COLORADO RIVER RECOVERY PROGRAM
FY 2011 ANNUAL PROJECT REPORT

RECOVERY PROGRAM
PROJECT NUMBER: C-18/19

I. Project Title: Chemically Fingerprinting Nonnative Fishes in Reservoirs

II. Principal Investigator(s):

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III. Project Summary:

This project addresses movement of nonnative fish into river reaches of critical habitat from reservoirs known to support cool- and warmwater species of nonnative fish. These species include northern pike, smallmouth bass, largemouth bass, black crappie, and walleye. These species are believed to pose a significant predatory threat to the young life stages of endangered and other native fishes (Tyus and Saunders 1996; Martinez et al. 2001; Johnson et al. 2005). However, it is uncertain to what extent the presence of nonnative species in critical habitat is the result of escapement or illicit transfers from reservoirs. We have found that strontium isotope ratio ($^{87}Sr/^{86}Sr$, "Sr ratio") is an excellent natural tracer for studying fish origins and movements. This tracer is very consistent among species in a given reservoir, is temporally stable and all reservoir signatures (i.e., the Sr ratio of fish from a given reservoir) we have examined are unique and thus can be used to determine provenance of fish in critical habitat.

IV. Study Schedule: FY06-FY12

V. Relationship to RIPRAP:

General Recovery Program Support Action Plan:

III. Reduce negative impacts of nonnative fishes and sport fish management activities.

III.A.2. Identify and implement viable control measures.

Colorado River Action Plan: Main stem

III. Reduce negative impacts of nonnative fishes and sport fish management activities.

III.A.4.a. Evaluate sources of nonnative fishes and make recommendations.

VI. Accomplishment of FY 2011 Tasks and Deliverables, Discussion of Initial Findings and Shortcomings:

Tasks 1. Field Collections
Work completed.
All otolith samples inventoried and archived for long-term storage.

Task 2. Microchemical Analysis of Otoliths
Work completed.
Manuscript in review:
Wolff, B.A., B.M. Johnson, A. Breton, P.J. Martinez, and D.L. Winkelman. In review. Origins and movements of invasive piscivores determined from strontium isotope (87Sr/86Sr) ratio of otoliths. Canadian Journal of Fisheries and Aquatic Sciences.

Presentations:
Wolff, B.A., B.M. Johnson, P.J. Martinez, and D.L. Winkelman. 2011. Strontium isotope ratios (87Sr:86Sr) as tracers of origins and movements of nonnative piscivores in the Upper Colorado River Basin. Nonnative Fish Workshop, Grand Junction, CO.

Wolff, B.A., B.M. Johnson, P.J. Martinez, and D.L. Winkelman. 2011. Tracing origins of invasive piscivores using strontium isotope ratios (87Sr/86Sr) in otoliths. National meeting of the American Fisheries Society, Seattle, WA.

Task 3. Reservoir Emigration Risk Assessment
Work to be completed for final report in January 2012
Presentations:
Johnson, B.M., B.A. Wolff, and P.J. Martinez. 2011. Emigration risk of nonnative fish in Upper Colorado River Basin reservoirs. Upper Colorado River Basin Endangered Fish Recovery Program Researcher's Meeting, Moab, UT.

Task 4. Refine Understanding of Strontium Ratios of Nonnative Fish Captured in Rivers
Work to be completed for final report in January 2012
Background/Rationale

Tracing reservoir origins of fish captured in critical habitat requires knowledge of the chemical fingerprints of potential source reservoirs and an understanding of how reservoirs differ from rivers downstream. Our previous worked showed that fish sampled in 13 Upper Basin reservoirs could be distinguished from each other and that fish that escaped from reservoirs on tributaries to the Colorado or Green rivers could be distinguished from other fish in these rivers, using strontium isotope signatures of otoliths. However, two issues created some uncertainty in assigning river fish origins: 1) managers suspected that walleye, and potentially other species of nonnative fish, could be migrating upstream from Lake Powell into the Upper Green River, and no strontium data existed for fish from Lake Powell; and 2) some questioned whether fish captured downstream of Flaming Gorge Reservoir in the Green River could be distinguished as either resident river fish or fish that emigrated from Flaming Gorge Reservoir to the Green River.

In 2011 we added Task 4 to the project to a) determine the strontium signature of nonnative fish sampled from Lake Powell, and b) address uncertainty about distinctiveness of reservoir fish and river fish. Because fish sampled from rivers may have originated at some river location or they may have emigrated from a reservoir into the river, we needed an independent measure of river strontium isotope signature. Crayfish and snails are widely distributed throughout the Upper Basin, their exoskeletons contain strontium, and they are relatively sedentary. Preliminary research showed that strontium ratios in crayfish carapaces differed greatly across reservoirs and were similar to strontium ratios of fish from the same location but carapaces are difficult to analyze. Thus, these invertebrates appear to be site-specific sentinels of strontium ratios, but analysis of gastroliths ("stomach stones"; Figure 1) would be cheaper and easier. Snails provide a surrogate at locations where crayfish are not found.

