

THESIS

ORIGINS AND MOVEMENTS OF INVASIVE PISCIVORES DETERMINED FROM  
OTOLITH ISOTOPIC MARKERS

Submitted by

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## ABSTRACT

### ORIGINS AND MOVEMENTS OF INVASIVE PISCIVORES DETERMINED FROM OTOLITH ISOTOPIC MARKERS

I examined the utility of the strontium isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) ratio in fish otoliths to determine the origins and movements of invasive piscivores in the Upper Colorado River Basin (UCRB). My goal was to determine if invasive piscivores entered riverine habitat of endangered fishes in the UCRB by escaping from reservoirs, and if so, determine their likely origins. Fishes were collected from 14 reservoirs and rivers directly downstream. My objectives were to examine the distinctiveness of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from fishes in different reservoirs, and temporal stability and interspecies variability of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of fishes within reservoirs. Variance components analysis and model selection using an information theoretic approach were used to rank relative importance of reservoir location, species and year on  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. My results showed that in most cases  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were unique across reservoirs, overlapped among species in a given reservoir, and were temporally stable across years. I was able to identify the likely reservoir of origin of river-caught fish, and I was able to determine, in some cases, the year of escapement. Overall, my results demonstrate the utility of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios for tracking fish movement and origin in river-reservoir systems and provide important insights into processes that affect escapement risk such as dam operations, weather conditions, fish behavior and fish physiology. Analysis of stable isotopes hydrogen ( $\delta^2\text{H}$ ), carbon ( $\delta^{13}\text{C}$ ), and oxygen ( $\delta^{18}\text{O}$ ) were performed on a sub-set of otoliths from the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio analysis to determine if additional

markers aid in discriminating between groups, particularly when  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are similar among different locations. A canonical discriminant function analysis was used to visually differentiate different locations using natural isotopes. These results showed that  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  provide very useful separation among different groups, particularly when in conjunction with  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios.

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# Table of Contents

<b>Chapter 1</b> .....	<b>1</b>
<b>Introduction</b> .....	<b>1</b>
<b>Materials and methods</b> .....	<b>4</b>
<i>Study area</i> .....	4
<i>Fish collections</i> .....	5
<i>Otolith preparation</i> .....	5
<i>Strontium isotope analysis</i> .....	6
<i>Statistical analysis</i> .....	7
<i>Origins of river fishes</i> .....	10
<b>Results</b> .....	<b>11</b>
<i>Reservoir analysis</i> .....	11
<i>Origins of river fishes</i> .....	13
<b>Discussion</b> .....	<b>16</b>
<b>Acknowledgments</b> .....	<b>21</b>
<b>References</b> .....	<b>22</b>
<b>Chapter 2</b> .....	<b>41</b>
<b>Introduction</b> .....	<b>41</b>
<b>Materials and methods</b> .....	<b>42</b>
<i>Stable Isotope analysis</i> .....	42
<i>Statistical analysis</i> .....	43
<b>Results</b> .....	<b>44</b>
<b>Discussion</b> .....	<b>46</b>
<b>References</b> .....	<b>48</b>
<b>Appendix A</b> .....	<b>56</b>
<b>Appendix B</b> .....	<b>63</b>

## Chapter 1

# ORIGINS AND MOVEMENTS OF INVASIVE PISCIVORES DETERMINED FROM THE STRONTIUM ISOTOPE ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) RATIO OF OTOLITHS

### 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 require tools that can differentiate individuals or groups of fishes. In parts of Canada and in the U.S. west of the continental divide, native sport fishes are uncommon relative to nonnative sport fishes (Nico and Fuller 1999). Agencies have created sport fisheries in discrete locations by introducing piscivores such as northern pike *Esox lucius*, large- and smallmouth bass *Micropterus salmoides* and *Micropterus dolomieu*, and walleye *Sander vitreus*. Natural 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* (U.S. 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 when reservoirs were created, are dispersing throughout the UCRB by emigration from

reservoirs and inhibiting the recovery of native fishes (Martinez et al. 1994; Tyus and Saunders 2000). Stocking to create recreational fisheries has been instrumental in declines of native species worldwide (Cambray 2003; Eby et al. 2006). Furthermore, habitat alternations caused by impoundments provide a greater opportunity for multiple invader species compared to 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\$1M 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. 2007). 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 2009) and seasonal and annual variability can be high (Gillanders 2002; Bacon et al. 2004; Walther and Thorrold 2009). The strontium isotope ratio  $^{87}\text{Sr}/^{86}\text{Sr}$  (hereafter, “Sr ratio”) 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). The Sr ratio varies among water bodies because it is driven by the underlying geology across landscapes and weathering effects (Kennedy et al. 2000), but it is unaffected by changes in ambient Sr concentration induced by flow fluctuations. The Sr ratio in otoliths is strongly

correlated with the Sr ratio in ambient water (Kennedy et al. 2000; Walther and Thorrold 2006; Barnett-Johnson et al. 2008), with virtually no biotic fractionation (Kennedy et al. 2000). Few studies have evaluated interspecies differences in Sr ratios or Sr ratios among reservoirs, which typically have larger watersheds than natural lakes, but exhibit less hydrologic variability than rivers (Thornton et al. 1990).

In this study, I examined the utility of Sr ratios in otoliths to determine origins of piscivores throughout the UCRB. My goals were to evaluate: 1) distinctiveness of otolith Sr ratios from different reservoirs and subbasins (containing one or more reservoirs); 2) inter-annual variability of otolith Sr ratios within reservoirs; and 3) inter-species differences of otolith Sr ratios within reservoirs. Some studies have suggested that chemical signatures in otoliths may differ among species via different vital effects on elemental concentrations in otoliths (Hamer and Jenkins 2007), also species with similar phylogeny and ecology had more consistent chemistries compared to fishes with different lineages and behaviors (Swearer et al. 2003; Reis-Santos et al. 2008). To my knowledge, none have compared otolith Sr ratios of the species in this study, many of which are popular sport fishes. Testing species effects is important to determine if signatures of different species can be compared among different water bodies, particularly when some species are difficult to collect or when dealing with sensitive species. Finally, I tested the utility of Sr ratios for tracing origins of suspected reservoir escapees sampled in rivers below study reservoirs. This work is important to managers dealing with invasive species, who could benefit from a tool that uses a chemical ‘fingerprint’ to reveal the source location of immigrant fish.

## **Materials and methods**

### *Study area*

My study area encompassed the UCRB (Figure 1). The basin extends from southwestern Wyoming through western Colorado and eastern Utah, functionally terminating at Glen Canyon Dam in southern Utah, with an area around 289,540 km<sup>2</sup>. There are many potential source locations of invasive piscivores within and outside the UCRB. However, it was not feasible to test all locations where invasive piscivores are found. Therefore, I focused on reservoirs deemed by the Upper Colorado River Endangered Fish Recovery Program as likely sources of escapement into rivers of the UCRB. Sampling sites included 14 reservoirs in six tributary subbasins of the UCRB (Table 1), 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.

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 (Figure 1). 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 (2,100 m – 4,000 m above sea level) dictating annual hydrologic variability (Poff and Ward 1989). Most reservoirs here are located below 2,100 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 this 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 Division of Wildlife, CDOW; Colorado State University Larval Fish Lab, CSU-LFL; U.S. Fish and Wildlife Service, USFWS; Utah Division of Wildlife Resources, UDWR; and Wyoming Game and Fish Department, WGF) conducting sampling for the monitoring and management of nonnative piscivores during 2007-2009 from reservoirs (Table 1) and river sites throughout the basin. The goal was to sample the full complement of nonnative piscivores present in each system, collectively including: black crappie *Pomoxis nigromaculatus*, burbot *Lota lota*, largemouth bass, northern pike, smallmouth bass and walleye. Captured fishes were euthanized with a lethal dose of 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.

### *Otolith preparation*

Sagittal otoliths were removed with non-metallic forceps and sonicated in Milli-Q water for five minutes. Left otoliths were used for all Sr ratio 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 μm 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 five minutes. Mounted otoliths were dried for 24 hours 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 Sr ratios 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  $\mu\text{m}$  per second 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 Sr ratios. For reservoir fishes, each otolith was ablated 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 Sr ratio (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 Sr ratios 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. I 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 Ca argides, Ca dimers and doubly charged Er and Yb. However, it has been demonstrated that rubidium (Rb) and krypton (Kr) isotopes create the main interferences in other Sr ratio laser ablation ICP-MS analyses (Barnett-Johnson et al. 2005; Jackson and Hart 2006; Walther et al. 2008). I 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 Sr ratio of 0.71024) using the formula:  $^{87}\text{Sr}/^{86}\text{Sr}_{\text{normalized}} = (0.71024 \div \text{SRM 987}_{\text{measured}}) \times ^{87}\text{Sr}/^{86}\text{Sr}_{\text{sample}}$ . Solutions of JRS and SRM 987 produced daily mean ( $\pm 1$  SD; sample size) Sr ratios 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 Sr ratio of 0.70918 ( $\pm 0.00003$ ; n = 4).

### *Statistical analysis*

Preliminarily, I visually examined three bivariate plots to assess the importance of subbasin, reservoir, species and year as factors affecting variation in Sr ratios. A plot of Sr ratio from each reservoir, averaged across years and species, was examined to determine differences among subbasins and reservoirs. Average otolith Sr ratio of all fish in each reservoir was examined for inter-annual trends and compared among reservoirs within subbasins. Finally, average Sr ratios among species within reservoirs were also examined.

Following the visual assessment of the data, I assessed normality of the Sr ratios for each reservoir using box-and-whisker plots and conditional studentized residuals diagnostic plots from mixed regression models (described below). Diagnostic plots did not reveal any major departures from normality. Additionally, Shapiro-Wilk tests (PROC UNIVARIATE, SAS<sup>®</sup>)

failed to reject normality of studentized residuals of Sr ratios from each reservoir, with the exception of Flaming Gorge Reservoir ( $p = 0.02$ ); therefore no data transformations were performed.

I used mixed regression models (PROC MIXED, SAS<sup>®</sup>) to estimate the relative importance of reservoir, species and year as effects contributing to variation in Sr ratios. I performed my analysis in three phases, principally because the sampling protocol resulted in an unbalanced dataset, as species and years were not sampled equally among reservoirs. I specified the full maximum likelihood estimation option in PROC MIXED to accommodate phase three, an  $AIC_c$  analysis involving competing models and different fixed effects (Singer 1998). In the first phase I considered two models fitted with only one fixed effect, an intercept, and up to two random effects. The purpose of this phase of analysis was to determine important sources of variance (variance components) in Sr ratios. In the first model I 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 I again included FishID as a random effect, but this time I added a second random effect (hereafter, “Res\_Spp\_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 ( $AIC_c$ ). The best random effects structure was the model that included the Res\_Spp\_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 analysis, I 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\_Spp\_Year model from phase one. The approach, deploying variance

components, enabled us to determine the amount of variation attributable to (explained by) each of the fixed effects (Singer and Willet 2003).

In the third phase I evaluated, using  $AIC_c$  and associated statistics, a suite of models encompassing all possible combinations of fixed effects – reservoir, species and year. *Post hoc*, I also assessed a dichotomous species effect (DSE) where walleye Sr ratios were specified as different than all other species using a 0 or 1 dichotomous variable (0 = walleye, 1 = non-walleye). To determine the relative importance of each model, delta  $AIC_c$  ( $\Delta_i$ ) and Akaike weights ( $w_i$ ) were calculated for each model  $i$ . Delta  $AIC_c$  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  $AIC_c$  value:  $\Delta_i = AIC_{c(i)} - AIC_{c(top)}$ . Akaike weights or model probabilities, calculated using model likelihoods (ML), sum to one across the full model set thus provide relative support for each model:  $w_i = ML_i \div \sum ML$ , where  $ML_i = e^{(-0.5 \times \Delta_i)}$ . 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.

To measure the ability to assign fish to their reservoir of origin using Sr ratios alone I supplemented the PROC MIXED analysis with a linear discriminant function analysis (DFA; cross-validated, leave-one-out and prior probabilities equal; PROC DISCRIM, SAS<sup>®</sup>). Individual fish Sr ratios were averaged across all years because inter-annual variability was deemed negligible based on the model selection and variance components analysis described above. Linear DFA comparisons were confined to within subbasins because many of the reservoir comparisons are sufficiently separated geographically that regardless of whether they

shared a similar Sr ratio, their mutual consideration as a potential escapement source would be unrealistic.

### *Origins of river fishes*

Known fish histories based on tagging and recapture data from the Yampa River, in particular, allowed us to test the effectiveness of otolith Sr ratios for tracing origins of fish emigrating from reservoirs into rivers. Smallmouth bass in the Yampa River have been tagged with Floy t-bar anchor tags since 2003 (Hawkins et al. 2009). Some of these fish were subsequently transplanted to Elkhead Reservoir on a tributary to the Yampa River and recaptured in Elkhead Reservoir (hereafter, “transplants”). Others that had not been transferred were recaptured in the Yampa River (hereafter, “residents”). I examined Sr ratios from fishes exhibiting each of these capture histories.

For other species and river/reservoir systems I needed to determine, in the absence of known histories, if Sr ratios of river-resident fish differed from those in upstream reservoirs before implicating reservoir escapement. I compared predictions of each reservoir’s Sr ratio from the top ranked model (phase three from above; reservoir and dichotomous species fixed effects) to the measured core and edge Sr ratios of river-captured fish. There were two prediction interval estimates in the top ranked model, one estimate for walleye and the other estimate for all other species. These comparisons assumed that 1) reservoir emigrants possessed a core Sr ratio similar to a reservoir’s signature unless it emigrated at a very early age before accreting a detectable reservoir signature; and 2) 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 Sr ratio of a river-caught fish was within the 95% confidence limits of the predicted estimates (top ranked model, phase three) of an upstream reservoir's signature, I concluded that the fish had likely moved to the river from that reservoir. In circumstances where river-caught fishes had overlapping Sr ratios of upstream reservoir for their entire lifetime (core and edge), then these fish were treated as potential escapees that had recently moved from the reservoir. However, with the exception of tagged Yampa River fish, I were unable to conclusively determine if such fish were simply river-residents from an environment that shared the same Sr ratio as the reservoir upstream.

