

**COLORADO RIVER RECOVERY PROGRAM**  
**FY-2007 -2012 PROPOSED SCOPE OF WORK for:**  
Chemically Fingerprinting Nonnative Fishes in Reservoirs

Project No.: C-18/19

Lead Agency: Colorado State University

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Category:

Ongoing Project

Ongoing-revised project

Requested new project

Unsolicited proposal

Expected Funding Source:

Annual funds

Capital funds

Other (explain)

**I. Title of Proposal:** Chemically Fingerprinting Nonnative Fishes in Reservoirs

**II. Relationship to RIPRAP:**

This proposal 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. Overall, this study is intended to assess escapement risk and develop chemical fingerprints of nonnative fishes in 11 reservoirs that are potential sources of nonnative fishes to the critical habitat of Upper Colorado River Basin through microchemical analysis of otoliths. Understanding of escapement risk and development of chemical fingerprints will provide the means to assess the proportion of nonnative fishes in these rivers that originate from reservoirs and thereby guide management efforts to reduce this influx of nonnative fishes.

### **III. Study Background/Rationale and Hypotheses:**

#### **Background/Rationale:**

Nonnative fishes are present throughout the Upper Basin (Martinez 2002, Trammel et al. 2002), and can adversely impact the recovery of endangered fishes through predation or competition at critical life stages or in critical locales. However, the recruitment sources and origins of nonnative fishes are not well known. Immigration of nonnative fishes from nearby reservoirs has been demonstrated in some cases by the recapture of fishes that had been tagged as part of other studies. However, large scale tagging efforts to address the growing concern about escapement of nonnative piscivores from multiple reservoirs throughout the Upper Basin is impractical. This Scope of Work seeks to verify fish escapement from reservoirs as a source of nonnative fish entering critical habitat by applying newly developed techniques for identifying naturally occurring markers via microchemical analysis of otoliths.

Otolith microchemistry provides a means to trace the origins and movements of fishes in marine (Humphreys et al. 2005, Campana et al. 2000; Bath et al. 2000) and freshwater environments (Brazner et al. 2004, Bronte et al. Wells et al. 2003). In freshwater systems differences in underlying geology can result in water chemistry that varies among watersheds. Limnological processes and chemical transformations within reservoirs impart further distinctiveness to water chemistry among lentic and lotic water bodies. Chemical composition of ambient water is imparted to otoliths of resident fish in a highly predictable and temporally referenced manner. Because otoliths are physiologically inert structures their chemical composition does not change after material is accreted. Thus, otoliths record the environmental history of a fish and that information can be used to determine the fish's provenance (origin and movements).

Recent work by Whitley et al. (2006, 2007) has demonstrated that otolith microchemistry has excellent potential for tracing the provenance of nonnative fishes in the Upper Colorado River Basin. Further, graduate work by CSU students Ryan Fitzpatrick and Daniel Gibson-Reinemer is showing that many water bodies and hatcheries (Gibson-Reinemer et al. 2009) in Colorado possess unique chemical fingerprints, and that these fingerprints are imparted to the otoliths of fish originating from each location. It also appears that transfers of fish can be detected in otoliths as shifts in the chemical composition along laser transects performed with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS; Johnson et al. 2007). These findings coupled with the highly heterogeneous nature of the Colorado Plateau's geology suggest that otolith microchemistry is likely to reveal new insights into the movements of nonnative fishes within the Upper Colorado River Basin.

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. We do not currently have data on the chemical fingerprint of one potential source reservoir of interest to managers, Lake Powell. Nor can we definitively determine if reservoir fish and river fish captured immediately downstream possess different signatures specific to the environments they inhabit. A new task (4) has been added to this study to remedy this deficiency and improve our understanding of fish escapement from reservoirs.

## **Hypotheses:**

We hypothesize that:

- a. configuration of outlet structures, dam operations, and hydro-climatic variability interact to cause inter-site and inter-annual differences in risk of nonnative fish emigration from reservoirs,
- b. the chemical composition (fingerprints) of otoliths from nonnative fishes will differ among reservoirs,
- c. inter-annual variation in otolith fingerprints will be small relative to inter-reservoir differences,
- d. otolith core signatures of fishes that were reared in reservoirs and immigrated to rivers in critical habitat will be distinct from signatures of fishes inhabiting rivers since hatching, and
- e. otolith core signatures can be used to identify fishes as having originated from a particular reservoir.
- f. crayfish, as relatively sessile animals with a calcium-based exoskeleton, will serve as “sentinels” of Sr ratio expected at specific locations in rivers.