Study Area

This research was conducted in Lake Powell, Flaming Gorge Reservoir and its tailwater plus three locations downstream in the Upper Green River (Figure 2). Additional crayfish and otolith samples were obtained from Blue Mesa Reservoir and Elkhead Reservoir. We also looked for crayfish in Vermillion Creek, Brown's Park, Colorado.

Methods

We collected samples of crayfish, New Zealand mudsnails *Potamopyrgus antipodarum* and water at several locations in the Upper Green River and Flaming Gorge Reservoir (Figure 2) to evaluate river-reservoir signatures. Crayfish were collected with baited traps set overnight near the boat ramp at Swinging Bridge in Brown's Park, Colorado and near the dam in Flaming Gorge Reservoir during September 11-16, 2011. No crayfish were found at the tailwater or at the Little Hole and Vermillion Creek sites. Water samples were collected, using ultra-clean sampling methods (Shiller 2003), from Flaming Gorge Reservoir, the Green River at the Flaming Gorge tailwater, Little Hole and Swinging Bridge. We also collected samples of New Zealand mudsnails at this time, from the three river sites, to use as surrogates for crayfish where crayfish were not found. Samples of age-0 smallmouth bass were provided by K. Bestgen, CSU Larval Fish Laboratory. These SMB were collected 09/20/2011 from the Green River, Moffat County, CO; RM 349.3, below Wild Mountain Campground (Lodore Canyon) by K. Zelasko and T. Wilcox.

Crayfish were euthanized upon collection by separating the abdomen from the cephalothorax, and were placed in individual Whirl-pak bags. New Zealand mudsnails were also sealed in Whirl-paks. All samples were stored on dry ice to insure complete mortality. Water samples were collected in pre-cleaned 250 mL Nalgene high-density polyethylene bottles and subsequently rinsed and filtered through a 50 mL polyethylene syringe coupled with a 0.45 μm polypropylene filter. The filtered water was then collected in a 15 mL Nalgene high-density polyethylene bottle and placed on ice until they were brought to the laboratory.

In the laboratory, we identified crayfish and sexed them prior to dissection for removal of gastroliths, if present. Water samples were kept under refrigeration until being shipped to the Woods Hole Oceanographic Institution (WHOI) Plasma Mass

Spectrometer Facility, Woods Hole, Massachusetts, for strontium analysis by lab manager Jurek Blusztajn. Crayfish gastroliths were extracted, ultrasonically cleaned in Milli-Q water, sanded flat and attached to petrographic slides using double sided tape. Smallmouth bass otoliths were extracted using acid washed plastic forceps. Extracted otoliths were ultrasonically cleaned and sanded on the dorsal surface to reveal the inner core. Sanded otoliths were then placed onto petrographic slides, the same as the crayfish. Mudsnail shells were cleared of all soft tissue and ultrasonically cleaned in Milli-Q water. Cleaned shells were embedded in epoxy and cut longitudinally through the midplane to get a flat surface exposed for laser ablation.

Otoliths, gastroliths and snails were analyzed by B. Wolff, CSU, at the Neptune LA-ICP-MS mass spectrometry facility at the Woods Hole Oceanographic Institution, in Woods Hole, Massachusetts during October 2011. Samples were analyzed using a Thermo Finnigan Neptune Multi-collector Inductively Coupled Plasma Mass Spectrometer coupled with a UP 193 nm laser (LA-MC-ICP-MS). The laser was set at 80% intensity, 10Hz, 35 µm ablation spot size, 5 µm/sec ablation path speed and 525 µm ablation paths. All otoliths were ablated near the core providing a signature for time corresponding to early-juvenile development. Gastroliths were ablated near the center and snail shells were ablated in the widest (thickest) area of the shell to prevent ablation of surrounding epoxy.

We analyzed otoliths of smallmouth bass *Micropterus dolomeiu* that were collected from Lake Powell in April, 2004 to determine if fish from that system could be distinguished from fishes from other Upper Basin reservoirs and the Green River. Otolith sample preparation followed methods developed during this project (see Johnson and Martinez 2010). We examined previously collected data on carbon (^{13}C) and oxygen (^{18}O) isotopes of smallmouth bass otoliths from two time periods to evaluate if these other markers could be used to distinguish river fish (Green, Yampa, and Colorado rivers) from potential reservoir emigrants (Lake Powell, and Flaming Gorge, Starvation, and Rifle Gap reservoirs). All samples were analyzed by the same laboratory (University of Alaska-Fairbanks stable isotope facility), but at two different times (2005, 2010).