## **Results**

### *Reservoir analysis*

With the exception of the Colorado subbasin, reservoir Sr ratios were not visibly clustered within subbasins (Figure 2). Consistent with this result, pre-analysis suggested that the fixed effect of subbasin was not statistically or biologically significant, i.e., variation in Sr ratios was a function of reservoir with no effect of subbasin. Given these observations, subbasin effects were excluded from subsequent analyses. Reservoir Sr ratios averaged across all years and species were distinct, particularly within subbasins (Figure 2). Annual variation in reservoir Sr ratio was small in comparison to the differences I observed between reservoirs in the same subbasin (Figure 3). Lake Catamount showed the most annual variability of any reservoir in my study; however, Lake Catamount's signature still remained distinct from the other reservoirs (Elkhead and Stagecoach) in the Yampa River subbasin. When I visually examined possible species effects within reservoirs, only one species (walleye) showed consistent differences in Sr

ratios compared to the others (Figure 4). These differences were observed in Juniata, McPhee, Rifle Gap and Starvation reservoirs (Figure 4).

Of the two random effects models examined (Table 2a), the top ranked model included the Res\_Spp\_Year ( $AIC_c = -4524.20$ ;  $w_i = 1.00$ ). The competing model with only the FishID random effect had no support ( $\Delta = 103.50$ ;  $w = 0.00$ ). I maintained the random effects from the top model in subsequent analyses. Total variance in the top random effects model was reduced by 96%, 50%, and 5% with the addition of reservoir, species and year, respectively (Table 3).

From the analysis of fixed effects (Table 2b), the top ranked model included the fixed effects reservoir and species. This model attained virtually all of the model weight ( $AIC_c = -4922.60$ ;  $w = 0.98$ ). The second highest ranked model included reservoir, species and year ( $\Delta = 7.70$ ;  $w = 0.02$ ) followed by a model with only reservoir effects ( $\Delta = 9.20$ ;  $w = 0.00$ ), but note the sum of the weights for these models was 0.02. Thus, given the data, my 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 2b). These results indicate that the effect of reservoir, which was also in the only model supported in the analysis of fixed effects, was strongly supported by the data. Although species had an equivalent cumulative weight as reservoirs, this effect nonetheless performed poorly in models that did not include reservoir (best species model that did not include reservoir,  $\Delta \geq 361.40$ ). The cumulative weight for year is strong evidence that Sr ratios varied independent of year.

My visual assessment suggested that walleye was the only species that showed differences in Sr ratios compared to 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. When the DSE model was added to the same set of models in phase two, it was ranked as the top model ( $AIC_c = -4930.30$ ;  $w = 0.98$ ). The next best model consisted of reservoir and species (non-dichotomous) fixed effects ( $\Delta_i = 7.70$ ;  $w = 0.02$ ) and all of the other models had a  $\Delta > 20$  and a combined  $w$  of 0.00 (Table 2c). Thus, only the top model was supported by the data and the species effect was largely driven by differences in walleye compared to other species. In summary, both phases two (variance components) and three (fixed effects) suggested that the effect of year was negligible compared to reservoir and species.

Linear DFA of reservoirs within their respective subbasin had classification rates with a mean ( $\pm$  SD) of 88% accuracy ( $\pm 21$ ). Nine of 14 reservoirs had 100% classification rates: Lake Catamount, Rio Blanco Lake, Crawford, Elkhead, Flaming Gorge, Kenney, Paonia, Red Fleet and Starvation reservoirs. Harvey Gap and Rifle Gap reservoirs had classification rates of 53% and 43%, and misclassifications went to Rifle Gap and Harvey Gap reservoirs, respectively, i.e., they were classified to an adjacent reservoir (Figure 1). Juniata Reservoir had a classification rate of 60%, the other 40% were incorrectly classified to Paonia Reservoir, 75 km east of Juniata Reservoir. Stagecoach Reservoir had a classification rate of 92%, 8% were incorrectly classified to Elkhead Reservoir. McPhee reservoir was the only reservoir examined within the Dolores River subbasin, thus there were no other locations to compare.

### *Origins of river fishes*

Changes in Sr ratios of transplanted smallmouth bass ( $N = 3$ ) from the Yampa River into Elkhead Reservoir could be detected in all cases (Figure 5). All of these transplants had edge Sr ratios lower than the Yampa River, but only one fish's Sr ratio overlapped with Elkhead Reservoir fish. I believe the higher than expected Sr ratio 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.

Ablation time series of smallmouth bass captured in the Yampa River (Figure 6) suggested that these fish originated from somewhere within the Yampa River and not Elkhead Reservoir, a suspected source of reservoir fish to the river (Hawkins and Nesler 1991; Nesler 1995), i.e., all smallmouth bass core Sr ratios were outside the upper 95% prediction limits of the mean Elkhead Reservoir signature using the top ranked model (analysis, phase three). As noted above, only one transplanted smallmouth bass overlapped with the 95% prediction limits of the mean Elkhead Reservoir signature (Figure 6). The higher than expected Sr ratio of transplanted fishes is better explained when accounting for the length of time they likely resided in Elkhead Reservoir (Figure 6). The two transplants outside the Elkhead Reservoir signature had been transplanted only two and five months prior to recapture.

Six of 10 northern pike caught in the Yampa River had core Sr ratios that indicated Yampa River or Lake Catamount origin. Their core Sr ratios overlapped with resident smallmouth bass from the Yampa River and 95% prediction limits of the mean Lake Catamount signature. The remaining four fish examined had core Sr ratios that were most consistent with several ponds in that region (R.M. Fitzpatrick, Colorado Division of Wildlife, unpublished data).

In the Colorado River subbasin, all of the smallmouth bass I examined (N = 4) appeared to be of river origin but all walleye (N = 7) appeared to have emigrated from Rifle Gap Reservoir (Figure 7). Examination of the time series of Sr ratios of individual walleye showed the time of movement occurred during 1996-1998, a period with unusually high inflow to and spills from Rifle Gap Reservoir (B.M. Johnson, unpublished data).

In the Green River subbasin, four of five smallmouth bass captured from the Duchesne River were classified as escapees from Starvation Reservoir based on core Sr ratios. However, three of five edge Sr ratios were also consistent with Starvation Reservoir implying that the signatures of the Duchesne River and Starvation Reservoir may be too similar to distinguish. In the Upper Green River, three of five smallmouth bass were classified as having originated from Flaming Gorge Reservoir (Figure 8). Note, however, that smallmouth bass collected from the Yampa River have Sr ratios similar to Flaming Gorge Reservoir and overlap slightly with edge Sr ratios of fishes caught from the Upper Green River. Therefore, I cannot rule out the possibility that these fish came from the Yampa River or the Green River. 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 or a source not examined. It is likely that these fish originated from the Yampa River subbasin where northern pike are much more abundant. One of these six northern pike had been floy tagged in Lake Catamount in 2003, at the upper end of the Yampa River basin. Therefore I know that northern pike can move between these locations. All of the walleye examined ( $N = 13$ ) from the Upper Green River show clear trend of emigration from a reservoir source. Five of thirteen walleye from the Upper Green River were classified to Starvation Reservoir, using the predicted Sr ratio of walleye from the top ranked model from phase three of the analysis and the remaining individuals fell just outside that range (Figure 8) However, as stated above, Starvation Reservoir and the Duchesne River share similar Sr signatures, so it is possible that these walleye originated from the Duchesne River.

In the Dolores and White River subbasins, Sr ratios of the core and edge of river-caught smallmouth bass overlapped with the predicted Sr ratios of upstream reservoirs McPhee and Kenney Reservoirs, respectively. Rio Blanco Reservoir was not indicated as an escapement source of smallmouth bass captured from the White River.

## **Discussion**

Given the general distinctiveness of reservoirs, temporal stability, and lack of inter-specific effects, the Sr ratio in otoliths proved to be an excellent tracer for reservoir piscivores. This research also provided convincing evidence that reservoirs are major sources of invasive piscivores in the UCRB. This was particularly true for walleye, where in every case I detected clear movement patterns from a reservoir source. This evidence suggests that walleye reproduction in these rivers is very limited, and therefore that this species could be controlled in critical habitat by containment of reservoir sources. In some other cases I 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.

Within subbasins, reservoirs had unique Sr signatures that could be distinguished with relatively high accuracy (> 92%), for all locations except Harvey Gap, Rifle Gap, and Juniata reservoirs. Additionally, Harvey Gap and Rifle Gap reservoirs share the same water supply and underlying geology and are within a few km of each other, and thus not surprisingly similar Sr ratios. The ability to classify fishes to their geographic origin was consistent with other studies using the Sr ratio in otoliths. Barnett-Johnson et al. (2008) reclassified Chinook salmon *Oncorhynchus tshawytscha* to their natural and hatchery spawning grounds in the California Central Valley with 82% accuracy. Although their accuracy was higher than my results, the range of reservoir Sr ratios was much smaller (0.70895-0.71085) compared to theirs (0.70386-

0.71025). Feyrer et al. (2007) presented 71% classification accuracy of splittail *Pogonichthys macrolepidotus* to natal river spawning sites near the Sacramento-San Joaquin River delta when using Sr ratios and Sr/Ca ratios. Gibson-Reinemer et al. (2009) found that classification rates increased from 63% to 96% by adding Sr ratios to Ba/Ca and Sr/Ca markers of Colorado hatchery trout.

I found that inter-annual variability in the Sr ratio of reservoir fish from most reservoirs was negligible, at least over the nearly decadal scale of this 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 signatures is not surprising because reservoirs gather water from relatively large drainage areas and dampens 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 the use of Sr ratios 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 Sr ratio signatures. The temporal stability of reservoir Sr ratios has important implications for managers. Under the right circumstances, relatively few samples, even in a single year, would be necessary to characterize the Sr ratio 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 I had less opportunity to evaluate the stability of river Sr ratios since river fishes were generally of unknown provenance, Sr ratios of tagged smallmouth bass in the Yampa River had inter-annual variability comparable to reservoir temporal variability; suggesting that river Sr

ratios remain stable over many years. Additionally, Sr ratios of Colorado River fishes in the study had edge signatures that were very similar to the Sr ratio of water sampled from the Colorado River near Hoover Dam ( $0.71075 \pm 0.00003$ ,  $2\sigma$ ) two decades earlier (Goldstein and Jacobsen 1987), providing further evidence that Sr ratios fluctuate little over many years in some river-reservoir systems. These findings are consistent with some other studies utilizing Sr ratios to determine the environmental history of fishes that inhabited freshwater locations. Kennedy et al. (2000) found minimal seasonal variation of Sr ratios in streams and Atlantic salmon *Salmo salar* otoliths from tributaries of the Connecticut River. Barnett-Johnson et al. (2008) found that Sr ratios of otoliths were similar to water Sr ratios from the same locations collected many years earlier by Ingram and Weber (1999). However, Walther and Thorrold (2009) found significant differences in Sr ratios among years of American shad *Alosa sapidissima* otoliths from the Hudson and Pamunkey rivers in the Eastern U.S. Variance components analysis showed that Sr ratios accounted for only 5-19% of the total variability within each river there. Feyrer et al. (2007) also found inter-annual differences of Sr ratios, but the effect of year had little impact on discrimination of natal splittail locations. While more investigation of river variability is needed in the study area, it appears that Sr ratios remain relatively stable over many years, making annual location 'fingerprint' updates unnecessary.

I found that Sr ratios of piscivorous species within a reservoir did not differ, with one exception. In some reservoirs, Sr ratios of walleye were lower than sympatric species. Reasons for the disparity are unknown but differential habitat use or physiological differences could be responsible. If walleye inhabited particular areas of reservoirs not frequented by other species (e.g., the hypolimnion) and these areas differed in Sr ratio a disparity among species could arise. Dufour et al. (2007) suggested that spatial heterogeneity in Sr ratios within lakes could explain

the lack of correspondence between fish remains and ambient water Sr ratios. Alternatively, walleye may exhibit a physiological difference that alters the water/otolith Sr ratio relationship. While species differences in uptake of trace elements including Sr have been shown (Hamer and Jenkins 2007), I am unaware of cases of such differences in Sr ratios. The fact that I did not see a bias in Sr ratios of walleye in every reservoir cast doubt on this explanation. 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 Sr ratios among species within a given reservoir contributes to the utility of this marker for fingerprinting potential source locations. In general, Sr ratio signatures of suspected immigrant fish could be compared to 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 Sr ratio of reservoir fishes statistically allowed us to evaluate likely origins of unknown-history river-caught piscivores. Those river fish with core Sr ratios within the 95% prediction interval for a reservoir could have come from that location. Perhaps equally useful, I could use the same approach to exclude sources and thereby determine water bodies that posed lower escapement risk. However, I also showed that main stem reservoirs might have Sr ratios indistinguishable from the river signatures immediately downstream. Thus, this 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 escapees are captured sufficiently downstream to be beyond the geochemical influence of the reservoir. Additional markers (e.g.,  $\delta^{18}\text{O}$ ) could improve discrimination of reservoir and river habitats. 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 2006, 2007; Walther et al. 2008).

The ambiguities I encountered in some of the known-history fish studied in the Yampa River system highlight two challenges when comparing edge and core Sr ratios 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 than spots ablated closer to the otolith's core. Second, without known river-resident fish, it is difficult to infer the expected Sr ratio 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 Sr ratio could improve inference in provenance studies involving rivers and other systems where fish can move throughout heterogeneous Sr ratio environs.

The utility of natural markers to identify nonnative fish that have emigrated from reservoirs relies upon their distinctiveness among locations and long-term temporal stability. When considering distinctness of reservoir signatures, the likelihood of overlapping Sr ratios increases as additional suspected sources are added. Therefore, for this technique to be most effective, comparisons of otolith Sr ratios 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 Sr ratios 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. My findings also support the use of Sr ratios as a deterrent against illicit transfer of aquatic organisms. In a 2011 Grand County, CO criminal case Sr ratios were used to rule out source locations where a suspect was accused of illegal harvest and transportation of crayfish (B.M. Johnson, unpublished data). Managers seeking to discourage illegal introductions can promote among stakeholders 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. My 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 ratios provide UCRB managers with the means to evaluate what reservoirs pose the greatest escapement risk and focus their control efforts most efficiently.

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## 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. *Canadian Journal of Fisheries and Aquatic Sciences* 61:2425-2439. 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. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 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. *Limnology and Oceanography*. 53: 1633-1642.
- 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. *Geochimica et Cosmochimica Acta* 64: 1705-1714.
- Cambray, J.A. 2003. Impact on indigenous species biodiversity caused by globalization of alien recreational freshwater fisheries. *Hydrobiologia* 500: 217-230.
- Campana, S.E., and Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 30-38. doi: 10.1139/cjfas-58-1-30.

