

# Origins of invasive piscivores determined from the strontium isotope ratio ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) of otoliths

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**Abstract:** We examined strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) in fish otoliths to determine the origins of invasive piscivores in the Upper Colorado River Basin (UCRB, western USA). We examined  $^{87}\text{Sr}/^{86}\text{Sr}$  from fishes in different reservoirs, as well as the temporal stability and interspecies variability of  $^{87}\text{Sr}/^{86}\text{Sr}$  of fishes within reservoirs, determined if  $^{87}\text{Sr}/^{86}\text{Sr}$  would be useful for “fingerprinting” reservoirs where invasive piscivores may have been escaping into riverine habitat of endangered fishes in the UCRB, and looked for evidence that such movement was occurring. Our results showed that in most cases  $^{87}\text{Sr}/^{86}\text{Sr}$  was unique among reservoirs, overlapped among species in a given reservoir, and was temporally stable across years. We identified the likely reservoir of origin of river-caught fish in some cases, and we were also able to determine the year of possible escapement. The approach allowed us to precisely describe the  $^{87}\text{Sr}/^{86}\text{Sr}$  fingerprint of reservoir fishes, trace likely origins of immigrant river fish, and exclude potential sources, enabling managers to focus control efforts more efficiently. Our results demonstrate the potential utility of  $^{87}\text{Sr}/^{86}\text{Sr}$  as a site-specific and temporally stable marker for reservoir fish and its promise for tracking fish movements of invasive fishes in river–reservoir systems.

**Résumé :** Nous examinons les isotopes de strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) dans les otolithes de poissons afin de déterminer les origines des piscivores qui envahissent le bassin supérieur du Colorado (UCRB, ouest des É.-U.). Nous avons déterminé le rapport  $^{87}\text{Sr}/^{86}\text{Sr}$  chez les poissons de différents réservoirs ainsi que la stabilité dans le temps et la variabilité entre les espèces des  $^{87}\text{Sr}/^{86}\text{Sr}$  des poissons dans les réservoirs; nous avons cherché à savoir si le  $^{87}\text{Sr}/^{86}\text{Sr}$  est utile pour identifier les réservoirs d'où s'échappent peut-être les piscivores envahisseurs pour aller dans les habitats de rivière des espèces menacées du UCRB et si de tels déplacements se produisent. Nos résultats indiquent que, dans la plupart des cas, les  $^{87}\text{Sr}/^{86}\text{Sr}$  sont particuliers à chaque réservoir, qu'ils se chevauchent chez les différentes espèces d'un même réservoir et qu'ils sont stables dans le temps au cours des années. Dans certains cas, nous avons pu identifier le réservoir d'origine probable de poissons retrouvés dans la rivière et avons pu aussi déterminer l'année probable de la fuite. Cette méthodologie nous a permis de décrire avec précision l'empreinte  $^{87}\text{Sr}/^{86}\text{Sr}$  des poissons des réservoirs, retracer l'origine probable des poissons immigrants dans la rivière et exclure des sources potentielles, ce qui permet aux gestionnaires de centrer leurs efforts de contrôle de manière plus efficace. Nos résultats illustrent l'utilité potentielle du  $^{87}\text{Sr}/^{86}\text{Sr}$  comme marqueur stable dans le temps et spécifique au site pour les poissons des réservoirs et son potentiel pour suivre les déplacements des poissons envahisseurs dans les systèmes de rivières–réservoirs.

[Traduit par la Rédaction]

## Introduction

An understanding of fish origins and movements, or provenance, is important to many applied issues in fisheries. Accurately distinguishing fish of wild vs. hatchery origin, discriminating evolutionarily significant units in mixed stock fisheries, tracking movements of migratory fishes, and determining sources of illegally introduced or invasive fishes re-

quire tools that can differentiate individuals or groups of fishes. In parts of Canada and in the USA west of the continental divide, native sport fishes are uncommon relative to nonnative sport fishes (Nico and Fuller 1999). Agencies have developed sport fisheries in discrete locations by introducing piscivores such as northern pike (*Esox lucius*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), and walleye (*Sander vitreus*). Natu-

Received 24 May 2011. Accepted 20 January 2012. Published at [www.nrcresearchpress.com/cjfas](http://www.nrcresearchpress.com/cjfas) on 16 March 2012. J2011-0225

Paper handled by Associate Editor Bronwyn Gillanders.

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ral dispersal and unauthorized transplantation by anglers is increasing the range of these species, sometimes with undesirable consequences (Johnson et al. 2009). Better methods for tracking origins of fishes arriving in locations where they are unwanted would be helpful for containment efforts.

In the Upper Colorado River Basin (UCRB), there are vast stretches of critical habitat for the endemic, river-dwelling bonytail (*Gila elegans*), humpback chub (*Gila cypha*), Colorado pikeminnow (*Ptychocheilus lucius*), and razorback sucker (*Xyrauchen texanus*) (US Fish and Wildlife Service 1994). These native species became imperiled by habitat alteration from reservoir impoundments, diversions, and river channelization (Valdez and Muth 2005). However, nonnative piscivores, originally introduced by agencies to supplement sport fisheries in reservoirs, are dispersing throughout the UCRB by emigration from reservoirs to rivers and inhibiting the recovery of native fishes (Martinez et al. 1994; Tyus and Saunders 2000). Stocking to develop recreational fisheries has been instrumental in declines of native species worldwide (Cambrey 2003; Eby et al. 2006). Furthermore, habitat alterations caused by impoundments provide a greater opportunity for multiple invader species compared with those found in natural lakes (Johnson et al. 2008). Therefore, native species downstream of reservoirs throughout the UCRB could be at considerable risk. Control of nonnatives is a major objective of the native fish recovery program in the UCRB, with about US\$1 million spent annually on removal and containment (Johnson et al. 2009). Control would be facilitated if biologists better understood the sources of nonnative fish found in critical habitat, but conventional marking and tagging methods have proven impractical given the tremendous geographic scale of the problem.

The chemical composition of otoliths has proven useful in addressing fish provenance at large spatial scales, such as determining origins of anadromous fishes and stock identification in mixed-stock fisheries (Barnett-Johnson et al. 2008; Walther et al. 2008). Trace element analysis has been used frequently in studies of fish origins, movements, and connectivity among aquatic habitats (Campana and Thorrold 2001; Munro et al. 2005; Clark et al. 2010). However, only a few elements (e.g., Sr and Ba) appear to be reliably correlated with ambient water (Bath et al. 2000; Wells et al. 2003; Gibson-Reinemer et al. 2009), and seasonal and annual variability can be high (Gillanders 2002; Bacon et al. 2004; Walther and Thorrold 2009). The use of strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) is emerging as one of the more useful otolith constituents for tracing fish provenance in freshwater fishes (Bacon et al. 2004; Kennedy et al. 2005; Walther et al. 2008). Strontium isotopes vary among water bodies because  $^{87}\text{Sr}/^{86}\text{Sr}$  is driven by the underlying geology across landscapes and weathering effects (Kennedy et al. 2000). Strontium isotopes in otoliths are strongly correlated with the  $^{87}\text{Sr}/^{86}\text{Sr}$  in ambient water (Kennedy et al. 2000; Walther and Thorrold 2006; Barnett-Johnson et al. 2008), suggesting negligible fractionation by biotic processes. The temporal stability of  $^{87}\text{Sr}/^{86}\text{Sr}$  is typically greater than that of the trace elements mentioned above, mainly because  $^{87}\text{Sr}/^{86}\text{Sr}$  is not influenced by environmental factors that modify concentrations of trace elements (e.g., river discharge) or their incorporation into otoliths (e.g., temperature and salinity; Fowler et

al. 1995). However, seasonal flow fluctuations may alter  $^{87}\text{Sr}/^{86}\text{Sr}$  if these affect the relative contributions of water derived from geologically distinct portions of the watershed (Walther et al. 2011).

In our study, we examined the utility of  $^{87}\text{Sr}/^{86}\text{Sr}$  in otoliths to determine origins of piscivores throughout the UCRB. Our primary goals were to evaluate (i) distinctiveness of otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  from different reservoirs and subbasins (containing one or more reservoirs); (ii) interannual variability of otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  within reservoirs; and (iii) interspecies differences of otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  within reservoirs due to differential habitat use. We also tested the utility of  $^{87}\text{Sr}/^{86}\text{Sr}$  for tracing origins of suspected reservoir escapees sampled in rivers below study reservoirs. Establishing the likely origin of river-caught fish in river-reservoir systems through the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature of an otolith could be a highly valuable aid for managers dealing with invasive species in these systems.