Results and Discussion

We intended to use crayfish gastroliths for laser ablation analysis for 87Sr/87Sr. However, no gastroliths were found from any samples collected from Flaming Gorge Reservoir (09/13/11; N = 43), the Green River Brown's Park, Colorado (09/15/11; N = 8) and Elkhead Reservoir, Craig, Colorado (09/24/11; N=6). Given this limitation, we analyzed gastroliths and otoliths collected from Blue Mesa Reservoir and compared Sr ratios of the two structures as an evaluation of the methodology. We are analyzing crayfish carapaces from samples from the study area and will report on these findings in the final report.

As far as we know, ours is the first study anywhere to measure strontium ratio in crayfish gastroliths. We found that gastroliths have plenty of strontium for accurate measurements of Sr ratio, and are very amenable to laser ablation, the primary technique we use on strontiuum analysis of otoliths. Results from the comparison of gastroliths and otoliths at Blue Mesa Reservoir were mixed (Figure 3). There was no significant difference in 87Sr/86Sr between gastroliths (N = 7) and brown trout otoliths (N = 2) or yellow perch otoliths (N = 2) but lake trout (N = 2) N= 2) had significantly lower

87Sr/86Sr. Because our sample size for comparisons was so limited and outside our study location with different species, we will analyze carapaces (instead of gastroliths) of the crayfish from the study locations. This will be a more definitive test for the correspondence between crayfish Sr ratio and fish otolith Sr ratio, and of the potential for crayfish to serve as site-specific sentinels of water Sr ratio in the Green River.

Stronium analysis ($^{87}Sr/^{86}Sr$) of water and otolith samples from Flaming Gorge Reservoir and downstream to Brown's Park, CO suggests that the strontium signatures of the Green River above the confluence with the Yampa River are similar to those from Flaming Gorge Reservoir (Figure 4). Comparison of water samples collected from Flaming Gorge Reservoir (FGR; N = 1) and the Green River near Brown's Park, CO (N = 1) showed that these sites had very similar 87Sr/86Sr, at the time of sampling. Comparison of the above water samples with smallmouth bass otoliths from FGR and Lodore Canyon (downstream from Brown's Park) also suggest that locations downstream from FGR are not distinguishable from each other or the reservoir using 87Sr/86Sr. We are waiting for results of strontium analysis of water samples from the tailwater and Little Hole, which will improve the strength of our conclusions about strontium signatures of Flaming Gorge Reservoir and the Green River below. We will update our findings in the final report.

Snail shells showed much greater variability than other structures and had higher strontium than other types of samples. Thus, they do not appear to be reliable as indicators of ambient water chemistry without further research. Reasons for the lack of correspondence we saw (Figure 4) are currently unknown but could be due to 1) temporal variation in river Sr ratios, 2) very low levels of Sr concentrations in snail shells which can lead to reduced precision in 87Sr/86Sr and 3) possible incorporation of Sr from the substrate that the snails were attached to, biasing the measurement of ambient water signature. Regardless of the speculation, we do not presently have enough information to reliably use snail shells as sentinels of river water chemistry in the future, and crayfish (carapaces or gastroliths) appear to be better indicators of water Sr ratio. Ongoing analysis of carapaces of crayfish that did not possess gastroliths will provide a better assessment of the full potential of crayfish as sentinels, and we will report on these new data in the final report.

Samples of walleye otoliths from Lake Powell were not available before our trip to WHOI in October 2011. Instead, we used archived smallmouth bass otoliths for ablation at WHOI. Our work has shown that temporal variability of reservoir signatures is minimal so comparing Lake Powell otolith signatures with those from reservoirs sampled at a different time should not be a problem. However, our other work also found that walleye otolith signatures were different from other species, in some reservoirs. Our latest results indicate that Lake Powell smallmouth bass Sr signatures are different from the signatures of smallmouth bass (and water) found for the Green River above the Yampa River confluence and for Flaming Gorge Reservoir (Figure 5). However, Lake Powell smallmouth bass have Sr signatures very similar to those of smallmouth bass from Starvation Reservoir, the primary hypothesized source of walleyes to the Green River. This suggests that walleye immigrating to the Green River from Lake Powell would be difficult to distinguish from immigrants from Starvation Reservoir without analysis of other chemical markers.

Bivariate plots of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope ratios showed that these two markers are not spatially or temporally stable (Figure 6). Thus, these two isotopes are not useful for discriminating river fish origins, given our understanding at this time. Other markers (Sr/Ca ratio, $\delta^{2}H$ hydrogen isotopes) could be useful but we have no data. For the time being, the questions of walleye immigration from Lake Powell to the Green River, and origins of smallmouth bass in the Green River (river vs. Flaming Gorge Reservoir) are unresolved.

VII. Recommendations:

1. Complete data analysis and final report preparation, integrating results from Tasks 1-4, per scope of work.
2. Additional research on crayfish gastroliths and carapaces is needed to evaluate crayfish as sentinels of river strontium isotope signatures.
3. Consider funding additional work to look for other markers that could help discriminate Lake Powell and Starvation Reservoir walleyes, and Green River vs. Flaming Gorge Reservoir smallmouth bass.