- 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*. *Marine Biology* 149: 1227-1235. doi: 10.1007/s00227-006-0280-x.
- 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. *Journal of Archaeological Science* 1226-1239. doi: 10.1016/j.jas.2006.10.014.
- Eby, L.A., W.J. Roach, L.B. Crowder, and J.A. Stanford. 2006. Effects of stocking-up freshwater food webs. *Trends in Ecology and Evolution* 21:576-584.
- 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. *Oceanography and Marine Biology* 46: 297-330.
- 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. *Transactions of the American Fisheries Society* 136: 469-476. doi: 10.1577/T06-044.1.
- 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. *Canadian Journal of Fisheries and Aquatic Sciences* 66: 513-524. doi: 10.1577/T06-044.1.

- Gillanders, B.M. 2002. Temporal and spatial variability in elemental composition of otoliths: implications for determining stock identity and connectivity of populations. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 669-679.
- 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. *Chemical Geology* 66: 245-272.
- Hamer, P.A., and Jenkins, G.P. 2007. Comparison of spatial variation in otolith chemistry of two fish species and relationships with water chemistry and otolith growth. *Journal of Fish Biology* 71:1035-1055. doi: 10.1111/j.1095-8649.2007.01570.x.
- Hawkins, J.A., and Nesler, T.P. 1991. Nonnative fishes of the upper basin: an issue paper. Final Report of Colorado State University Larval Fish Laboratory to Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado.
- Hawkins, J., Walford, C., and Hill, A. 2009. Smallmouth bass control in the middle Yampa River, 2003-2007. Contribution 154 of the Larval Fish Laboratory, Colorado State University, Fort Collins, Colorado.
- 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. *Hydrological Processes* 24: 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* 27:851-854.
- Jackson, M.G., and Hart, S.R. 2006. *Earth and Planetary Science Letters* 245: 260-277. doi: 10.1016/j.epsl.2006.02.040.

- Johnson, P.T.J., Olden J.D., and Vander Zanden, M.J. 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. *Frontiers in Ecology and the Environment* 6: 359-365.
- 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* 34: 389-394. doi: 10.1577/1548-8446-34.8.389.
- Kendall, C., and Coplen, T.B. 2001. Distribution of oxygen-18 and deuterium in river waters across the United States. *Hydrological Processes* 15: 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. *Canadian Journal of Fisheries and Aquatic Sciences* 57:2280-2292.
- 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*). *Canadian Journal of Fisheries and Aquatic Sciences* 62: 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. *Transactions of the American Fisheries Society* 137: 1674-1682. doi: 10.1577/T07-249.1.
- Martinez, P.J., Chart, T.E., Trammel, M.A., Wullschleger, J.G., and Bergersen, E.P. 1994. Fish species composition before and after construction of a main stem reservoir on the White River, Colorado. *Environmental Biology of Fishes* 40: 227-239.

- 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. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 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* 24: 16-27. doi: 10.1577/1548-8446(1999)0242.0.CO.
- Poff, N.L., and Ward, J.V. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1805-1818. doi: 10.1139/f89-228.
- Reed, J.C., and Bush, C.A. 2005. Generalized Geologic Map of the United States, Puerto Rico and the Virgin Islands [online]. Available from <http://pubs.usgs.gov/atlas/geologic/> (accessed April 4, 2000).
- Reis-Santos, P., Vasconcelos, R.P., Ruano, M., Latkoczy, C., Gunter, D., Costa, M.J., and Cabral, H. 2008. Interspecific variations of otolith chemistry in estuarine fish nurseries. *Journal of Fish Biology* 72:2595-2614. doi: 10.1111/j.1095-8649.2008.01871.x.
- 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. *Transactions of the American Fisheries Society* 137: 818-829. doi: 10.1577/T06-023.1.

- Singer, J.D. 1998. Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. *Journal of Educational and Behavioral Statistics* 24: 323-355.
- Singer, J.D., and Willet, J.B. 2003. *Applied Longitudinal Data Analysis: modeling change and event occurrence*. Oxford University Press, New York, New York.
- Swearer, S.E., Forrester, G.E., Steele, M.A., Brooks, A.J., and Lea, D.W. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. *Estuarine Coastal and Shelf Science* 56:1111-1123. doi: 10.1016/S0272-7714(02)00317-7.
- Thornton, K.W., Kimmel, B.L., and Payne, F.E. 1990. *Reservoir Limnology: Ecological Perspectives*. John Wiley & Sons, Somerset, New Jersey, USA.
- Tyus, H.M., and Saunders, J.F. 2000. Nonnative fish control and endangered fish recovery: Lessons from the Colorado River. *Fisheries* 25:17-24. doi: 10.1577/1548-8446(2000)0252.0.CO.
- U.S. 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. *Federal Register* 59:54 (21 March 1994): 13374-13400.
- U.S. Census Bureau. 2000. Cartographic Boundary Files [online]. Available from <http://www.census.gov/geo/www/cob/index.html> (accessed April 4, 2001).
- Valdez, R.A., and Muth, R.T. 2005. Ecology and conservation of native fishes in the Upper Colorado River Basin. Pages 157-204 in J.N. Rinne, R.B. Hughes, and B. Calamusso, editors. *Historical changes in large river fish assemblages of the Americas*. American Fisheries Symposium 45, Bethesda, Maryland.

- 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. *Marine Ecology-Progress Series* 311:125-130.
- Walther, B.D., Thorrold, S.R., and Olney, J.E. 2008. Geochemical signatures in otoliths record natal origins of American shad. *Transactions of the American Fisheries Society* 137: 57-69. doi: 10.1139/F08-164.
- Walther, B.D., and Thorrold, S.R. 2009. Inter-annual variability in isotope and elemental ratios recorded in otoliths of an anadromous fish. *Journal of Geochemical Exploration* 102: 181-186. doi: 10.1016/j.gexplo.2008.10.001.
- Wells, B.K., Reiman, 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. *Transactions of the American Fisheries Society* 132: 409-424.
- Whitledge, G.W., Johnson, B.M., and Martinez, P.J. 2006. Stable hydrogen isotopic composition of fishes reflects that of their environment. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 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. *Transactions of the American Fisheries Society* 136:1263-1275. 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. *Journal of Analytical Atomic Spectrometry* 20: 22-27. doi: 10.1039/b412730g.

Table 1. Characteristics of reservoirs from which fish were sampled for isotopic examination of otoliths. Subbasin codes:

CRB = Colorado, DOB = Dolores, GRB = Green, GUB = Gunnison, WHB = White and YAB = Yampa. N/A = not applicable, some reservoirs were off channel. No data on hydraulic residence time available for Rio Blanco Reservoir. CV = coefficient of variation.

Reservoir	Reservoir code	Subbasin code	Stream impounded	Surface elevation (m)	Surface area (ha)	Volume (ha-m)	Max depth (m)	Mean HRT (years)	CV HRT
Harvey Gap	HGR	CRB	N/A	1 951	116	723	12.5	0.54	0.13
Rifle Gap	RGR	CRB	Rifle Creek	1 817	145	13	26.5	0.53	0.34
McPhee	MCP	DOB	Dolores R.	2 110	1 809	47 020	85.3	1.70	0.75
Flaming Gorge	FGR	GRB	Green R.	1 841	17 005	467 354	133.0	4.14	0.42
Red Fleet	RFR	GRB	Brush Creek	1 709	210	3 210	44.0	1.15	0.60
Starvation	STA	GRB	Strawberry R.	1 741	1 117	20 081	47.2	1.53	0.39
Crawford	CRA	GUB	Smith Fork Creek	1 997	164	1 776	36.6	0.73	0.24
Juniata	JUR	GUB	N/A	1 740	58	847	16.5	1.31	0.05
Paonia	PAO	GUB	N. Fork Gunnison R.	1 965	135	2 584	42.7	0.31	0.64
Kenney	KER	WHR	White	1 609	243	1 702	21.6	0.03	0.31
Rio Blanco Lake	RBR	WHR	N/A	1 754	47	128	5.5	-	-
Lake Catamount	CAT	YAB	Yampa R.	2 142	214	915	13.0	0.06	0.33
Elkhead	ELK	YAB	Elkhead Creek	2 142	162	3 056	17.7	0.48	0.28
Stagecoach	SCR	YAB	Yampa R.	2 196	316	4 104	39.6	0.86	0.62

Table 2. A) Results from 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 ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) in each reservoir. Model FishID is for individual fishes with multiple measures. B) Results from  $\text{AIC}_c$  analysis of the top ranked random effects model with all combinations of fixed effects reservoir (Res), species (Spp) and year. C)  $\text{AIC}_c$  results when a dichotomous species effect, Spp (DSE), was added in place of the all species are different effect (Spp).  $\Delta_i$  = Delta  $\text{AIC}_c$ ,  $\text{ML}_i$  = Maximum Likelihood,  $w_i$  = Akaike Weight and  $W_{+(j)}$  = Cumulative Akaike Weight.

Model	$\text{AIC}_c$	$\Delta_i$	$\text{ML}_i$	$w_i$	$W_{+(j)}$
A) Res_Spp_Year*	-4524.20	0.00	1.00	1.00	-
FishID	-4409.60	114.60	0.00	0.00	-
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
C) Res; Spp (DSE)	-4930.30	0.00	1.00	0.98	-
Res; Spp	-4922.60	7.70	0.02	0.02	-

\*Model includes the FishID random effect

Table 3. Results from variance components analysis for each fixed effect from otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis.

Fixed effects	Proportion of total variance (%)			Total variance	Total variance decrease (%)
	FishID random effect	Res_Spp_Year random effect	Residual		
Reservoir	24	31	45	$1.22 \times 10^{-8}$	96
Species	93	3	4	$1.40 \times 10^{-7}$	50
Year	1	97	2	$2.66 \times 10^{-7}$	5
Intercept	1	97	2	$2.79 \times 10^{-7}$	-

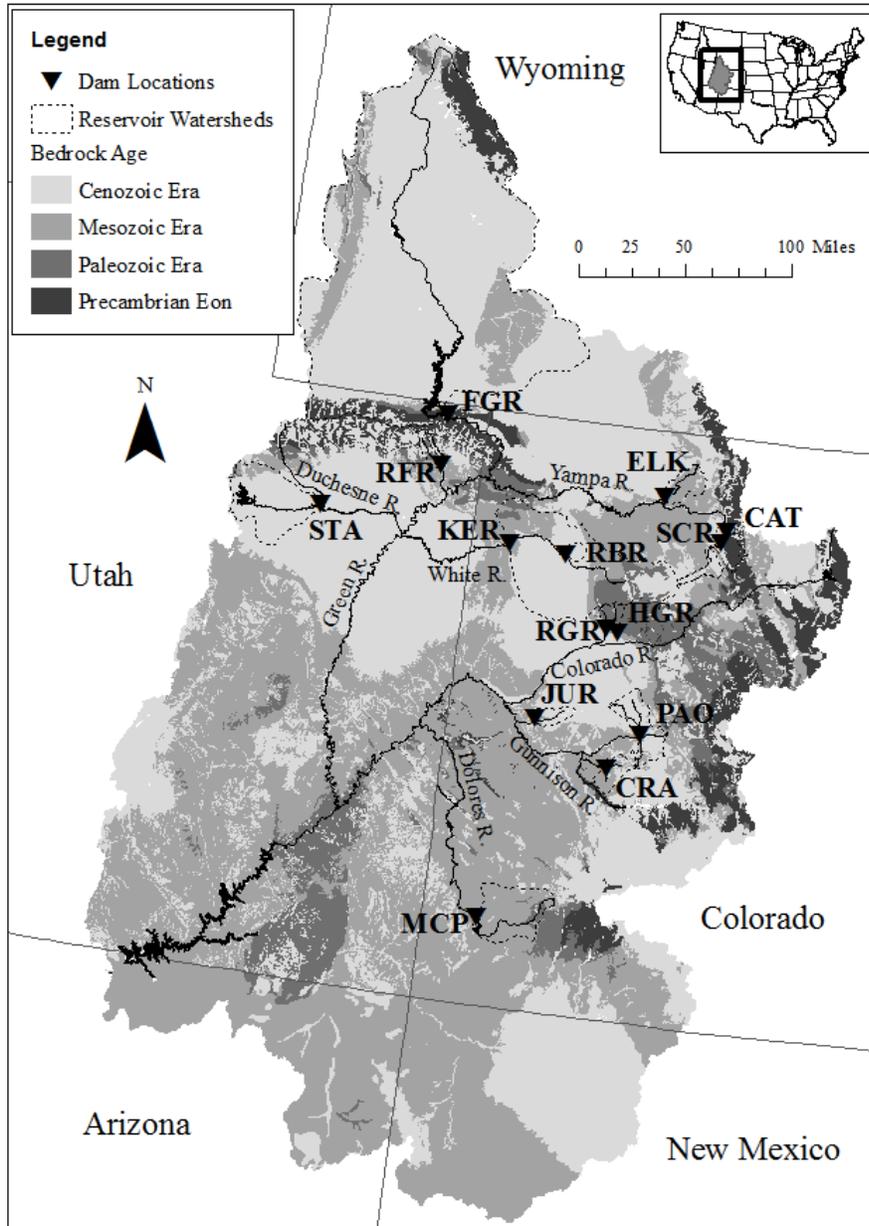


Figure 1. Map of bedrock age and reservoir locations (mentioned in the text) within the Upper Colorado River Basin. Reservoir watersheds delineate the effective area of water flow into each reservoir. Bedrock ages and state lines were obtained through Reed and Bush (2005) and U.S. Census Bureau (2000), respectively. Refer to Table 1 for reservoir codes

