

Elemental signatures in otoliths of hatchery rainbow trout (*Oncorhynchus mykiss*): distinctiveness and utility for detecting origins and movement

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Abstract: Otolith chemistry in freshwater has considerable potential to reveal patterns of origin and movement, which would benefit traditional fisheries management and provide a valuable tool to curb the spread of invasive and illicitly stocked species. We evaluated the relationship between otolith and water chemistry for five markers (Ba/Ca, Mn/Ca, Sr/Ca, Zn/Ca, and $^{87}\text{Sr}/^{86}\text{Sr}$) in rainbow trout (*Oncorhynchus mykiss*) using the existing hatchery system in Colorado and Wyoming, USA, to provide controlled, seminatural conditions. Otolith Ba/Ca, Sr/Ca, and $^{87}\text{Sr}/^{86}\text{Sr}$ reflected ambient levels, whereas Mn/Ca and Zn/Ca did not. Using only the markers correlated with water chemistry, we classified fish to their hatchery of origin with up to 96% accuracy when element and isotope data were used together. Large changes in $^{87}\text{Sr}/^{86}\text{Sr}$ were evident in otolith transects, although subtler changes in Sr/Ca were also detectable. Our results suggest the relatively few otolith markers that reflect ambient chemistry can discriminate among locations and track movements well enough to provide valuable insight in a variety of applied contexts.

Résumé : La chimie des otolithes en eau douce présente un potentiel considérable pour révéler les patrons d'origine et de déplacement, ce qui pourrait être bénéfique pour la gestion traditionnelle des pêches et fournir un outil pour réduire la dispersion des espèces envahissantes ou introduites de façon illicite. Nous évaluons les relations entre la chimie des otolithes et celle de l'eau pour cinq marqueurs (Ba/Ca, Mn/Ca, Sr/Ca, Zn/Ca et $^{87}\text{Sr}/^{86}\text{Sr}$) chez la truite arc-en-ciel (*Oncorhynchus mykiss*) dans le réseau actuel de piscicultures du Colorado et du Wyoming, É.-U., afin d'obtenir des conditions semi-naturelles contrôlées. Les rapports Ba/Ca, Sr/Ca et $^{87}\text{Sr}/^{86}\text{Sr}$, mais non Mn/Ca et Zn/Ca, correspondent aux valeurs du milieu ambiant. En n'utilisant que les marqueurs qui sont corrélés avec la chimie de l'eau, nous avons réparti les poissons en fonction de leur pisciculture d'origine avec une exactitude pouvant atteindre 96 % lorsque les données élémentaires et isotopiques sont utilisées conjointement. D'importants changements de $^{87}\text{Sr}/^{86}\text{Sr}$ sont évidents dans les transects d'otolithes, bien que des changements plus subtils de Sr/Ca soient aussi décelables. Nos résultats indiquent que le nombre relativement restreint de marqueurs des otolithes qui représentent la chimie du milieu ambiant permet de séparer les sites et de suivre les déplacements assez bien pour fournir des perspectives intéressantes dans une variété de contextes d'application.

[Traduit par la Rédaction]

Introduction

Otolith chemistry is emerging as a powerful method to track the origins and movement, or provenance, of fish in a variety of taxa and ecosystems. Otoliths are uniquely suited for investigations of provenance because they are continuous growing structures that incorporate elements from the surrounding water in a chronological structure not affected by

resorption and, therefore, record information about the environmental history of fishes over their entire lifespan (Campana and Thorrold 2001). Using otolith chemistry to deduce past environmental histories requires consistent and predictable incorporation of elements from the ambient water. Thus, determining the elemental markers in otoliths that reflect environmental conditions is essential for this technique to be effective.

Received 13 April 2008. Accepted 30 December 2008. Published on the NRC Research Press Web site at cjfas.nrc.ca on 17 March 2009. J20512

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Although most research to date has focused on marine or anadromous populations, freshwater applications are increasingly common and can yield insight into origin and movement (Kennedy et al. 2002; Wells et al. 2003; Whitledge et al. 2007). Short- and long-distance movements can occur exclusively in freshwater (Lucas and Baras 2001) and are increasingly viewed as critical for understanding and managing aquatic ecosystems (e.g., Fausch et al. 2002). Using elemental otolith signatures to track movements of threatened (Brenkman et al. 2007; Hogan et al. 2007), invasive, or illicitly stocked species (Munro et al. 2005) is one of the most promising applications of otolith chemistry.

To date, relatively few chemical markers in otoliths have been shown to reflect ambient water chemistry. Among element/Ca ratios, only Ba/Ca and Sr/Ca are strongly supported as markers that are correlated with ambient chemistry (Bath et al. 2000; Wells et al. 2003; Elsdon and Gillanders 2006). Although several factors may affect the rate of elemental incorporation, water chemistry appears to be the most important for Sr and Ba (Farrell and Campana 1996; Walther and Thorrold 2006). Several isotope ratios have been useful for stock discrimination, although $^{87}\text{Sr}/^{86}\text{Sr}$ may be the most tightly tied to geology and, hence, water chemistry (Kennedy et al. 2000; Bacon et al. 2004). High classification accuracy among locations has been achieved with some of these markers in freshwater (Wells et al. 2003; Brazner et al. 2004; Bickford and Hannigan 2005), but increasing the suite of chemical markers correlated with ambient water chemistry would improve the ability to differentiate among watersheds and may allow greater use of the technique. Further, spatial variation and within-site stability of some chemical markers in freshwater may equal or exceed those in seawater. Given the relatively linear connectivity of rivers and streams and geologic diversity that sometimes occurs over short distances, there is potential for otolith chemistry to contribute to knowledge of movement patterns that occur entirely within freshwater areas.

We evaluated three established markers (Ba/Ca, Sr/Ca, and $^{87}\text{Sr}/^{86}\text{Sr}$) and two less-studied markers (Mn/Ca, and Zn/Ca) across a broad range of values to determine their potential as environmental tracers. We observed the uptake of elements from ambient water using rainbow trout (*Oncorhynchus mykiss*) in hatcheries distributed across two states to control for the potential influence of diet on otolith chemistry. Hatcheries provided an environment with natural ranges of water chemistry and temperature, which simulate conditions for wild fish but also allowed us to observe changes in otolith chemistry associated with known movements between chemically distinct locations. Simulating the chemical changes experienced by invasive or illicitly stocked species under controlled conditions is an important step towards the more effective use of otolith chemistry in applied contexts. Herein, we evaluate otolith elemental signatures as they relate to water chemistry, with emphasis on the application of this technique to reconstruct natural movements or illicit introductions.

Materials and methods

Description of hatcheries and collection of fish and water

Fish were collected from 11 hatcheries operated by the

Colorado Division of Wildlife (CDOW) and one hatchery operated by the Wyoming Game and Fish Department (WGF; Table 1). Surface water was the only source for Chalk Cliffs (CCL), Poudre Rearing Unit (PRU), and Watson Lake (WAT). Both PRU and WAT used water from the Cache La Poudre River (Fig. 1), although WAT is downstream of both PRU (42 km) and a reservoir that receives water diverted across the Continental Divide (6 km). Glenwood Springs (GSU) used spring water for the hatch house and surface water for raceways. The remaining seven CDOW hatcheries all used water from springs, wells, or a mix of the two. Hatcheries with multiple water sources (e.g., several springs) mixed the sources well, often in unpressurized packed columns, before water reached the fish.