VIII. Project Status:

This project will continue into FY 2012 and it should be considered on track and ongoing. All work on the project will be completed and a final report prepared in January, 2012.

IX. FY 2011 Budget Status

A. Funds Provided: $64,495

B. Funds Expended: $59,495

C. Difference: $ 5,000

D. Percent of the FY 2011work completed, and projected costs to complete: Tasks 1-2 are completed. Task 3 is 75% complete. Task 4 is 75% complete. Remaining funds will be expended in FY12 to complete all tasks and prepare the final report.

E. Recovery Program funds spent for publication charges: $0
A manuscript is in review at Canadian Journal of Fisheries and Aquatic Sciences but there will be no page charges.

X. Status of Data Submission (Where applicable): Not applicable.

XI. Signed:

Brett M. Johnson 11/30/11
Principal Investigator Date

Patrick J. Martinez 11/30/11
Principal Investigator Date

XII. References:

Johnson, B.M. and P.J. Martinez. 2010. Chemically Fingerprinting Nonnative Fishes in Reservoirs.

Johnson, B. M., G. Whitledge, M. Sullivan, and D. Gibson-Reinemer. 2005. Stable isotopes and statistics. Progress report, Colorado Division of Wildlife, Grand Junction, Colorado, 22 pages.

Martinez, P. J., B. M. Johnson, and J. D. Hobgood. 2001. Stable isotope signatures of native and nonnative fishes in Upper Colorado River backwaters and ponds. The Southwestern Naturalist 46: 311-322.

Shiller, A.M. 2003. Syringe filtration methods for examining dissolved and colloidal trace element distributions in remote field locations. Environ. Sci. Technol. 37: 3953-3957.

Tyus, H. M., and J. F. Saunders, III. 1996. Nonnative fishes in natural ecosystems and a strategic plan for control of nonnatives in the Upper Colorado River basin. Recovery Implementation Program DRAFT REPORT. Cooperative Agreement No. 14-48-006-95-923. U.S. Fish and Wildlife Service, Denver, Colorado.