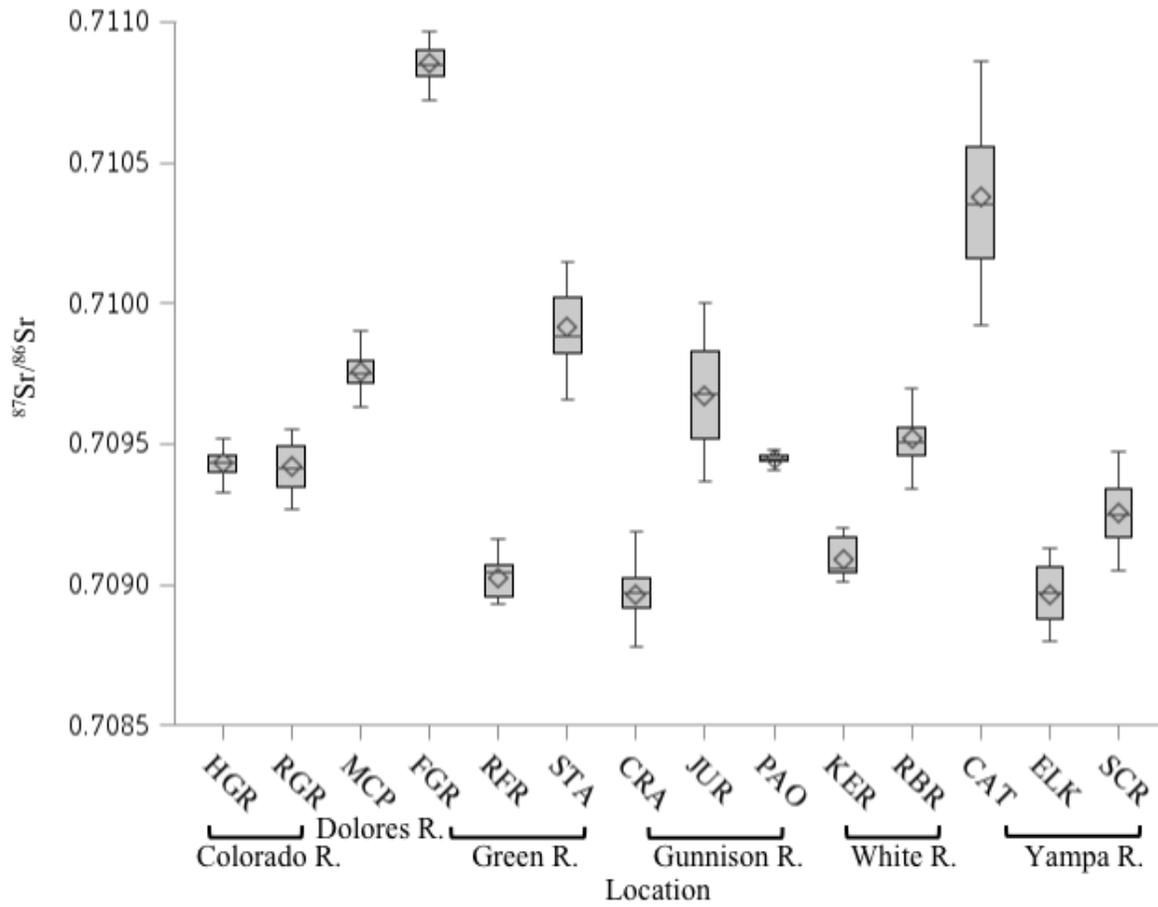


Figure 2. Box-and-whisker plots of strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) among reservoirs in six subbasins of the Upper Colorado River Basin, including all species and years. For each box-and-whisker plot, the bottom and top edges are the 25th and 75th percentiles respectively, 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. Refer to Table 1 for reservoir codes.

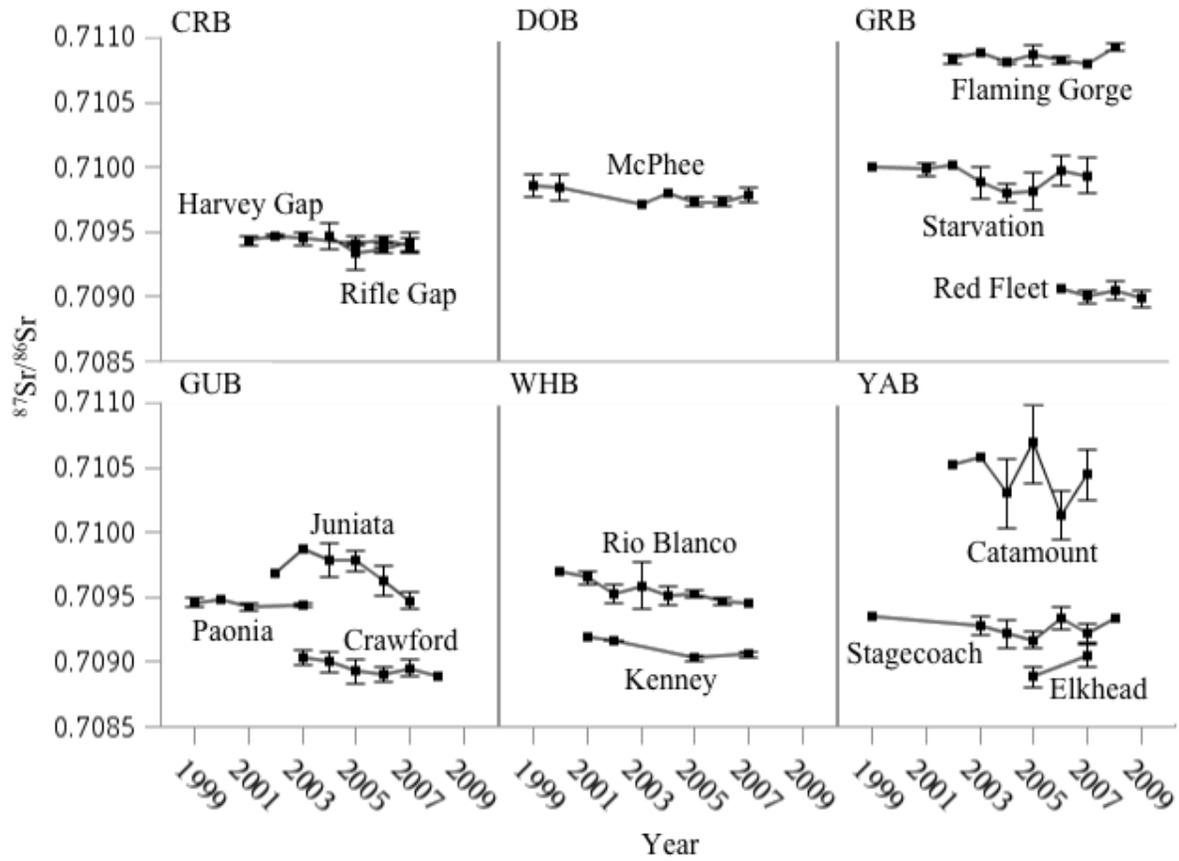


Figure 3. Strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr} \pm 2 \text{ SE}$ ) of reservoirs from 1999 to 2009, averaged for each individual and species in a given year. Reservoirs are separated by river subbasins. Refer to Table 1 for river basin codes.

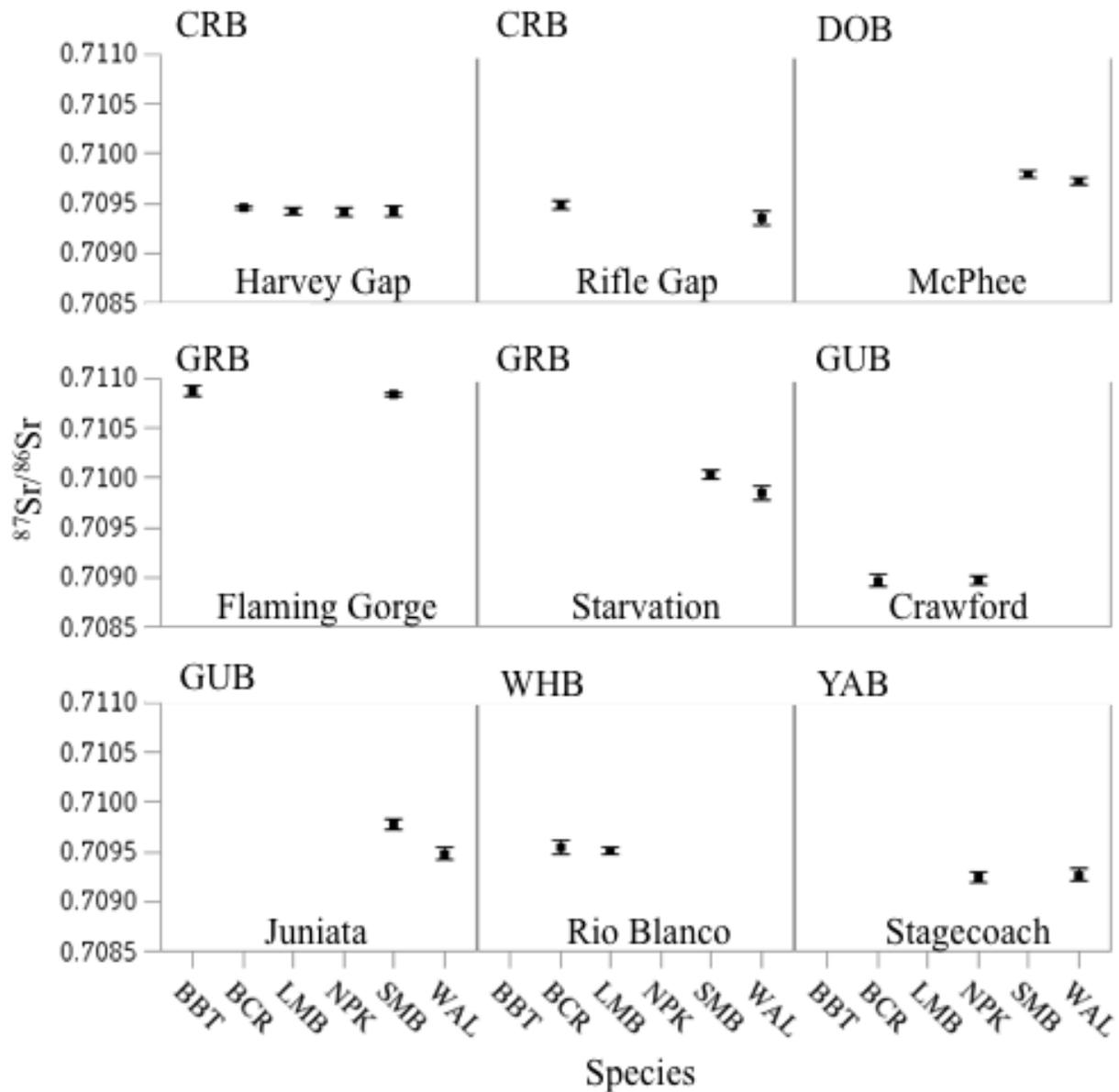


Figure 4. Strontium isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) ratios among species within reservoirs with 95% confidence limits, including all years of data. Species codes: BBT = burbot, BCR = black crappie, LMB = largemouth bass, NPK = northern pike, SMB = smallmouth bass, WAL = walleye. Reservoirs are separated by river subbasins. Refer to Table 1 for river basin codes.

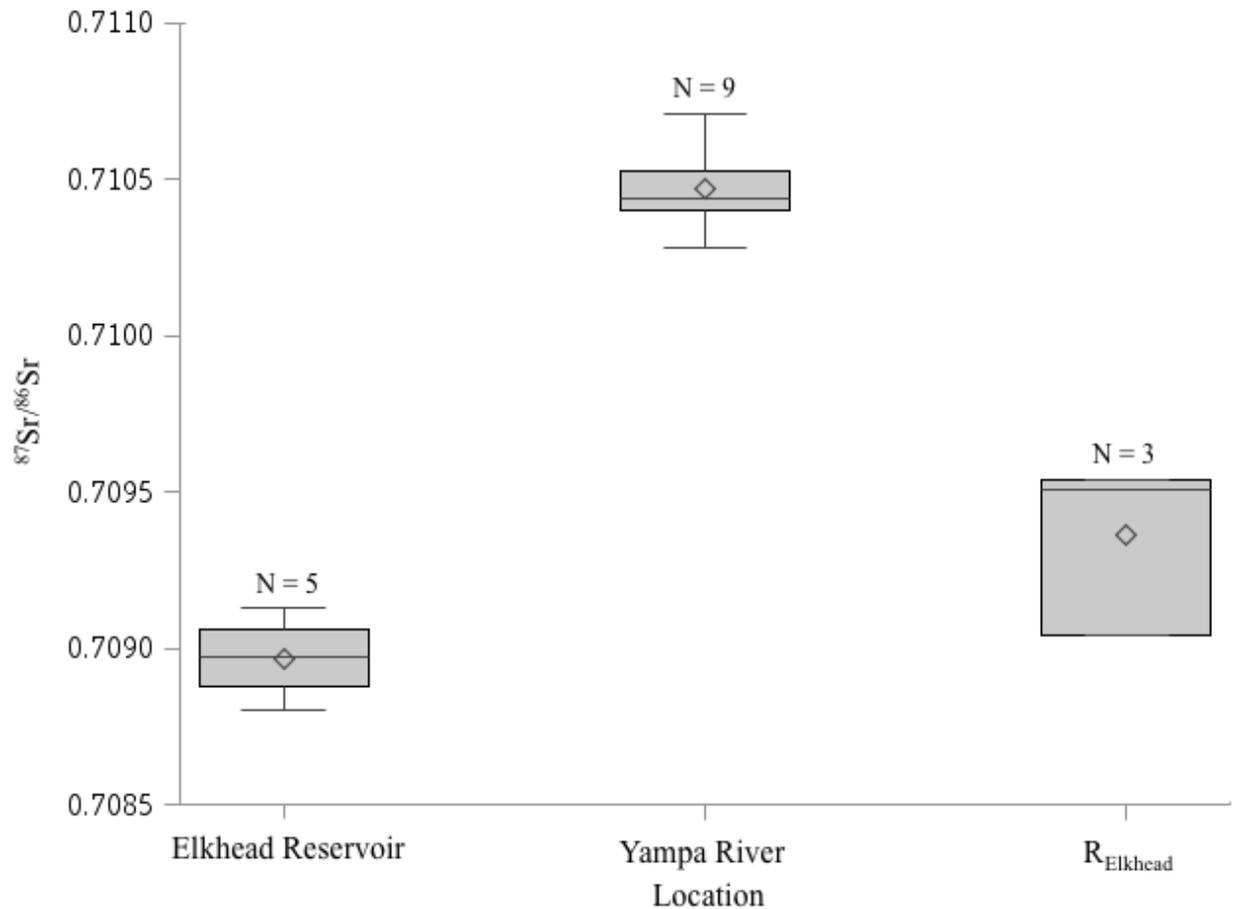


Figure 5. Box-and-whisker plots of strontium isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) ratios of transplanted smallmouth bass from the Yampa River Basin. The Yampa River boxplot is from smallmouth bass that had been tagged and released in the Yampa River, where they were recaptured at least a year later.  $R_{\text{Elkhead}}$  refers to edge Sr ratios of fish that were transplanted from the Yampa River into Elkhead Reservoir and recaptured from Elkhead Reservoir.