We collected water samples at all 11 CDOW hatcheries once per year in 2004, 2005, and 2006. To maximize the ability to examine temporal variation in water chemistry given a budgetary constraint of three site visits per hatchery, we sampled in a different season each year: summer in 2004, late winter in 2005, and fall in 2006. Samples were taken from water supplies after mixing occurred in acid-washed 250 mL polyethylene bottles and filtered into 15 mL bottles using a 0.45 μm syringe filter as in Shiller (2003). We initially assumed sampling error was negligible due to the well-mixed nature of the water supplies, and thus, a single sample was collected at each hatchery in 2004 and 2005. In 2006, we collected three to six samples per site to verify that assumption and to examine analytical error. We also collected 11 samples of the commercial feeds used by CDOW (Rangen Inc., Buhl, Idaho).

To compare water and otolith chemical signatures, samples of 10 rainbow trout or rainbow \times cutthroat trout hybrids (*O. mykiss* \times *Oncorhynchus clarkii*) were collected at the same set of CDOW hatcheries and at the same time as the water collections in summer 2004 and late winter 2005 and at the Wyoming hatchery in October 2005 (Table 1). Fish had been moved between hatcheries per agency policy in several instances (Table 2). All other fish used in the study were reared exclusively at the hatcheries from which they were collected. In the field, sampled fish were immediately euthanized with an overdose of tricaine methanesulfonate (MS-222), bagged, and frozen on dry ice. In the laboratory, they were stored at -23°C until otoliths were removed.

Water and feed chemistry analysis

We quantified the molar concentrations of 20 elements (Na, Mg, Si, S, Ca, K, P, Li, Ce, Pb, U, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Ba, and Se) and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in hatchery water sources. In a laminar flow clean bench, water samples were acidified with ultrapure HCl. Internal standards of Sc, In, and Th were run every eight samples, with In generally used to compensate for instrument drift. Molar concentrations and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were measured with a double-focusing Thermo-Finnigan Element 2 high-resolution inductively coupled plasma mass spectrometer (ICP-MS), which is not the most precise instrument for measuring $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. To account for the potential contribution of Rb to the $^{87}\text{Sr}/^{86}\text{Sr}$ data, we used an internal standard of Rb and applied a correction factor based on the $^{85}\text{Rb}/^{87}\text{Rb}$ ratio. Feed samples

Table 1. Hatcheries sampled for water and fish during 2004–2006 indicating water source for each hatchery and the date and mean total length (TL, mm) of each sample of 10 fish.

Hatchery abbreviation	Hatchery name	Water source	Fish sampled			
			2004	TL	2005	TL
BLV	Bellvue	Well	3 Sept.	116 (15) [‡]	8 Apr.	56 (4)
CCL	Chalk Cliffs	Chalk Creek	21 July	294 (13)	15 Mar.	251 (13)
CRU	Crystal River	Spring, well	19 July	311 (19)	17 Mar.	288 (39)
CRU	Crystal River	Spring, well	—	—	17 Mar.	70 (8)
DUR	Durango	Springs	20 July	276 (20)	14 Mar.	244 (18)
GSU	Glenwood Springs	Spring,* Mitchell Creek [†]	19 July	221 (14)	17 Mar.	121 (14)
MSH	Mount Shavano	Spring	21 July	139 (15) [‡]	15 Mar.	154 (19)
PRU	Poudre Rearing Unit	Cache la Poudre River	10 Sept.	231 (27) [‡]	8 Apr.	230 (20)
RIF	Rifle Falls	Springs	20 July	283 (24)	17 Mar.	230 (22)
ROJ	Roaring Judy	Spring, well	9 Sept.	230 (27)	16 Mar.	236 (34)
SLS	San Luis Valley	Well	21 July	223 (12)	15 Mar.	208 (17)
TFH	Tillet Springs	Springs	—	—	24 Oct.	303 (24)
WAT	Watson	Cache la Poudre River	3 Sept.	276 (23)	8 Apr.	236 (16)

Note: All hatcheries except TFH were operated by the Colorado Division of Wildlife; TFH was operated by Wyoming Game and Fish Department. Fish collected from TFH originated at the Wyoming Game and Fish Department's Tensleep hatchery (TSP), which also used spring water. No fish were collected from TFH in 2004. Values for TL are means with SDs given in parentheses.

*Hatch house.

[†]Raceways.

[‡]Rainbow trout (*Oncorhynchus mykiss*) × cutthroat trout (*Oncorhynchus clarkii*) hybrids.

were dissolved in ultrapure nitric acid and hydrogen peroxide and were analyzed for Ba, Ca, Mn, Sr, Zn, and ⁸⁷Sr/⁸⁶Sr on the same instrument. Elemental concentrations of water and feed data were measured in grams per litre and converted to molar concentrations to produce element/Ca ratios that were used in data analyses.

Otolith chemistry analysis

Sagittal otoliths were removed with plastic forceps, cleaned of adhering tissue using Kimwipes, and stored in individual polypropylene tubes. Right otoliths were used in >95% of all samples; for the remainder, left otoliths substituted for right otoliths that had been cracked or lost during extraction. Transverse thin sections were prepared following the methods in Whitley et al. (2007). Polished thin sections were mounted on glass slides, sonicated for 5 min in ultrapure water, and dried under a laminar flow hood overnight.

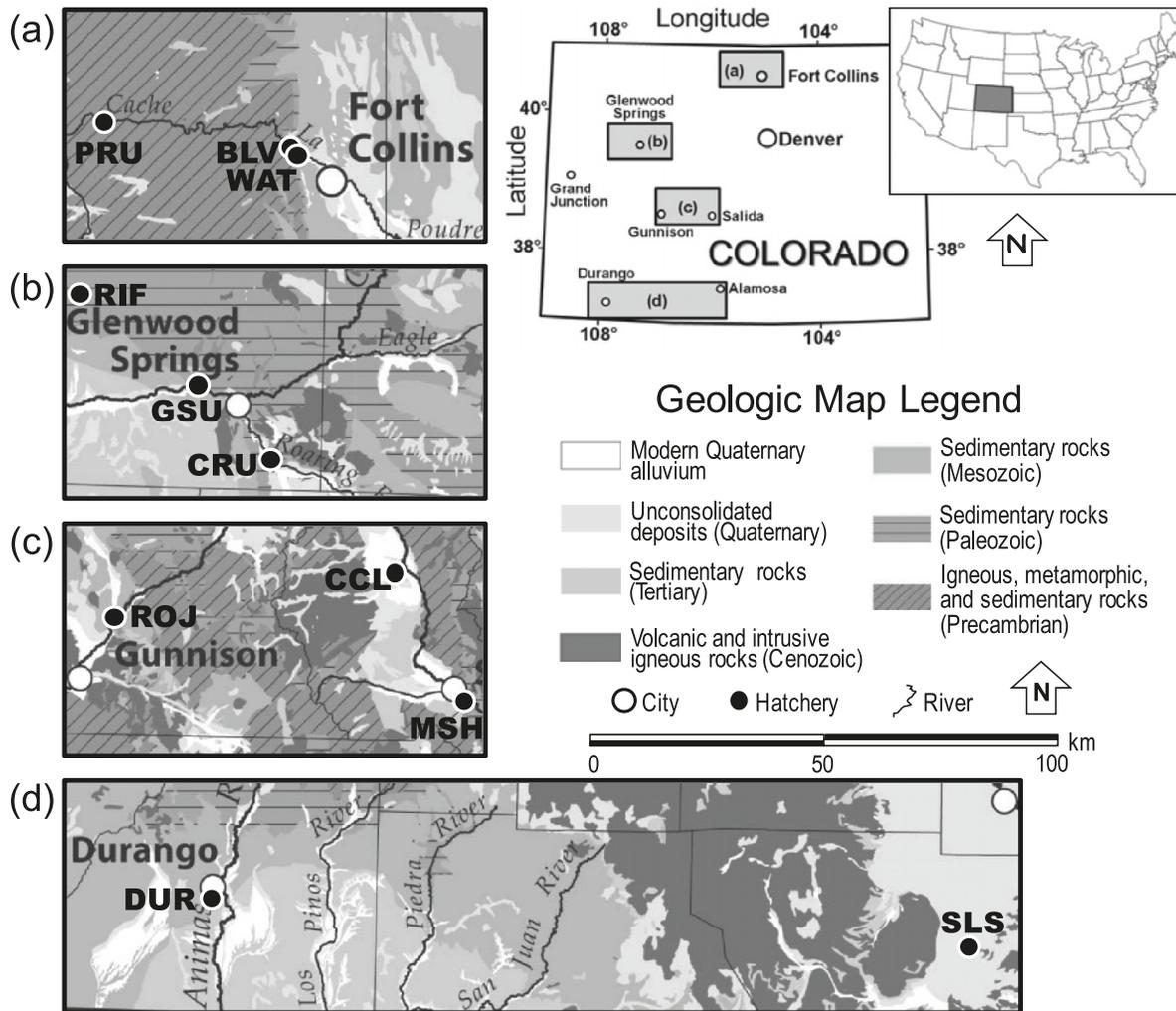
We quantified the abundance of up to 24 elements (B, C, Na, Mg, Al, P, Cl, Ca, Ti, Cr, Fe, Mn, Ni, Cu, Zn, As, Kr, Rb, Sr, Cd, Ba, Ce, Pb, and U) along a transect from the natal zone to edge of otoliths using a Perkin Elmer ELAN6000 ICP-MS coupled to a CETAC Technologies LSX-500 laser ablation (LA) system. Hereafter, we refer to the natal zone as the region corresponding to the size of the otolith from the time fish hatch until they reach 75–125 mm total length (TL) because no fish were moved between hatcheries before reaching this size. Instrumental conditions and concentration calculations described in Whitley et al. (2007) are identical to those we used (beam diameter = 25 μm, scan rate = 10 μm·s⁻¹, wavelength = 266 nm). External calibration of the system was conducted using the prototype US Geological Survey calcium carbonate reference material MACS-1 (S. Wilson, US Geological Survey, Denver Federal Center MS 964, Denver, Colorado 80225, USA, personal communi-