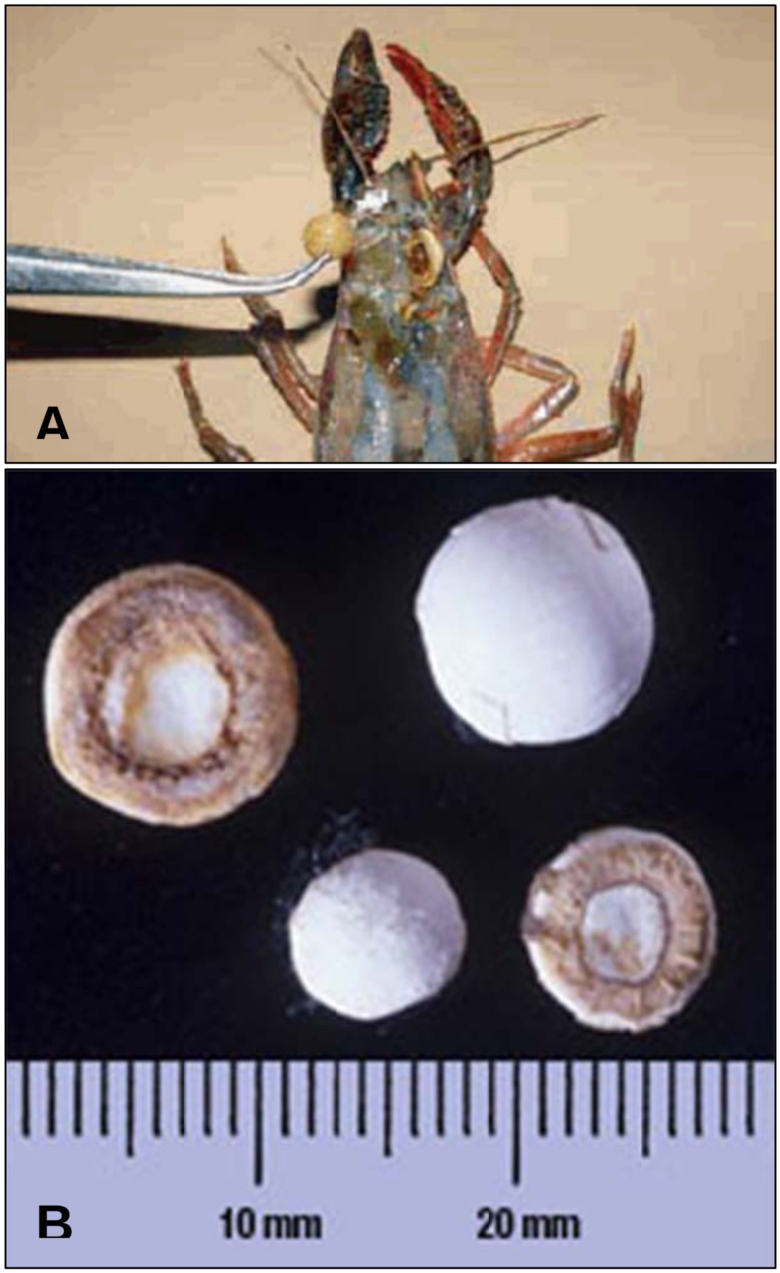

Figure 1. Crayfish gastroliths (literally 'stomach stones') are paired, round, calcareous concretions in the cephalothorax. Gastroliths are produced in the lining of the stomach when the crayfish is preparing to molt. After the crayfish molts the gastroliths are dissolved by the stomach where their calcium is resorbed into the blood. (Images: (A) http://www.thefishsite.com/articles/832/crawfish-biology, (B) http://sandboxtests.blogspot.com/2008/04/new-suit-of-armor-for-pinchy.html)

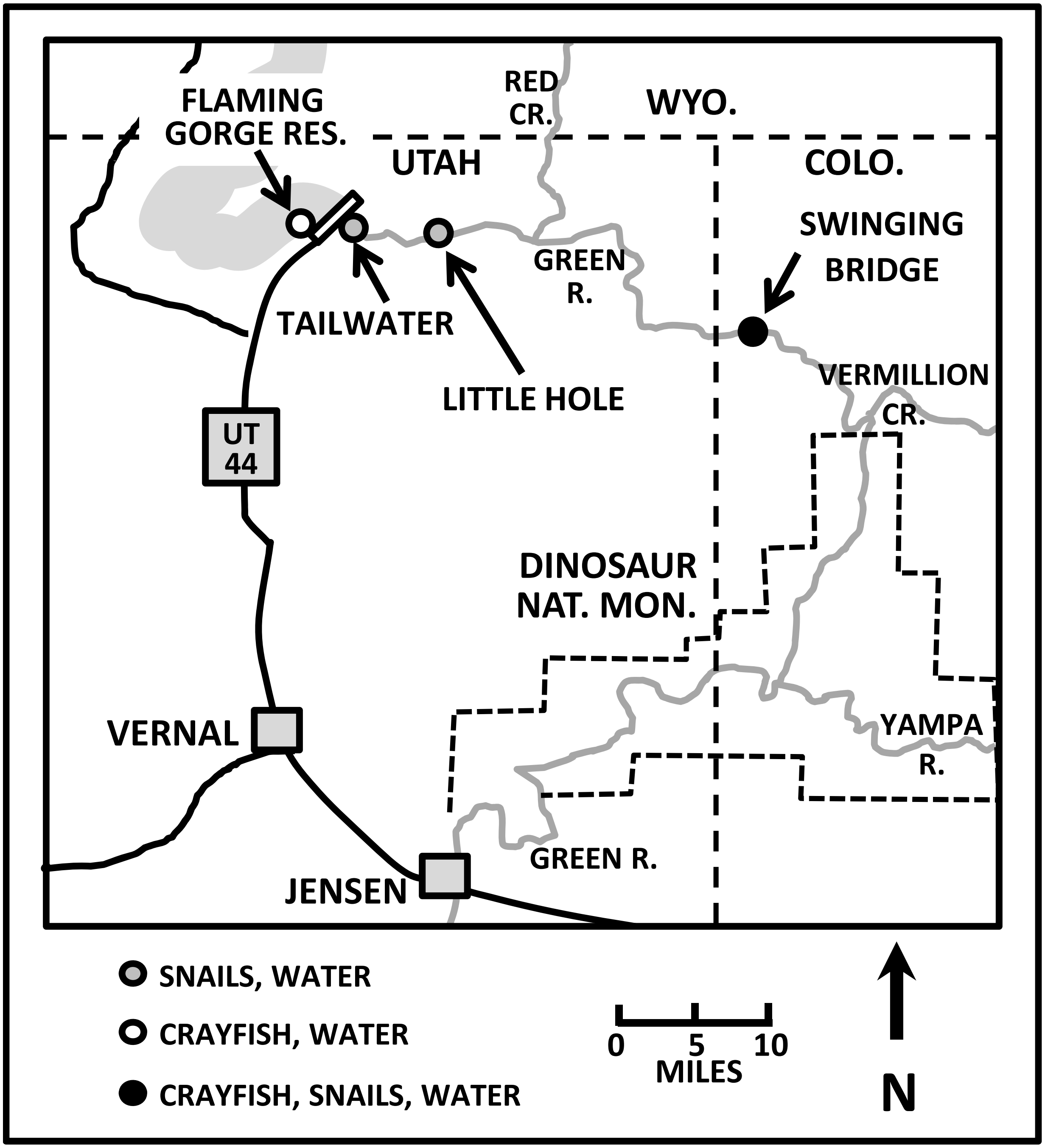

Figure 2. Sampling locations on the Green River where water, crayfish and snails were collected for strontium analysis. No snails were captured in Flaming Gorge Reservoir; no crayfish were captured at two river sites, only water and snails were collected at those (gray circles). Crayfish, water and snails were all collected at the Brown's Park, CO, site (black circle).