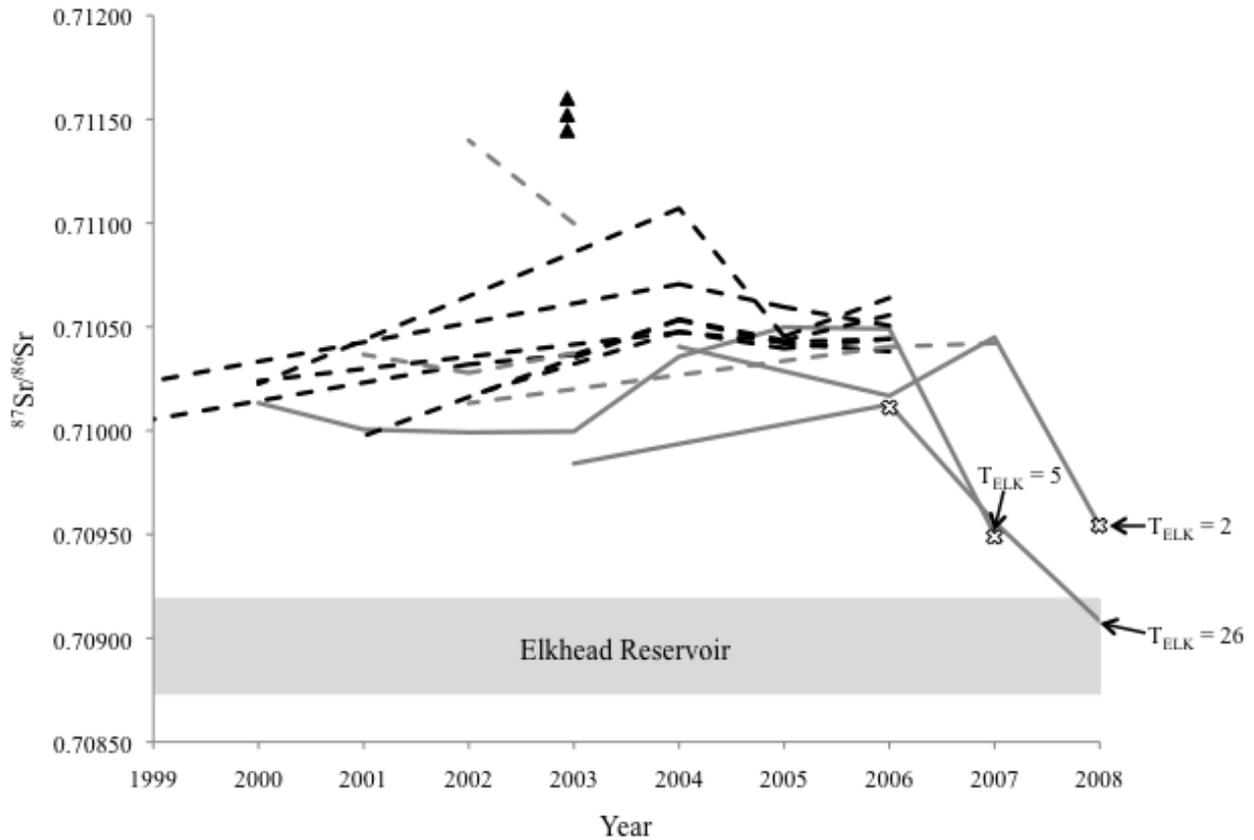


Figure 6. Movements of tagged smallmouth bass determined by Sr isotope ratios from year-specific ablations, Yampa River, CO. Solid gray lines are fish that were captured from the Yampa River and transplanted into Elkhead Reservoir, where they were finally recaptured. Black dashed lines are fish that were tagged and released within the Yampa River and had at spent at least one year in the river prior to capture, 2004-2007. Gray dashed lines and triangles are ablations from untagged fish. The open X marks indicate the year when each fish was tagged and relocated to Elkhead Reservoir.  $T_{ELK}$  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 three).

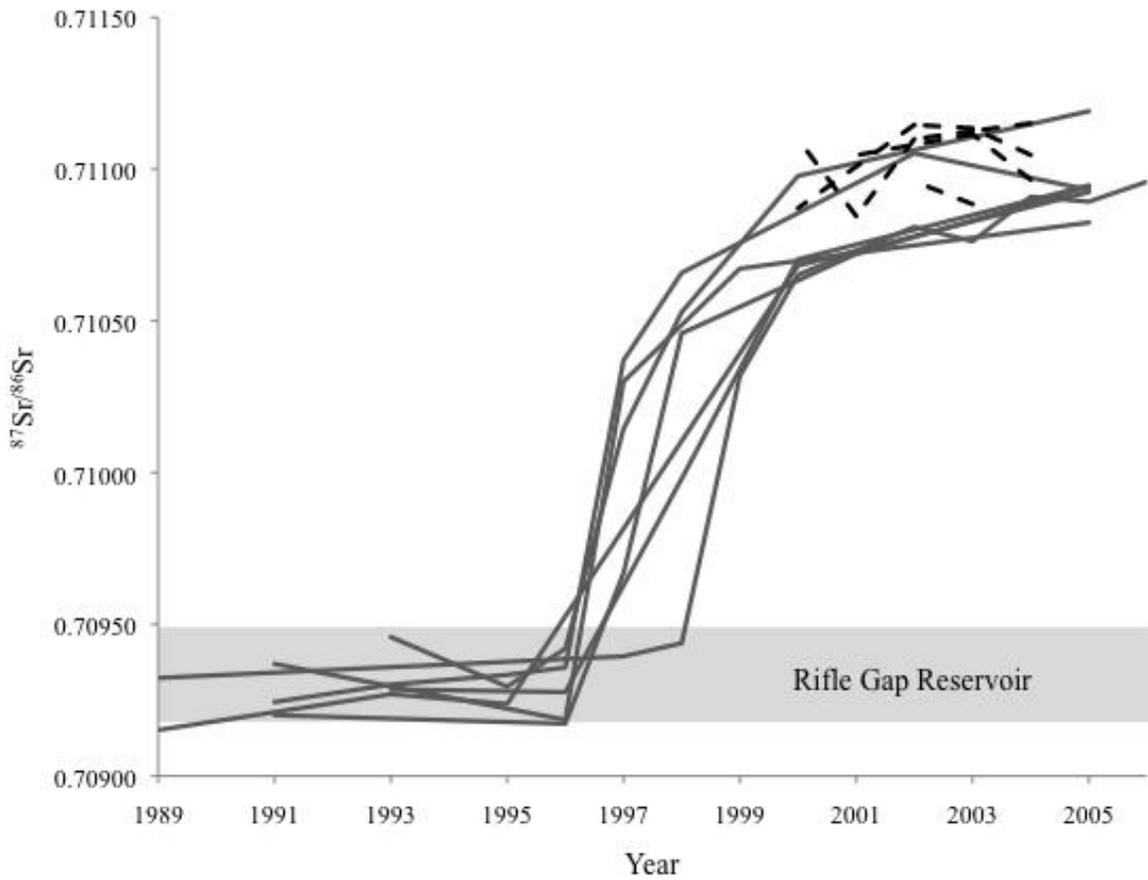


Figure 7. Movements of four smallmouth bass (dashed lines) and seven walleye collected from the Colorado River, collected downstream of Rifle Gap Reservoir near Rulison, CO, determined by year-specific ablations of otoliths. The gray bar represents the 95% confidence interval of the Rifle Gap Reservoir signature of walleye determined by predictions from the top ranked model (analysis, phase three).

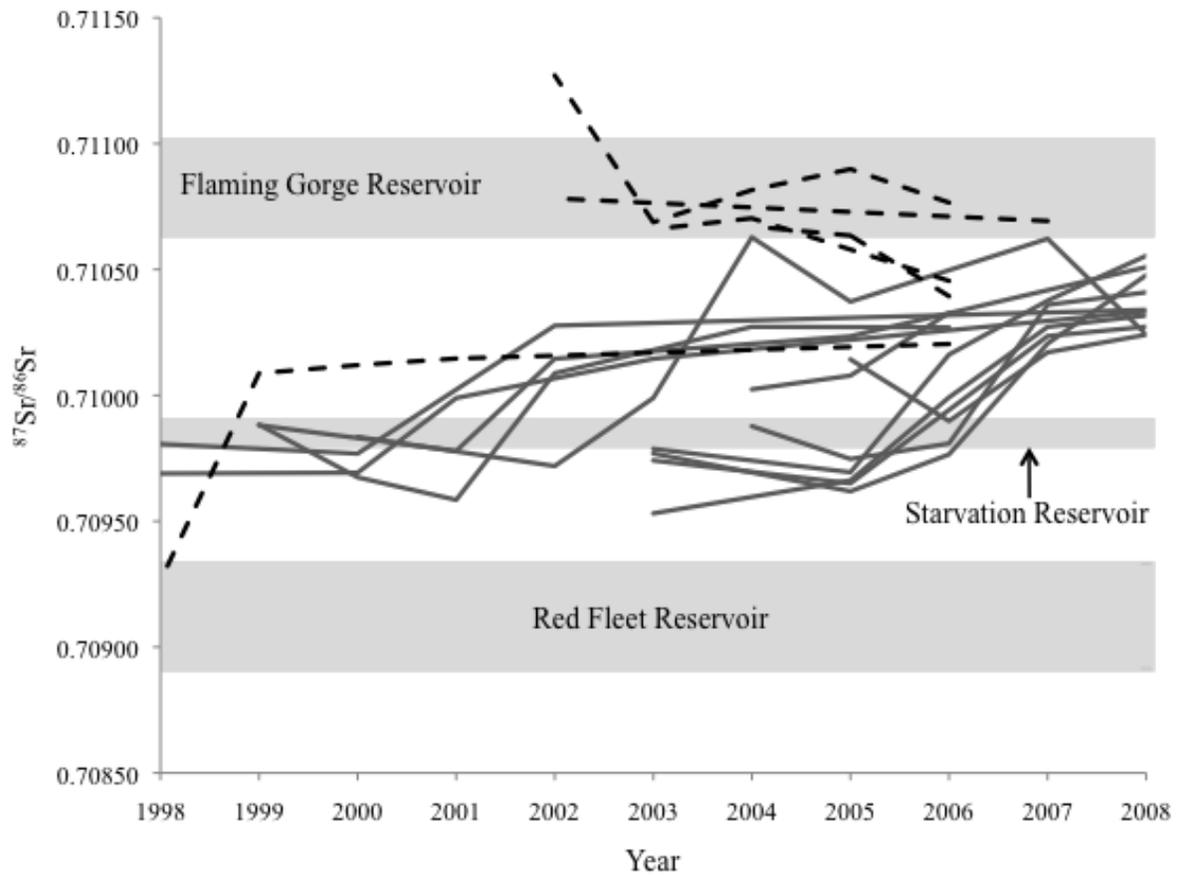


Figure 8. Movements of five smallmouth bass (dashed lines) and twelve walleye (solid lines) collected from the Upper Green River, UT, determined by year-specific ablations of otoliths. The gray bars represent the 95% confidence intervals of each reservoir signature determined by predictions from the top ranked model (analysis, phase three). The Starvation Reservoir bar is the modeled prediction of walleye from that reservoir.

## Chapter 2

# DO ADDITIONAL STABLE ISOTOPES ( $\delta\text{D}$ , $\delta^{13}\text{C}$ AND $\delta^{18}\text{O}$ ) IN OTOLITHS PROVIDE DISCRIMINATION AMONG GROUPS?

### Introduction

Stable isotopes of hydrogen ( $\delta\text{D}$ ; Deuterium), carbon ( $\delta^{13}\text{C}$ ), and oxygen ( $\delta^{18}\text{O}$ ) were examined to determine if other natural markers would be effective in distinguishing locations, particularly in cases where strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ; hereafter, “Sr ratio”) alone cannot. These markers have been shown to be effective at separating potential locations of unknown sources of fishes (Walther et al. 2008; Whitley et al. 2009; Zeigler and Whitley 2011).

Oxygen isotopes have been particularly useful in determining temperatures experienced by fishes throughout their lifetimes (Devereux 1967; Patterson 1998). Otoliths incorporate  $\delta^{18}\text{O}$  near isotopic equilibrium with ambient waters (Devereux 1967; Iacumin et al. 1992; Thorrold et al. 1997), where observed  $\delta^{18}\text{O}$  values can be translated to temperatures of waters fishes inhabit. As water evaporates, generally more of the heavy isotope  $^{18}\text{O}$  is retained in a water body and the lighter isotope  $^{16}\text{O}$  is removed in the water vapor. This fractionation results in a predictable pattern of relatively depleted  $\delta^{18}\text{O}$  values at higher latitudes (Criss 1999) and elevations (Rose et al. 1996).

Hydrogen isotopes have been used for studies examining habitat use and migration patterns of terrestrial animals (Bowen et al. 2005; Wunder et al. 2005; Sellick et al. 2009), but only a few studies have examined  $\delta\text{D}$  in fish otoliths (Whitley et al. 2006; 2007). Deuterium is dependent upon similar factors as  $\delta^{18}\text{O}$ , where evaporation enriches the  $\delta\text{D}$  of the water body. Fish incorporate ambient  $\delta\text{D}$  in their otoliths without further fractionation (Whitley et al. 2006).

Carbon isotope fractionation in the environment is mostly due to differences in photosynthetic pathways of plants, where C3 plants typically are depleted around 22 ‰ and C4 plants are depleted ca. 4 ‰ (Peterson and Fry 1987). There is evidence that  $\delta^{13}\text{C}$  in otoliths is controlled largely by metabolic effects, where there is typically a negative relationship of temperature and a positive relationship of somatic growth on  $\delta^{13}\text{C}$  (Thorrold et al. 1997). However, some  $\delta^{13}\text{C}$  in otoliths is derived from dissolved inorganic carbon (Kalish 1991), so different locations may have levels of DIC that providing unique  $\delta^{13}\text{C}$  in otoliths (Whitledge 2009).

