

I. Project Title: Stable Isotope Analysis of Centrarchid Concentration Areas

II. Principal Investigators:

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III. Project Summary:

Non-native centrarchids, including largemouth bass, bluegill, black crappie, and green sunfish, occur in a variety of aquatic habitats throughout the Grand Valley reach of the Colorado River and represent a significant predatory threat to young life stages of endangered fishes. However, it is uncertain whether centrarchid presence in critical riverine habitats is primarily the result of escapement from off-channel ponds or resident “in-stream” reproduction. The goal of this project is to identify centrarchid sources to critical riverine habitats. Results to date indicate that identification of centrarchid origins is possible through isotopic analysis of fish otoliths. Analysis of otolith samples to determine origins of centrarchids collected from riverine habitats is ongoing. This project will conclude in FY2005 and will facilitate fiscal and ecological efficiency of centrarchid control through improved knowledge of centrarchid sources.

IV. Study schedule:

FY 2003:

Task 1. Hire a post-doctoral research associate to identify sampling intensity, oversee isotopic analyses, evaluate data and provide findings.

An annual report was submitted to Pat Nelson by December 15, 2003.

FY 2004:

Task 1. Anita Martinez, CDOW Nonnative Fish Control biologist, will lead field sampling access and collection efforts in cooperation with Pat Martinez, CDOW Aquatic Researcher and field technicians. Consultation with the CSU post-doc will guide all sampling efforts. Sampling site selection and intensity for the study will be based on several factors:

1) in part on findings of prior isotopic work by Martinez et al. (2001),

- 2) on preliminary floodplain pond/riverine biota isotopic data from 2001-2002 (P. Martinez, CDOW, and B. Johnson, CSU, in progress),
- 3) on results of A. Martinez's work on centrarchid concentration areas (Hot Spots),
- 4) on the GIS analysis of fish distributions resulting from the Nonnative Fish Regulation evaluation (Martinez and Nibbelink 2004), and
- 5) on obtaining access from private/municipal landowners.

Task 2. Sampling will be conducted approximately two weeks every month or as needed.

Task 3. Dr. Brett Johnson at CSU will select a post-doctoral research associate to perform analyses and interpretation of isotopic samples. Work will involve year round sample and data analysis, the post-doctoral research associate will advise CDOW on field sample collection and ongoing sample preparation for isotopic analyses. The post-doctoral research associate will submit quarterly reports to Pat Martinez, CDOW.

An annual report will be submitted to Pat Nelson by November 11, 2004.

FY 2005:

Task 1. Anita Martinez, CDOW Nonnative Fish Control biologist, will lead field sampling access and collection efforts in cooperation with Pat Martinez, CDOW Aquatic Researcher and field technicians. Consultation with the CSU post-doc will guide all sampling efforts. Sampling site selection and intensity for the study will be based on several factors:

- 1) in part on findings of prior isotopic work by Martinez et al. (2001),
- 2) on preliminary floodplain pond/riverine biota isotopic data from 2001-2002 (P. Martinez, CDOW, and B. Johnson, CSU, in progress),
- 3) on results of A. Martinez's work on centrarchid concentration areas (Hot Spots),
- 4) on the GIS analysis of fish distributions resulting from the Nonnative Fish Regulation evaluation (Martinez and Nibbelink 2004),
- 5) on obtaining access from private/municipal landowners, and
- 6) results from 2004 will guide 2005 sampling efforts.

Task 2. Sampling will be conducted approximately two weeks every month or as needed.

Task 3. CSU post-doctoral research associate will perform analyses and interpretation of isotopic samples. Post-doctoral research associate will submit quarterly reports to Pat Martinez, CDOW. Post-doctoral research associate will also present preliminary findings at Upper Basin Researcher's Meeting and prepare Draft Final Report in RIP format in conjunction with Dr. Brett Johnson, CSU and A. Martinez and P. Martinez, CDOW.

Reporting: Draft final report to Pat Nelson – December 15, 2005
1st revised draft final report to peer review – January 15, 2006
(peer reviews due to author – February 15, 2006; BC
comments due – March 3, 2006)
2nd revised draft final report to Biology Committee – April 3, 2006

V. Relationship to RIPRAP:

This project addresses the movement of nonnative fish into river reaches of critical habitat from floodplain habitats known to support large numbers of Centrarchidae fish species. Nonnative fishes, including largemouth bass, green sunfish, bluegill, and black crappie, are known to occur in floodplain ponds, backwaters, beaver ponds, washes and irrigation drainage ditches throughout the Grand Valley reach of the Colorado River. In riverine habitats, these fish species are most commonly associated with backwaters or slow-moving side channels. It is in these low-velocity riverine habitats that centrarchids are believed to pose a significant predatory threat to the young life stages of endangered and other native fishes. However, it is uncertain to what extent the presence of centrarchid species in low-velocity riverine habitats is the result of escapement from off-channel ponds or resident “in-stream” reproduction. Overall, this study is intended to identify the source of nonnative fishes in the Colorado River through isotopic analysis.

