

I. Project Title: Chemically Fingerprinting Nonnative Fishes in Reservoirs

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III. Project Summary:

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.

IV. Study Schedule: FY06-FY11

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 2008 Tasks and Deliverables, Discussion of Initial Findings and Shortcomings:

The graduate student on the project (Phillip Brinkley) resigned to pursue other interests in January, 2008. A considerable amount of time was spent planning how to complete the study and a new Scope of Work was prepared in February, 2008 to extend the duration of the study and add funds to accommodate the timing needed to recruit and complete a new Masters student. A national search was conducted and an outstanding graduate student was recruited, Brian Wolff. Brian began working on the project in mid August, 2008. Brian has selected and met with his graduate advisory committee and he is taking a full load of courses this fall semester.

The former grad student was making good progress when he decided to quit and left the project fish and otolith collections in good condition. His databases were less well organized and we have spent a considerable amount of time creating a new Access database, merging all information on otolith collections since 2004, including all those collected for the previous study under Project C-18/19, Analysis of Centrarchid Concentration Areas.

Task 1. Field Collections

Colorado State University took over lead responsibility for coordinating field collections and reservoir and river sampling was conducted by state and federal crews during May through September, 2008. We requested samples with an emphasis on waters and species where target sample sizes were not achieved in FY07. Elkhead Reservoir and its tailwater (stilling basin) received particular attention because of low sample size obtained in 2007 and because this is a system where tagged, known provenance fish that have resided in both a river (Yampa River) and a reservoir (Elkhead Reservoir) are present. Agencies providing fish to the project included the Colorado Division of Wildlife (CDOW), Utah Division of Wildlife Resources (UDWR), U.S. Fish and Wildlife Service (USFWS), and the Colorado State University Larval Fish Lab (LFL).

We completed a thorough inventory of the otoliths that have been extracted from fish samples collected from study waters in 2006-2008. Since 2006 we have gathered at total of 850 sets of otoliths from the five nonnative species of primary concern (Table 1). Some of the fish samples collected during 2008 are in possession of agencies, pending availability of storage space in freezers at CSU and are thus not included in the totals in Table 1. A large number of otoliths were collected, some of which were analyzed by destructive (whole otolith, solution-based) or semi-destructive (laser ablation) techniques, prior to 2006 and have been included in sample sizes reported in previous reports (Martinez and Johnson 2006, 2007). We have also collected several hundred sets of otoliths from other nonnative species (including bluegill, burbot, gizzard shad, grass carp, sunfish, yellow perch); these samples have also been disclosed in previous reports and are not repeated here.

Table 1. Number of nonnative fish of primary concern collected for microchemical analysis of otoliths during 2006-2008. 2008 data do not include otoliths yet to be retrieved from fish samples in CSU or agency freezers. 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.

Water Body	2006						2007						2008						Sum
	BCR	LM B	NPK	SM B	WA L	Total	BCR	LM B	NPK	SM B	WA L	Total	BCR	LM B	NPK	SM B	WA L	Total	
Colorado River	0	0	0	0	6	6	3	18	0	30	2	53	0	0	0	0	0	0	59
Crawford Reservoir	0	0	0	0	0	0	0	0	4	1	0	5	0	0	36	0	0	36	41
Dolores River	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Duchesne River	NA	NA	0	16	0	16	NA	NA	0	0	0	0	NA	NA	0	0	0	0	16
Elkhead Reservoir	0	0	0	0	NA	0	3	1	11	11	NA	26	0	0	0	0	NA	0	26
Flaming Gorge Reservoir	NA	NA	NA	0	NA	0	NA	NA	NA	20	NA	20	NA	NA	NA	0	NA	0	20
Green River	2	NA	0	0	9	11	0	NA	12	26	35	73	0	NA	0	0	0	0	84
Harvey Gap Reservoir	0	0	0	0	NA	0	6	12	1	8	NA	27	0	0	0	0	NA	0	27
Juniata Reservoir	NA	NA	NA	0	0	0	NA	NA	NA	16	10	26	NA	NA	NA	0	0	0	26
Kenney Reservoir	0	NA	NA	NA	NA	0	3	NA	NA	NA	NA	3	0	NA	NA	NA	NA	0	3
McPhee Reservoir	NA	0	NA	3	0	3	NA	0	NA	0	0	0	NA	0	NA	0	0	0	3
Paonia Reservoir	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	0
Rifle Gap Reservoir	0	0	28	2	7	37	5	21	65	6	8	105	0	0	24	22	2	48	190
Rio Blanco Reservoir	0	0	0	0	NA	0	13	21	1	0	NA	35	0	0	0	0	NA	0	35
Stagecoach Reservoir	NA	NA	0	NA	3	3	NA	NA	9	NA	3	12	NA	NA	0	NA	0	0	15
Starvation Reservoir	NA	NA	NA	0	0	0	NA	NA	NA	17	20	37	NA	NA	NA	0	0	0	37
Yampa River	29	NA	50	160	0	239	7	NA	4	18	0	29	0	NA	0	0	0	0	268
Total	31	0	78	181	25	315	40	73	107	153	78	451	0	0	60	22	2	84	850