## **Materials and methods**

### *Stable Isotope analysis*

For stable isotope analysis, the whole right sagittal otolith from each fish was ground to a fine powder. Analysis of  $\delta\text{D}$  required different laboratory techniques than those used for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  because the  $\delta\text{D}$  analysis is performed on the organic portion of the otolith, whereas  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  analysis is done on the inorganic portion of the otolith. Since the organic portion of the otolith is ca. 10% of the otolith, the majority of the otolith had to be used for  $\delta\text{D}$  analysis.

For  $\delta\text{D}$  analysis, 2 mg otolith powder from each fish was placed into a tin capsule, where it was sealed and sent to the Alaska Stable Isotope Facility (ASIF) of University of Alaska Fairbanks. All otolith samples were allowed to equilibrate to ambient lab conditions at ASIF for at least two weeks before analysis. The otolith samples were then assayed for  $\delta\text{D}$  using a Thermo Finnigan MAT high temperature elemental analyzer (TC/EA) and a ConFlo III interfaced with a DeltaXP Mass Spectrometer (Pyrolysis-EA-IRMS). The otolith capsules were placed into the TC/EA autosampler, pyrolyzed into  $\text{H}_2$  and  $\text{CO}_2$  gases then separated chromatographically. These gases were then transferred to the IRMS, where the isotopes are measured. Blanks were

analyzed every twenty samples and working standards (benzoic acid) every ten samples. Additionally, the laboratory working standards were compared to the National Institute of Standards and Technology (NIST) standards NBS22, NBS30, PEF1 and sucrose ANU each day.

For  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  analysis, otolith samples were assayed using a Thermo Finnigan GasBench II analyzer with a Delta<sup>Plus</sup>XP Mass Spectrometer (Gas-IRMS). Otolith samples (0.50 mg) were first placed into clean round bottom exetainer tubes. The tubes were purged for 20 minutes with UHP Helium and then 0.2 mL of 85%  $\text{H}_3\text{PO}_4$  was added by syringe to the sample through the septum. The headspace gases were then transferred to the GasBench II where the water was removed through a nafion dryer. Carbon dioxide was separated chromatographically from other gases present and then transferred to the IRMS, where the isotopes of oxygen and carbon were measured. Blanks were analyzed every twenty samples and NIST standards NBS18, NBS19 and LSVEC every ten samples.

All three stable isotopes are expressed in standard  $\delta$  notation, defined as parts per mil ( $\text{‰}$ ) deviation of an isotope sample compared to standard material (Pee Dee Belemite for otolith  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  and Vienna Standard Mean Ocean Water for otolith  $\delta\text{D}$ ):  $\delta\text{R} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$ , where R represents the stable isotope ratios  $^2\text{H}/^1\text{H}$ ,  $^{13}\text{C}/^{12}\text{C}$ , or  $^{18}\text{O}/^{16}\text{O}$  (Fry 2006).

### *Statistical analysis*

Canonical discriminant function analysis (DFA; PROC CANDISC, SAS<sup>®</sup>) was used to visualize the ability of  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and Sr ratios to discriminate between different locations in the Upper Colorado River Basin. Fishes in this analysis were either captured from a reservoir, or were river-caught fishes that had Sr ratios that appeared stable throughout their lifetimes, implying river-residence. The Pillai's Trace (Johnson 1998) statistic was used to test the significance of each variable. Canonical correlations and Eigenvalues were used to assess the

dimensional space necessary to separate groups. Total canonical variate coefficients were used to examine the relative importance of each variable in observed separation among locations. Post hoc, a linear discriminant function analysis (DFA) was used to determine the ability to reclassify individuals to their origin using  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and Sr ratios, excluding  $\delta\text{D}$ .

Variance components analysis was performed on each stable isotope ( $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) separately to determine the relative importance of location and species effects; since otoliths had to be ground whole, I was unable to examine temporal effects for the stable isotope analysis. This variance components analysis was performed using the same method explained in Chapter 1 Sr ratio analysis (Wolff et al. in review), but without multiple measures within fish or year-specific isotopic signatures. Therefore, I used  $\text{AIC}_c$  to rank two models to be used for the variance components analysis: 1) no random effects and only a fixed intercept, Intercept, and 2) random effect for all location and species combinations, Loc\_Spp. Lastly, I performed regression analysis on each stable stable isotope to determined the predictability of isotopes with changes in reservoir elevation and latitude.

## **Results**

Canonical DFA revealed that all isotopes examined were significantly different across locations (Pillai's Trace,  $F = 17.56$ ;  $p < 0.0001$ ). Canonical correlations showed that the majority of the location assignments could be explained into two dimensions with Eigenvalue proportions of 76% and 18% (Eigenvalue = 33.82 and 7.81) for canonical variates 1 and 2, respectively. Total canonical variate coefficients showed that Sr ratios provided the greatest relative separation among groups, followed by  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$  and  $\delta\text{D}$ , respectively. Although  $\delta\text{D}$  was statistically significant ( $F = 7.77$ ;  $p < 0.0001$ ), this isotope marker was relatively poor at visually distinguishing different locations, i.e., the canonical DFA plot with the inclusion of  $\delta\text{D}$

looked essentially the same as when excluded. I found that  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and Sr ratios separate locations with high precision and very little overlap among locations (Figure 1). Additionally, when  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were used solely in the canonical DFA, many locations were still separated well in two-dimensional space (Figure 2). The addition of carbon and oxygen stable isotopes provided separation among locations where Sr ratios alone could not distinguish. For instance, the Sr ratios of fishes from Flaming Gorge Reservoir, Lake Catamount and the Yampa River had very similar Sr ratios, but were easily distinguished in the canonical DFA with the additional stable isotopes.

Linear DFA showed that most locations were correctly assigned to their location of origin using only  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and Sr ratios (Table 2). Individuals were correctly classified to origin by an average of 94.11% (SD = 9.31). Lake Catamount and Colorado and Yampa rivers had classification accuracies of 80%, 89%, and 79%, respectively. There were no misclassifications of reservoirs within the same subbasin.

The top random effects model chosen using  $\text{AIC}_c$  was the model with a random effect for all location and species combinations, Loc\_Spp. The model Loc\_Spp possessed 100% of the Akaike weight for  $\delta\text{D}$ ,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ , and there was no support for the model without a random effect with delta of 15.30, 77.50, and 99.40 for  $\delta\text{D}$ ,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ , respectively. The variance components analysis showed that location explains a much greater amount of variance in the model than species. Location explained 45% of the total variance from the random effects model for  $\delta\text{D}$ , and 82% for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . Species explained only 9%, 24%, and 18% of the total variance from the random effects model for  $\delta\text{D}$ ,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  respectively (Table 3). Therefore, I conclude fairly negligible species effects within location when using the additional stable isotope analysis.

Regression analysis of stable isotopes as a function of elevation and latitude revealed no statistically significant ( $p > 0.05$ ) relationships (Figure 3). However, trends of  $\delta^{18}\text{O}$  and  $\delta\text{D}$  were positively correlated with increasing latitude and  $\delta^{13}\text{C}$  was negatively correlated with latitude and elevation.

## **Discussion**

The addition of stable isotope markers to Sr ratios showed very high separation among different locations. I found that many locations with similar Sr ratios could be distinguished with the addition of stable isotopes  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{18}\text{O}$ , such as Flaming Gorge Reservoir and Colorado and Yampa rivers. Others have also found these to be useful markers to differentiate groups (Walther et al. 2008; Whitley 2009; and Zeigler and Whitley 2011). Stable isotopes  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  showed strong separation among different groups, whereas  $\delta\text{D}$  provided very minimal separation. Because  $\delta\text{D}$  analysis also consumes an entire otolith, eliminating the examination of temporal effects, this tracer may not be useful in this context. Both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  appear to be useful adjuncts to Sr ratios, especially when Sr ratios are similar among locations. Unfortunately, some reservoirs could not be differentiated (e.g., Stagecoach and Elkhead reservoirs), even with the addition of stable isotope markers, and remained closely grouped to each other.

Because the whole otolith was ground to a powder for all stable isotope analyses, I could not examine temporal stability of these additional markers. Previous researchers have found  $\delta^{18}\text{O}$  to be temporally unstable across years (Walter and Thorrold 2009). Since,  $\delta^{18}\text{O}$  is strongly influenced by precipitation rates (Bowen et al. 2005), it is very likely to fluctuate between seasons (e.g., base flow vs. runoff) and water years (e.g., drought conditions vs. high water). Therefore, caution must be used when applying this isotope as a marker of fishes from different

age classes. If the stable isotopes in this study are not temporally stable, samples would need to be collected yearly to establish an “archive” of stable isotope signatures for each location, particularly where there is limited Sr ratio separation.

Stable isotope  $\delta^{13}\text{C}$  is largely metabolically driven, ca. 45% of the fractionation (Thorrold et al. 1997). Therefore, this isotope is likely to vary by species more than  $\delta^{18}\text{O}$  and  $\delta\text{D}$  because many species have different metabolisms, thermal tolerances, and habitat preferences that may alter  $\delta^{13}\text{C}$  within a water body. However, variance components showed only 25% of the total variance was caused species differences in with a location. Therefore, it is possible that species differences are relatively small compared to DIC differences among locations when assigning origins of fishes.

Stable isotope signatures did not vary with latitude or elevation as has been shown in other studies. Thorrold et al. (1997) found that  $\delta^{13}\text{C}$  in fish became more enriched with elevation and latitude but I found the opposite pattern. Alternatively,  $\delta^{18}\text{O}$  and  $\delta\text{D}$  are typically more depleted with increasing elevation and latitude (Criss 1999), and again I found the opposite trend. Regression coefficients in my data were not strong, and it may also be true that the scale of the study area was too small to expect clear patterns with elevation and altitude.

In conclusion, I observed high classification accuracies and relatively low inter-specific variability using  $\delta^{18}\text{O}$  and  $\delta\text{D}$  differences among reservoirs. The additional stable isotope markers clearly have benefits of distinguishing groups in situations where Sr ratios alone cannot.

## References

- Bowen, G.J., Wassenaar, L.I., and Hobson, K.A. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* 143: 337-348.
- Criss, R.E. 1999. Principles of stable isotope distribution. Oxford University Press, New York, New York.
- Devereux, I. 1967. Temperature measurements from oxygen isotope ratios of fish otoliths. *Science* 155: 1684-1685.
- Fry, B. 2006. Stable isotope ecology. Springer, New York, New York.
- Iacumin, P., Bianucci, G., and Longinelli, A. 1992. Oxygen and carbon isotopic composition of fish otoliths. *Marine Biology* 113: 537-542.
- Johnson, D.E. 1998. Applied multivariate methods for data analysts. Brooks/Cole Publishing Company, Pacific Grove, California.
- Patterson, W.P. 1998. North American continental seasonality during the last millennium: high-resolution analysis of sagittal otoliths. *Palaeogeography, Palaeoclimatology, Palaeoecology* 138: 271-303.
- Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18: 293-320.
- Rose, T.P., Davisson, M.L., Criss, R.E. 1996. Isotope hydrology of voluminous cold springs in fractured rock from an active volcanic region, northeastern California. *Journal of Hydrology* 179: 207-236.
- Sellick, M.J., Kyser, T.K., Wunder, M.B., Chipley, D., and Norris, D.R. 2009. Geographic variation of strontium and hydrogen isotopes in avian tissue: implications for tracking migration and dispersal. *PLoS ONE* 4 (3): 1-11.

- Thorrold, S.R., Campana, S.E., Jones, C.M., and Swart, P.K. 1997. Factors determining  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  fractionation in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* 61: 2909-2919.
- Walther, B.D., Thorrold, S.R., and Olney, J.E. 2008. Geochemical signatures in otoliths record natal origins of American shad. *Transactions of the American Fisheries Society* 137: 57-69.
- Whitledge, G.W., Johnson, B.M., and Martinez, P.J. 2006. Stable hydrogen isotopic composition of fishes reflects that of their environment. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 1746-1751.
- 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. *Transactions of the American Fisheries Society* 136:1263-1275.
- Whitledge, G.W. 2009. Otolith microchemistry and isotopic composition as potential indicators of fish movement between the Illinois River drainage and Lake Michigan. *Journal of Great Lakes Research* 35: 101-106.
- Wunder, M.B., Kester, C.L., Knopf, F.L., and Rye, R.O. 2005. A test of geographic assignment using isotope tracers in feathers of known origin. *Oecologia* 144: 607-617.
- Zeigler, J.M., and Whitledge, G.W. 2011. Otolith trace element and stable isotopic compositions differentiate fishes from the Middle Mississippi River, its tributaries, and floodplain lakes. *Hydrobiologia* 661: 289-302.

Table 2.1. Total canonical variate coefficients from canonical discriminant function analysis to separate among fishes in different water bodies of the Upper Colorado River Basin, 2007-2009. The value of each coefficient indicates the relative importance of each isotope in separation among locations. Isotopes are ranked from highest relative importance (top) to lowest (bottom).

Isotope ratio	Variate 1	Variate 2
$^{87}\text{Sr}/^{86}\text{Sr}$	5.52	0.22
$\delta^{18}\text{O}$	-0.09	2.02
$\delta^{13}\text{C}$	-0.22	-1.66
$\delta\text{D}$	0.16	-0.09

Table 2.2. Linear discriminant function analysis classification accuracy results from fishes in different locations of Upper Colorado River Basin using  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopes. All species were combined within reservoirs. Location codes: CAT = Lake Catamount, COR = Colorado River, ELK = Elkhead Reservoir, FGR = Flaming Gorge Reservoir, RGR = Rifle Gap Reservoir, RFR = Red Fleet Reservoir, SCR = Stagecoach Reservoir, STA = Starvation Reservoir, and YAR = Yampa River. Subbasins listed above location. Sub-basin codes: CRB = Colorado River Basin, GRB = Green River Basin, and YAB = Yampa River Basin.

Reservoir classifications, in percent (sample size)									
From reservoir	CRB	CRB	GRB	GRB	GRB	YAB	YAB	YAB	YAB
	COR	RGR	FGR	RFR	STA	CAT	ELK	SCR	YAR
COR	89 (8)	-	-	-	-	-	-	-	11 (1)
RGR	-	100 (10)	-	-	-	-	-	-	-
FGR	-	-	100 (8)	-	-	-	-	-	-
RFR	-	-	-	100 (5)	-	-	-	-	-
STA	-	-	-	-	100 (8)	-	-	-	-
CAT	-	-	-	-	20 (1)	80 (4)	-	-	-
ELK	-	-	-	-	-	-	100 (5)	-	-
SCR	-	-	-	-	-	-	-	100 (8)	-
YAR	22 (2)	-	-	-	-	-	-	-	78 (7)

Table 2.3. Results from variance components analysis for each fixed effect from otolith stable isotope analysis: A)  $\delta D$ , B)  $\delta^{13}C$ , and C)  $\delta^{18}O$ . Loc\_Spp is model with on fixed effect, an intercept, and a random effect for location and species.