## **IV. Study Goals, Objectives, End Product:**

**Study Goals:** a) determine how configuration of outlet structures, dam operations, and hydro-climatic variability affect the likelihood of nonnative fish emigration from reservoirs, b) determine chemical “fingerprints” of nonnative fishes in reservoirs that are potential sources of nonnative fishes to critical habitat.

### **Study Objectives:**

Primary objectives of the investigation will be to:

1. identify species/water-years/locations with the highest risk of emigration from reservoirs,
2. quantify chemical “fingerprints” of fishes within study reservoirs and evaluate the degree of inter-annual variation in those fingerprints.
3. determine if fish sampled in rivers in the vicinity of study reservoir possess otolith core signatures that identify them as having originated from one of the study reservoirs.
4. improve our understanding of the degree to which immigration or transfers from reservoirs contributes to the load of nonnative fishes in critical habitat of the Upper Colorado River basin.
5. provide recommendations to guide management efforts to reduce the influx of nonnative fishes from reservoirs.

### **End Products:**

1. A quantitative tool to determine the proportion of nonnative fishes in critical habitat that originate from reservoirs.

2. A forensic tool to assist conservation officers in prosecuting individuals engaged in the illegal transfer of nonnative fishes from reservoirs.
3. Identification of the origin and contributing sources of target nonnative fishes to critical habitat, to facilitate the fiscal and ecological efficiency of nonnative fish control.
4. Recommendations for altered dam operations, and other management measures to minimize nonnative fish emigration into critical habitat.

## **V. Study Area:**

The principal area of study for this SOW will be large reservoirs within the Upper Colorado River Basin, including those in northeastern Utah, southwestern Wyoming and western Colorado (Bottle Hollow, Crawford, Elkhead, Flaming Gorge, Harvey Gap, Kenney, McPhee, Paonia, Red Fleet, Ridgeway, Rifle Gap, Rio Blanco and Starvation reservoirs).

## **VI. Study Methods/Approach:**

This study will compliment recent work that estimated the degree of immigration of nonnative fishes to the Colorado River from floodplain ponds and backwaters (Martinez and Martinez 2004, Whitley et al. 2006, Whitley et al. 2007).

### **Task 1. Field Collections.**

Nonnative fishes will be collected by standard fisheries sampling techniques, collateral to ongoing sampling by state, federal or university efforts. The number of species varies by reservoir and river, but will include northern pike, smallmouth bass, largemouth bass, black crappie and walleye. Other nonnative species will be collected opportunistically and tissue samples will be collected and archived for possible analysis at a later date.

### **Task 2. Microchemical Analysis of Otoliths.**

We will extract sagittal otoliths from up to 20 individuals of each species from each site. Otoliths will be removed from fishes using non-metallic forceps, rinsed with distilled water, and stored dry in polyethylene vials until preparation for analyses. A range of fish sizes/ages will be collected to allow us to examine otolith core (first year of life) signatures across a number of year classes, and thereby assess inter-annual variation in those signatures. Otoliths will be embedded in Epo-fix® epoxy, sectioned in a transverse plane using an ISOMET low-speed saw, and polished to reveal annuli. Otolith thin sections will be mounted on acid-washed glass slides using double-sided tape, ultrasonically cleaned for 5 min in ultrapure water, and dried for 24 h under a laminar flow hood. We will employ well-established methods for the microchemical analysis using LA-ICP-MS (Campana 1999) in addition to new techniques developed with Recovery Program funding by Whitley et al. (2006). Dr. Brett Johnson of the Department of Fish, Wildlife and Conservation Biology at CSU will hire and supervise a graduate research associate (M.S.) to identify sampling intensity, conduct and oversee microchemical analyses, evaluate data and provide findings. CSU will maintain oversight of this project and will coordinate field sample collection with assistance of Pat

Martinez and CDOW. Analytical work will be conducted under the guidance of Alan Koenig, U.S.G.S. Research Scientist, using the LA-ICP-MS instrument at the U.S.G.S. Mineral Resources Laboratory in Denver, Colorado. Additional laboratory analyses will be contracted with facilities providing services unavailable at the Denver U.S.G.S. laboratory (e.g., University of Melbourne, Australia, the University of Alaska-Fairbanks, and Woods Hole Oceanographic Institution).