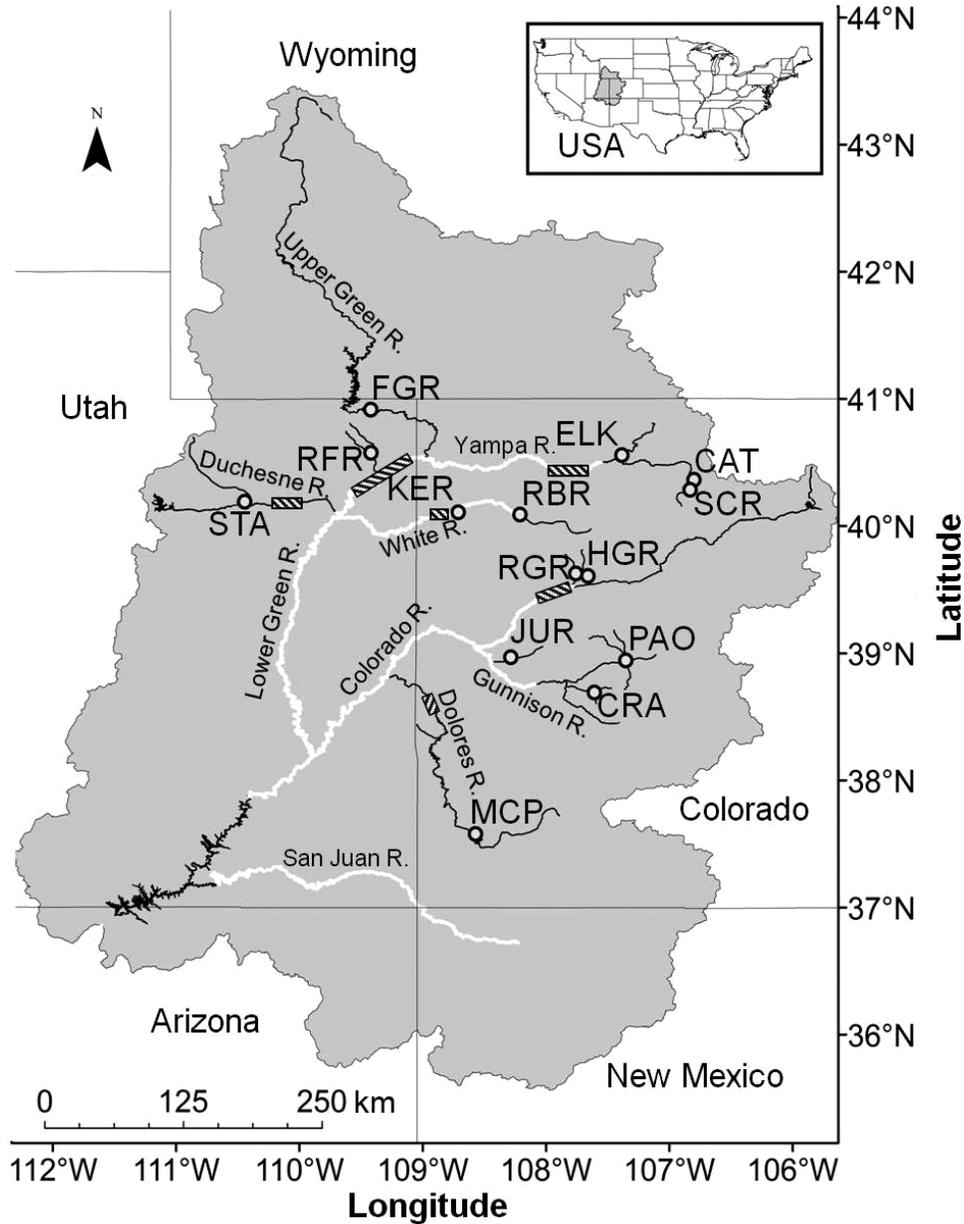
## Materials and methods

### Study area

Our study area encompassed the UCRB (Fig. 1). The basin extends from southwestern Wyoming through western Colorado and eastern Utah, USA, functionally terminating at Glen Canyon Dam near Page, Arizona, with an area around 290 364 km<sup>2</sup>. For our reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis, data were available, with only five exceptions, from all reservoirs  $\geq 50$  ha in Colorado, Utah, and Wyoming that are connected to critical habitat and contained one or more of the following invasive species, smallmouth bass, walleye, and northern pike, species considered in the "Origins of river-caught fishes" section of our analysis. Exceptions were Midview (also called Lake Boreham, contains walleye and smallmouth bass) and Cottonwood (contains smallmouth bass) reservoirs in Utah. Samples were also not available from William's Fork and Wolford Mountain (each contains northern pike) reservoirs in Colorado. William's Fork and Wolford Mountain are high elevation reservoirs far above ( $\sim 200$  km) critical habitat on the Upper Colorado River. At the initiation of the study it was believed that Lake Powell (Arizona and Utah), more than 350 km downstream of other reservoirs in the study, was not a source of nonnative fish to the Green River. Most of the reservoirs from which samples were available have been subject to illegal introductions of warm water fish, making knowledge about their microchemical signatures useful for managers seeking to better understand and curtail this activity. Available data provided samples from 14 reservoirs in six tributary subbasins of the UCRB, including the Colorado River (Harvey Gap and Rifle Gap reservoirs), Dolores River (McPhee Reservoir), Green River (Flaming Gorge, Starvation, and Red Fleet reservoirs), Gunnison River (Crawford, Juniata, and Paonia reservoirs), White River (Kenney Reservoir and Rio Blanco Lake), and Yampa River (Elkhead and Stagecoach reservoirs and Lake Catamount) basins (Fig. 1).

Most of these reservoirs are located on the periphery of the Colorado Plateau, a relatively stable physiographic province west of the Rocky Mountains. The majority of exposed rock of the basin consists of beds of Mesozoic sandstone as well as younger Cenozoic sedimentary, shale, and volcanic rock.

**Fig. 1.** Study area and reservoir locations (circles) within the Upper Colorado River Basin (western USA). The parts of the rivers outlined in white are designated critical habitat for the endangered bonytail (*Gila elegans*), Colorado pikeminnow (*Ptychocheilus lucius*), humpback chub (*Gila cypha*), and razorback sucker (*Xyrauchen texanus*). Collection sites of river-caught fishes are shown with boxes with diagonal lines. Refer to Table 1 for reservoir names.



Lake Catamount, Elkhead, Flaming Gorge, and Stagecoach reservoirs are within the Wyoming Basin province; Crawford and Paonia reservoirs lie within the Southern Rocky Mountains province. In general, rivers in this region are snowmelt driven, with mountain snowpack (2100–4000 m above sea level) dictating annual hydrologic variability (Poff and Ward 1989). Most reservoirs here are located below 2100 m and were built to capture spring runoff for agricultural and domestic use during typically dry summers. Hydraulic residence time (HRT) averaged about 1 year in our study, but ranged 0.03–4.14 years, generally increasing with reservoir size (Table 1).

#### Fish collections

Fishes were collected opportunistically from agency biologists in cooperation with the Upper Colorado River Endangered Fish Recovery Program (Colorado Parks and Wildlife; Colorado State University Larval Fish Lab; US Fish and Wildlife Service (USFWS); Utah Division of Wildlife Resources; and Wyoming Game and Fish Department) conducting sampling for the monitoring and management of nonnative piscivores during 2004–2009 from reservoirs and rivers (Table 2) throughout the basin. The goal was to sample the full complement of nonnative piscivores present in each system, collectively including black crappie (*Pomoxis nigro-*

*maculatus*), burbot (*Lota lota*), largemouth bass, northern pike, smallmouth bass, and walleye. River-caught fish, which we used in the “Origins of river-caught fishes” section of our analysis, were collected throughout endangered fish critical habitat in each river system in the study area (Fig. 1), with the exception of the San Juan River subbasin, an isolated basin managed by the San Juan River Recovery Implementation Program (USFWS). The San Juan flows over a waterfall that effectively precludes upstream fish migration just before draining into Lake Powell. Captured fishes were euthanized with a lethal dose of tricaine methanesulfonate (MS-222), placed on ice in the field, subsequently frozen, and transferred to the Colorado State University Fisheries Ecology Laboratory in Fort Collins, Colorado, where otoliths were extracted.

Special circumstances allowed us to test the effectiveness of otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  for tracing origins of reservoir fish emigrating into rivers in the Yampa River – Elkhead Reservoir system. In this system, investigators have been Floy-tagging smallmouth bass in the river and transplanting some of them (hereafter, “transplants”) into Elkhead Reservoir in a tributary to the Yampa River since 2003 (Hawkins et al. 2009). Some of these transplanted fish subsequently escaped back into the Yampa River and were recaptured. Others, that had been tagged and released into the Yampa River were recaptured in the Yampa River (hereafter, “residents”). We examined  $^{87}\text{Sr}/^{86}\text{Sr}$  from fishes exhibiting each of these known transplant and resident capture histories.

### Otolith preparation

Sagittal otoliths were removed with nonmetallic forceps and sonicated in Milli-Q water for 5 min. Left otoliths were used for all  $^{87}\text{Sr}/^{86}\text{Sr}$  analytical work; right otoliths were only used if the left otolith was broken or lost. Otoliths were embedded in Epo-Fix epoxy prior to being sectioned through the core in a transverse plane on an Isomet low-speed saw. Sections were sanded to a thickness of approximately 200–250  $\mu\text{m}$  to expose the inner annuli, mounted onto glass slides using double-sided tape, washed with 5% ultrapure nitric acid, and sonicated in ultrapure water for 5 min. Mounted otoliths were dried for 24 h under a Class 100 laminar flow hood and subsequently enclosed in polystyrene Petri dishes prior to ablation analysis.

### Strontium isotope analysis

Otolith thin sections were assayed for  $^{87}\text{Sr}/^{86}\text{Sr}$  using a Thermo Finnigan Neptune multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) coupled to a New Wave Research UP 193 nm excimer laser ablation system at the Woods Hole Oceanographic Institution (WHOI) Plasma Mass Spectrometry Laboratory in Woods Hole, Massachusetts. The laser ablation MC-ICP-MS was configured to run at 80% intensity, 10 Hz pulse rate, 35  $\mu\text{m}$  laser beam spot size, 7  $\mu\text{m}\cdot\text{s}^{-1}$  laser scan speed, and 550  $\mu\text{m}$  laser ablation distance within annuli. Using this method, ablated otolith material was carried from the laser cell to the MC-ICP-MS via helium gas, where it was then mixed in a spray chamber with argon gas and a wet aerosol, at which time a suite of isotopes were measured:  $^{83}\text{Kr}$ ,  $^{84}\text{Sr}$ ,  $^{85}\text{Rb}$ ,  $^{86}\text{Sr}$ ,  $^{87}\text{Sr}$ ,  $^{88}\text{Sr}$ .

Spots on otoliths were ablated within annuli to obtain year-specific  $^{87}\text{Sr}/^{86}\text{Sr}$ . For reservoir fishes, each otolith was ab-

**Table 1.** Characteristics of reservoirs from which fish were sampled for otolith  $^{87}\text{Sr}/^{86}\text{Sr}$ .

Reservoir	Reservoir code	Stream impounded	Surface elevation (m)	Surface area (ha)	Volume (ha-m)	Max. depth (m)	Mean HRT (years)	CV HRT
Harvey Gap	HGR	NA	1 951	116	723	12.5	0.54	0.13
Rifle Gap	RGR	Rifle Creek	1 817	145	13	26.5	0.53	0.34
McPhee	MCP	Dolores River	2 110	1 809	47 020	85.3	1.70	0.75
Flaming Gorge	FGR	Green River	1 841	17 005	467 354	133.0	4.14	0.42
Red Fleet	RFR	Brush Creek	1 709	210	3 210	44.0	1.15	0.60
Starvation	STA	Strawberry River	1 741	1 117	20 081	47.2	1.53	0.39
Crawford	CRA	Smith Fork Creek	1 997	164	1 776	36.6	0.73	0.24
Junjata	JUR	NA	1 740	58	847	16.5	1.31	0.05
Paonia	PAO	North Fork Gunnison River	1 965	135	2 584	42.7	0.31	0.64
Kenney	KER	White River	1 609	243	1 702	21.6	0.03	0.31
Rio Blanco Lake	RBR	NA	1 754	47	128	5.5	—	—
Lake Catamount	CAT	Yampa River	2 142	214	915	13.0	0.06	0.33
Elkhead	ELK	Elkhead Creek	1 952	364	3 152	17.7	0.48	0.28
Stagecoach	SCR	Yampa River	2 196	316	4 104	39.6	0.86	0.62

**Note:** NA, not applicable, some reservoirs were off channel; HRT, hydraulic residence time; CV, coefficient of variation. No data on HRT available for Rio Blanco Reservoir.

**Table 2.** Sampling summary of fishes collected from reservoirs and rivers in the Upper Colorado River Basin, 2004–2009.

