apparently recently moved to the stream from another location and therefore were omitted from the regression analysis. Solid line represents least squares regression line, shaded area represents 95% confidence intervals for the regression line, and the gray dashed line shows 1:1 relationship between water and otolith values.



Otolith profiles

We measured Sr:Ca and ^{87}Sr:^{86}Sr ratios from the otolith cores to the edges of juvenile cutthroat trout (Fig. 5). The results, visualized using the same format as the Sr isoscapes used for the water sampling, showed the movements of individual fish across the Sr isoscape through time. Data were adjusted using $D_{\mathrm{Sr:Ca}}$ to account for the lower Sr:Ca values in otoliths compared with water. In the South Fork basin, we found evidence for both limited and substantial movement of juveniles away from natal streams. One individual from Youngs Creek, one from Little Salmon Creek, and both individuals from Danaher Creek reared in their natal stream until capture (Fig. 5*a*). All the other juveniles showed evidence of movement across the Sr isoscape into waters with different Sr:Ca and ^{87}Sr:^{86}Sr ratios. The two juveniles collected in White River showed the most movement, with both apparently spawned in Danaher Creek before moving into the main stem and then only into White River immediately before they were captured.

Juveniles from the North Fork drainage also showed evi-

Fig. 5. Profile plots of Sr:Ca and ^{87}Sr:^{86}Sr ratios measured in otoliths from juvenile westslope cutthroat trout collected in streams of the South Fork (*a*) and North Fork (*b*) Flathead rivers. The sample from the edge of the otolith is represented as a diamond, and subsequent samples across the axis of otolith growth for individual fish are represented as squares, with lines connecting consecutive point samples from individual fish otoliths. Circles represent the strontium isoscapes in the water samples from the streams where fish were collected, and the ellipses are the range of water chemistry values from each stream. Colors represent individual streams. Panel (*a*): green, Youngs Creek; magenta, Little Salmon Creek; red, Danaher Creek; and blue, Whites River. Panel (*b*): magenta, Camas Creek; red, Hay Creek; blue, Sage Creek; green, Tuchuck Creek; and black, Langford Creek.

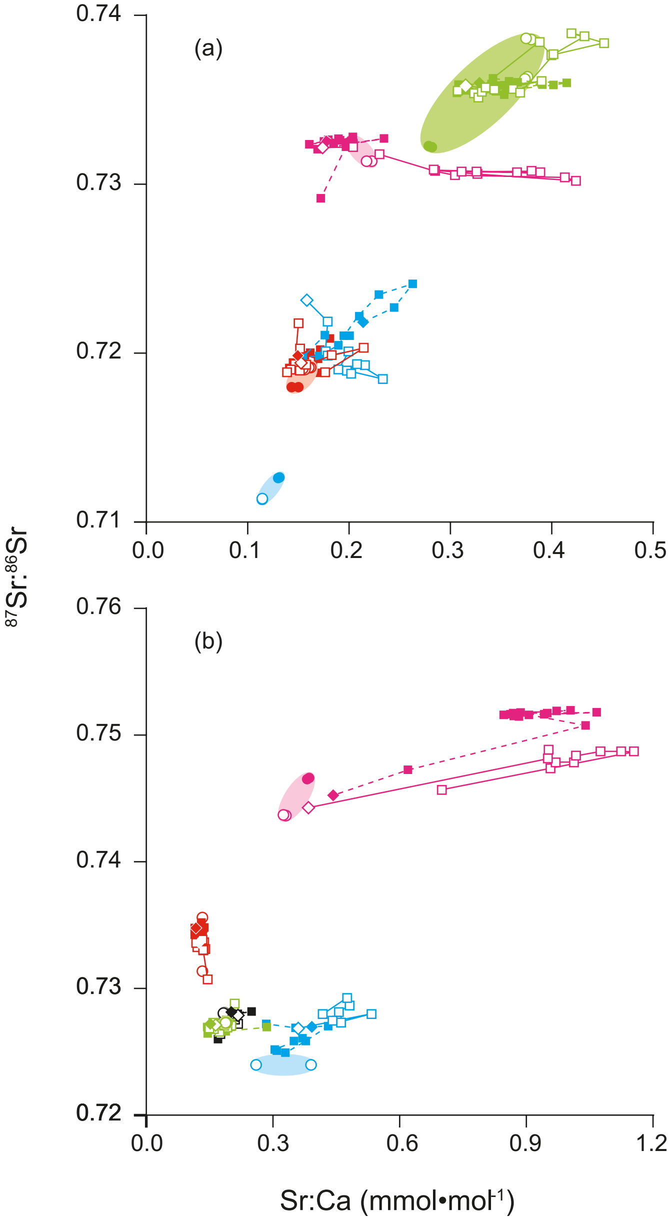
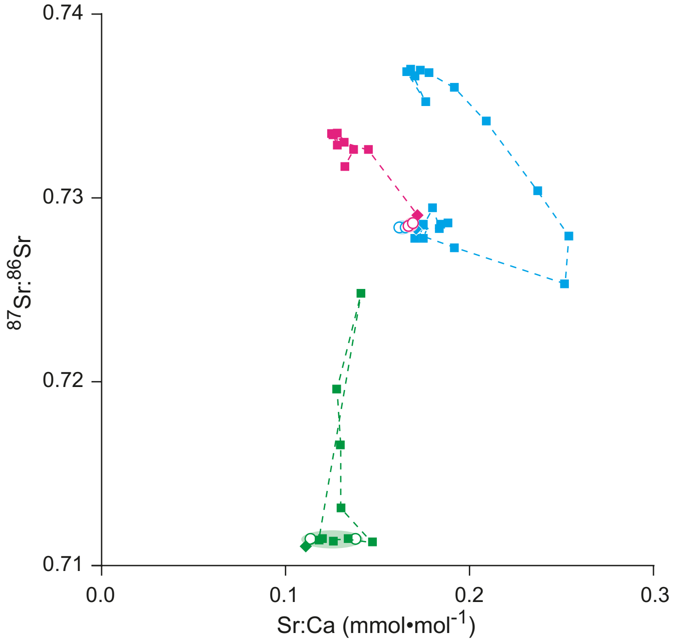