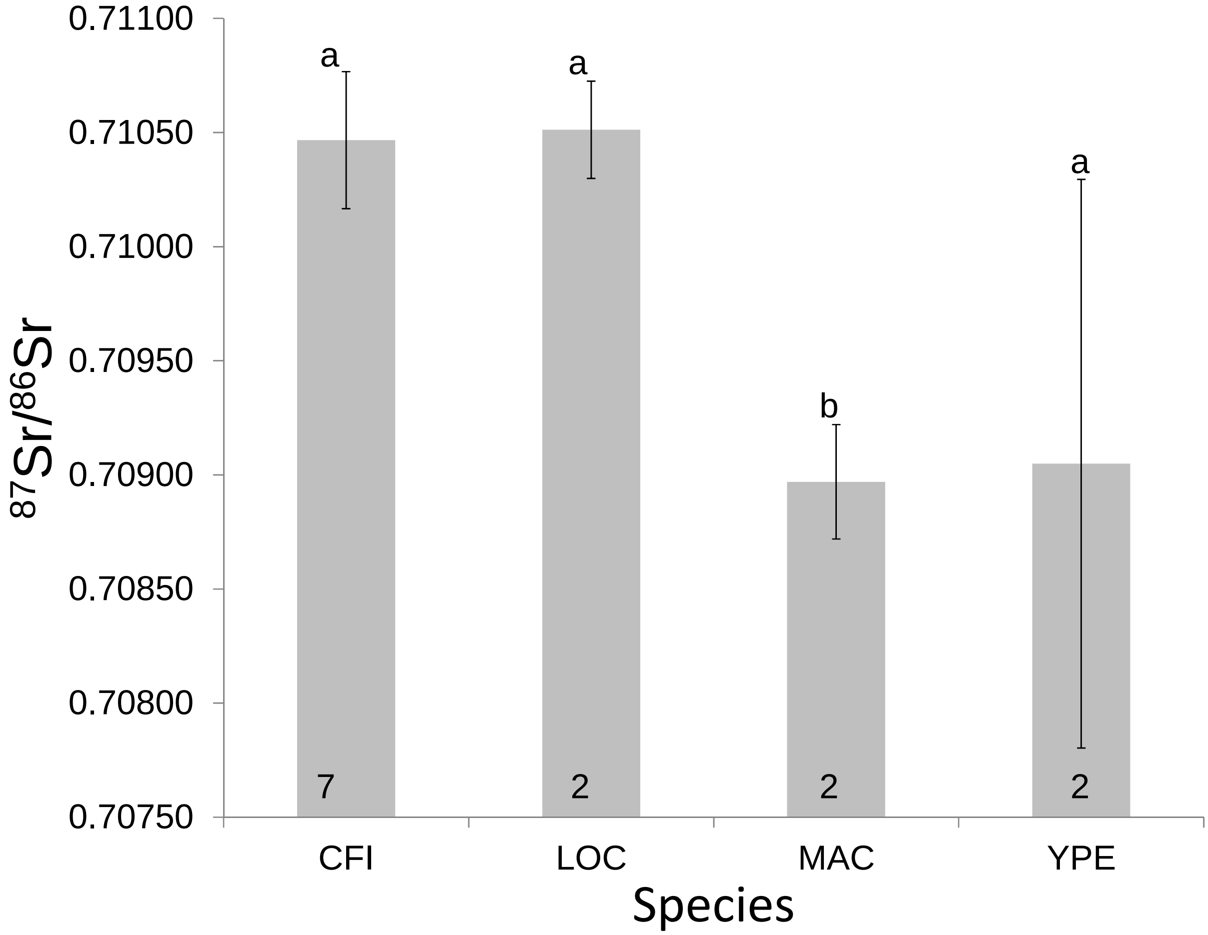

Figure 3. Strontium isotope ratio of crayfish gastroliths (CFI), brown trout (LOC), lake trout (MAC) and yellow perch (YPE) otoliths from Blue Mesa Reservoir. Error bars show ±2SE. Numbers inside each bar are sample sizes; sample means with the same letter above are not significantly different from one another.