cation, 2006). Drift was monitored using periodic analyses throughout the day using a known reference material (MACS-1). This reference material is a near match for the aragonite matrix of the otoliths.

All otolith transects were initially evaluated without prior knowledge of movement history (these analyses are hereafter referred to as blind integrations). Stable portions of transects were integrated to produce a mean concentration as in Longerich et al. (1996) and reported as parts per million (ppm). When changes were detected in the chemical composition within a transect, stable regions of each zone were integrated to produce a mean value while omitting the transition zones. We used our qualitative analysis of trends among elements to categorize stable portions of the otoliths in the blind integrations. The mean values of stable regions were used in multivariate analyses to characterize hatchery signatures. A subset of transects were reevaluated after multivariate analyses had been completed to maximize the likelihood of detecting chemical changes between the natal zone and edge portions (these analyses are hereafter referred to as post hoc analyses).

The post hoc analyses focused on otoliths of fish that had moved between hatcheries, which should have displayed two chemically distinct portions. In contrast to the blind integrations, we focused primarily on Sr abundance and sought the smallest area of the otolith in which it was possible to quantify Sr signatures. Post hoc analyses compared a small portion of the otolith in the natal zone, representing growth before fish were moved, with a small portion near the otolith edge, representing growth that occurred after fish were large enough to have moved between hatcheries. This was an objective measure that used the same fixed transect length on all otoliths. Whereas the blind integrations considered a number of elements and were conducted with no prior knowledge of fish movements (as would be the case with

Fig. 1. Locations of the 11 rainbow trout (*Oncorhynchus mykiss*) hatcheries sampled in Colorado, USA, shown with local geologic types at four regions of the state (a–d) corresponding to the shaded rectangles in the state map.



fish of unknown origins), the post hoc analyses were analyzed with knowledge of fish movement.

We examined patterns of Sr and Mg abundance within otoliths to identify chemical abnormalities caused by a change in the crystal form of calcium carbonate from aragonite to vaterite. We frequently observed vaterite portions within otolith transects and could identify them easily on the basis of their characteristically low levels of Sr and high levels of Mg (Gauldie 1996; Melancon et al. 2005), which typically occurred outside the core. If vaterite completely obscured the chemical signature of otoliths, they were excluded from further analyses. Natal zone signatures from otoliths with discernable aragonite portions but vaterite edges were included in classification analyses.

To increase the classification accuracy and aid environmental reconstructions, we analyzed $^{87}\text{Sr}/^{86}\text{Sr}$ in a subset of otoliths after examining trends in elemental abundance. The subset was selected using three objectives: to create a data set with a wide range of $^{87}\text{Sr}/^{86}\text{Sr}$ values using water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios as a guide; to determine the effectiveness of $^{87}\text{Sr}/^{86}\text{Sr}$ for discriminating sites that were frequently misclassified on the basis of elemental data alone; and to track move-

ments of fish in cases where elemental abundance alone was inconclusive. Otoliths were cleaned as described above to remove debris from the first ablation. A transect approximately parallel to that of the first was ablated to measure $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. A Nu Plasma MC-ICPMS was employed coupled to a 193 nm excimer LA system (beam size = 70 μm) as described in Woodhead et al. (2005). Integrations of $^{87}\text{Sr}/^{86}\text{Sr}$ transects were completed without prior knowledge of fish movement. It was not necessary to perform post hoc analyses of these data.

Data analysis

We used linear discriminant function analysis (DFA) to evaluate the extent to which hatcheries had distinguishing multielement signatures (Manly 2005). We used a cross-validated, leave-one-out approach to classify fish to their location of origin (e.g., Wells et al. 2003). Prior probabilities were equal, although sample sizes varied among hatcheries because of vaterite deposits within some otoliths that rendered them unusable. The criteria for including chemical markers in classification analyses were the correlation between ambient and otolith chemistry and occurrence above

Table 2. Otolith Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ measurements (means with SDs given in parentheses) from fish that remained at a single hatchery (first four rows) and fish that were moved between hatcheries (remaining rows) and were collected from the destination hatchery in the year shown.

Origin	Destination	Year	Sr (ppm)			$^{87}\text{Sr}/^{86}\text{Sr}$			
			<i>n</i>	Whole	Natal zone	Edge	<i>n</i>	Natal zone	Edge
CCL	NA	2004	9	555 (38)	578 (34)	582 (58)	0	NA	NA
CCL	NA	2005	7	627 (77)	642 (101)	597 (116)	5	0.7089 (0.0001)	
MSH	NA	2004	5	1013 (67)	1029 (90)	1023 (67)	0	NA	NA
MSH	NA	2005	2	1073 (8)	1189 (88)	962 (22)	5	0.7106 (0.0003)	
BLV	PRU	2004	9	562 (68)	580 (33)	624 (45)	0	NA	NA
BLV	PRU	2005	6	481 (75)	449 (54)	524 (64)	3	0.7112 (0.0002)	0.7170 (0.0027) [†]
BLV	WAT	2004	4	604 (76)	423 (24)	556 (61)*	0	NA	NA
BLV	WAT	2005	9	538 (30)	529 (66)	627 (96)*	0	NA	NA
MOH	SLS	2004	3	925 (7)	986 (54)	839 (63)*	0	NA	NA
MSH	SLS	2005	2	843 (30)	871 (54)	770 (63)	5	0.7105 (0.0011)	0.7085 (0.0005) [†]
TSP	TFH	2005	10	—	412 (95)	860 (41)*	0	NA	NA

Note: Whole otolith transect values for Sr were derived from blind integrations where there was no difference detected along the length of the transect. The only otolith transects to display distinct natal zone and edge Sr signatures in the blind integrations were those collected from TFH; a whole otolith Sr concentration was not computed for those fish. All other natal zone–edge comparisons were from post hoc analyses. Otoliths collected from CCL and MSH in 2005 showed no within-otolith differences in $^{87}\text{Sr}/^{86}\text{Sr}$ so values reported are for the whole otolith transect. See Table 1 for hatchery abbreviations. NA, data are not available for the given measurement.