Location	Species	No. of fish collected per year						Total collected	Mean total length (mm)	Mean age (years)
		2004	2005	2006	2007	2008	2009			
<b>Reservoirs</b>										
CAT	NPK	—	—	—	5	—	—	5	463	3
CRA	BCR	—	—	—	5	—	—	5	196	4
CRA	NPK	—	—	—	—	5	—	5	816	5
ELK	NPK	—	—	—	—	5	—	5	334	3
FGR	BBT	—	—	—	—	6	—	6	453	3
FGR	SMB	—	—	—	8	—	—	8	210	5
HGR	BCR	—	—	—	6	—	—	6	215	8
HGR	LMB	—	—	—	3	—	—	3	329	4
HGR	NPK	—	—	—	5	—	—	5	652	4
HGR	SMB	—	—	—	3	—	—	3	321	5
JUR	SMB	—	—	—	5	—	—	5	241	4
JUR	WAL	—	—	—	5	—	—	5	206	1
KER	BCR	—	—	—	—	5	—	5	184	5
MCP	SMB	—	—	5	—	3	—	8	262	5
MCP	WAL	—	—	—	5	1	—	6	301	2
PAO	NPK	5	—	—	—	—	—	5	312	4
RBR	BCR	—	—	—	5	—	—	5	190	6
RBR	LMB	—	—	—	8	—	—	8	247	5
RFR	WAL	—	—	—	—	—	5	5	410	2
RGR	BCR	—	—	—	3	1	—	4	157	4
RGR	WAL	—	—	1	1	1	—	3	534	14
SCR	NPK	—	—	—	8	—	—	8	542	4
SCR	WAL	—	—	3	—	—	2	5	449	5
STA	SMB	—	—	—	5	—	—	5	284	6
STA	WAL	—	—	—	5	—	—	5	305	3
Sum		5	0	9	85	27	7	133		
Mean									345	5
<b>Rivers</b>										
COR	SMB	—	4	—	—	—	—	4	261	4
COR	WAL	—	—	5	2	—	—	7	525	16
DOL	SMB	—	—	—	5	—	5	10	182	3
DUC	SMB	—	—	—	—	—	5	5	238	6
GRU	NPK	—	—	—	3	—	4	7	523	3
GRU	SMB	—	—	—	5	—	—	5	324	5
GRU	WAL	—	—	—	2	—	11	13	520	7
WHB	SMB	—	—	—	3	—	—	3	248	2
YAR	NPK	2	—	1	6	—	1	10	586	5
YAR	SMB*	5	—	—	—	1	—	6	182	2
YAR	SMB <sup>†</sup>	—	—	—	9	—	—	9	362	7
YAR	SMB <sup>‡</sup>	—	—	—	—	3	—	3	353	6
Sum		7	4	6	35	4	26	82		
Mean									359	6

**Note:** Refer to Table 1 for reservoir codes. Species codes: BCR, black crappie; BBT, burbot; LMB, largemouth bass; NPK, northern pike; SMB, smallmouth bass; WAL, walleye. River codes: COR, Colorado River; DOL, Dolores River; DUC, Duchesne River; GRU, Upper Green River; WHB, White River; YAR, Yampa River.

\*Untagged, unknown history smallmouth bass.

<sup>†</sup>Tagged smallmouth bass with known residence within the Yampa River.

<sup>‡</sup>Tagged smallmouth bass transplanted into Elkhead Reservoir.

lated within the outermost annulus (hereafter, “edge”) and the innermost annulus (hereafter, “core”). All core ablations were outside the primordium to minimize potential developmental or maternal influence on the otolith’s <sup>87</sup>Sr/<sup>86</sup>Sr (Chittaro et al. 2006; Macdonald et al. 2008). For river-caught fishes, each

otolith (as above) was ablated at the core and edge. In addition, if the core and edge <sup>87</sup>Sr/<sup>86</sup>Sr were >150 ppm different, then spots in additional annuli between the core and edge were ablated to establish the year when the change occurred. That year was presumed to be the time when the fish moved

from a reservoir to the river. We used a compound light microscope to determine fish ages from otolith annuli and from capture date, the year corresponding with within-annulus ablations.

According to Woodhead et al. (2005), interference on Sr isotopes can be caused by calcium (Ca) argides, Ca dimers, and doubly charged erbium (Er) and ytterbium (Yb). However, it has been demonstrated that rubidium (Rb) and krypton (Kr) isotopes create the main interferences in other  $^{87}\text{Sr}/^{86}\text{Sr}$  laser ablation ICP-MS analyses (Barnett-Johnson et al. 2005; Jackson and Hart 2006; Walther et al. 2008). We adjusted for Kr and Rb interferences following the techniques described by Jackson and Hart (2006) and Walther et al. (2008), respectively. All otolith samples and standards were normalized to a daily mean of the National Institute of Standards and Technology (NIST) standard reference material 987 (SRM 987; accepted  $^{87}\text{Sr}/^{86}\text{Sr}$  of 0.71024) using the following formula:  $^{87}\text{Sr}/^{86}\text{Sr}_{\text{normalized}} = (0.71024 \div \text{SRM } 987_{\text{measured}}) \times ^{87}\text{Sr}/^{86}\text{Sr}_{\text{sample}}$ . Dissolved otolith certified reference material (CRM; accepted  $^{87}\text{Sr}/^{86}\text{Sr}$  of 0.70918; Yoshinaga et al. 2000) and SRM 987 produced daily mean ( $\pm 1$  standard deviation, SD; sample size)  $^{87}\text{Sr}/^{86}\text{Sr}$  of 0.70916 ( $\pm 0.00001$ ;  $n = 5$ ) and 0.71029 ( $\pm 0.00006$ ;  $n = 9$ ), respectively, and ablations of marine sclerosponge produced a daily mean  $^{87}\text{Sr}/^{86}\text{Sr}$  of 0.70918 ( $\pm 0.00003$ ;  $n = 4$ ).

### Statistical analysis

Based on analysis suggestions provided by Singer and Willet (2003), we visually examined three bivariate plots to assess the importance of subbasin, reservoir, species, and year as factors affecting variation in  $^{87}\text{Sr}/^{86}\text{Sr}$ . A plot of  $^{87}\text{Sr}/^{86}\text{Sr}$  from each reservoir was examined to determine differences among subbasins and reservoirs; each plot shows the distribution of mean core and edge  $^{87}\text{Sr}/^{86}\text{Sr}$  for each individual fish per location. A second plot of mean  $^{87}\text{Sr}/^{86}\text{Sr}$  of reservoirs over time — by taking the average  $^{87}\text{Sr}/^{86}\text{Sr}$  from all ablations corresponding to a given year, including all species — was examined for interannual trends and compared among reservoirs within subbasins. Finally, a third plot of  $^{87}\text{Sr}/^{86}\text{Sr}$  among species within reservoirs was also examined — generated by averaging core and edge  $^{87}\text{Sr}/^{86}\text{Sr}$  of each individual and then averaging these values for all individuals of a given species.

Following our visual assessment, we performed a statistical analysis in three phases using mixed regression models (PROC MIXED, SAS, SAS Institute Inc., Cary, North Carolina). Our analysis strategy was based on suggestions provided by Singer and Willet (2003) and Burnham and Anderson (2002; see also Breton et al. 2008). We specified the full maximum likelihood estimation option in PROC MIXED to accommodate phase 3, an Akaike information criterion (AIC) analysis (Burnham and Anderson 2002) involving competing models and different fixed effects. We assessed normality of the  $^{87}\text{Sr}/^{86}\text{Sr}$  for each reservoir using box-and-whisker plots and conditional Studentized residuals diagnostic plots. Diagnostic plots did not reveal any major departures from normality. Additionally, Shapiro–Wilk tests (PROC UNIVARIATE, SAS, SAS Institute Inc., Cary, North Carolina) failed to reject normality of Studentized residuals of  $^{87}\text{Sr}/^{86}\text{Sr}$  from each reservoir, with the exception of Flam-

ing Gorge Reservoir ( $p = 0.02$ ); therefore, no data transformations were performed.

In the first phase of our regression analysis, we considered two models fitted with only one fixed effect, an intercept, and up to two random effects. The purpose of this phase of our analysis was to determine important sources of variance (variance components) in  $^{87}\text{Sr}/^{86}\text{Sr}$ . In the first model we fitted a random effect for each individual fish (hereafter, “FishID”), as fish could contribute data points from multiple ablations (one per annuli, core and edge). In the second model we again included FishID as a random effect, but this time we added a second random effect (hereafter, “Res  $\times$  Spp  $\times$  Year”) consisting of all unique combinations of reservoir, species, and year. The two random effects models were ranked using Akaike’s information criterion adjusted for small sample size ( $\text{AIC}_c$ ). The best random effects structure was the model that included the Res  $\times$  Spp  $\times$  Year random effects (more below). The random effects structure from this model was maintained in all fixed effects models considered in subsequent analyses (phases 2 and 3).

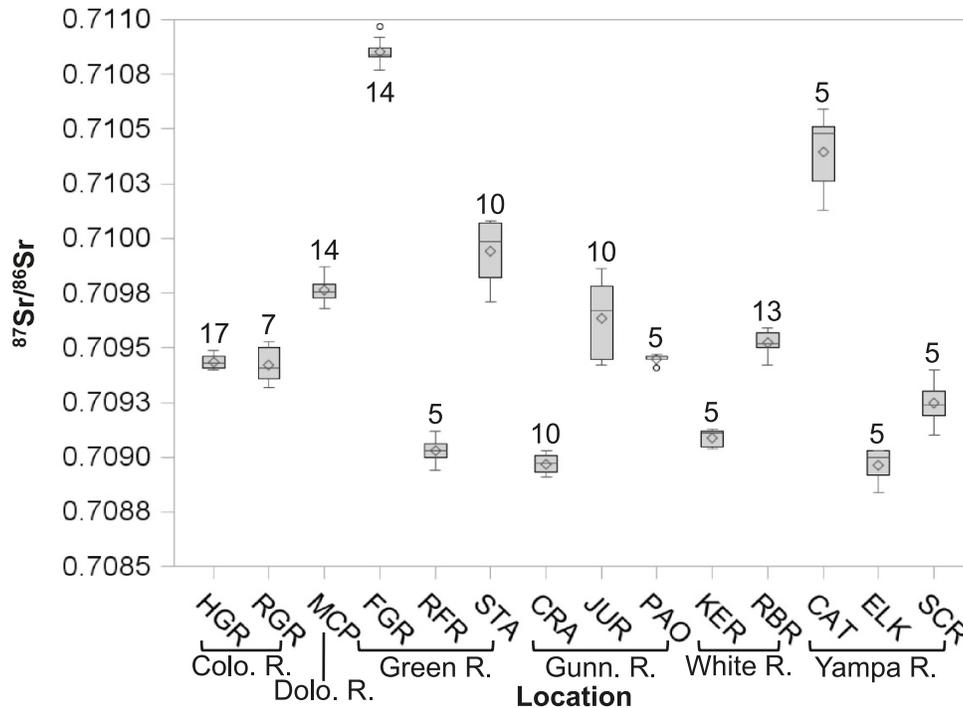
In the second phase of our analysis, we separately estimated the variance explained by each fixed effect (reservoir, species, and year) as the proportional change in the total variance when each fixed effect was added to the Res  $\times$  Spp  $\times$  Year model from phase 1. The approach, deploying variance components, enabled us to determine the amount of variation explained by each of our fixed effects (Singer and Willet 2003).

In the third phase we evaluated, using  $\text{AIC}_c$  and associated statistics, a suite of models encompassing all possible combinations of our fixed effects: reservoir, species, and year. Post hoc, we also assessed a dichotomous species effect (DSE) where walleye  $^{87}\text{Sr}/^{86}\text{Sr}$  was specified as different than all other species using a 0 or 1 dichotomous variable (0 = non-walleye, 1 = walleye). Additionally, we included a reservoir  $\times$  DSE interaction effect because observed differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  between walleye and other fishes were not consistent among reservoirs in our bivariate species plot (phase 1). To determine relative support for each model, delta  $\text{AIC}_c$  ( $\Delta_i$ ) and Akaike weights ( $w_i$ ) were calculated for each model  $i$  (Burnham and Anderson 2002). Delta  $\text{AIC}_c$  can be thought of as the amount of information lost (about the true process that produced the data) relative to the top-ranked model, where the top (or best) model has the lowest  $\text{AIC}_c$  value:  $\Delta_i = \text{AIC}_{c(i)} - \text{AIC}_{c(\text{top})}$ . Akaike weights or model probabilities, calculated using model likelihoods, sum to one across the full model set and thus provide relative support for each model (Burnham and Anderson 2002). To determine the relative importance of each fixed effect, cumulative Akaike weights ( $W_{+(j)}$ ) were calculated for all fixed effects by summing the  $w_i$  from each model that contained each respective fixed effect.