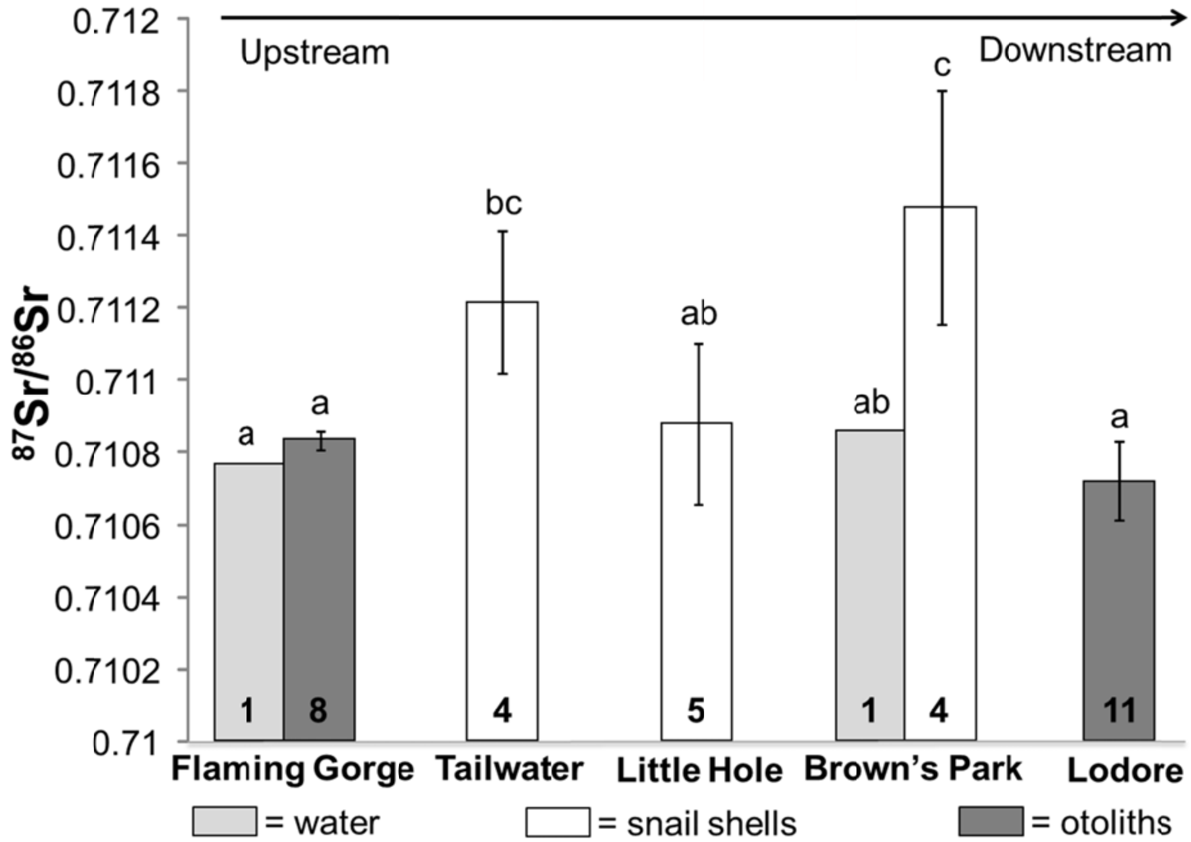

Figure 4. Strontium isotope ratio ($^{87}Sr/^{86}Sr$) of water, otoliths and snail shells collected at several locations on the Green River, including Flaming Gorge Reservoir. Error bars show ±2SE. Numbers inside each bar are sample sizes; sample means with the same letter above are not significantly different from one another.

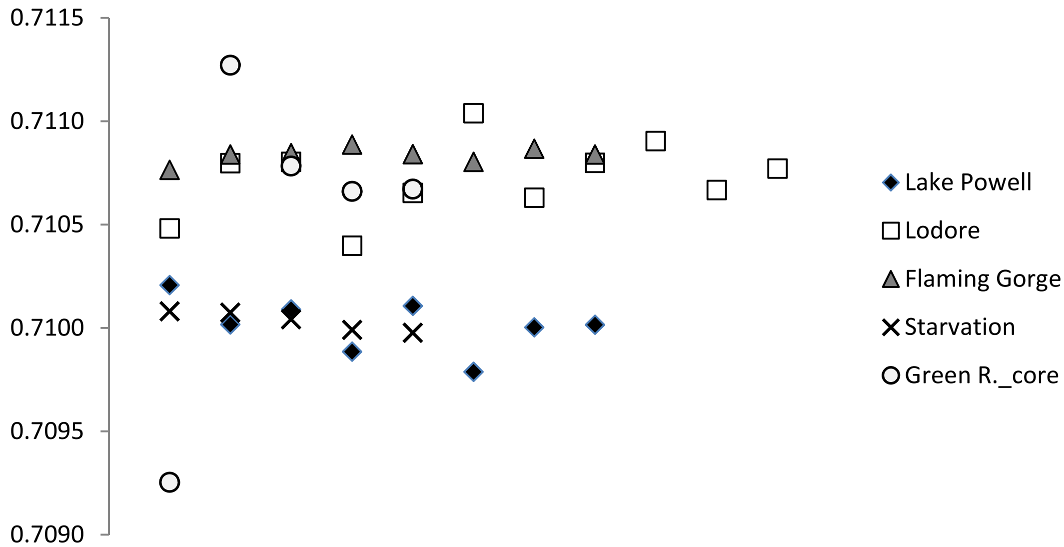

Figure 5. Strontium isotope ratio of smallmouth bass otolith cores collected from five locations in the Upper Colorado River Basin.