*Significantly different natal zone and edge mean Sr values ($p < 0.05$).

[†]Significantly different natal zone and edge mean $^{87}\text{Sr}/^{86}\text{Sr}$ values ($p < 0.0001$).

reporting limits in >90% of otolith transects. Elemental abundances were \log_e transformed to meet assumptions of homogeneity of variance, but $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were not transformed. Otolith data were pooled within a location across years if there was no significant year effect. When fish were moved between locations, we used the post hoc edge signatures to characterize the signature of the location from which the fish were collected. Data from the blind integrations were used for fish that did not move. All 11 CDOW hatcheries were classified in a DFA using elemental data, but $^{87}\text{Sr}/^{86}\text{Sr}$ data were not available for all hatcheries. To compare the increase in classification accuracy using $^{87}\text{Sr}/^{86}\text{Sr}$ data, the five hatcheries for which $^{87}\text{Sr}/^{86}\text{Sr}$ data were available were analyzed in both a DFA using only elemental data and a DFA using elemental and $^{87}\text{Sr}/^{86}\text{Sr}$ data. The accuracy of the models was compared with that expected by chance alone, assuming random chance will classify otoliths accurately with a percentage inversely proportional to the number of locations classified (White and Ruttenberg 2007).

To assess the degree of variation in otolith chemistry, we analyzed elemental abundance data in an analysis of variance (ANOVA) with hatchery and year as fixed effects. Hatchery and year were set as fixed effects to determine whether elemental signatures in otoliths within a hatchery varied among years. If there was no significant difference for elements between years within hatcheries, we pooled years for the DFA. Within-site variance tended to increase with increasing elemental abundance in water samples, so we fit a linear regression to the relationship for the 2006 water samples and used that equation to estimate error terms for samples collected in 2004 and 2005.

Because hatchery fish receive a diet that differs chemically from natural foods, we examined the degree to which this synthetic source obscured the relationship between otolith and water chemistry that exists in wild fish. To determine the relative contributions of diet and water to otolith

Sr, we used the equations describing the percent contributions from each source given in Kennedy et al. (2000). Using the difference in $^{87}\text{Sr}/^{86}\text{Sr}$ between the mean feed value and the water at each hatchery and the difference in $^{87}\text{Sr}/^{86}\text{Sr}$ between the mean feed value and each otolith within a hatchery, these equations allow us to calculate the influence of dietary Sr on otolith Sr. The percent contributions were calculated for each otolith within a hatchery using the mean feed value from 11 feed samples. We used the water values from 2005 for each hatchery because all otoliths came from the 2005 collection. Mean percent contributions from feed and water were calculated for each hatchery, and the means from each hatchery were averaged to produce an overall estimate of the percent contribution of feed and water to otolith Sr.

To determine if dietary Sr/Ca influenced otolith Sr/Ca, we used the percent contributions of diet and water calculated from $^{87}\text{Sr}/^{86}\text{Sr}$ data to predict otolith Sr/Ca for each hatchery. We made two predictions based on different assumptions about the sources of otolith Sr. “Mixture” predictions were calculated assuming the dietary ($\text{Sr}/\text{Ca}_{\text{diet}}$) and water Sr/Ca ($\text{Sr}/\text{Ca}_{\text{water}}$) levels contributed to otolith levels according to the percent contributions from $^{87}\text{Sr}/^{86}\text{Sr}$ data:

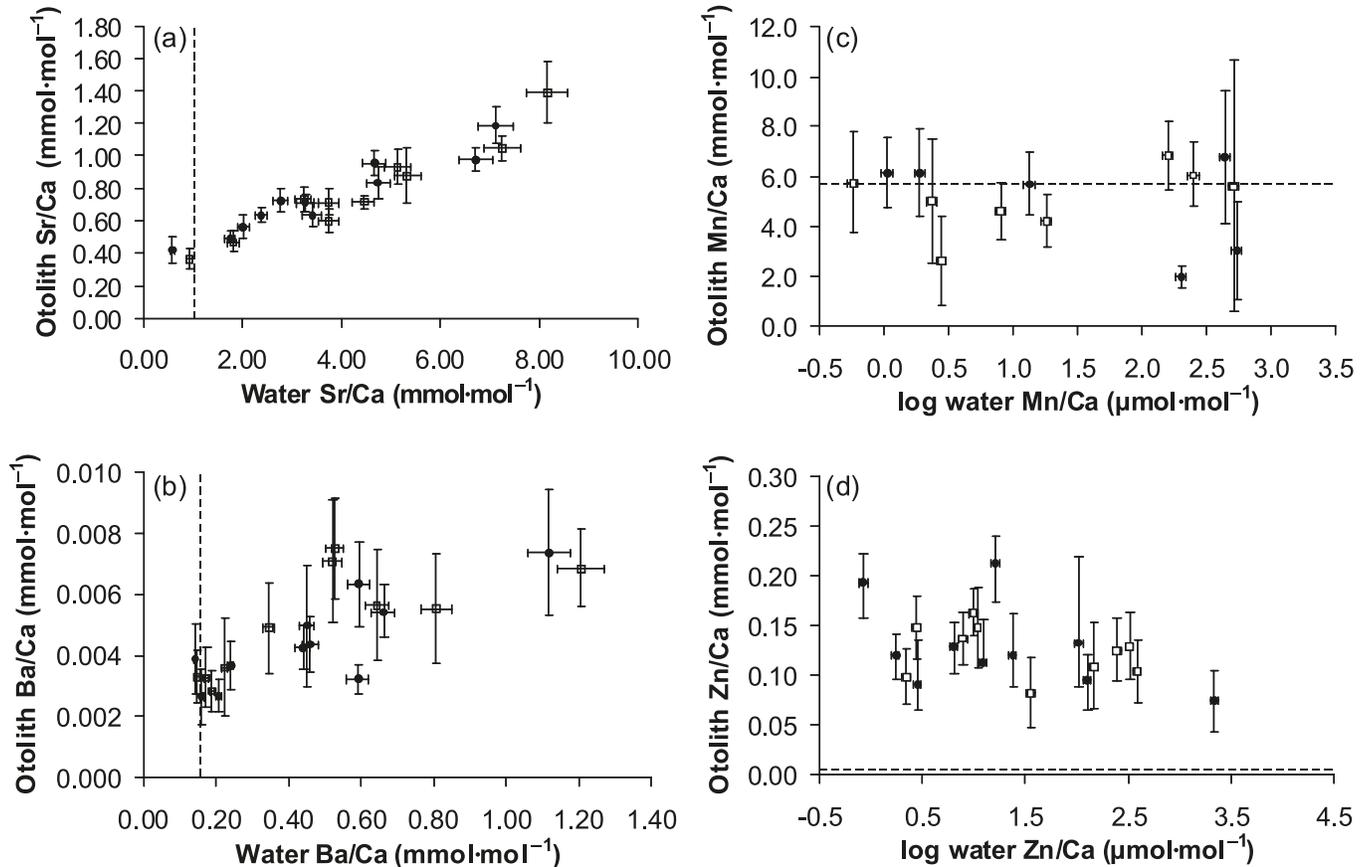
$$(1) \quad \text{Sr}/\text{Ca}_{\text{otolith}} = D_{\text{Sr}}(0.66 \times \text{Sr}/\text{Ca}_{\text{water}}) + (0.34 \times \text{Sr}/\text{Ca}_{\text{diet}})$$

where D_{Sr} is the distribution coefficient. “Water” predictions assumed otolith Sr/Ca was a strict function of water Sr/Ca and D_{Sr} :

$$(2) \quad \text{Sr}/\text{Ca}_{\text{otolith}} = D_{\text{Sr}} \times \text{Sr}/\text{Ca}_{\text{water}}$$