### Origins of river-caught fishes

In the absence of known histories from tagging that were available only from the Yampa River – Elkhead Creek system mentioned previously, we compared predictions of each reservoir’s  $^{87}\text{Sr}/^{86}\text{Sr}$  from the top-ranked model (phase 3 from above; reservoir and dichotomous species fixed effects with interaction) with the measured core and edge  $^{87}\text{Sr}/^{86}\text{Sr}$  of river-captured fish to assess their possible origins (i.e.,

**Fig. 2.** Box-and-whisker plots of  $^{87}\text{Sr}/^{86}\text{Sr}$  among reservoirs in six subbasins of the Upper Colorado River Basin, including all species and years. Each box-and-whisker plot shows the distribution of  $^{87}\text{Sr}/^{86}\text{Sr}$  for each individual's mean core and edge value per location. The bottom and top edges are the 25th and 75th percentiles, respectively, the middle line is the median, the diamond symbol within the box is the mean, and the whiskers contain values that are within 1.5 times the interquartile range. The number above or below each box plot is the total number of fishes ablated from each location. Refer to Table 1 for reservoir codes.



river resident or reservoir escapee). There were two prediction interval estimates in the top-ranked model: one estimate for walleye, when sampled from a reservoir (i.e., not all reservoirs had a predicted walleye  $^{87}\text{Sr}/^{86}\text{Sr}$ ), and the other estimate for all other species. These comparisons assumed that (i) reservoir emigrants possessed a core  $^{87}\text{Sr}/^{86}\text{Sr}$  similar to a reservoir's signature unless it emigrated at a very early age before accreting a detectable reservoir signature; and (ii) river-caught reservoir emigrants exhibited a river signature in their otolith edge that differed from the reservoir signature in their core unless it emigrated shortly before capture. Since many of the river-caught fishes were collected in early summer (June and July), otolith growth in that year would likely be too narrow to accommodate the ablation laser's beam diameter. Thus, when the edge was too narrow, edge signatures measured by the ablation may actually reflect the fish's environment in the year prior to capture. This complication notwithstanding, if the core  $^{87}\text{Sr}/^{86}\text{Sr}$  of a river-caught fish was within the 95% confidence limits of the predicted estimates (top-ranked model, phase 3) of an upstream reservoir's signature, we concluded that the fish could have moved to the river from that reservoir. In circumstances where river-caught fishes had overlapping  $^{87}\text{Sr}/^{86}\text{Sr}$  with the upstream reservoir for their entire lifetime (core and edge), we assumed that these fish could have recently moved from the reservoir. However, with the exception of tagged fish in the Yampa River, we were unable to conclusively determine if fish we classified as "recently moved" were instead river residents from an environment that shared the same  $^{87}\text{Sr}/^{86}\text{Sr}$  as the upstream reservoir. Especially problematic were those cases where a river-caught fish was sampled below a main-

stem dam, such as smallmouth bass caught in the Green River below Flaming Gorge Dam.

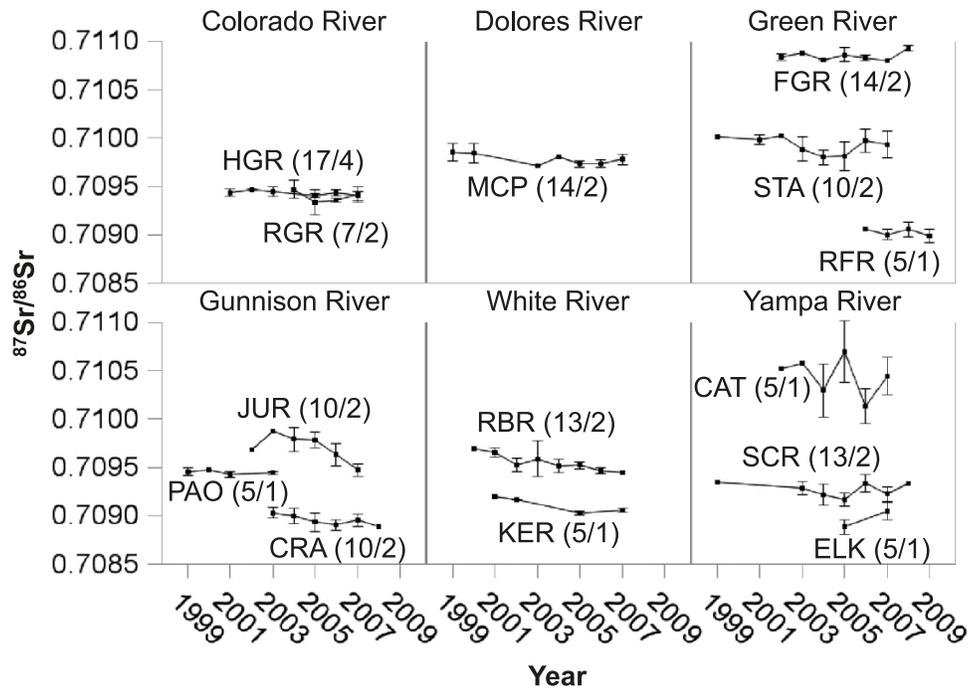
## Results

### Reservoir analysis

With the exception of the Colorado River subbasin, reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$  were not visibly clustered within subbasins (Fig. 2). Consistent with this result, our visual analysis of bivariate plots suggested that the fixed effect of subbasin was not statistically or biologically significant (i.e., variation in  $^{87}\text{Sr}/^{86}\text{Sr}$  was a function of reservoir with no effect of subbasin). Given these observations, subbasin effects were excluded from the regression analysis. Reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$  averaged across all individuals were distinct, particularly within subbasins (Fig. 2). By averaging all ablations in a given year in each reservoir, we found that annual variation in reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$  was small in comparison with the differences we observed between reservoirs in the same subbasin (Fig. 3). Lake Catamount showed the most annual variability of any reservoir in our study; however, Lake Catamount's signature still remained distinct from the other reservoirs (Elkhead and Stagecoach) in the Yampa River subbasin. When we visually examined possible species effects within reservoirs, only one species (walleye) showed consistent differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  compared with the others (Fig. 4). These differences were observed in Juniata and Starvation reservoirs (Fig. 4).

Of the two random effects models examined (Table 3, part i), the top-ranked model included the random Res  $\times$

**Fig. 3.** Mean  $^{87}\text{Sr}/^{86}\text{Sr} \pm 2$  standard errors (SE) of reservoirs in the Upper Colorado River Basin, 1999–2009. Each point on a line represents the average  $^{87}\text{Sr}/^{86}\text{Sr}$  from all ablations corresponding to a given year, including all species; core and edge signatures were not averaged for each individual, since they occur in different years. Reservoirs were grouped by river subbasin, shown in the upper part of each cell. Refer to Table 1 for reservoir names. The numbers next to each reservoir code refer to the number of fish collected and the number of species included, respectively.



Spp  $\times$  Year and FishID effects ( $\text{AIC}_c = -4524.20$ ;  $w_i = 1.00$ ). The competing model with only the FishID random effect had no support ( $\Delta_i = 114.60$ ;  $w_i = 0.00$ ). We maintained the random effects from the top model in our subsequent analyses. Total variance in our top random effects model was reduced by 96%, 50%, and 5% with the addition of reservoir, species, and year, respectively (Table 4).

From our analysis of fixed effects (Table 3, part *ii*), the top-ranked model included the fixed effects of reservoir and species. This model attained virtually all of the model weight ( $\text{AIC}_c = -4922.60$ ;  $w_i = 0.98$ ). The remaining models had delta  $\text{AIC}_c$  values  $> 7$  and the sum of the weights for these models was 0.02 or 2%. Thus, given the data, our analysis of fixed effects supported only one model, the model with reservoir and species.

Cumulative Akaike weights for reservoir, species, and year effects were 1.00, 1.00, and 0.02, respectively (Table 3, part *ii*). These results indicate that the effect of reservoir, which was also in the only model supported in our analysis of fixed effects, was strongly supported by the data. Although species had an equivalent cumulative weight as reservoir, this effect nonetheless performed poorly in models that did not include reservoir (best species model that did not include reservoir,  $\Delta_i \geq 361.40$ ). The cumulative weight for year is strong evidence that  $^{87}\text{Sr}/^{86}\text{Sr}$  varied independent of year.

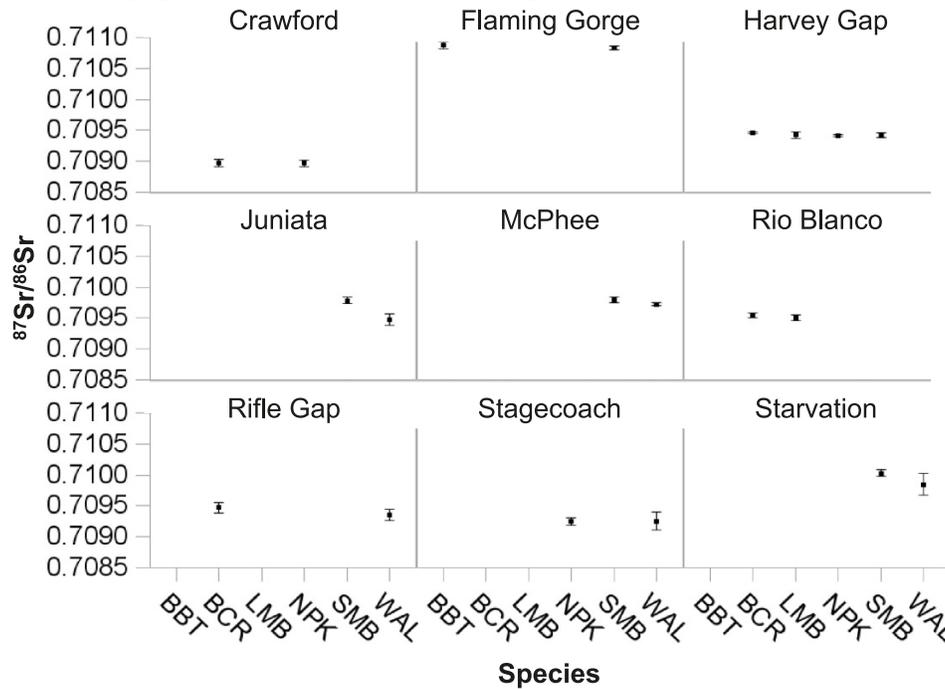
Our visual assessment suggested that walleye was the only species that showed differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  compared with other species. This suggests an alternative and more parsimonious structure for the species effect that is likely more consistent with the data: namely, a dichotomous species effect

(DSE), where walleye differs from all other species examined rather than all species are different. Since the observed differences with walleye appeared in only two of five reservoirs containing walleye (Juniata and Starvation reservoirs), we also added a model with a reservoir and species interaction term. When the DSE models were added to the same set of models in phase 2, the top-ranked model was reservoir and DSE fixed effects with interaction ( $\text{AIC}_c = -4940.70$ ;  $w_i = 0.99$ ). All of the other models had a  $\Delta_i > 10$  and a combined  $w_i$  of 0.01 (Table 3, part *iii*). Thus, only the top model was supported by the data, and the species effect was largely driven by differences in walleye compared with all other species. In summary, our visual and statistical analyses suggested that the effect of year was negligible compared with reservoir and a dichotomous species effect.