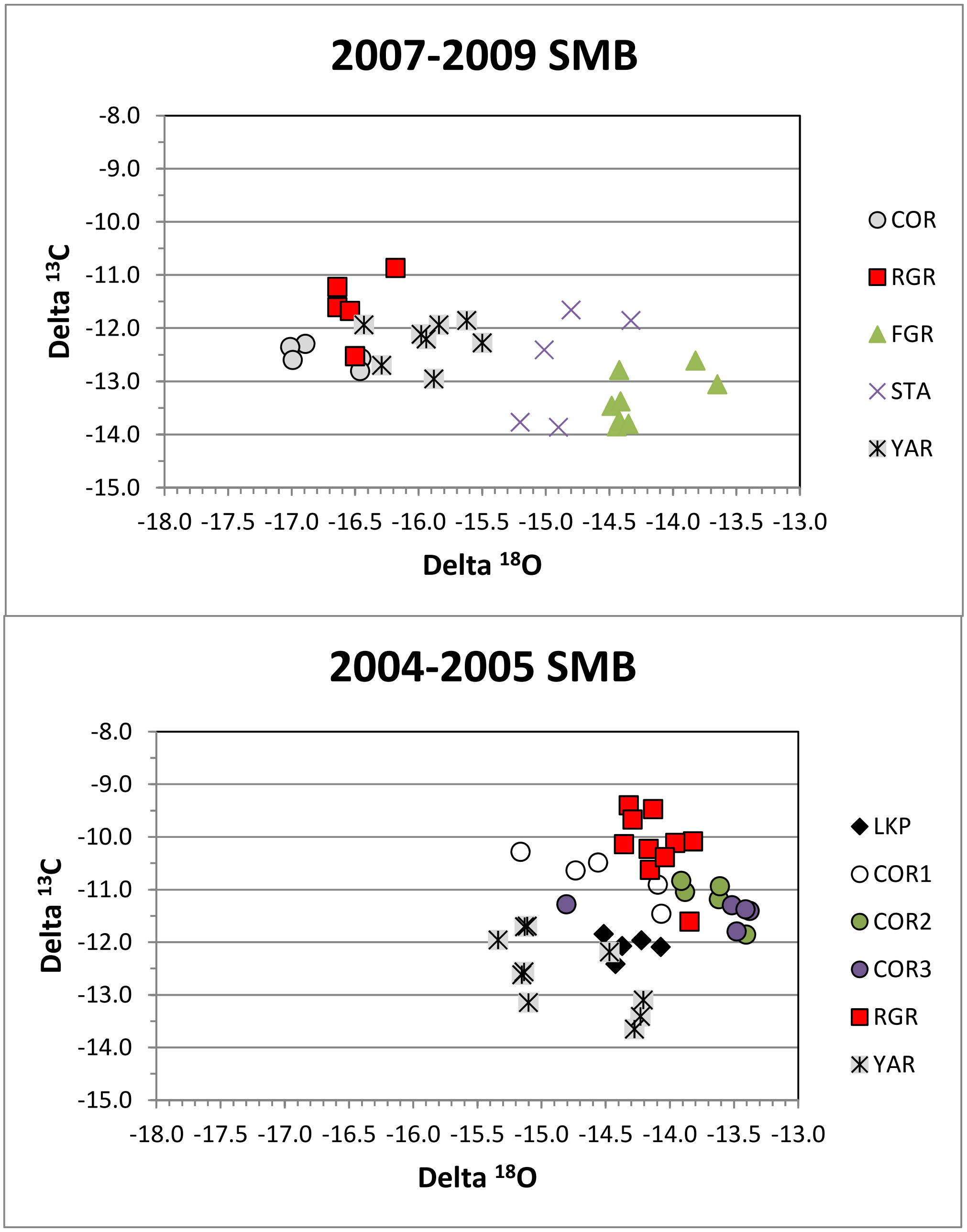

Figure 6. Bivariate plots of carbon (^{13}C) and oxygen (^{18}O) isotope ratios measured in smallmouth bass otoliths sampled from several rivers and reservoirs during 2007-2009 and 2004-2005. COR=Colorado River (at several sites above confluence with Green), RGR=Rifle Gap Reservoir, FGR=Flaming Gorge Reservoir, STA=Starvation Reservoir, YAR=Yampa River.