Fixed effects	Proportion of total variance (%)		Total variance	Decrease in total variance (%)
	Loc_Spp random effect	Residual		
A) Location	0	100	51.11	45
Species	31	69	85.21	9
Intercept	37	63	93.62	-
B) Location	88	12	0.74	82
Species	30	70	3.05	27
Intercept	83	17	4.15	-
C) Location	89	11	0.13	81
Species	40	60	0.57	13
Intercept	87	13	0.66	-

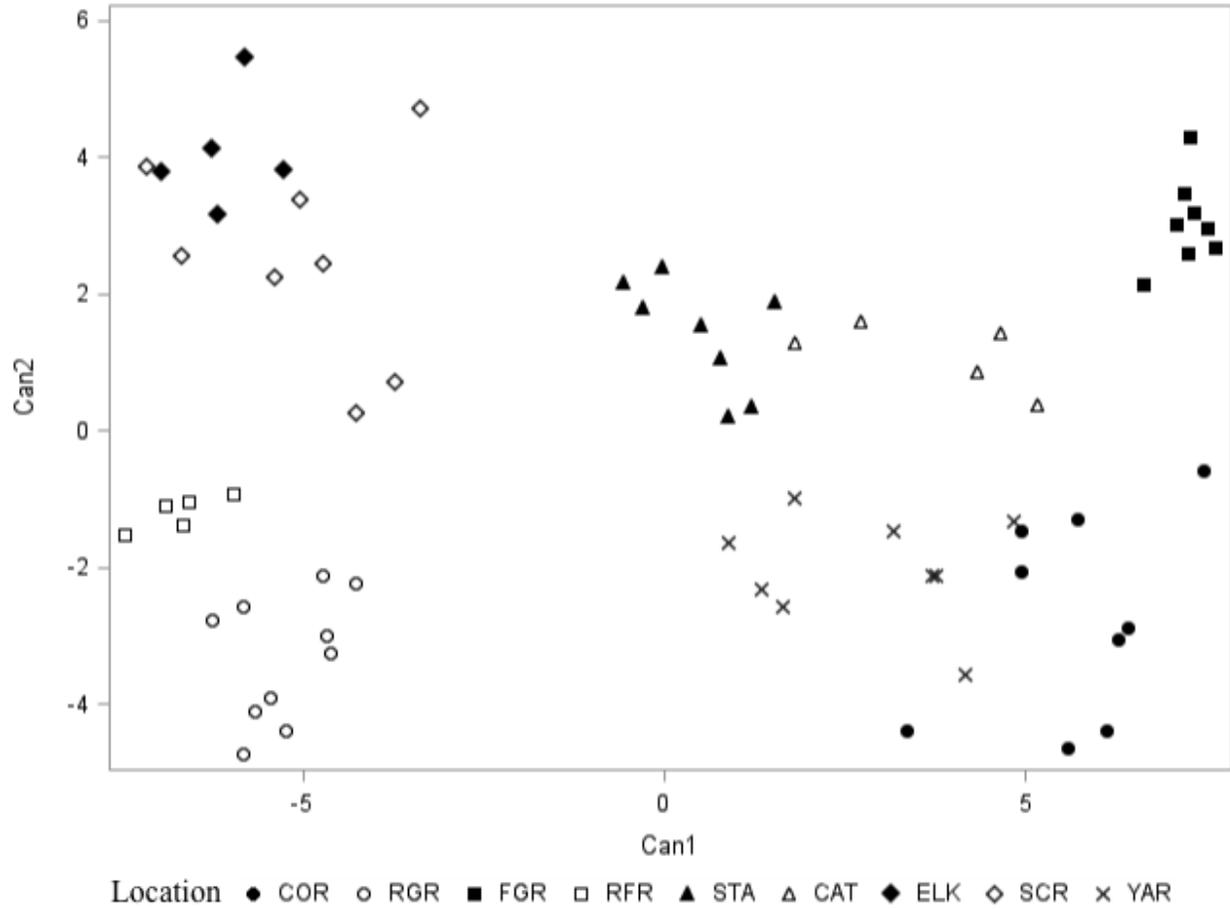


Figure 2.1. Canonical Discriminant Function analysis of  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  in otoliths from study locations in the Upper Colorado River Basin. Can1 and Can 2 are canonical variates 1 and 2, respectively. Location codes, in order from left to right from legend above: COR = Colorado River, RGR = Rifle Gap Reservoir, FGR = Flaming Gorge Reservoir, RFR = Red Fleet Reservoir, STA = Starvation Reservoir, CAT = Lake Catamount, ELK = Elkhead Reservoir, SCR = Stagecoach Reservoir and YAR = Yampa River.

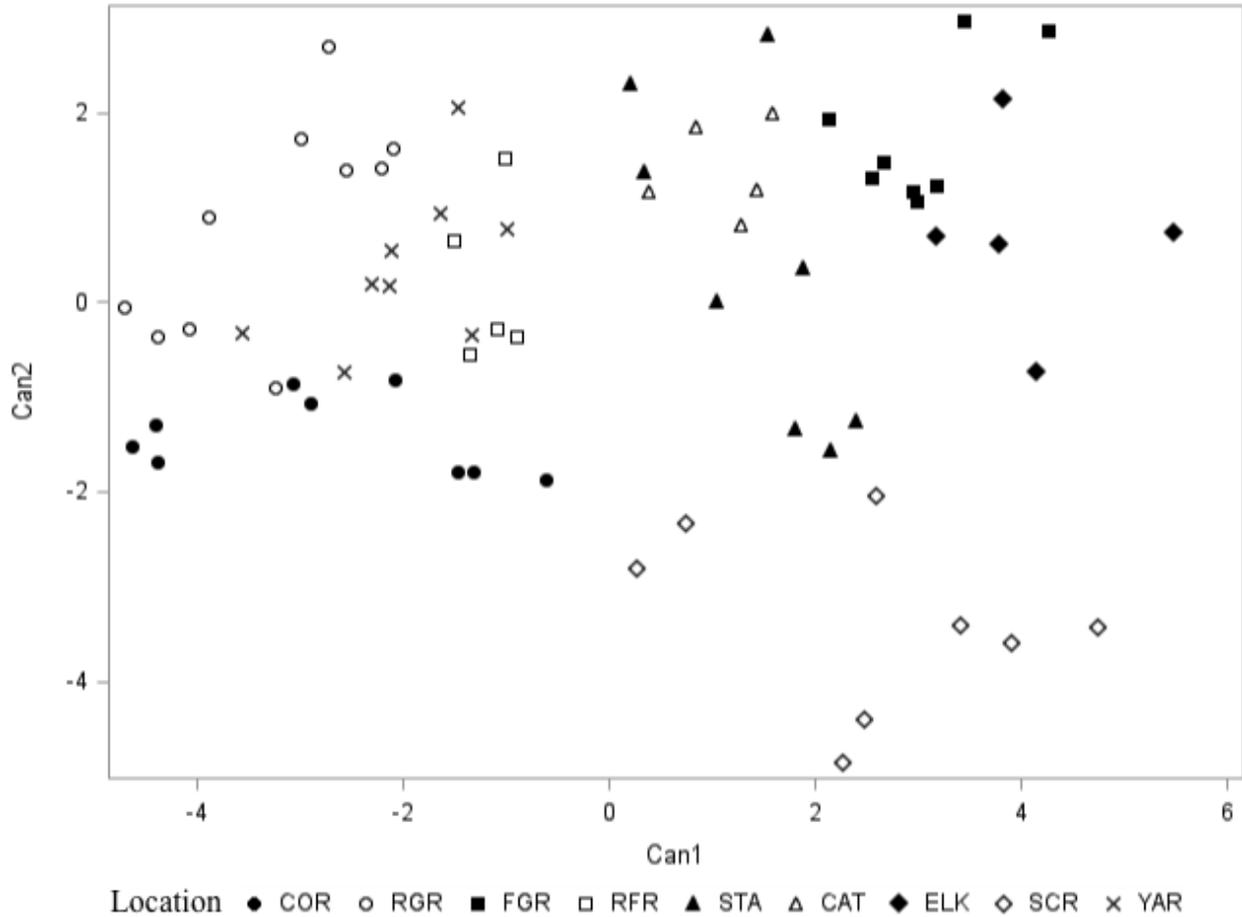


Figure 2.2. Canonical Discriminant Function analysis of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in otoliths from study locations in the Upper Colorado River Basin. Can1 and Can 2 are canonical variates 1 and 2, respectively. Refer to Figure 2.1 for location codes.

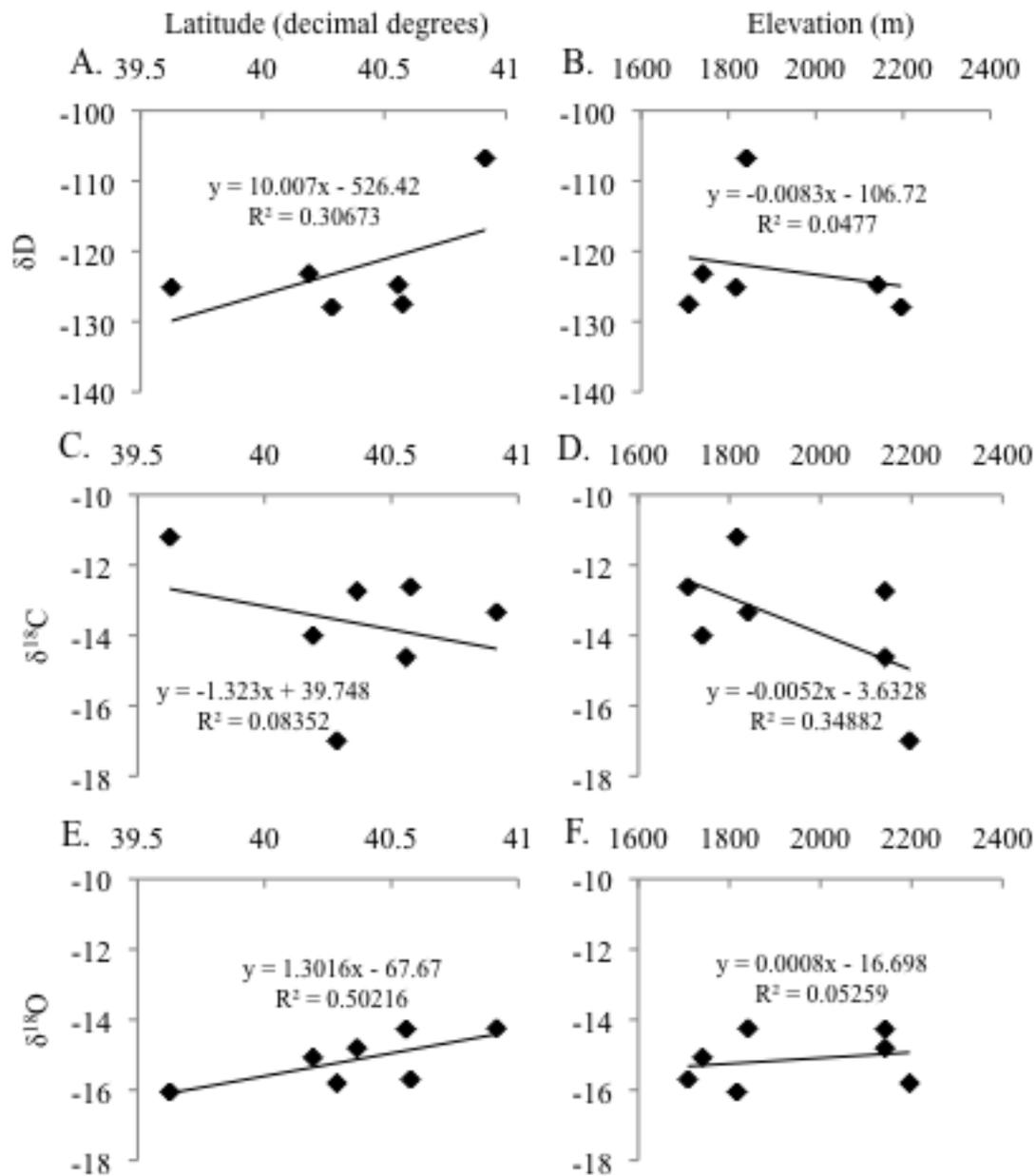


Figure 2.3. Regression analysis of stable isotopes for reservoirs in the Upper Colorado River Basin. A and B are δD versus latitude and elevations, respectively. C and D are δ<sup>13</sup>C versus latitude and elevation, respectively. E and F are δ<sup>18</sup>O versus latitude and elevations, respectively. R<sup>2</sup> refers to the fit of the observed versus predicted values. The equation is the slope and intercept of the regression line.

## Appendix A

Table 1A. Number of nonnative fish ablated/collected for microchemical analysis of otoliths during 2006-2010. N/A indicates that that species is not known to occur in that water body, or it is not targeted for sampling at that location. Species codes are: BCR = black crappie, LMB = largemouth bass, NPK = northern pike, SMB = smallmouth bass, WAL = walleye, BGL = bluegill, BUR = burbot, YPE = yellow perch, WHS = white sucker. Refer to Table 1 for water body codes.

Water Body	BCR	LMB	NPK	SMB	WAL	BGL	BUR	YPE	WHS	Grand Total
Colorado R.	0/5	5*/99	0	9‡/113	7/8	0/23	N/A	N/A	0/20	21/268
RGR	4/45	0	5*/135	5*/49	6/14	N/A	N/A	0/157	0	20/400
HGR	6/10	3/12	3/7	3/9	N/A	0/3	N/A	0/16	0	15/57
Dolores R.	0	0	0	10/15	0	0	N/A	N/A	0	10/15
MCP	N/A	0	N/A	11†/29	6/13	0	N/A	0	0	17/42
Duchesne R.	0	0	0	5/17	0	0	N/A	N/A	0	5/17
STA	N/A	N/A	N/A	5/22	5/35	N/A	N/A	N/A	0	10/57
Midview Reservoir (MVR)	0	0	0	0	1/1	0	N/A	0	0	1/1

\*Not included in final analysis because the otolith was ablated by a single transect from the core to edge, and thus, able to obtain year-specific Sr ratios.