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 FY2004 Tasks and Deliverables, Discussion of Initial Findings and Shortcomings:

Anita Martinez (CDOW Nonnative Fish Control Biologist) led field sampling access and collection efforts in cooperation with Pat Martinez (CDOW Aquatic Researcher), field technicians, and Greg Whitlege (the postdoctoral research associate at CSU). Field sampling was conducted to meet the following project objectives:

1. Determine whether the origins and movement patterns (collectively termed provenance) of centrarchids in the Grand Valley reach of the Colorado River can be identified using stable isotope and/or microchemical analyses.
2. Determine the proportion of centrarchids in backwater and main channel habitats within the study area that originated from out-of-channel ponds versus in-channel habitats.
3. If feasible, pinpoint “hotspots” where centrarchids present in connected backwaters and main channel habitats have originated by narrowing the list of possible sources (e.g. from “off-channel ponds” to specific ponds or groups of ponds).

Sampling from November 2003 through early July 2004 was primarily directed at addressing objective 1. Water samples were collected from off-channel ponds, beaver ponds, backwaters, and the Colorado River main channel within our study area (Rifle downstream to Horsethief Canyon) to determine whether these habitats possess distinct chemical signatures that could be used as natural markers of fish provenance. Table 1 shows locations and dates of water sample collections. Water samples were collected in November 2003, April 2004, and July 2004 to assess the extent of seasonal variation in water chemistry within and among habitats. Samples collected during November 2003 were analyzed for hydrogen isotopic composition ($\delta^2\text{H}$) and strontium isotope ratios. Results from these analyses indicated that $\delta^2\text{H}$ values of off-channel ponds and “riverine” (beaver pond, backwater, and main channel) habitats were distinct throughout our study area, but strontium isotope ratios did not differ among pond and river samples or longitudinally within our study area. Therefore, strontium isotope ratios were abandoned as a potentially useful natural marker of fish provenance. Greg Whitlege gave a presentation reporting preliminary results from November water sampling at the Upper Basin Researchers meeting in January 2004. April and July 2004 water samples were analyzed for $\delta^2\text{H}$ only. Results from April water samples indicated that temporal variation in $\delta^2\text{H}$ signatures was minimal for all habitat types and that off-channel ponds and “riverine” habitats continued to possess distinct $\delta^2\text{H}$ signatures. July 2004 water samples are currently being analyzed; we expect to see data for these samples in November.

Additional field sampling directed at addressing objective 1 involved collecting fishes of known environmental history in order to establish and quantify relationships between values of natural chemical markers (e.g. $\delta^2\text{H}$) in the environment and in fish (especially otoliths). Understanding how environmental values of each chemical marker being considered are related to values observed in fishes whose environmental history is known is necessary in order to interpret values for each marker measured in fishes of unknown provenance (e.g. centrarchids in Colorado River backwaters). This is particularly true for $\delta^2\text{H}$, which has not previously been applied as a natural marker of fish provenance. Locations sampled for fishes of known provenance are shown in Table 2. Some of these locations are outside of the Grand Valley because we had difficulty finding sufficient locations within our study area that contained fishes of **known** environmental history. Fishes of known environmental history were collected from 12 locations and muscle and

otolith samples were taken from each fish along with water samples for $\delta^{2}\text{H}$ analysis. Results from analysis of these samples indicate that: 1) there is a strong linear relationship between $\delta^{2}\text{H}$ values in fishes (for either otolith or muscle) and the water they inhabit ($r^2 > 0.9$, $p < 0.001$), and 2) the relationship between water and fish $\delta^{2}\text{H}$ values is unaffected by fish species or size. Fishes have also been collected from 5 locations to date for analysis of otolith strontium concentrations (note that this is different from strontium isotope ratios mentioned previously in the paragraph on water sampling). These analyses were conducted because a scoping analysis of otoliths taken from centrarchids collected at Skipper's Island backwater in October 2003 by Pat and Anita Martinez and the USGS indicated that some of these fish had unusually high strontium concentrations in their otolith core regions. Such high strontium concentrations are characteristic of fishes exposed to high-salinity environments. Therefore, we began analyzing strontium concentrations in otoliths taken from centrarchids collected in Grand Valley ponds that differ in salinity. Otolith thin-sectioning required for this analysis was performed by CDOW technicians supervised by Pat and Anita Martinez. Results of these analyses to date indicate that there is a strong correlation between otolith strontium concentration and water salinity ($r^2 = 0.92$). This is consistent with results of previous studies that have used otolith strontium concentration as an indicator of anadromy. A third natural marker that we are considering is selenium concentration, which is known to vary among locations within our study area. Based on discussions with Barb Osmundson (USF&WS), we have collected fishes from high- and low-selenium locations. Unfortunately, mass spectrometer problems have prohibited us from analyzing for selenium in sectioned otoliths from these fish yet. We hope to be able to perform these analyses this fall.