### Origins of river-caught fishes

Change in  $^{87}\text{Sr}/^{86}\text{Sr}$  of smallmouth bass transplanted from the Yampa River into Elkhead Reservoir ( $n = 3$ ) was detected in each case (Fig. 5). These fish also allowed us to evaluate transitional dynamics of the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature. A fish moved from the river and residing for just 2 months in the reservoir had an  $^{87}\text{Sr}/^{86}\text{Sr}$  between those of the two locations; and a transplanted fish residing in the reservoir for 5 months also had an intermediate signature (Fig. 5). We believe the intermediate  $^{87}\text{Sr}/^{86}\text{Sr}$  was due to the intrusion of the river signature into measurements when the laser ablated portions of the otolith that included periods of both river and reservoir occupancy. The  $^{87}\text{Sr}/^{86}\text{Sr}$  of a fish that resided in the reservoir for 26 months overlapped the reservoir's  $^{87}\text{Sr}/^{86}\text{Sr}$ , implying that a fish would need to reside in a new loca-

**Fig. 4.** Mean  $^{87}\text{Sr}/^{86}\text{Sr} \pm 95\%$  confidence limit among species within reservoirs. Means were generated by averaging core and edge  $^{87}\text{Sr}/^{86}\text{Sr}$  of each individual and then averaging these values for all individuals of a given species. Refer to Table 2 for species codes.



tion for  $>5$  and  $\leq 26$  months to acquire a pure  $^{87}\text{Sr}/^{86}\text{Sr}$  of that location, given our methods.

Ablation time series of untagged (unknown history) smallmouth bass captured in the Yampa River (Fig. 5) provided evidence that these fish originated (core) and resided (edge) from somewhere within the Yampa River itself and not from Elkhead Reservoir, a known source of smallmouth bass escapement into the upper basin (Hawkins and Nesler 1991; Nesler 1995; Hawkins et al. 2009). Core  $^{87}\text{Sr}/^{86}\text{Sr}$  of all untagged, river-caught smallmouth bass were well outside the 95% prediction limits of the mean Elkhead Reservoir signature using the top-ranked model (analysis, phase 3) and did not overlap with any other reservoir signature from our analysis. Consistency in the signatures from these river-caught fish, which were captured from different years and sections of the Yampa River, provides evidence that otoliths from these fish record the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature of the Yampa River itself rather than reservoirs and other off-channel water bodies not included in our analysis.

Two of 10 northern pike caught in the Yampa River had core  $^{87}\text{Sr}/^{86}\text{Sr}$  that fell within the 95% confidence limits of the Lake Catamount  $^{87}\text{Sr}/^{86}\text{Sr}$  (0.71035–0.71049), a reservoir located within the main stem of the Yampa River and a known source of northern pike escapement (Finney and Haines 2008). Given its mainstem status, not surprisingly the Lake Catamount  $^{87}\text{Sr}/^{86}\text{Sr}$  was similar to the  $^{87}\text{Sr}/^{86}\text{Sr}$  of smallmouth bass known to have been residing downstream in the Yampa River. Therefore, it is possible that these 2/10 fish originated from within the Yampa River itself below Lake Catamount. Of the remaining eight northern pike examined, four had core  $^{87}\text{Sr}/^{86}\text{Sr}$  that were most consistent with several ponds in that region (R.M. Fitzpatrick, Colorado Parks and Wildlife, Fort Collins, Colorado, unpublished data, 2009), and those remaining were slightly outside the

predicted 95% confidence limits of Lake Catamount's signature and may have come from that source or the Yampa River downstream of Lake Catamount. Taking into consideration the standard error associated with each ablation's  $^{87}\text{Sr}/^{86}\text{Sr}$ , three of the four previously mentioned fish would have overlapped with the Lake Catamount  $^{87}\text{Sr}/^{86}\text{Sr}$  prediction interval.

In the Colorado River subbasin, all of the river-caught smallmouth bass that we examined ( $n = 4$ ), sampled in different years and reaches of the river, had overlapping signatures that fell well outside of reservoir signatures from our study, suggesting that these fish carried the signature of the Colorado River in this region. In contrast, all walleye ( $n = 7$ ) appeared to have emigrated from a suspected source, Rifle Gap Reservoir, where four of seven otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  were within the 95% confidence limits of Rifle Gap's  $^{87}\text{Sr}/^{86}\text{Sr}$  (Fig. 6). The core  $^{87}\text{Sr}/^{86}\text{Sr}$  of the remaining fish were just outside the predicted Rifle Gap Reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$ , but subsequent growth bands fell within Rifle Gap's predicted signature for all but one case, until the fish appeared to move from the reservoir to the Colorado River (Fig. 6). Additionally, when considering the standard error of each ablation's  $^{87}\text{Sr}/^{86}\text{Sr}$ , all Colorado River walleye would have been classified to Rifle Gap Reservoir as a source location. Examination of the time series of  $^{87}\text{Sr}/^{86}\text{Sr}$  of individual walleye showed the time of movement occurred during 1996–1998, a period with unusually high flows in the Colorado River subbasin (Fig. 6).

In the Green River subbasin, four of five smallmouth bass captured from the Duchesne River were classified as escapees from Starvation Reservoir, a known source of smallmouth bass and walleye escapement (Brunson et al. 2007), based on otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$ . However, three of four edge

**Table 3.** Model ranking results using Akaike's information criterion (AIC) for reservoir fishes.

Model	AIC <sub>c</sub>	$\Delta_i$	ML <sub>i</sub>	w <sub>i</sub>	W <sub>+(j)</sub>
<b>(i) Random effects with a fixed intercept.</b>					
Res × Spp × Year*	-4524.20	0.00	1.00	1.00	—
FishID	-4409.60	114.60	0.00	0.00	—
<b>(ii) Top-ranked random effects model with all combinations of fixed effects.</b>					
Res; Spp	-4922.60	0.00	1.00	0.98	—
Res; Spp; Year	-4914.90	7.70	0.02	0.02	—
Res	-4909.60	13.00	0.00	0.00	1.00
Res; Year	-4905.70	16.90	0.00	0.00	—
Spp; Year	-4561.20	361.40	0.00	0.00	—
Intercept	-4524.20	398.40	0.00	0.00	—
Spp	-4515.90	406.70	0.00	0.00	1.00
Year	-4504.10	418.50	0.00	0.00	0.02
<b>(iii) DSE in place of "all species are different effect".</b>					
Res × DSE <sup>†</sup>	-4940.70	0.00	1.00	0.99	—
Res; DSE	-4930.30	10.40	0.01	0.01	—

**Note:** (i) Analysis of random effects with a fixed intercept. Model Res × Spp × Year is for all possible combinations of species and year-specific strontium isotope ratios (<sup>87</sup>Sr/<sup>86</sup>Sr) in each reservoir. Model FishID is for individual fishes with multiple measures. (ii) Top-ranked random effects model with all combinations of fixed effects reservoir (Res), species (Spp), and year. (iii) Dichotomous species effect (DSE; 0 = non-walleye, 1 = walleye) added in place of the "all species are different effect" (Spp).  $\Delta_i$ , delta AIC<sub>c</sub>; ML<sub>i</sub>, maximum likelihood; w<sub>i</sub>, Akaike weight; and W<sub>+(j)</sub>, cumulative Akaike weight.

\*Model included the FishID random effect.

<sup>†</sup>Model included reservoir and DSE species fixed effects.

**Table 4.** Results from variance components analysis for each fixed effect from otolith <sup>87</sup>Sr/<sup>86</sup>Sr analysis.

Fixed effects	Percentage of total variance (%)				Total variance decrease (%)
	FishID random effect	Res × Spp × Year random effect	Residual	Total variance	
Reservoir	24	31	45	1.22×10 <sup>-8</sup>	96
Species	93	3	4	1.40×10 <sup>-7</sup>	50
Year	1	97	2	2.66×10 <sup>-7</sup>	5
Intercept	1	97	2	2.79×10 <sup>-7</sup>	—

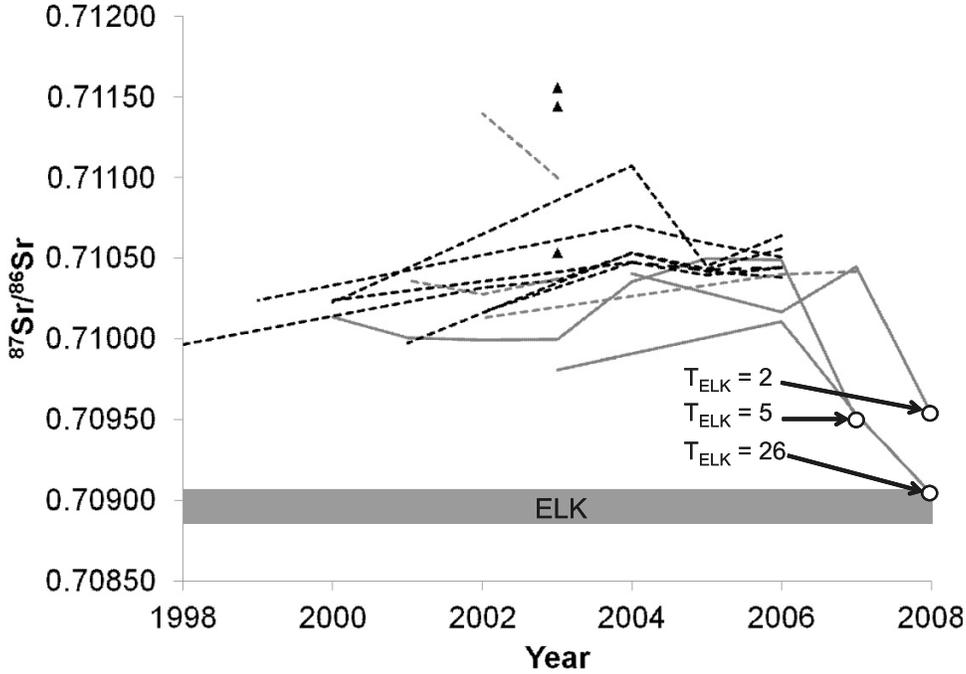
<sup>87</sup>Sr/<sup>86</sup>Sr were also consistent with the Duchesne River, suggesting that these two potential sources may be too similar to distinguish. In the Upper Green River, one of five smallmouth bass was classified as having originated from Flaming Gorge Reservoir (Fig. 7). Note, however, that smallmouth bass collected from the Yampa River have <sup>87</sup>Sr/<sup>86</sup>Sr similar to the isotope ratios of smallmouth bass collected from the Upper Green River. Therefore, we cannot rule out the possibility that these fish came from the Yampa River, a known source of smallmouth bass moving into Green River (Hawkins et al. 2009). Another smallmouth bass had an <sup>87</sup>Sr/<sup>86</sup>Sr profile that indicated a source not measured in our study.