#### Task 3. Reservoir Emigration Risk Assessment.

The risk of nonnative fishes emigrating downstream from reservoirs into critical habitat via entrainment in dam releases will be evaluated by considering the interplay of three fish-related factors: 1) proximity, 2) vulnerability, and 3) survival, and three reservoir-related factors: 1) physico-chemical conditions, dam configuration, and 3) dam operations. To address fish-related factors, we will gather basic life history information from the literature including reproductive chronology, and larval and juvenile stage growth and behavior, and susceptibility of adults to entrainment. We will also conduct a literature review of survival rates of each species and lifestage passing through dams of various configurations. To address dam-related factors we will examine the configuration of outlet works at each of the study reservoirs, and gather historical data on operations. In a subset of reservoirs we will construct operations scenarios for wet, dry and normal hydro-climatic years, as well as associated limnological information corresponding to each hydro-climatic year type. Dam configuration, operations and reservoir conditions will be coupled with fish reproductive and distribution predictions to generate risk of emigration for each species-reservoir combination in wet, dry, and normal water years. Finally, recommendations for alternative dam operations and other management measures that could reduce fish emigration through dam releases will be generated.

#### Task 4. Refine Understanding of Sr Ratios of Fish Captured in Rivers.

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. We do not currently have data on the chemical fingerprint of one potential source reservoir of interest to managers, Lake Powell. Nor can we definitively determine if reservoir fish and river fish captured immediately downstream possess different signatures specific to the environments they inhabit. We are proposing to gather, prepare and analyze otoliths from nonnative piscivores sampled from Lake Powell. We will determine their strontium isotope fingerprint for comparison to samples of fish from the Upper Green River that are already analyzed. We will also gather samples of crayfish from the Upper Green River to use as site-specific sentinels of strontium ratios. Crayfish are widely distributed throughout the UCRB, their exoskeleton contains strontium, and they are relatively sedentary. Preliminary research showed that crayfish strontium ratios differ greatly across reservoirs and are similar to strontium ratios of fish from the same location.

## **VII. Task Description and Schedule:**

FY 2008:

Task 1.

Pat Martinez, CDOW Aquatic Researcher, and Brett Johnson, CSU Professor, will coordinate collection efforts performed by various state and federal sampling teams.

Task 2.

Brett Johnson will recruit a new MS student to resume the microchemistry work. CSU will process whole fish samples, prepare otoliths for microchemical analysis and perform analyses and interpretation of otolith samples. CSU will submit quarterly activity reports to Pat Martinez.

Task 3. Reservoir Emigration Risk Assessment.

Brett Johnson will recruit a student from the CSU College of Engineering to assist with developing dam operations and hydro-climate scenarios (Spring-Fall 2008). CSU will begin gathering basic life history information on the five priority nonnative species, in addition to limnological data from study reservoirs (Spring-Fall 2008).

Reporting: An annual report will be submitted to Tom Chart by December 15, 2007.

FY 2009:

Task 1.

Brett Johnson, CSU Professor, and Pat Martinez, CDOW Aquatic Researcher, will coordinate any additional collection efforts deemed necessary, to be performed by various state and federal sampling teams. New collections will be made to investigate walleye emigration from Red Fleet Reservoir.

Task 2.

CSU will process whole fish samples, prepare otoliths for microchemical analysis and perform analyses and interpretation of otolith samples, including samples collected from Red Fleet Reservoir, other nearby source populations and recipient rivers.

Task 3.

CSU will overlay ecological information with dam configuration and operations scenarios. Emigration risk analysis will be completed.

Reporting: Tasks 1, 2, 3.

An annual report will be submitted to Tom Chart by December 15, 2008. CSU will present preliminary findings at Upper Basin Researcher's Meeting in January 2009.

FY 2010:

Task 1.

CSU will coordinate any additional collection efforts deemed necessary, to be performed by various state and federal sampling teams.

Task 2.

CSU will process whole fish samples, prepare otoliths for microchemical analysis and perform analyses and interpretation of otolith samples, including samples collected from Red Fleet Reservoir, other nearby source populations and recipient rivers.