We used $D_{\text{Sr}} = 0.25$ based on the calculations from our samples and those of wild rainbow trout in Montana (Munro 2004), as well as generally similar values across a range of species (Bacon et al. 2004; Zimmerman 2005). The pre-

Fig. 2. Mean water and otolith ratios for (a) Sr/Ca, (b) Ba/Ca, (c) Mn/Ca, and (d) Zn/Ca based on fish and water collections from hatcheries in 2004 (solid circles) and 2005 (open squares). Error bars are SDs. Water data were log-transformed in Figs. 2c and 2d to enhance legibility. The vertical broken lines in Figs. 2a and 2b are mean feed ratios, whereas Figs. 2c and 2d have horizontal broken lines that show the mean water ratios. Mean feed ratios in Figs. 2c and 2d were at least an order of magnitude below the lowest water ratio to allow comparison with mean otolith ratios. Seven ratios in Fig. 2c and one ratio in Fig. 2d were excluded because concentrations of Mn and Zn, respectively, in the water were below detection limits.



dicted values of these two equations were plotted together with observed values, and the 95% confidence limits around observed data points were weighted by inverse variance.

Results

Otolith Ba/Ca and Sr/Ca were correlated with the same ratios in water, but there was no correlation between water and otolith Mn/Ca or Zn/Ca (Fig. 2). Water Mn/Ca and Zn/Ca varied over several orders of magnitude, whereas measured ranges of Ba/Ca and Sr/Ca were narrower. Water samples for Mn and Zn were below detection limits at several hatcheries, but Ba and Sr were consistently reported at levels more than 25 times the detection limits. Barium and Sr were the only elements retained in classification analyses. Water Sr/Ca and Ba/Ca did not differ significantly among years within hatcheries (ANOVA type 3 test of fixed effects, $p = 0.070$ for Sr/Ca and $p = 0.202$ for Ba/Ca). Levels of elemental markers in hatchery feed were generally low relative to water and otolith levels (Table 3).

Otolith Ba and Sr were stable within hatcheries over time (ANOVA type 3 test of fixed effects, $p = 0.158$ for Ba and $p = 0.177$ for Sr) and, thus, were pooled among years within hatcheries for classification analyses. Log_e-transformed Ba

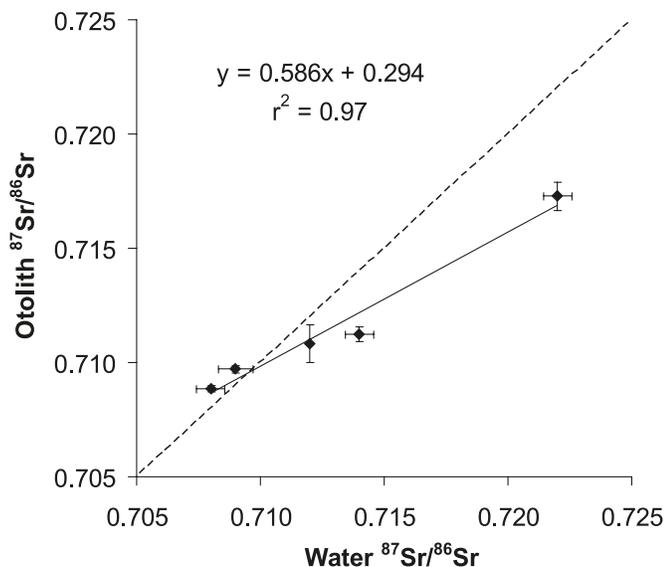
Table 3. Mean (with SD given in parentheses) ratios of Ba/Ca, Mn/Ca, Sr/Ca, Zn/Ca and ⁸⁷Sr/⁸⁶Sr in feed samples from CDOW hatcheries ($n = 11$ for all markers).

Marker	Ratio
Sr/Ca	1.045 (0.328)
Ba/Ca	0.158 (0.060)
Mn/Ca	0.006 (0.002)
Zn/Ca	0.005 (0.002)
⁸⁷ Sr/ ⁸⁶ Sr	0.7076 (0.0020)

Note: Ratios for Sr are moles per mole, and the remaining element/Ca ratios are millimoles per mole.

and Sr met assumptions for homogeneity of variance (Levene's test, $p = 0.586$ for Ba and $p = 0.216$ for Sr). Magnesium was the only other trace element that occurred in >90% of ablated transects but was not examined further because the MACS-1 standard did not include suitable amounts of Mg to permit quantification. Of the remaining 19 elements measured in otolith transects, C, Na, P, Cl, and Ca were biologically unsuitable for use as environmen-

Fig. 3. Mean water $^{87}\text{Sr}/^{86}\text{Sr}$ in relation to mean otolith $^{87}\text{Sr}/^{86}\text{Sr}$ for five hatcheries in Colorado. Error bars are SDs. The broken line indicates the 1:1 relationship between otolith and water $^{87}\text{Sr}/^{86}\text{Sr}$ expected in wild fish. The solid line shows the fitted linear regression.



tal markers, and the other 13 were below detectable limits too often to meet our criteria for further analysis.

Otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios varied with ambient water but departed from a 1:1 relationship (Fig. 3). Water $^{87}\text{Sr}/^{86}\text{Sr}$ accounted for 66% of otolith $^{87}\text{Sr}/^{86}\text{Sr}$, and diet accounted for 34%. Although otolith $^{87}\text{Sr}/^{86}\text{Sr}$ showed an influence from diet, a proportional relationship between ambient water and otolith $^{87}\text{Sr}/^{86}\text{Sr}$ remained. Thus, the otolith $^{87}\text{Sr}/^{86}\text{Sr}$ data were retained in classification models because they yielded information about the past environmental history of fish. Patterns of Sr/Ca levels in otoliths were better explained by the mixture predictions; water predictions tended to be too high (Fig. 4).

Otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were the best indicators of provenance (Fig. 5). Movement between hatcheries was indicated by changes in 9 of 10 otolith $^{87}\text{Sr}/^{86}\text{Sr}$ transects, whereas blind integrations of Sr abundance revealed movement between hatcheries in only one group of fish (TSP to TFH; Table 2). Post hoc analyses of fish that had moved between locations showed relatively small, but significant, differences in Sr abundance occurred between the natal zone and edge of most groups of fish that moved between locations.