Northern pike captured from the Upper Green River had very low classification rates; two of seven were classified to Flaming Gorge and Starvation reservoirs, but neither of these locations had known populations of northern pike. This suggests that most of the northern pike examined emigrated from a location outside the Green River subbasin. It is likely that these fish originated from the Yampa River subbasin, where northern pike are much more abundant, and Floy tagging work showed that such movements do occur (Martin et al.

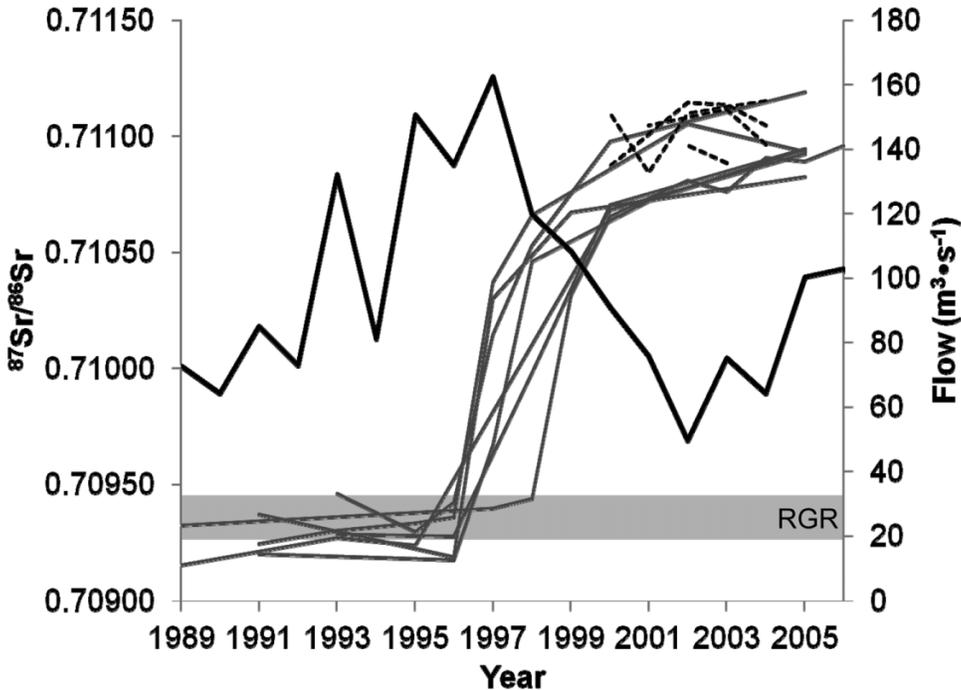
2010). When considering potential sources from the Yampa River subbasin, four of seven pike had an ablation that matched Lake Catamount before the edge portion of the otolith. The remaining three northern pike had <sup>87</sup>Sr/<sup>86</sup>Sr indicating a source not sampled in this study.

All of the walleye examined ( $n = 13$ ) from the Upper Green River showed a clear trend suggesting emigration from a reservoir source. Eight of 13 walleye from the Upper Green River were classified to Starvation Reservoir, a known source of escapement of nonnative piscivores into the upper basin (Brunson et al. 2007), using the predicted <sup>87</sup>Sr/<sup>86</sup>Sr of walleye from the top-ranked model from phase 3 of the analysis, and the remaining individuals fell just outside that range (Fig. 7). When taking into account the standard error of each ablation's <sup>87</sup>Sr/<sup>86</sup>Sr, all but two walleye would have been classified to Starvation Reservoir as a source location. However, as stated above, Starvation Reservoir and the Duchesne River share similar <sup>87</sup>Sr/<sup>86</sup>Sr, so it is possible that these walleye originated from the Duchesne River. Examination of river flows just upstream of Starvation Reservoir showed some evidence that most of the apparent escapement from

**Fig. 5.** Otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  profiles of smallmouth bass collected from the Yampa River, Colorado. Solid gray lines are fish that were captured from the Yampa River and transplanted into Elkhead Reservoir, where they were finally recaptured. Black dashed lines show fish that were tagged and released within the Yampa River and had spent at least 1 year in the river prior to capture, 2004–2007. The dashed gray lines and triangles are untagged fish. Each fish that was tagged and relocated to Elkhead Reservoir is highlighted with an open circle.  $T_{\text{ELK}}$  refers to the number of months spent in Elkhead Reservoir after transfer to the latest otolith ablation. The gray bar represents the 95% confidence interval of the Elkhead Reservoir signature determined by predictions from the top-ranked model (analysis, phase 3).

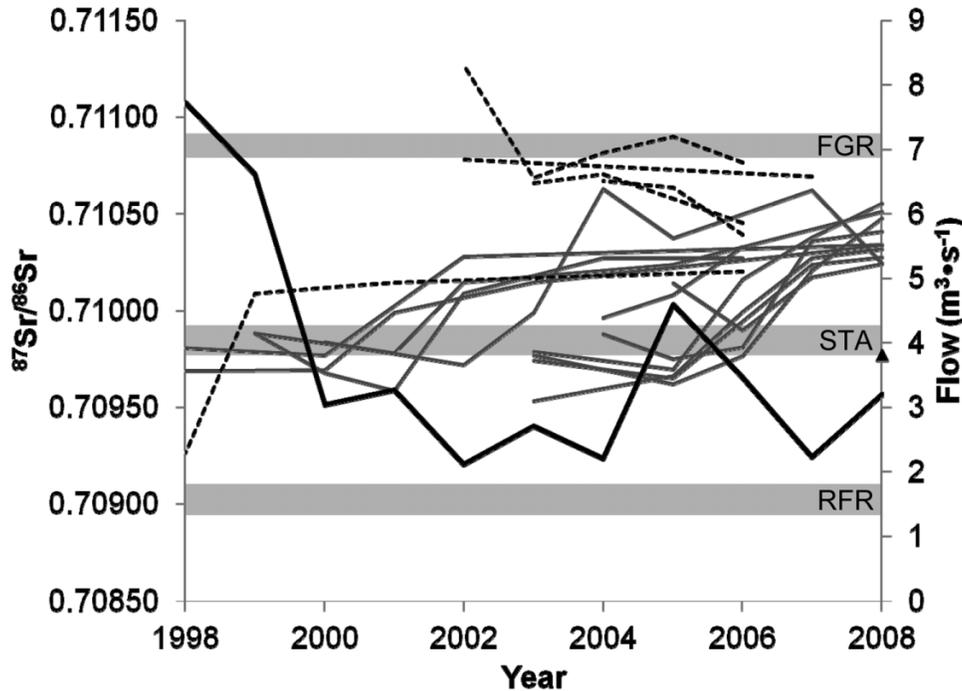


**Fig. 6.** Otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  profiles of four smallmouth bass (dashed lines) and seven walleye (solid gray lines) collected from the Colorado River, collected downstream of Rifle Gap Reservoir near Rulison, Colorado. The gray bar represents the 95% confidence interval of the Rifle Gap Reservoir signature of walleye determined by predictions from the top-ranked model (analysis, phase 3). The solid black line is the hydrograph of the Colorado River taken from US Geological Survey stream gauge station 09095500 near Cameo, Colorado, approximately 80 km downstream from Rifle Gap Reservoir.



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**Fig. 7.** Otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  profiles of five smallmouth bass (dashed lines) and 13 walleye (solid gray lines and triangle) collected from the Upper Green River, Utah. The gray bars represent the 95% confidence intervals of each reservoir signature determined by predictions from the top-ranked model (analysis, phase 3). The Starvation Reservoir bar is the modeled prediction of walleye from that reservoir. The solid black line is the hydrograph of the Strawberry River taken from US Geological Survey stream gauge station 09288180 near Duchesne, Utah, approximately 0.61 km upstream of Starvation Reservoir.



Starvation Reservoir occurred following a high flow event and concurrent high spill event over the unscreened Starvation Reservoir spillway (Fig. 7). However, some escapement still occurred during low flow periods, which suggests that either these fish escaped through an outlet that doesn't require reservoir spillover or they represent anthropogenic transplants.

In the Dolores and White River subbasins,  $^{87}\text{Sr}/^{86}\text{Sr}$  of the core and edge of river-caught smallmouth bass overlapped with the predicted  $^{87}\text{Sr}/^{86}\text{Sr}$  of upstream (main stem) reservoirs McPhee and Kenney Reservoirs, respectively, and are thus indistinguishable from these rivers. Nearby Rio Blanco Reservoir was not indicated, in our analysis, as an escapee source of smallmouth bass captured from the White River.

## Discussion

Given the general distinctiveness of reservoirs, temporal stability, and lack of interspecific effects in our findings, the  $^{87}\text{Sr}/^{86}\text{Sr}$  in otoliths showed great potential as a natural tracer for provenance of reservoir piscivores. Our research also provided evidence that some reservoirs are potentially important sources of invasive piscivores in the UCRB. This was particularly true for walleye, where in every river subbasin containing the species, we detected clear movement patterns from a likely reservoir source. This evidence suggests that walleye reproduction in these rivers may be limited and therefore that this species could be controlled in critical habitat by containment of reservoir sources. However, the relative contribution of potential reservoir escapees to riverine stocks has yet

to be determined. In some other cases we could not conclusively distinguish river from reservoir signatures, but the method was still useful for eliminating some potential sources, allowing managers to better focus their control efforts.

We found that interannual variability in the  $^{87}\text{Sr}/^{86}\text{Sr}$  of reservoir fish from most reservoirs was negligible, at least over the nearly decadal scale of our study. Models with a year term were not well supported, based on low cumulative Akaike weight and the variance components analysis. Temporal stability of reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$  is not surprising because reservoirs gather water from relatively large drainage areas, which dampen effects of hydro-climatic variation on their water storage and presumably water chemistry. Thus, reservoirs integrate spatial and temporal variation present in the characteristics of their inflows. The integrative nature of reservoirs along with our use of  $^{87}\text{Sr}/^{86}\text{Sr}$  rather than elemental markers such as Sr–Ca, which are known to exhibit more temporal variability (Bacon et al. 2004; Schaffler and Winkelman 2008; Walther and Thorrold 2009), likely accounts for the stability of reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures. The temporal stability of reservoir  $^{87}\text{Sr}/^{86}\text{Sr}$  has important implications for managers. Under the right circumstances, relatively few samples, even in a single year, would be necessary to characterize the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature of fish in potential source reservoirs, obviating the need to match known signatures from specific cohorts to unknown-history fishes (Schaffler and Winkelman 2008; Elsdon et al. 2008).