†3 of 11 otoliths were not included in the final analysis because the otolith was ablated by a single transect from the core to edge, and thus, able to obtain year-specific Sr ratios.

‡Five of nine otoliths were not included in the final analysis because the otolith was ablated by a single transect from the core to edge, and thus, able to obtain year-specific Sr ratios.

Table 1A (continued). Number of nonnative fish collected for microchemical analysis of otoliths during 2006-2010. N/A indicates that that species is not known to occur in that water body, or it is not targeted for sampling at that location. Species codes are: BCR = black crappie, LMB = largemouth bass, NPK = northern pike, SMB = smallmouth bass, WAL = walleye, BGL = bluegill, BUR = burbot, YPE = yellow perch, WHS = white sucker. Refer to Table 1 for water body codes.

Water Body	BCR	LMB	NPK	SMB	WAL	BGL	BUR	YPE	WHS	Grand Total
Green R. - Lower	0	N/A	0	0/23	0/11	0	N/A	N/A	0	0/34
Green R. - Upper	0/33	N/A	7/40	5/57	13/55	0/1	N/A	N/A	0/49	25/235
RFR	0	0	0	0	5/18	0	N/A	N/A	0	5/18
FGR	N/A	N/A	N/A	8/20	0	N/A	6/23	N/A	0	14/43
Gunnison R.	0/1	0	0	0	0	0/1	N/A	0	0	0/2
JUR	0	0	0	5/16	5/10	0	N/A	0	0	10/26
PAO	N/A	N/A	5/6	N/A	N/A	N/A	N/A	N/A	0	5/6
CRA	5/20	0/1	5/35	0	N/A	0	N/A	0/23	0	10/79

Table 1A (continued). Number of nonnative fish collected for microchemical analysis of otoliths during 2006-2010. N/A indicates that that species is not known to occur in that water body, or it is not targeted for sampling at that location. Species codes are: BCR = black crappie, LMB = largemouth bass, NPK = northern pike, SMB = smallmouth bass, WAL = walleye, BGL = bluegill, BUR = burbot, YPE = yellow perch, WHS = white sucker.

Water Body	BCR	LMB	NPK	SMB	WAL	BGL	BUR	YPE	WHS	Grand Total
White R.	0	0	0	4/5	0	0	N/A	N/A	0	4/5
KER	5/20	N/A	N/A	N/A	N/A	0	N/A	0	0	5/20
RBR	5/13	8/20	1*/1	0	N/A	0	N/A	N/A	0	14/34
Yampa R.	0/114	0/1	10/67	30*/235	0/1	0/57	N/A	N/A	0/21	40/496
SCR	0	0	11§/14	0	5/11	N/A	N/A	N/A	0	16/25
CAT	N/A	N/A	5  /5	N/A	0	N/A	N/A	N/A	0	5/5
ELK	6*/23	1*/1	15†/38	8/44	N/A	0/5	N/A	N/A	0	30/111
Loudy Simpson Pond (LSP)	N/A	N/A	5/24	N/A	N/A	N/A	N/A	N/A	N/A	5/24
All waters	31/284	17/134	72/372	108/654	53/177	0/90	6/23	0/196	0/90	287/2020

\*11 of 30 not included in final analysis because the otolith was ablated by a single transect from the core to edge, and thus, able to obtain year-specific Sr ratios.

†10 of 15 not included in final analysis because the otolith was ablated by a single transect from the core to edge, and thus, able to obtain year-specific Sr ratios.

‡4 of 8 not included in final analysis because the otolith was ablated by a single transect from the core to edge, and thus, able to obtain year-specific Sr ratios.

§3 of 11 not included in final analysis because the otolith was ablated by a single transect from the core to edge, and thus, able to obtain year-specific Sr ratios. Five of 14 fish were provided by D.L. Winkelman and R.M. Fitzpatrick.

||fish were provided by D.L. Winkelman and R.M. Fitzpatrick.

Table 2A. Escapement rates and sources of river-caught piscivores in the Upper Colorado River Basin, 2007-2009. N refers to the sample size. UNK is an origin that did not match an  $^{87}\text{Sr}/^{86}\text{Sr}$  signature from examined reservoir fishes. DOL = Dolores R., DUC = Duchesne R., GRU = Green River – Upper, YAR = Yampa River. Refer to Table 1A for source and species codes.

River	Species	Number Sampled	% Escaped (N)	Suspected Source	Years of movement (N)
Colorado R.	SMB	4	0	-	-
Colorado R.	WAL	7	100 (7)	RGR	1996-1997 (5), 1998-1999 (1), 1995-2000 (1)
Dolores R.	SMB	10	100	MCP or DOL	-
Duchesne R.	SMB	5	100 (5)*	STA or DUC	-
Green R.	NPK	7	71 (5)	CAT† or YAR	2002-2003 (1), 2004-2007 (4)
			29 (2)	UNK	2008-2009 (2)
Green R.	SMB	5	80 (4)	FGR, YAR or GRU	2004-2005 (1), 2005-2006(1), 2006-2007 (1), 2007-2008 (1)
			20 (1)	RFR	1998-1999
Green R.	WAL	13	85 (11)	STA	2000-2001 (2), 2001-2002 (2), 2002-2003 (1), 2005-2006 (3), 2006-2007 (3)
			15 (2)	UNK	2005-2006 (2)
White R.	SMB	3	100 (3)*	KER or WHR	-
Yampa R.	NPK	10	60 (6)*	CAT or YAR	-
			40 (4)	UNK‡	1998-1999 (1), 2005-2007 (2), 2007-2008 (1)
Yampa R.	SMB	6	0	-	-

\*Reservoir source may be indistinguishable from river, core (origin) otolith signatures overlapped with edge signatures (capture).

†Tagging data confirmed Lake Catamount origin for one northern pike.

‡Close match with Sr ratios of pond signatures in that region (R.M. Fitzpatrick, unpublished data).

Table 3A. Results of Linear Discriminant Function Analysis to classify reservoir of origin within the Upper Colorado River Basin. All species were combined within reservoirs. Core and edge ablations were averaged for each individual fishes due to a lack of temporal effects between years. N refers to the sample size for each classification. Refer to Table 1 for reservoir codes.

		Reservoir classifications, in percent (N)													
From Reservoir	HGR	RGR	MCP	FGR	RFR	STA	CRA	JUR	PAO	KER	RBR	CAT	ELK	SCR	
HGR	18 (3)	47 (8)	-	-	-	-	-	-	29 (5)	-	6 (1)	-	-	-	
RGR	-	43 (3)	-	-	-	-	-	-	14 (1)	-	29 (2)	-	-	14 (1)	
MCP	-	-	79 (11)	-	-	7 (1)	-	14 (2)	-	-	-	-	-	-	
FGR	-	-	-	100 (14)	-	-	-	-	-	-	-	-	-	-	
RFR	-	-	-	-	20 (1)	-	20 (1)	-	-	40 (2)	-	-	20 (1)	-	
STA	-	-	40 (4)	-	-	60 (6)	-	-	-	-	-	-	-	-	
CRA	-	-	-	-	30 (3)	-	20 (2)	-	-	-	-	-	50 (5)	-	
JUR	-	10 (1)	40 (4)	-	-	10 (1)	-	10 (1)	30 (3)	-	-	-	-	-	
PAO	-	20 (1)	-	-	-	-	-	-	80 (4)	-	-	-	-	-	
KER	-	-	-	-	40 (2)	-	-	-	-	60 (3)	-	-	-	-	
RBR	-	8 (1)	-	-	-	-	-	8 (1)	8 (1)	-	77 (10)	-	-	-	
CAT	-	-	-	-	-	20 (1)	-	-	-	-	-	80 (4)	-	-	

ELK	-	-	-	-	60 (3)	-	40 (2)	-	-	-	-	-	0	-
SCR	-	23 (3)	-	-	-	-	-	-	-	15 (2)	-	-	-	62 (8)

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Table 4A.  $^{87}\text{Sr}/^{86}\text{Sr}$  summary of fishes sampled from rivers, 2007-2009. Species codes: NPK = northern pike, SMB = smallmouth bass and WAL = walleye. N = number of fish analyzed from each river. TL = total length of each fish. N/A = core ablations were not taken, only ablated regions of the otolith corresponding to known Yampa River residence

Location	Species	$^{87}\text{Sr}/^{86}\text{Sr}$				N	Mean TL (mm)	Mean age (years)
		Core	SD core	Edge	SD edge			
Colorado R.	SMB	0.71099	0.00010	0.71101	0.00011	4	261	4
Colorado R.	WAL	0.70929	0.00011	0.71099	0.00019	7	525	16
Dolores R.	SMB	0.70989	0.00009	0.70987	0.00007	10	182	3
Duchesne R.	SMB	0.70991	0.00007	0.71002	0.00016	5	238	6
Green R.	NPK	0.71032	0.00053	0.71031	0.00025	7	523	3
Green R.	SMB	0.71053	0.00075	0.71050	0.00023	5	324	5
Green R.	WAL	0.70983	0.00015	0.71036	0.00011	13	520	7
White R.	SMB	0.70914	0.00012	0.70905	0.00005	3	248	2
Yampa R.	NPK	0.71279	0.00402	0.71132	0.00322	10  /9¶	586	5
Yampa R.	SMB*	0.71091	0.00063	0.71060	0.00035	6  /3¶	182	2
Yampa R.	SMB†	N/A	N/A	0.71048	0.00007	9	362	7
Yampa R.	SMB‡	0.71039	0.00002	0.70982	0.00060	3	370	7
Elkhead	SMB§	0.71012	0.00030	0.70936	0.00028	3	353	6
Total						86  /82¶		
Grand mean							361	6

\*untagged smallmouth bass

†tagged smallmouth bass with known residence in the Yampa River

‡tagged smallmouth bass transplanted into Elkhead Reservoir and recaptured in the Yampa River

§tagged smallmouth bass transplanted into Elkhead Reservoir

||core

¶edge

## Appendix B

### Methods

To determine if Sr ratios are predictably correlated with geology of surrounding reservoir watersheds I compared observed Sr ratios with predicted Sr ratios from Beard and Johnson (2000). Beard and Johnson (2000) used age of bedrock to model Sr ratio using the equation:  $^{87}\text{Sr}/^{86}\text{Sr}_P = (^{87}\text{Sr}/^{86}\text{Sr}_I) + (^{87}\text{Rb}/^{86}\text{Sr})(e^{\lambda t} - 1)$ , where  $P$  and  $I$  represent the sample at present time and some time in the past respectively,  $t$  is time (years),  $\lambda$  is the decay constant for  $^{87}\text{Rb}$  ( $1.42 \times 10^{-11} \text{ yr}^{-1}$ ), and  $^{87}\text{Sr}/^{86}\text{Sr}_I$  was set as a constant 0.7050. The Sr ratio was then converted to  $\epsilon^{87}\text{Sr}$  for easier comparison of small differences of Sr ratios using the equation:  $\epsilon^{87}\text{Sr} = [ (^{87}\text{Sr}/^{86}\text{Sr}_{\text{measured}} \div ^{87}\text{Sr}/^{86}\text{Sr}_{\text{bulk earth}}) - 1 ] \times 10,000$ , where bulk earth equals 0.7045. Using a geographic information system (GIS) geology layer of the UCRB (Reed and Bush 2005),  $\epsilon^{87}\text{Sr}$  was added as an attribute for all bedrock time periods. The updated geology layer was intersected with a GIS layer of reservoir watershed boundaries (upstream from each dam) to derive a weighted  $\epsilon^{87}\text{Sr}$  average, based on percent area of each bedrock age for each reservoir.

### Results

Observed  $\epsilon^{87}\text{Sr}$  of reservoirs were not significantly correlated ( $r^2 = 0.20$ ;  $p = 0.13$ ;  $N = 13$ ) with predictions from the Beard and Johnson (2000) model (Table 1B). Observed  $\epsilon^{87}\text{Sr}$  was higher than predicted by an average of 48.04 (RMSE = 49.93). Lake Catamount was the only reservoir that had lower observed  $\epsilon^{87}\text{Sr}$  than predicted with a  $\epsilon^{87}\text{Sr}$  of 83.68 and 111.39, respectively. Therefore, bedrock age did not appear to predict Sr ratios reliably over the range of  $\epsilon^{87}\text{Sr}$  observed in my study reservoirs.

## References

Beard, B.L., and Johnson, C.M. 2000. Strontium isotope composition of skeletal material can determine the birthplace and geographic mobility of humans and animals. *Journal of Forensic Sciences* 45: 1049-1061.

Reed, J.C., and Bush, C.A. 2005. Generalized Geologic Map of the United States, Puerto Rico and the Virgin Islands [online]. Available from <http://pubs.usgs.gov/atlas/geologic/> (Accessed April 4, 2000).

Table 1B. Watershed characteristics and observed versus predicted  $\epsilon^{87}\text{Sr}$  results for reservoirs in the Upper Colorado River Basin. Refer to Table 1A for reservoir codes.

Reservoir	Watershed area (km <sup>2</sup> )	Bedrock age area (%)				Observed mean $^{87}\text{Sr}/^{86}\text{Sr}$	Observed mean $\epsilon^{87}\text{Sr}$	Predicted mean $\epsilon^{87}\text{Sr}$	Observed minus predicted
		Cenozoic Era	Mesozoic Era	Paleozoic Era	Precambrian Eon				
CAT	777	48	15	0	37	0.71040	83.68	111.39	-27.71
CRA	501	32	68	0	0	0.70897	63.42	16.57	46.85
ELK	575	17	83	0	0	0.70897	63.39	18.26	45.13
FGR	37 991	79	13	2	6	0.71085	90.16	32.38	57.78
JUR	323	81	19	0	0	0.70963	72.86	9.96	62.90
KER	7 035	61	23	16	0	0.70909	65.11	19.44	45.67
MCP	1 944	3	89	8	0	0.70976	74.65	25.37	49.28
PAO	1 095	80	20	0	0	0.70945	70.21	10.94	59.27
RBR	161	100	0	0	0	0.70952	71.25	9.94	61.31
RFR	235	34	17	36	13	0.70903	64.28	40.91	23.37
RGR	297	2	24	74	0	0.70942	69.85	43.21	43.21
SCR	334	73	27	0	0	0.70925	67.40	12.39	55.01
STA	2 492	92	7	1	0	0.70994	77.22	13.56	63.66