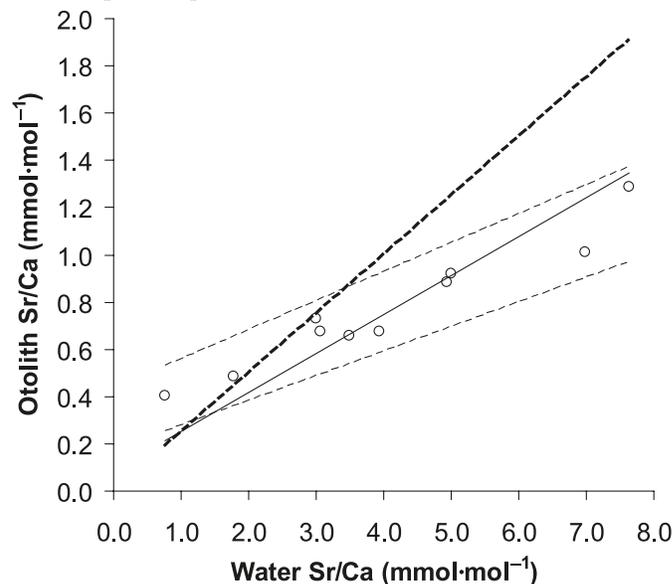
Otolith chemistry classified fish to their hatchery of origin with the greatest accuracy when Ba, Sr, and $^{87}\text{Sr}/^{86}\text{Sr}$ data were included in DFA models. Classification accuracy among 11 locations using only Sr and Ba was 61%. Classification accuracy was 96% in the subset of five hatcheries for which all three markers were available but decreased to 63% without $^{87}\text{Sr}/^{86}\text{Sr}$ data (Table 4). Model accuracy was consistently greater than chance alone would predict.

Discussion

Element water–otolith relationships

Clear relationships between otolith and water chemistry are vital for reconstructing movement patterns. The con-

Fig. 4. Predicted and observed relationships between otolith Sr/Ca and water Sr/Ca for hatcheries in Colorado. The solid line shows the predicted relationship based on the combined influence of diet and water (mixture), whereas the heavy broken line shows the relationship predicted from the influence of water alone. The light broken lines are approximate 95% confidence limits around the observed points (open circles).

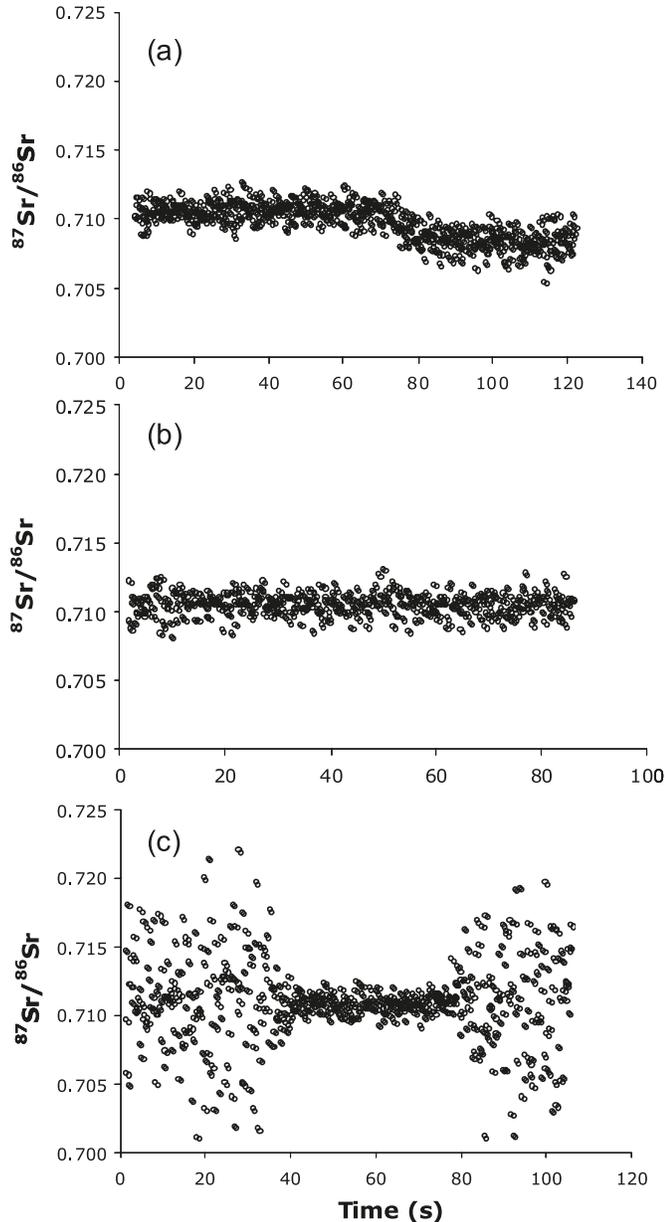


trolled hatchery conditions and large natural range of water chemistry among hatcheries allowed us to evaluate this aspect of otolith chemistry under conditions close to those experienced by wild fish. Central and western Colorado, with volcanic and nonvolcanic rocks, was particularly fertile ground for examining the water–otolith relationship for Mn/Ca (Hem 1985). The lack of a predictable relationship between ambient and otolith chemistry in a region with high variation in water chemistry suggests Mn/Ca is unlikely to be useful for illuminating origin and movement in at least some freshwater fishes.

The absence of a correlation between otolith and water chemistry for Zn/Ca is consistent with other studies and is probably due to the physiological importance of Zn. Zinc serves essential functions in teleost fishes and, therefore, is subject to strong regulation (Bury et al. 2003). In a previous transect study of Zn, Halden et al. (2000) observed oscillations of Zn along the axis of growth in Arctic char (*Salvelinus alpinus*) otoliths that occurred independently of changes in Sr. We frequently observed a similar pattern in our analyses, which may partially account for the large within-site variance of otolith Zn/Ca. Because Zn uptake is highly regulated and otolith Zn is not correlated with ambient water or other demonstrated element markers, other mechanisms are more likely to explain its abundance in otoliths. Variable Zn distribution within aragonite otoliths appears to be due to the association of Zn with otolith protein and the much higher levels of Zn within otolith proteins relative to the aragonite matrix (R. Hannigan, Arkansas State University, PO Box 847, State University, AR 72467, USA, personal communication, 2008).

Similarly, Mn/Ca levels in otoliths may be primarily a

Fig. 5. Otolith $^{87}\text{Sr}/^{86}\text{Sr}$ transects from (a) a fish (222 mm total length, TL) that was moved from Mount Shavano (MSH) to San Luis Valley (SLS), (b) a fish (145 mm TL) that hatched at MSH and was not moved between hatcheries, and (c) a fish (180 mm TL) that was hatched at MSH and was not moved between hatcheries, but vaterite portions at the beginning and end of the transect have prevented precise measurements of the signature. The fish in Fig. 5a was 10 months old at collection, whereas fish in Figs. 5b and 5c were 7 months old at collection and were from the same lot. Time is used as a proxy for distance from the otolith core. See Table 2 for a summary of $^{87}\text{Sr}/^{86}\text{Sr}$ in otolith transects.



function of physiological processes. Sharp increases in Mn have been reported near otolith primordia (Brophy et al. 2004). We detected a similar pattern in some transects, although it was less commonly observed than oscillation in Zn. Although the mechanism responsible for this pattern is currently unknown, it implicates the physiology of the developing fish or chemical changes associated with the struc-

Table 4. Mean classification accuracy of discriminant function analysis models with Ba/Ca and Sr/Ca ratios alone compared with accuracy with the addition of $^{87}\text{Sr}/^{86}\text{Sr}$ data and expected classification rate, which is the percentage of sites that would be expected to be classified correctly by chance alone.

Markers in model	No. of locations	Mean classification accuracy (%)	Expected classification rate (%)
Ba/Ca, Sr/Ca	11	61.1	9.1
Ba/Ca, Sr/Ca, $^{87}\text{Sr}/^{86}\text{Sr}$	5	63.0	20.0
Ba/Ca, Sr/Ca, $^{87}\text{Sr}/^{86}\text{Sr}$	5	96.0	20.0

ture of the nascent otolith. The lack of a correlation between water and otolith Mn/Ca is not surprising given previous work (Martin and Thorrold 2005). Although previous studies examining relatively small ranges of water Mn/Ca have yielded correlations with otolith Mn/Ca (Clarke et al. 2007; Dorval et al. 2007), otolith Mn/Ca tended to be homogenous in an experiment in which water Mn/Ca increased 16 times above ambient conditions (Elsdon and Gillanders 2003), and an observational study of freshwater fish (Thorrold et al. 1998) found no correlation. Sanchez-Jerez et al. (2002) noted a correlation between otolith and dietary Mn/Ca, and we plotted the mean Mn/Ca in hatchery feed in Fig. 2 to highlight the fact that otolith Mn/Ca appeared to be more closely tied to dietary, rather than water, Mn/Ca. We do not necessarily argue that dietary uptake is the mechanism controlling otolith Mn but, rather, suggest the lack of correlation between otolith Mn/Ca and water Mn/Ca in this study fits well with previous work and may deserve further inquiry.