While we had less opportunity to evaluate the stability of river  $^{87}\text{Sr}/^{86}\text{Sr}$ , since river fishes were generally of unknown provenance,  $^{87}\text{Sr}/^{86}\text{Sr}$  of tagged smallmouth bass in the Yampa River had interannual variability comparable to reser-

voir temporal variability, suggesting that river  $^{87}\text{Sr}/^{86}\text{Sr}$  remain stable over many years. Additionally,  $^{87}\text{Sr}/^{86}\text{Sr}$  of Colorado River fishes in our study had edge signatures that were very similar to the  $^{87}\text{Sr}/^{86}\text{Sr}$  of water sampled from the Colorado River near Hoover Dam ( $0.71075 \pm 0.00003$ , 2SD) two decades earlier (Goldstein and Jacobsen 1987), providing further evidence that  $^{87}\text{Sr}/^{86}\text{Sr}$  fluctuates little over many years in some river–reservoir systems. These findings are consistent with some other studies utilizing  $^{87}\text{Sr}/^{86}\text{Sr}$  to determine the environmental history of fishes that inhabited freshwater locations. Kennedy et al. (2000) found minimal seasonal variation of  $^{87}\text{Sr}/^{86}\text{Sr}$  in streams and Atlantic salmon (*Salmo salar*) otoliths from tributaries of the Connecticut River. Barnett-Johnson et al. (2008) found that  $^{87}\text{Sr}/^{86}\text{Sr}$  of otoliths were similar to water  $^{87}\text{Sr}/^{86}\text{Sr}$  from the same locations collected many years earlier by Ingram and Weber (1999). However, Walther and Thorrold (2009) found significant differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  among years of American shad (*Alosa sapidissima*) otoliths from the Hudson and Pamunkey rivers in the eastern USA. Nonetheless, these among-year differences explained only 5%–19% of the total variability in  $^{87}\text{Sr}/^{86}\text{Sr}$  detected in the two rivers. Feyrer et al. (2007) also found interannual differences of  $^{87}\text{Sr}/^{86}\text{Sr}$ , but the effect of year had little impact on discrimination of natal locations of splittail (*Pogonichthys macrolepidotus*). While more investigation of river variability is needed in our study area, it appears that  $^{87}\text{Sr}/^{86}\text{Sr}$  remains relatively stable over many years, making annual location “fingerprint” updates unnecessary.

We found that  $^{87}\text{Sr}/^{86}\text{Sr}$  of piscivorous species within a reservoir did not differ, with one exception. In two out of five reservoirs,  $^{87}\text{Sr}/^{86}\text{Sr}$  of walleye was lower than that of sympatric species. The literature does not support differential fractionation among species as a plausible explanation (Blum et al. 2000; Bentley 2006). Regardless, any possible fractionation of  $^{87}\text{Sr}/^{86}\text{Sr}$  would be masked by the normalization of  $^{88}\text{Sr}/^{86}\text{Sr}$  (specified value of 8.375) used in this study to correct for instrumental fractionation (Faure and Powell 1972). Reasons for the disparity are unknown, but differential habitat use is likely responsible. If walleye inhabited particular areas of reservoirs not frequented by other species (e.g., the hypolimnion) and these areas differed in  $^{87}\text{Sr}/^{86}\text{Sr}$ , a disparity among species could arise. Dufour et al. (2007) suggested that spatial heterogeneity in  $^{87}\text{Sr}/^{86}\text{Sr}$  within lakes could explain the lack of correspondence between fish remains and ambient water  $^{87}\text{Sr}/^{86}\text{Sr}$ . Fortunately, even in cases where walleye signatures differed from other species in the same reservoir, classification rates of individual walleye to their correct location were very high. Overall, the strong concordance of otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  among species within a given reservoir contributes to the utility of this marker for fingerprinting potential source locations. In general,  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures of suspected immigrant fish could be compared with signatures of any species from source locations, making the job of fingerprinting source locations much easier. The substitutability of species for fingerprinting sources could also alleviate the need for lethal sampling of a protected species.

Estimating the expected  $^{87}\text{Sr}/^{86}\text{Sr}$  of reservoir fishes statistically allowed us to evaluate potential origins of unknown-history river-caught piscivores. Those river fish with core  $^{87}\text{Sr}/^{86}\text{Sr}$  within the 95% prediction interval for a reservoir

could have come from that location. Perhaps equally useful, we could use the same approach to exclude sources and thereby determine water bodies that posed lower escapement risk. However, we also showed that mainstream reservoirs might have indistinguishable  $^{87}\text{Sr}/^{86}\text{Sr}$  from the river signatures immediately downstream. Thus, at present, our approach might be most suited to situations where the emigration source is on a tributary to the river reach of interest, is in an off-channel location, or else escapees are captured sufficiently downstream to be beyond the geochemical influence of the reservoir. Targeted water sampling to compare  $^{87}\text{Sr}/^{86}\text{Sr}$  of reservoirs and rivers below could identify cases where signatures of reservoir and river fish would be different or the same. Additional markers (e.g.,  $\delta^{18}\text{O}$ ) could potentially improve discrimination of reservoir and river habitats and help identify reservoir escapees in rivers immediately downstream of dams. The  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of surface waters vary predictably with latitude and elevation (Kendall and Coplen 2001), appear to differ between rivers and lakes (Henderson and Shuman 2010), and have been useful in fish provenance studies (Whitledge et al. 2006, 2007; Walther et al. 2008).

The ambiguities we encountered in some of the known-history fish studied in the Yampa River system highlight two challenges when comparing edge and core  $^{87}\text{Sr}/^{86}\text{Sr}$  to infer habitat switching. First, otolith growth bands can become compressed as a fish ages, leaving a smaller amount of material deposited during that period of life. Given a fixed ablation beam diameter, spots ablated in later years of life may have less temporal resolution (here, slightly larger than 35  $\mu\text{m}$  or approximately 1-year resolution) than spots ablated closer to the core. Second, without known river-resident fish, it is difficult to infer the expected  $^{87}\text{Sr}/^{86}\text{Sr}$  of a river below a reservoir source population from otoliths. Fish below dams may be permanent local residents or be recent immigrants from downstream or the reservoir upstream. The use of stationary, integrative “sentinels” of river  $^{87}\text{Sr}/^{86}\text{Sr}$  could improve inference in provenance studies involving rivers and other systems where fish can move throughout heterogeneous  $^{87}\text{Sr}/^{86}\text{Sr}$  environments.

The utility of natural markers to identify nonnative fish that have emigrated from reservoirs to rivers below relies upon their distinctiveness among locations and long-term temporal stability. When considering distinctness of reservoir signatures, the likelihood of overlapping  $^{87}\text{Sr}/^{86}\text{Sr}$  increases as additional suspected sources are added. Therefore, for this technique to be most effective, comparisons of otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  should be restricted to specific escapement hypotheses. For instance, if a suspected escapement source has been mitigated via screens or impingement devices, subsequent sampling of river fishes downstream and analysis of  $^{87}\text{Sr}/^{86}\text{Sr}$  could be performed to evaluate barrier effectiveness. Conversely, this method may be well suited to determine locations that do not appear to be source locations and thus eliminating the need of barriers or screens in those locations. With high-resolution laser ablation MC-ICP-MS, the year of escapement may be established and could be related to environmental conditions or dam operations that may have contributed to escapement. Our findings also support the use of  $^{87}\text{Sr}/^{86}\text{Sr}$  as a deterrent against illicit transfer of aquatic or-

ganisms. In a 2011 Grand County, Colorado criminal case,  $^{87}\text{Sr}/^{86}\text{Sr}$  was used to rule out source locations where a suspect was accused of illegal harvest and transportation of crayfish (B.M. Johnson, Colorado State University, unpublished data). Managers seeking to discourage illegal introductions can inform stakeholders of the forensic utility of this technology for determining provenance of aquatic organisms.

The relatively high proportion of suspected escapees captured from rivers should raise concern for managers in this region and for managers of reservoir–river systems in general. Our results suggest that efforts to control nonnative fishes in riverine critical habitat will need to continue indefinitely unless reservoir sources are contained. Because many nonnative piscivores sampled from rivers did not appear to have recruited from their location of capture, containment of reservoir sources coupled with removals in rivers may be an effective strategy to reduce the density of invasive piscivore populations. Strontium isotope ratios provide UCRB managers with the means to evaluate what reservoirs pose the greatest escapement risk and focus their control efforts most efficiently.

## Acknowledgements

Many thanks are extended to the biologists working in the Upper Colorado River Endangered Fish Recovery Program for providing fish samples. We thank Scot Birdwhistell and Jurek Blusztajn at Woods Hole Oceanographic Institution (WHOI) Plasma Mass Spectrometry Facility for their assistance with otolith ablation analysis. Simon Thorrold, WHOI, provided valuable advice regarding isotopic analysis. Thanks go to the Recovery Program and the Bureau of Reclamation for providing financial support. Lastly, we thank the three anonymous reviewers, Bronwyn Gillanders (Associate Editor), and Rolf Vinebrooke (Co-Editor), whose thoughtful comments greatly increased the overall quality of our manuscript.

## References

- Bacon, C.R., Weber, P.K., Larsen, K.A., Reisenbichler, R., Fitzpatrick, J.A., and Wooden, J.L. 2004. Migration and rearing histories of Chinook salmon (*Oncorhynchus tshawytscha*) determined by ion microprobe Sr isotope and Sr/Ca transects of otoliths. *Can. J. Fish. Aquat. Sci.* **61**(12): 2425–2439. doi:10.1139/f04-167.
- Barnett-Johnson, R., Ramos, F.C., Grimes, C.B., and MacFarlane, R. B. 2005. Validation of Sr isotopes in otoliths by laser ablation multicollector inductively coupled plasma mass spectrometry (LA–MC–ICPMS): opening avenues in fisheries science applications. *Can. J. Fish. Aquat. Sci.* **62**(11): 2425–2430. doi:10.1139/f05-194.
- Barnett-Johnson, R., Pearson, T.E., Ramos, F.C., Grimes, C.B., and MacFarlane, R.B. 2008. Tracking the natal origins of salmon using isotopes, otoliths, and landscape geology. *Limnol. Oceanogr.* **53**(4): 1633–1642. doi:10.4319/lo.2008.53.4.1633.
- Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J. W., and Lam, J.W.H. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta*, **64**(10): 1705–1714. doi:10.1016/S0016-7037(99)00419-6.
- Bentley, B.A. 2006. Strontium isotopes from the Earth to the archaeological skeleton: a review. *J. Archaeol. Method Theory*, **13**(3): 135–187. doi:10.1007/s10816-006-9009-x.
- Blum, J.D., Taliaferro, H., Weisse, M.T., and Holmes, R.T. 2000. Changes in Sr/Ca, Ba/Ca, and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios between two forest ecosystems in the northeastern USA. *Biogeochemistry*, **49**(1): 87–101. doi:10.1023/A:1006390707989.
- Breton, A.R., Kildaw, D., Murra, K., and Buck, C.L. 2008. Multilevel models reveal no cohort-level variation in time spent foraging to account for a collapse in kittiwake (*Rissa tridactyla*) breeding success. *Ecol. Model.* **212**(3–4): 233–243. doi:10.1016/j.ecolmodel.2007.10.003.
- Brunson, R.E., Christopherson, K.D., and Hedrick, T.N. 2007. Evaluation of nonnative fish escapement from Starvation Reservoir [online]. Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado. Available from <http://www.coloradoriverrecovery.org/documents-publications/technical-reports/nna/Starvation.pdf> [accessed 29 December 2011].
- Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. Springer, New York.
- Cambray, J.A. 2003. Impact on indigenous species biodiversity caused by globalization of alien recreational freshwater fisheries. *Hydrobiologia*, **500**(1–3): 217–230. doi:10.1023/A:1024648719995.
- Campana, S.E., and Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.* **58**(1): 30–38. doi:10.1139/f00-177.
- Chittaro, P.M., Hogan, J.D., Gagnon, J., Fryer, B.J., and Sale, P.F. 2006. In situ experiment of ontogenetic variability in the otolith chemistry of *Stegastes partitus*. *Mar. Biol.* (Berl.), **149**(5): 1227–1235. doi:10.1007/s00227-006-0280-x.
- Clark, L.M., Munch, S.B., Thorrold, S.R., and Conover, D.O. 2010. High connectivity among locally adapted populations of a marine fish (*Menidia menidia*). *Ecology*, **91**: 3526–3537. doi:10.1890/09-0548.1.
- Dufour, E., Holmden, C., Van Neer, W., Zazzo, A., Patterson, W.P., Degryse, P., and Keppens, E. 2007. Oxygen and strontium isotopes as provenance indicators of fish at archaeological sites: the case study of Sagalassos, SW Turkey. *J. Archaeol. Sci.* **34**(8): 1226–1239. doi:10.1016/j.jas.2006.10.014.
- Eby, L.A., Roach, W.J., Crowder, L.B., and Stanford, J.A. 2006. Effects of stocking-up freshwater food webs. *Trends Ecol. Evol.* **21** (10): 576–584. doi:10.1016/j.tree.2006.06.016. PMID:16828522.
- Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C. M., Limburg, K.E., Secor, D.H., Thorrold, S.R., and Walther, B.D. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. *Oceanogr. Mar. Biol. Annu. Rev.* **46**: 297–330. doi:10.1201/9781420065756.ch7.
- Faure, G., and Powell, J.L. 1972. Strontium isotope geology. Springer-Verlag, New York.
- Feyrer, F., Hobbs, J., Baerwald, M., Sommer, T., Yin, Q., Clark, K., May, B., and Bennett, W. 2007. Otolith microchemistry provides information complementary to microsatellite DNA for a migratory fish. *Trans. Am. Fish. Soc.* **136**(2): 469–476. doi:10.1577/T06-044.1.
- Finney, S.T., and Haines, G.B. 2008. Northern pike removal, smallmouth bass monitoring, and native fish monitoring in the Yampa River, Hayden to Craig Reach, 2004–2006 [online]. Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado. Available from <http://www.coloradoriverrecovery.org/documents-publications/technical-reports/nna/FinalReport98b.pdf> [accessed 29 December 2011].
- Fowler, A.J., Campana, S.E., Jones, C.M., and Thorrold, S.R. 1995. Experimental assessment of the effect of temperature and salinity