Task 2. Microchemical Analysis of Otoliths

Departure of the first graduate student on the project was a significant setback that delayed sample analysis. We have also experienced difficulties gaining access to the U.S.G.S. Mineral Resources Laboratory in Denver to do otolith analyses. Given these problems, the high cost of microchemical analysis and the fact that the current graduate student is just now completing his research proposal, we have so far opted to forgo chemical analyses and focus on database development and refining our otolith sectioning techniques.

After consulting with otolith experts at Cornell University and Woods Hole Oceanographic Institute, we have refined our sectioning, polishing and photomicrographic methods to allow us to obtain optimum images of our otolith sections for age and growth determination prior to chemical analysis. Age and growth information is required for the reservoir emigration risk (Task 3) portion of the study.

Task 3. Reservoir Emigration Risk Assessment.

We have been unable to recruit a student from the CSU College of Engineering to assist with developing dam operations and hydro-climate scenarios because students there are currently fully funded with their own graduate projects. We will continue to seek an engineering student assistant but have begun gathering dam operations inputs ourselves.

We have accumulated basic life history information on the five priority nonnative species, plus other nonnative species of interest (Table 2). This information is needed to relate the phenology of spawning, rearing and juvenile development in each reservoir to reservoir operations. Coupling knowledge of the reproductive timing of each species with release patterns in a particular reservoir will allow us to estimate young-of-year entrainment likelihoods. As we accumulate better information on age-growth of the nonnative fishes in study reservoirs (see Task 2 above) we will use that knowledge to develop first year growth trajectories and examine year-class-strength as a function of reservoir conditions and hydrology.

Table 2. Reproductive ecology of nonnative fishes in Upper Colorado River Basin reservoirs; DD is degree days. Sources for ecological information are as follows: a) Becker (1983), b) McPhail and Paragamian (2000), c) Scott and Crossman (1973), d) UMMZ (2008), e) Auer (1982).

Species	Reproductive Guild	Spawning substrate	Spawning depth (m)	Spawning season	Initiation of spawning (°C)	Peak of spawning (°C) ^e	Incubation time (d or DD)	Size at hatching (mm) ^e	Rearing, larval behavior
Black crappie	Nest building, guarding polyphil	Sand, mud, gravel, vegetation	0.25 to >2 ^c	Late spring ^c	14.4 ^a	18.7	3-5 d ^c	2.3	Larvae guarded until first feeding ^c
Large-mouth bass	Nest building, guarding polyphil	Sand, mud	0.6 ^a	Early summer ^c	15.6 ^a	19.9	3-4 d ^d	3.9	Larvae guarded by father for at least one month (till 30 mm) ^d
Northern pike	Scattering phytophil	Vegetation	0.2 ^a	Early spring ^c	2.2-2.8 (spawning migration) ^a	7.5	210-270 DD above 32°F ^a	8	Emigrate from marsh at 20 mm (18-24 d after hatch) ^c
Small-mouth bass	Nest building, guarding lithophil	Sand, gravel, cobble	0.4-3.7 ^a	Early summer ^c	15 (nest construction) ^a	18.3	12.8°: 9.5d, 23.9°: 2.3d ^a	4.8	Larvae are by male guarded for 14 d ^d
Walleye	Scattering lithophil	Gravel, cobble, rock	<1.0 ^a	Spring ^c	3.3-6.7 (spawning migration) ^a	8.8	4.4°: 26d, 11.5°: 21d, 13.9°: 7d ^a	7.5	Pelagic until 25-30 mm, then move inshore ^c
Yellow perch	Phytophil	Vegetation	0.6-3 ^a	Spring ^c	8.9 ^c	9.2	10.3°: 27d ^a 8-10 d ^d	5.6	Near surface for 3-4 weeks post hatch (till 25 mm, when they swim to bottom) ^c
Bluegill	Nest building, guarding lithophil	Fine gravel	0.8 ^c	Late spring thru August ^c	19.4 ^a	N/A	3 d ^d 3-5 d ^c	2.7	Nest guarded by father until larvae are 3-4 d old ^d
Burbot	Scattering litho-pelagophil	Sand, gravel	<3 ^c	Winter ^c		1.6 (surface) ^c	60-120 d ^d	4.5	No nest, no parental care, semi-pelagic eggs ^c