Sr/Ca ratios reflected water chemistry better than Ba/Ca, but this may be due to the relatively narrow range of water Ba/Ca in this study. Kraus and Secor (2004) compiled water Sr/Ca values from over 900 coastal-draining rivers and streams; the range of Sr/Ca in the waters we observed covered the majority of those points. In contrast, two of the three northern Idaho streams reported in Wells et al. (2003) had Ba/Ca ratios higher than all hatcheries in our study. This suggests that regional differences in water chemistry may be the primary factor in determining which markers are best suited for tracking movement. Importantly, otolith and water Ba/Ca were positively correlated despite relatively low abundance of Ba/Ca in hatchery water sources. Although Ba/Ca alone was less effective as an elemental otolith marker in our study, the fact that it occurred at low levels but still showed a positive correlation with water levels suggests it might be more effective in areas where levels of Ba/Ca are higher and more variable among locations.

Strontium and Ba may passively substitute for Ca during otolith formation because of their similar ionic properties (Campana 1999), explaining why they occur in otoliths in proportion to the ambient water. Such relatively simple pathways from the environment to the otolith have not been well described for other elements, which may be the reason no other element/Ca ratios have been shown to reflect ambient water to the extent of Sr/Ca and Ba/Ca. Our observations of Sr/Ca are consistent with a large body of research in a variety of systems (Bath et al. 2000; Wells et al. 2003; Els-

don and Gillanders 2005). Less research has focused on Ba/Ca, although it is also supported as an effective marker (Gillanders 2005; Hamer and Jenkins 2007). The general agreement among studies in a diverse range of environments suggests Sr/Ca and Ba/Ca are incorporated into otoliths from the ambient environment through a relatively passive process, making them rather ideal markers in most systems. Our results show that they reflect ambient water well and may have substantial variation in interior, freshwater systems.

Discrimination against uptake of many other trace elements is possible and may prevent the number of element/Ca ratios useful for environmental reconstructions from increasing. Trace elements can be subject to physiological regulation because of their biological importance (e.g., Mg and Cl) or toxicity (e.g., Pb and Hg). Elemental discrimination may occur at several points along the pathway from water to the otolith (Campana 1999) and could be expected to be greater for elements that are toxic or biologically important. If elements face strong discrimination, it is unlikely that their levels in otoliths will reflect levels in water. In addition to these problems, few elements are as ionically similar to Ca as are Sr and Ba and may be less bioavailable. In sum, it appears likely that complex, less predictable relationships between ambient and otolith concentrations may exist for nearly all elements outside group IIA (i.e., those not in the same column of the periodic table as Ca). However, investigations of novel trace elements such as La (Dorval et al. 2007) and Se (Palace et al. 2007) may prove fruitful, and heavily regulated elements may be useful if they occur beyond some threshold (e.g., “elevated” vs. “baseline” levels of some toxic metals).

Incorporation of element/Ca ratios in otoliths is affected by several factors, including temperature and salinity (Elsdon and Gillanders 2004), but this study had low power to detect such influences. However, water chemistry appears to be the overriding factor determining the incorporation of Ba and Sr (Gillanders 2005; Elsdon and Gillanders 2006). We sought to control movement and diet while allowing factors like temperature to vary as they would in wild fish so that the inferences would be more applicable to wild fish; any influence of temperature in this study should approximate that in wild fish. Thus, temperature varied among locations and seasonally within locations, as all fish were reared in outdoor raceways after they were fingerling size. Salinity was low across locations and unlikely to have played a large role in otolith chemistry. We acknowledge temperature and salinity may have had small effects on otolith chemistry but believe it is inevitable that such influences will arise in natural populations. Additionally, element incorporation may be impacted by variations in water chemistry. We tried to maximize the variation in water chemistry by sampling over 3 years in different seasons; however, otolith Ba/Ca and Sr/Ca were not significantly different within hatcheries among years. Temporal stability of water Ba/Ca and Sr/Ca is implied in the stability of otolith signatures over time and supports our observations of limited within-site variation in water chemistry, as well as those of other small streams (Wells et al. 2003).

Isotope water–otolith relationships

Passive incorporation of isotopes may explain the strong correlation between ambient and otolith isotope ratios. Dietary Sr had a minor but noticeable influence on otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, although otoliths from fish in natural food webs reflect ambient water $^{87}\text{Sr}/^{86}\text{Sr}$ in a direct 1:1 relationship (Kennedy et al. 2002; Woodhead et al. 2005). Thus, with no discrimination during uptake from the environment (i.e., partition coefficient = 1.0), $^{87}\text{Sr}/^{86}\text{Sr}$ data are likely to be better predictors of past environmental history than Ba/Ca or Sr/Ca. We see considerable potential for this marker in studies of wild fish.

Measuring Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ in otoliths allowed us to calculate the proportion of otolith Sr derived from diet and water. Although water and feed both contributed to otolith Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$, water chemistry was the most important factor. Patterns in otolith Sr/Ca were best explained by models that allowed for about one-third of Sr/Ca to come from feed. Predictions based on water chemistry alone overestimated otolith Sr/Ca, particularly at higher levels. The mixture predictions fit the observed data well throughout the range of water Sr/Ca values, strengthening our confidence in the proportional contributions of diet and water from $^{87}\text{Sr}/^{86}\text{Sr}$ data. The D_{Sr} value needed to produce a fit as good as the mixture model is roughly 0.14, which is uncharacteristically low in relation to other studies of salmonids (Bacon et al. 2004; Zimmerman 2005). We are cautious in interpreting the results of this aspect of the study because it was not explicitly designed to test the relative contributions of diet and water and because we measured feed and water $^{87}\text{Sr}/^{86}\text{Sr}$ data on a somewhat less precise single collector instrument. Rather than a precise calculation, we offer these results as evidence that diet can provide a non-negligible portion of otolith Sr, which should be considered in studies of hatchery-reared fish and other circumstances where food and water differ in Sr and Sr isotope composition.

Diet clearly contributes to otolith Sr, but the relative amounts may vary by species, feeding rate, and a variety of other factors. Two experiments designed to evaluate the influence of diet on otolith Sr/Ca showed smaller effects of diet on otolith chemistry. In tilapia (*Oreochromis niloticus*), water Sr accounted for 88% of otolith Sr (Farrell and Campana 1996), whereas water Sr contributed 83% of otolith Sr in mummichog (*Fundulus heteroclitus*; Walther and Thorold 2006). However, Kennedy et al. (2000) calculated dietary Sr accounted for 70% of otolith Sr in Atlantic salmon (*Salmo salar*) reared in a hatchery environment similar to ours.

It is significant that otolith Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ reflected levels in ambient water in spite of the influence of diet, and it suggests an even stronger relationship between otolith and water chemistry may be observed in wild fish. Low values of Sr/Ca in hatchery feed decreased otolith Sr/Ca, particularly at higher water Sr/Ca levels. If otolith Sr/Ca decreased because of the low Sr/Ca in hatchery feed, it is possible that wild fish over the same range of water Sr/Ca may show higher otolith Sr/Ca and, thus, be more distinctive. All fish at CDOW hatcheries received the same diet, so it is unlikely that differences in feed signatures contributed to differences in otolith signatures among hatcheries.