- on elemental composition of otoliths, using laser ablation ICPMS. *Can. J. Fish. Aquat. Sci.* **52**(7): 1431–1441. doi:10.1139/f95-138.
- Gibson-Reinemer, D.K., Johnson, B.M., Martinez, P.J., Winkelman, D.L., Koenig, A.E., and Woodhead, J.D. 2009. Elemental signatures in otoliths of hatchery rainbow trout (*Oncorhynchus mykiss*): distinctiveness and utility for detecting origins and movement. *Can. J. Fish. Aquat. Sci.* **66**(4): 513–524. doi:10.1139/F09-015.
- Gillanders, B.M. 2002. Temporal and spatial variability in elemental composition of otoliths: implications for determining stock identity and connectivity of populations. *Can. J. Fish. Aquat. Sci.* **59**(4): 669–679. doi:10.1139/f02-040.
- Goldstein, S.J., and Jacobsen, S.B. 1987. The Nd and Sr isotopic systematics of river-water dissolved material: implications for the sources of Nd and Sr in seawater. *Chem. Geol.* **66**: 245–272.
- Hawkins, J.A., and Nesler, T.P. 1991. Nonnative fishes of the upper basin: an issue paper. Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado. Copies may be obtained from John Hawkins, Larval Fish Laboratory, Colorado State University, 1474 Campus Delivery, Fort Collins, CO 80523, USA.
- Hawkins, J., Walford, C., and Hill, A. 2009. Smallmouth bass control in the middle Yampa River, 2003–2007 [online]. Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado. Available from <http://www.coloradoriverrecovery.org/documents-publications/technical-reports/nna/125final.pdf> [accessed 29 December 2011].
- Henderson, A.K., and Shuman, B.N. 2010. Differing controls on river- and lake-water hydrogen and oxygen isotopic values in the western United States. *Hydrol. Processes*, **24**(26): 3894–3906. doi:10.1002/hyp.7824.
- Ingram, B.L., and Weber, P.K. 1999. Salmon origin in California's Sacramento – San Joaquin river system as determined by otolith strontium isotopic composition. *Geology*, **27**(9): 851–854. doi:10.1130/0091-7613(1999)027<0851:SOICSS>2.3.CO;2.
- Jackson, M.G., and Hart, S.R. 2006. Strontium isotopes in melt inclusions from Samoan basalts: implications for heterogeneity in the Samoan plume. *Earth Planet. Sci. Lett.* **245**(1–2): 260–277. doi:10.1016/j.epsl.2006.02.040.
- Johnson, B.M., Arlinghaus, R., and Martinez, P.J. 2009. Are we doing all we can to stem the tide of illegal fish stocking. *Fisheries* (Bethesda, Md.), **34**: 389–394.
- Johnson, P.T.J., Olden, J.D., and Vander Zanden, M.J. 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. *Front. Ecol. Environ.* **6**(7): 357–363. doi:10.1890/070156.
- Kendall, C., and Coplen, T.B. 2001. Distribution of oxygen-18 and deuterium in river waters across the United States. *Hydrol. Processes*, **15**(7): 1363–1393. doi:10.1002/hyp.217.
- Kennedy, B.P., Blum, J.D., Folt, C.L., and Nislow, K.H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. *Can. J. Fish. Aquat. Sci.* **57**(11): 2280–2292. doi:10.1139/f00-206.
- Kennedy, B.P., Chamberlain, C.P., Blum, J.D., Nislow, K.H., and Folt, C.L. 2005. Comparing naturally occurring stable isotopes of nitrogen, carbon, and strontium as markers for the rearing locations of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **62**(1): 48–57. doi:10.1139/f04-184.
- Macdonald, J.I., Shelley, J.M.G., and Crook, D.A. 2008. A method for improving the estimation of natal chemical signatures in otoliths. *Trans. Am. Fish. Soc.* **137**(6): 1674–1682. doi:10.1577/T07-249.1.
- Martin, L.M., Wright, B.F., Hawkins, J.A., and Walford, C. 2010. Middle Yampa River northern pike and smallmouth bass removal and evaluation; Colorado pikeminnow and roundtail chub evaluation: 2004–2007 [online]. Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado. Available from <http://www.coloradoriverrecovery.org/documents-publications/technical-reports/nna/YAR98aSynthesisFinal-CDOW-2010.pdf> [accessed 13 December 2011].
- Martinez, P.J., Chart, T.E., Trammell, M.A., Wullschlegel, J.G., and Bergersen, E.P. 1994. Fish species composition before and after construction of a main stem reservoir on the White River, Colorado. *Environ. Biol. Fishes*, **40**(3): 227–239. doi:10.1007/BF00002509.
- Munro, A.R., McMahon, T.E., and Ruzycki, J.R. 2005. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake. *Can. J. Fish. Aquat. Sci.* **62**(1): 79–87. doi:10.1139/f04-174.
- Nesler, T.P. 1995. Interactions between endangered fish and introduced gamefishes in the Yampa River, Colorado, 1987–1991. Colorado Division of Wildlife, Fort Collins, Colorado.
- Nico, L.G., and Fuller, P.L. 1999. Spatial and temporal patterns of nonindigenous fish introductions in the United States. *Fisheries* (Bethesda, Md.), **24**: 16–27.
- Poff, N.L., and Ward, J.V. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. *Can. J. Fish. Aquat. Sci.* **46**(10): 1805–1818. doi:10.1139/f89-228.
- Schaffler, J.J., and Winkelman, D.L. 2008. Temporal and spatial variability in otolith trace-element signature of juvenile striped bass from spawning locations in Lake Texoma, Oklahoma–Texas. *Trans. Am. Fish. Soc.* **137**(3): 818–829. doi:10.1577/T06-023.1.
- Singer, J.D., and Willet, J.B. 2003. Applied longitudinal data analysis: modeling change and event occurrence. Oxford University Press, New York.
- Tyus, H.M., and Saunders, J.F. 2000. Nonnative fish control and endangered fish recovery: lessons from the Colorado River. *Fisheries* (Bethesda, Md.), **25**: 17–24.
- US Fish and Wildlife Service. 1994. Endangered and threatened wildlife and plants; determination of critical habitat for the Colorado River endangered fishes: razorback sucker, Colorado squawfish, humpback chub, and bonytail. *Fed. Regist.* **59**(54): 13374–13400.
- Valdez, R.A., and Muth, R.T. 2005. Ecology and conservation of native fishes in the Upper Colorado River Basin. *In* Historical changes in large river fish assemblages of the Americas. *Edited by* J.N. Rinne, R.B. Hughes, and B. Calamusso. *Am. Fish. Soc. Symp.* **45**, Bethesda, Maryland. pp. 157–204.
- Walther, B.D., and Thorrold, S.R. 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. *Mar. Ecol. Prog. Ser.* **311**: 125–130. doi:10.3354/meps311125.
- Walther, B.D., and Thorrold, S.R. 2009. Inter-annual variability in isotope and elemental ratios recorded in otoliths of an anadromous fish. *J. Geochem. Explor.* **102**(3): 181–186. doi:10.1016/j.gexplo.2008.10.001.
- Walther, B.D., Thorrold, S.R., and Olney, J.E. 2008. Geochemical signatures in otoliths record natal origins of American shad. *Trans. Am. Fish. Soc.* **137**(1): 57–69. doi:10.1577/T07-029.1.
- Walther, B.D., Dempster, T., Letnic, M., and McCulloch, T. 2011. Movements of diadromous fish in large unregulated tropical rivers inferred from geochemical tracers. *PLoS ONE*, **6**(4): e18351. doi:10.1371/journal.pone.0018351. PMID:21494693.
- Wells, B.K., Rieman, B.E., Clayton, J.L., Horan, D.L., and Jones, C. M. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. *Trans. Am. Fish. Soc.* **132**(3): 409–424. doi:10.1577/1548-8659(2003)132<0409:RBWOAS>2.0.CO;2.

- Whitledge, G.W., Johnson, B.M., and Martinez, P.J. 2006. Stable hydrogen isotopic composition of fishes reflects that of their environment. *Can. J. Fish. Aquat. Sci.* **63**(8): 1746–1751. doi:10.1139/f06-076.
- Whitledge, G.W., Johnson, B.M., Martinez, P.J., and Martinez, A.M. 2007. Sources of nonnative centrarchids in the upper Colorado River revealed by stable isotope and microchemical analyses of otoliths. *Trans. Am. Fish. Soc.* **136**(5): 1263–1275. doi:10.1577/T06-045.1.
- Woodhead, J., Swearer, S., Hergt, J., and Maas, R. 2005. *In situ* Sr-isotope analysis of carbonates by LA-MC-ICP-MS: interference corrections, high spatial resolution and an example from otolith studies. *J. Anal. At. Spectrom.* **20**(1): 22–27. doi:10.1039/b412730g.
- Yoshinaga, J., Nakama, A., Morita, M., and Edmonds, J.S. 2000. Fish otolith reference material for quality assurance of chemical analyses. *Mar. Chem.* **69**: 91–97. doi:10.1016/S0304-4203(99)00098-5.