Results of water sampling and known provenance fish sampling in FY2004 collectively indicate that we will be able to determine origins of centrarchids in the Grand Valley reach of the Colorado River using stable hydrogen isotope analysis and analysis of otolith strontium concentrations. Stable hydrogen isotope ($\delta^{2}\text{H}$) analysis of otolith cores can be used to distinguish fishes collected from the Colorado River and its backwaters as either of off-channel pond or "riverine" (backwater, beaver pond, main channel) origin using the regression relationship we have established between water and otolith $\delta^{2}\text{H}$ values for known provenance fish and known $\delta^{2}\text{H}$ signatures of off-channel pond and "riverine" habitats. Thus, $\delta^{2}\text{H}$ analysis will be applied to address objective 2. Analysis of strontium concentrations in otoliths from riverine centrarchids can be employed to address objective 3 through use of the otolith strontium-water salinity relationship in order to identify fish that have originated from low-salinity (riverine habitats and some off-channel ponds) vs. high salinity habitats (e.g. some ponds and irrigation return ditches).

Sampling since mid-July 2004 has been primarily directed at collecting fishes from the Colorado River and its backwaters to address objectives 2 and 3. An intensive fish collection effort was made by Pat and Anita Martinez, CDOW technicians, and Greg Whitlege (Postdoc, CSU) during the week of 25 July 2004. A total of 220 centrarchids were collected from 19 backwaters in the Grand Valley during that week. We have also been coordinating with USF&WS biologists to obtain additional centrarchids from

backwaters and the Colorado River main channel that have been collected as part of their non-native fish control activities. Otoliths from >300 centrarchids collected by CDOW and the USF&WS have been selected for analysis. Sample preparation and $\delta^{2}\text{H}$ and strontium analysis of these samples are currently in progress. We anticipate having sufficient data from these samples to enable us to preliminarily address the question of centrarchid origins at the Upper Basin Researcher's Meeting in January 2005.

VII. Recommendations:

Given the success of this project to date and our anticipated high probability of success in meeting project objectives, it is our belief that the project should continue into FY2005 without modification. The success of this project indicates that otolith isotopic and microchemical analyses have great potential as tools for identifying origins and tracking movement patterns of non-native fishes that are impeding recovery efforts for native fishes in the Upper Colorado River basin. We recommend that the Recovery Program consider funding additional studies using these techniques to address questions of sources of other non-native fishes (e.g. smallmouth bass, northern pike) to critical habitats for endangered fishes in the Upper Basin.

VIII. Project Status:

This project should be considered "on track and ongoing". Results to date indicate a high probability of success in meeting project objectives and funding is sufficient to complete remaining project tasks. A presentation of project findings will be given by Greg Whitlege at the Upper Basin Researcher's Meeting in January 2005. We anticipate having some preliminary answers to the question of centrarchid origins at that time. A draft final report for this project in RIP format will be submitted by 15 December 2005.

IX. FY2004 Budget Status:

A. Funds Provided: \$185,768.00 to Colo. State Univ-CSU. (includes funds "rolled forward" from FY2003). Funds provided to the CDOW for A. Martinez's operations will be expended as outlined in Scope of Work.

B. Funds Expended: \$92,664.37 by CSU

C. Difference: \$93,103.63 (CSU)

The project started late due to a delay in hiring the post-doctoral research associate at CSU, so some funds from FY2003 were rolled forward into FY2004. We have not yet received invoices for most of the analyses performed to date (this explains the difference between work completed and percent of funds provided that have been expended). We also have a substantial number of samples that have not yet been analyzed and much additional work remains to complete this project.

D. Percent of FY2004 Work Completed and Projected Costs to Complete: 65%.
Projected costs to complete FY2004 work: \$45,000 (CSU)

E. Recovery Program Funds Spent for Publication Charges: \$0

X. Status of Data Submission: Capture records for fish captured by CDOW in backwaters in 2004 will be submitted to C. McAda.

XI. Signed: *Patrick J. Martinez, Anita M. Martinez* 11-12-04