Discrimination among locations

Multivariate models using elemental and isotopic data were able to classify fish to their hatchery of origin with up to 96% accuracy, illustrating the potential to investigate fish origin and movement with only those otolith markers that are correlated with ambient water chemistry. Previous studies of freshwater systems found limits to the number of distinct chemical signatures using element/Ca ratios and suggested isotopic data may increase classification accuracy (e.g., Wells et al. 2003). Our results confirm that isotopes can substantially increase model accuracy: the error rate of a five-location model using Ba, Sr, and $^{87}\text{Sr}/^{86}\text{Sr}$ increased from 4% to 37% when $^{87}\text{Sr}/^{86}\text{Sr}$ data were removed. Misclassifications using only Sr/Ca and Ba/Ca were most frequent at PRU and WAT, which is unsurprising because both hatcheries received fish from the same hatchery (BLV) and use the Cache la Poudre River as their water source. There were no other strong patterns among misclassifications at other hatcheries.

Although the relative contributions of elemental markers in a given environment depend primarily on local variation in underlying geology, the widespread utility of $^{87}\text{Sr}/^{86}\text{Sr}$ data in coastal rivers of the Atlantic (Kennedy et al. 2002), Pacific (Ingram and Weber 1999), and Indian (Milton and Chenery 2003) oceans, as well interior North America, imply that this marker has cosmopolitan applicability. Adding $^{87}\text{Sr}/^{86}\text{Sr}$ data to environmental reconstructions is likely to offer considerable improvement in situations where insufficient variation in Ba/Ca and Sr/Ca occurs. Including other isotope ratios (e.g., δD ; Whitley et al. 2006) may offer further improvements in some watersheds.

Detecting movement between hatcheries

Strontium isotope ratios best identified movement between hatcheries in our samples, but this may reflect a greater range in $^{87}\text{Sr}/^{86}\text{Sr}$ relative to Sr in the hatcheries examined. Clear and unambiguous changes between otolith portions corresponding to different hatcheries were present in all but the otolith $^{87}\text{Sr}/^{86}\text{Sr}$ transect from one fish. In the one otolith where otolith $^{87}\text{Sr}/^{86}\text{Sr}$ did not reflect movement, vaterite at the edge may have obscured the true signal. Decreased abundance of Sr in vaterite lowered the precision of the measurements and may have obscured true differences in $^{87}\text{Sr}/^{86}\text{Sr}$ between the natal zone and edge. Fish moved between BLV and PRU highlight this point. Both hatcheries are within similar geologic formations and have relatively similar Sr signatures. In contrast, the $^{87}\text{Sr}/^{86}\text{Sr}$ signatures of BLV and PRU were widely separated, and movement between the two hatcheries was clear in the $^{87}\text{Sr}/^{86}\text{Sr}$ data. Although PRU and BLV occur within similar geologic formations, PRU uses surface water, and BLV uses groundwater. The otolith and water $^{87}\text{Sr}/^{86}\text{Sr}$ signatures at PRU and BLV are distinct and allow for discrimination, emphasizing the differences between the elemental signatures from surface and groundwater even in geologically similar areas.

The difference between the success detecting movement with Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ emphasizes the importance of a priori knowledge of movement or careful selection of the most informative marker available. The blind integrations were in-

tended to simulate conditions like those in many applied contexts, such as identifying provenance of invasive and highly migratory species, where information about origin and movement may be scarce. The poor results from these blind analyses using Sr abundance indicate the difficulty of tracking movements when origin is unknown and chemical gradients are slight. In contrast, changes in $^{87}\text{Sr}/^{86}\text{Sr}$ were so clear that it was unnecessary to perform post hoc analyses. Because Ba occurred at low levels and showed less difference between hatcheries, we did not examine patterns of Ba in post hoc analyses. Thus, even when three otolith markers are correlated with water chemistry, detecting movement between locations may be obvious (as in $^{87}\text{Sr}/^{86}\text{Sr}$), difficult without a priori knowledge (as in Sr), or unlikely to succeed (as in Ba). Post hoc analyses were more successful at identifying known movements because we were able to focus our analysis on regions of the otolith where hatchery residence was known and maximize the likelihood of obtaining significantly different elemental signatures. Adding additional markers that display greater variation may help, as demonstrated by the increase in success with $^{87}\text{Sr}/^{86}\text{Sr}$ data. When fish origin is unknown, we suggest more intense effort to identify the most informative chemical marker may offset a lack of information about origin and movement.

Unlike other techniques (e.g., analysis of whole otoliths), line transect analyses are well suited for detecting chemical changes within otoliths due to movement or structural changes to otoliths. As our study demonstrates, line transect analyses can be used to obtain continuous life-history information and to compare discrete portions of otoliths corresponding to shorter time intervals. When large differences between environmental chemistry exist, line transects can identify these regions and the approximate age at which this movement occurred. Qualitative analyses of trends in otolith elemental abundance have yielded valuable insight to fish provenance (Brenkman et al. 2007; Clarke et al. 2007; Hogan et al. 2007). However, the characterization of distinct otolith regions achieved here may be necessary in management or legal contexts in which compelling quantitative evidence is needed to justify action. Additionally, line transect analysis can identify regions of vaterite within otoliths that may otherwise obscure true Sr signatures. Vateritic otoliths are sometimes obvious prior to sectioning due to their glassy appearance (e.g., Gaudie 1986), but smaller and less obvious intrusions are also possible (Brown and Severin 1999). Given the dramatic chemical difference between aragonite and vaterite (Melancon et al. 2005), even small amounts of vaterite have the potential to obscure the true Sr signature of individual otoliths. The ability of LA-ICP-MS line transects to identify the presence of vaterite and to be able to integrate data with or without the vaterite signature is a distinct advantage over bulk dissolution methods that dissolve the entire otolith. Additionally, the line transect approach is relatively quick (2 min for small samples and up to 4 min for the largest samples in this study) and provides a more complete life-history signal than individual spot analyses.

Otolith chemistry has been used to investigate some of the most pressing concerns in fisheries science: documenting migration of threatened species (Brenkman et al. 2007; Ho-

gan et al. 2007) and source of invasive (Whitledge et al. 2007) and illicitly stocked (Munro et al. 2005) species. Our results provide a valuable extension to the field of otolith chemistry by demonstrating the accuracy that can be achieved using only those markers correlated with ambient water chemistry. Although significant work has already demonstrated some of the advantages in marine otoliths, these results indicate the utility for freshwater environments and highlight the differences between marine and freshwater systems. Additionally, our observations of Mn/Ca and Zn/Ca, along with continuing research into the nature of element incorporation, should help to delimit the suite of markers that are useful for environmental reconstructions. We suggest Ba/Ca, Sr/Ca, and $^{87}\text{Sr}/^{86}\text{Sr}$ have considerable potential in freshwater and may be particularly valuable in a number of circumstances where more traditional methods are uninformative.

Acknowledgements

Two anonymous reviewers and the associate editor provided helpful reviews. We thank the CDOW and Wyoming Game and Fish managers and hatchery personnel for their cooperation and assistance. John Stednick, Colorado State University, provided valuable guidance. Allan Shiller, University of Southern Mississippi, analyzed water and feed samples. Steven Campana, Fisheries and Oceans Canada, gave helpful advice regarding water and dietary contributions to otolith chemistry. Larry Scott, Colorado Geological Survey, provided geologic map data. Financial support was provided by the Whirling Disease Initiative, CDOW Law Enforcement and Aquatic sections, and the West Denver Chapter of Trout Unlimited.

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