Reporting: Tasks 1, 2.

An annual report will be submitted to Tom Chart by December 15, 2009. CSU will present preliminary findings at Upper Basin Researcher's Meeting in January 2010.

Task 3.

Final report – schedule per Biology Committee peer review process.

FY 2011:

Task 1.

Completed

Task 2.

CSU will process whole fish samples, prepare otoliths for microchemical analysis and perform analyses and interpretation of otolith samples.

Task 3.

CSU completes analysis of emigration risk data.

Task 4.

CSU gathers crayfish (Green River, Flaming Gorge) and otolith samples (Lake Powell). CSU completes isotopic analysis of crayfish and fish samples.

Reporting: Tasks 1, 2, 3, 4

An annual report will be submitted to Recovery Program by November 15, 2010. CSU will present findings at Upper Basin Researcher's Meeting in January 2011.

FY 2012:

Task 1.

Task completed.

Task 2.

Task completed.

Task 3.

Task completed.

Task 4.

Prepare final report synthesizing findings from Tasks 1-3 with Task 4.

Reporting: An annual report (Task 4) will be submitted to Recovery Program by November 15, 2011.  
 CSU will present findings at Upper Basin Researcher's Meeting in January 2012.  
 Draft final report synthesizing findings from Tasks 1-3 with Task 4 to Program- January 2012.

Revised Project Timeline:

Month	2008	2009	2010	2011	2012
<b>Jan</b>		Upper Basin presentation	Upper Basin presentation	Upper Basin presentation	Upper Basin presentation, Final Report (Task 1-4)
<b>Feb</b>					
<b>Mar</b>	Tasks 1-3: seek students				
<b>Apr</b>	Task 3: work begins				
<b>May</b>	Field collections to fill in gaps	Field collections to fill in gaps, & investigate Red Fleet Reservoir		Tasks 1-3: MS student graduates Task 4: Crayfish, fish sampling, analysis	
<b>Jun</b>	↓	↓		↓	
<b>Jul</b>	↓	↓		↓	
<b>Aug</b>	Tasks 1-2: MS student begins	↓		↓	
<b>Sep</b>	↓		↓	4: Crayfish, fish analysis	
<b>Oct</b>			Analyses and interpretation of otolith data.		
<b>Nov</b>			↓	Interim report (Task 4)	
<b>Dec</b>	Interim report (Tasks 1-3)	Interim report (Tasks 1-2)	Interim report (Tasks 1-3)		

**VIII.**

**FY- 2008 through 2012 Work:**

FY 2008 Deliverables:

**Findings summarized in annual report to Program- December 2007.**

FY 2009 Deliverables:

**Findings (Tasks 1-3) summarized in annual report to Program- December 2008.**

**Presentation of findings at Upper Basin Researcher's Meeting- January 2009.**

**Draft Final Report (Task 3) to Program- September 30, 2009.**

FY 2010 Deliverables:

**Findings (Tasks 1-2) summarized in annual report to Program- December 2009.**

**Presentation of findings at Upper Basin Researcher's Meeting- January 2010.**

FY 2011 Deliverables:

**Findings summarized in annual report to Program- December 2010.**

**Presentation of findings at Upper Basin Researcher's Meeting- January 2011.**

**Final report (Tasks 1-3) to Program- September 2011.**

FY 2012 Deliverables:

**Findings (Task 4) summarized in annual report to Program- November 2011.**

**Presentation of findings at Upper Basin Researcher's Meeting- January 2012.**

**Final report synthesizing findings from Tasks 1-3 with Task 4 to Program- January 2012.**

**Budget**

**FY 2008 Costs:**

Task 1 - Field Collection

Supplies	500
Travel	500
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	150
Total	\$1,150

Task 2 - Otolith Analysis

Graduate Research Associate & fringe	3,224
Graduate tuition (1 semester)	2,444
CSU professor salary, fringe (0.2 months)	2,172
Student hourly & fringe (50 hours)	520
Mass spectrometer use fees	2,500
Travel	700
Lab supplies	1,000
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	1,884
Total	\$14,444

Task 3 – Emigration Risk

Graduate Research Associate & fringe	4,836
CSU professor salary, fringe (1.0 months)	10,858
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	2,354
Total	\$18,048

**TOTAL (FY 2008) \$33,642**

**FY 2009 Costs:**

Task 1 - Field Collection

Travel	1,800
Supplies	200
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	300
Total	\$2,300