Fig. 6. Profile plots of Sr:Ca and ^{87}Sr:^{86}Sr ratios measured from the otolith cores to the edges of three adult westslope cutthroat trout collected in three streams of the North Fork Flathead River. The sample from the otolith core (natal stream) is represented as a diamond, and subsequent samples across the axis of otolith growth are represented as squares, with lines connecting consecutive point samples from individual fish otoliths. Circles represent the strontium isoscapes in the water samples from the spawning streams where fish were collected, and the ellipses are the range of water chemistry values from each stream. Colors represent individual streams: blue, Moose Creek; magenta, Moran Creek; and green, Colts Creek.



dence for both limited and extensive movements (Fig. 5*b*). Individuals from Hay, Langford, and Tuchuck creeks appeared to have reared in their natal streams. However, juveniles from both Camas and Sage creeks showed evidence of movements away from natal creeks. The Camas Creek individuals had apparently moved from adjacent Anaconda or Dutch creeks based on the North Fork Sr isoscape (Fig. 2*a*). Sage Creek juveniles, on the other hand, could not be matched to any values in our sampled Sr isoscape (Fig. 2*a*) for much of their otolith profiles.

Finally, we analyzed Sr profiles across otoliths of three postspawning adults collected in Moose (female, 401 mm TL), Moran (female, 328 mm TL), and Colts (male, 191 mm TL) creeks (Fig. 6). These profiles revealed lifetime movements of the individuals from natal creeks to spawning locations that were occupied immediately before capture. All three adults had Sr values at the otolith core that matched signatures from the stream that they were collected in, confirming natal homing of these individuals. Additionally, all three exhibited a migratory life history strategy; they reared in natal streams for 1–3 years, migrated downstream as subadults to the North Fork (i.e., fluvial) and possibly Flathead Lake (i.e., adfluvial), where they remained for 1 to 2 years, and then migrated upstream and spawned in natal streams. Interestingly, none of the profiles overlapped after each of the fish had moved out of their natal stream.

Discussion

We used natural variation in the strontium concentration and isotope composition of stream waters and corresponding values recorded in otoliths of westslope cutthroat trout to examine movements during their life history in a large river network. We found large spatial differences in both Sr:Ca concentrations and ^{87}Sr:^{86}Sr isotopes among numerous spawning and rearing streams that remained relatively constant seasonally. Values in the otoliths of juvenile trout were highly correlated with those values in ambient water. Furthermore, strontium profiles measured along the axis of otolith growth revealed movements at high temporal resolution (several weeks to months) over relatively small scales (8–15 km). Combined, these results show that Sr isoscapes of stream waters recorded in fish otoliths may be used to accurately reconstruct environmental histories of freshwater fishes within large river networks.

Previous studies have used trace element and stable isotopic compositions in otoliths for reconstructing the environmental history of adult fishes primarily in marine environments (Thorrold et al. 2001). Most applications of this technique in freshwater systems have focused on movements of anadromous salmonids during their freshwater phases of their life history (e.g., Bacon et al. 2004; Barnett-Johnson et al. 2005; Kennedy et al. 2002). Kennedy et al. (2002) used micromilling techniques to determine ^{87}Sr:^{86}Sr ratios in otoliths of Atlantic salmon (*Salmo salar*) and found that isotopic signatures were distinguishable among major life cycle stages, including natal stream rearing, smolt emigration, and ocean residence phases, and were highly correlated with the ambient water at each stage. We expanded on this work and found that naturally occurring stable Sr isotopes and Sr:Ca elemental compositions in otoliths and the ambient water provide a complementary and powerful approach for understanding movements of westslope cutthroat trout wholly in fresh water within a large river system and thus will be useful for understanding life history traits and conservation needs of many freshwater fish species worldwide.