VII. Recommendations: Continue the project as outlined in the Scope of Work.

Task 1. Field Collections

Results from microchemical analyses (Task 2) performed on samples collected thus far should be used to determine what additional samples are needed. Reasons for additional sampling include 1) to bolster sample size of species/location combinations inadequately represented to date, and 2) to evaluate interannual variation in site-specific signatures. CSU should continue to coordinate sample acquisition with the respective state, university, and federal crews operating in the target reservoirs and river reaches.

Task 2. Microchemical Analysis of Otoliths.

Johnson and Wolff should begin microchemical analysis of otoliths as soon as Wolff completes his graduate research proposal and we can get access to laboratory facilities. We have a trip booked to travel to the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility during December 14-19, 2008 to perform elemental and isotopic analyses of otolith thin sections. Our priorities for analysis on this trip will be 1) a comparison of signatures among species from within a reservoir to evaluate whether taxonomic (phylogenetic) differences affect otolith signatures of fish exposed to the same water chemistry, 2) to compare signatures within a given species across a subset of reservoirs to examine geographic variation in chemical signatures, and 3) to compare signatures of fish from a subset of river/reservoir combinations to begin to address factors responsible for differences in lotic and lentic signatures. Results from these preliminary analyses should be used to develop more intensive analytical work on the remaining otolith sample set.

Task 3. Reservoir Emigration Risk Assessment.

We should continue to look for a graduate research assistant from the CSU Engineering program to assist with developing reservoir operations and hydrologic scenarios. In the meantime we should accumulate historic reservoir operations and stream flow data. We should also gather the available information on limnological conditions (particularly dissolved oxygen and temperature profiles) in study reservoirs. We need to obtain information on first year growth of nonnative fish in study reservoirs; this information may be estimated from ageing structures (including otoliths) removed from fish collected for microchemical analysis. It will be desirable to seek additional funds to support the age-growth work which was not originally anticipated but now appears to be important to this accomplishing task. CDOW has already committed \$2500 toward this age-growth work, but those funds are currently subject to a statewide budget freeze. All of these efforts should be completed before the final stage of this task, the emigration risk analysis, can be accomplished.

VIII. Project Status:

This project will continue through FY 2011 and it should be considered on track and ongoing. Despite delays, there have been no significant changes in project direction, probability of success, or alignment with RIPRAP objectives and deadlines.

IX. FY 2008 Budget Status

- A. Funds Provided: \$97,020.00 (since inception of project)
- B. Funds Expended: \$ 62,317.30
- C. Difference: \$ 33,702.70

The surplus arose because we focused on sample collection in FY07 and deferred sample processing until FY08 (sample processing is a large expense), then the graduate student resigned leaving us unable to make progress on sample processing. Thus we saved funds budgeted for analytical costs, laboratory assistants, and laboratory supplies. We were also unable to hire the engineering graduate assistant to work on Task 3 so those funds are also still available until we can find an appropriate candidate. We expect to spend most of these funds in FY09 in addition to the amount budgeted for FY09, as shown in the project SOW.

- D. Percent of the FY 2008 work completed, and projected costs to complete:
 Task 2- lab work (LA-ICPMS): 1% complete. We expect to complete all this lab work on 2006-8 samples with the leftover funds shown above, during FY09. Our trip to Woods Hole on December 12-19, 2008 should expend approximately \$10K in laboratory fees plus travel expenses. A large amount of additional lab work is also required, later in FY09 and FY10. Task 3: 30% complete. Remaining funds will be expended when an appropriate student identified.
- E. Recovery Program funds spent for publication charges: \$0

X. Status of Data Submission (Where applicable): N/A

XI. Signed:

<u>Patrick J. Martinez</u>	<u>11/19/08</u>
Principal Investigator	Date
<u>Brett M. Johnson</u>	<u>11/19/08</u>
Principal Investigator	Date

XII. References:

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