Task 2 - Otolith Analysis

CSU professor salary & fringe (1 month)	11,047
Graduate Research Associate & fringe (7.5 months)	12,552
Student hourly & fringe (300 hours)	3,766
Mass spectrometer use fees	13,000
Travel	2,000
Lab supplies	990
Graduate tuition (1 semester)	3,200
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	6,503
Total	\$53,057

Task 3 – Emigration Risk

CSU professor salary, fringe (0.75 months)	8,285
Student hourly and fringe (90 hours)	1,130
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	1,412
Total	\$10,827

**TOTAL (FY 2009) \$66,184**

**FY 2010 Costs:**

CSU Indirect cost rate increased to 17.5% beginning in FY 2010.

Task 1 - Field Collection

Supplies	500
Travel	500
University indirect cost @ 17.5% (funds passed through existing Larval Lab- or BMR-USBR agreement)	175

Total	\$1,175
<u>Task 2 - Otolith Analysis</u>	
CSU professor salary, fringe (1.0 months)	11,599
Graduate Research Associate & fringe	13,180
Student hourly & fringe (150 hours)	1,883
Mass spectrometer use fees	5,000
Travel	2,000
Lab supplies	1,000
Page charges	1,000
Graduate tuition (1 semester)	3,520
University indirect cost @ 17.5% (funds passed through existing Larval Lab- or BMR-USBR agreement)	6,241
Total	\$45,422
<u>Task 3 – Emigration Risk</u>	
Total	\$0
<b>TOTAL (FY 2010)</b>	<b>\$46,597</b>
<b><u>FY 2011 Costs:</u></b>	
<u>Task 1 - Field Collection</u>	
Travel	500
University indirect cost @ 17.5% (funds passed through existing Larval Lab- or BMR-USBR agreement)	88
Total	\$588
<u>Task 2 - Otolith Analysis</u>	
Graduate Research Associate & fringe	8,303
Graduate tuition (1 semester)	3,872
CSU professor salary, fringe (1.0 months)	11,877
Student hourly & fringe	1,883
Travel	700
University indirect cost @ 17.5% (funds passed through existing Larval Lab- or BMR-USBR agreement)	3,984
Total	\$30,619
<u>Task 3 – Emigration Risk</u>	
Total	\$0
<u>Task 4 – Crayfish</u>	
Non-student hourly & fringe	17,546
Student hourly & fringe	603
Travel	3322
Mass spectrometer use fees	6560
Field and lab supplies	800

University indirect cost @ 17.5% (funds passed through existing Larval Lab- or BMR-USBR agreement)	5,045
Total	\$33,876

**TOTAL (FY 2011) \$64,495**

**FY 2012 Costs:**

<u>Task 1 - Field Collection</u>	
Total	\$0

<u>Task 2 - Otolith Analysis</u>	
Total	\$0

<u>Task 3 – Emigration Risk</u>	
Total	\$0

<u>Task 4 – Escapee Sr ratios</u>	
Total	\$0

**TOTAL (FY 2012) \$0**

**IX. Budget Summary (FY08-FY12):**

**FY-2008**

1) Field Collection:	\$1,150
2) Otolith Analyses:	\$14,444
3) Reservoir Emigration:	\$18,048
<b>Total</b>	<b>\$33,642</b>

**FY-2009**

1) Field Collection:	\$2,300
2) Otolith Analyses:	\$53,057
3) Reservoir Emigration:	\$10,827
<b>Total</b>	<b>\$66,184</b>

**FY-2010**

1) Field Collection:	\$1,175
2) Otolith Analyses:	\$45,422
3) Reservoir Emigration:	\$0
<b>Total</b>	<b>\$46,597</b>

**FY-2011**

1) Field Collection:	\$588
2) Otolith Analyses:	\$30,619

3) Reservoir Emigration:	\$0
4) Crayfish	\$33,876
<b>Total</b>	<b>\$64,495</b>

**FY-2012**

1) Field Collection:	\$0
2) Otolith Analyses:	\$0
3) Reservoir Emigration:	\$0
4) Crayfish	\$0
<b>Total</b>	<b>\$0</b>

**Total Budget FY08-FY12** **\$210,918**

**X. Reviewers:**

**XI. References:**

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