Like most other potamodromous trout species, westslope cutthroat trout spawn and rear in geographically separate streams (Behnke 1992) that drain diverse lithologies that produce unique dissolved trace element and isotopic concentrations in the water (Barnett-Johnson et al. 2008; Wells et al. 2003). However, seasonal or annual variation in stream-specific water chemistries could limit the utility of Sr isotopes for correlating ambient waters with trout otolith chemistry. It was therefore important to determine if water chemistries differed within and among streams and whether these differences were manifested in trout otoliths. We found that elemental signatures of Sr:Ca and Sr isotopes in water samples remained generally constant in seasonal water samples from individual stream sites, which corroborates previous studies in other freshwater systems in North America (Dorval et al. 2007; Kennedy et al. 2000; Wells et al. 2003). The minor seasonal variation in Sr concentrations and isotope compositions of stream waters likely results from basin-specific precipitation and discharge trends and subsequent timing and magnitude of mineral weathering. Stream water chemistry reflects the geomorphic and geologic properties of watersheds (Fisher and Stueber 1976; Graustein and Armstrong 1983). Geochemical signatures are highly dependent upon dissolution of minerals and ion exchange reactions, and the duration stream substrates are subjected to those reactions. Strontium isotopic composition is likely controlled by a balance between weathering of radiogenic silicates that contributes to higher ^{87}Sr:^{86}Sr values and less radiogenic carbonates with lower ^{87}Sr:^{86}Sr values. For example, ^{87}Sr:^{86}Sr values at sites in the southwest portion of Glacier National Park (e.g., Camas, Dutch, and Anaconda creeks) had the highest ^{87}Sr:^{86}Sr values of all study sites. These basins drain areas that have more exposed bedrock that contain a greater amount of siliciclastic rocks, the quartzitic Grinnell, and Appekunny argillite than other drainages in the study system (Whipple et al. 1992). Other sites with relatively higher ^{87}Sr:^{86}Sr values (e.g., McDonald, Taylor, Youngs creeks) similarly resulted from higher silicate content in the bedrock and greater amount of exposed bedrock. On the other hand, streams with the lowest ^{87}Sr:^{86}Sr values were characterized by carbonate bedrocks (Whipple et al. 1992).

Strontium profiles measured along the axis of otolith growth revealed that almost half (44%) of the juvenile cutthroat trout moved at least some distance from their natal streams. Migratory westslope cutthroat trout spawn in small tributaries, and juveniles rear in natal streams 1–4 years before emigrating to a river or lake (e.g., subadult phase) to mature (Liknes and Graham 1988; Shepard et al. 1984). Tagging studies have provided useful but limited insight into the movements of individuals during the juvenile phase of their life history. Our results indicate that juvenile fish many undergo extensive movements within and among streams in river networks, which were previously undescribed in the literature. Furthermore, individual adults used what appeared to be nonoverlapping habitats over their lifetimes. Several studies have elucidated the importance of maintaining natural connections and a diversity of habitats over a large spatial scale to conserve the full expression of life traits and processes influencing the natural dispersal of westslope cutthroat trout populations (Shepard et al. 1984; Liknes and Graham 1988; Schmetterling 2001).

Strontium profiles from three postspawning westslope cutthroat trout adults confirmed homing to natal streams. These results corroborate previous studies that have inferred natal homing using genetic analyses or have demonstrated natal homing using conventional tagging techniques (Liknes and Graham 1988; Shepard et al. 1984). The ability of salmonid fishes to use olfactory homing cues to return to nursery streams is well known, and juvenile imprinting is common in most salmonid species that rear for extended periods of time in natal streams. The use of Sr isotopes in trout otoliths and ambient waters to examine natal homing in westslope cutthroat trout in the Flathead River drainage is supported by the results of our study.

Movements through Sr isoscapes that are recorded in otoliths provides a method that complements and extends the utility of conventional tagging and tracking techniques (e.g., mark and relocation using external or batch marking techniques, telemetry, genetics) for understanding life history and movements of fishes over small spatial and temporal scales in river networks. Telemetry is time-consuming and small fish cannot be tagged with transmitters that last the life span of the individual. Traditional marking techniques require handling and tagging of many individuals, usually with low recapture rates, and tag retention may be a problem. Genetic markers are useful to determine the origin of fishes when spawning and rearing is confined to limited geographic areas. We have shown that Sr isotopes and elemental concentrations can provide a detailed and accurate description of the environmental history of individual fish in a large river network. This approach, however, requires that fish must be sacrificed to recover the natural tag and thus may not be appropriate for fishes of conservation concern. For this reason, nonlethal sampling using fish scales (e.g., Muhlfeld et al. 2005) or external tissues should continue to be evaluated for research on sensitive species. Additionally, the discriminatory power of geochemical signatures may be limited when the number of sample sites increases and there is overlap in stream signatures and when the geologic variability of a study region limits the spatial scale.

Our study demonstrates that otolith geochemistry records movements of cutthroat trout through Sr isoscapes and therefore provides a method for understanding the life history and movements of fishes in river networks. However, we recommend that water chemistry analyses precede the otolith chemistry work for future applications of this technique in freshwater systems to determine if the ambient waters differ substantially enough to allow differentiating possible natural markers in fish tissues (e.g., otoliths, bones, scutes, scales). Future applications of this technique may allow biologists to identify stock composition, assess habitat restoration activities, investigate nonnative species invasions, and quantify life histories in freshwater environments.

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