COLORADO RIVER RECOVERY PROGRAM
FY 2005 ANNUAL PROJECT REPORT

RECOVERY PROGRAM
PROJECT NO. C-18/19

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

II. Principal Investigators:

Anita Martinez
Colorado Division of Wildlife
711 Independent Ave.
Grand Junction, CO 81505
Phone: 970-255-6143
FAX: 970-255-6111
anita.martinez@state.co.us

Patrick J. Martinez (lead)
Colorado Division of Wildlife
711 Independent Ave.
Grand Junction, CO 81505
Phone: 970-255-6141
FAX: 970-255-6111
pat.martinez@state.co.us

III. Project Summary:

Non-native centrarchids, including largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, green sunfish *L. cyanellus*, and black crappie *Pomoxis nigromaculatus* 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 has been uncertain whether centrarchid presence in critical riverine habitats was 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 and thereby facilitate fiscally and ecologically efficient control of centrarchids through improved knowledge of their sources. The draft final report for this project is due December 15, 2005. The format of the report will be two draft manuscripts prepared for submission to peer-review journals and a section including conclusions and recommendations.

IV. Study schedule:

<u>FY 2003</u>:

Task 1. Post-doctoral scientist Dr. Gregory Whitledge was hired at Colorado State University (CSU) under the supervision of Dr. Bret Johnson, professor in the Department of Fishery and Wildlife Biology. Dr. Whitledge prescribed sample sites and numbers, oversaw or performed isotopic/microchemical analyses of water and otolith samples, evaluated data and provided findings.

An annual report was submitted to Pat Nelson on 26 November 2003. Presentation on project methodology was delivered at Upper Basin Researchers Meeting in Moab, January 2004.

FY 2004:

Task 1. Anita Martinez, CDOW Nonnative Fish Control biologist led field sampling access and collection efforts in cooperation with Pat Martinez, CDOW Aquatic Researcher and field technicians. Consultation with Dr.s Whitledge and Johnson guided all sampling efforts. Cooperation with U.S. Fish and Wildlife Service – Colorado River Fishery Project personnel, under the supervision of Bob Burdick, greatly facilitated sample collection. Sampling site selection and number of samples were based on several factors:

1) the findings of prior isotopic work by Martinez et al. (2001),
2) preliminary floodplain pond/riverine biota isotopic data from 2001-2002 (Martinez 2003),
3) the results of work on centrarchid concentration areas (Martinez 2004),
4) the GIS analysis of fish distributions resulting from the Nonnative Fish Regulation evaluation (Martinez and Nibbelink 2004), and
5) obtaining access from private/municipal landowners.

Task 2. Sampling was conducted approximately two weeks per month, as needed.

Task 3. Dr. Whitledge oversaw or performed analyses and interpretation of isotopic/microchemical data obtained from water and otolith samples. Work involved year round sample and data analysis, including advising CDOW on field sample collection and ongoing sample preparation for isotopic analyses. Dr. Whitledge submitted brief quarterly reports to P. Martinez, CDOW, to maintain coordination and progress for this project. An annual report was submitted to Pat Nelson on 12 November 2004. Preliminary project findings were presented at Upper Basin Researchers Meeting in Grand Junction, January 2005.

FY 2005:

Task 1. A. Martinez maintained records of water and otolith samples sent to Dr. Whitledge for analyses. Final sample preparation and analyses were performed based on results from 2003 and 2004 sampling efforts and analyses results.

Task 2. Minimal field sampling was required in 2005.

Task 3. Dr. Whitledge performed analyses and interpretation of isotopic/microchemical data and submitted brief quarterly reports to P. Martinez to maintain project coordination and progress toward completion. Research and findings will be incorporated into two draft manuscripts for submission to peer-reviewed journals. These manuscripts will constitute the body of the draft final report along with project conclusions and recommendations. This annual report was submitted to Pat Nelson on 16 November 2005.

Reporting: Draft final report due to Pat Nelson – December 15, 2005. Note that this date will likely be delayed due to Dr. Johnson's involvement in a serious accident – if this occurs, subsequent dates may also be adjusted.

1st revised draft final report to peer review – January 15, 2006 (peer reviews due to author – February 15, 2006; Biology Committee comments due – March 3, 2006).

2nd revised draft final report to Biology Committee – April 3, 2006

V. Relationship to RIPRAP:

This project addressed 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, bluegill, green sunfish, 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 was 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 has identified the sources of nonnative fishes in the Colorado River through isotopic/microchemical analysis of water and otolith samples under the riverine flow and floodplain conditions that existed during the timeframe of this project. These conditions were drier than normal, thus riverine flows were lower and the communication of these flows with floodplain features, including ponds, were likely less than during conditions of average or above average discharge.

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 FY2005 Tasks and Deliverables, Discussion of Initial Findings and Shortcomings:

A. Martinez coordinated tracking/transfer of water/otolith samples from CDOW, USFWS, and CSU field collections to Dr. Whitledge at CSU. P. Martinez oversaw otolith sectioning and preparation for submission to CSU. Dr. Whitledge coordinated completion of water samples analyses at various labs and performed isotopic/microchemical analyses of otoliths. Dr. Whitledge analyzed data and provided interpretations in conjunction with Dr. Johnson. Dr. Whitledge produced two draft manuscripts, including contributions from co-authors Dr. Johnson, P. Martinez and A, Martinez, summarizing the results and findings of this project.

Field sampling and sample analyses provided data to address the key project components as indicated below:

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.

Stable hydrogen isotope ratio ($^{2}H/^{1}H$ or D/H, expressed as δD, where ^{2}H=D=deuterium or heavy hydrogen) represents a naturally occurring environmental marker that has not been applied in any published studies of fish provenance. Results from this project indicate that stable hydrogen isotopic composition in fish otoliths from the Grand Valley reach of the Colorado River can distinguish whether a specific fish spent the bulk of its life in riverine habitats (mainstem, backwater, beaver pond) vs. floodplain ponds. Pond water samples were enriched in ^{2}H compared to water collected in the three riverine habitats and ranges of pond and riverine water δD values did not overlap. Median water δD was significantly greater for ponds compared to beaver ponds, backwaters, and the river main channel. Water δD was enriched in ^{2}H in ponds compared to riverine habitats due to greater opportunity for evaporative fractionation to be expressed in ponds as a result of their longer water residence time. A highly significant linear relationship exists between fish otolith values and δD signatures of the waters fish inhabit. Water samples and one otolith from each pair were analyzed for δD using an isotope ratio mass spectrometer. Otoliths < 2.5 mg were analyzed whole while otoliths > 2.5 mg were ground to obtain a 2-2.5 mg core sample centered on the otolith nucleus. The development of this methodology is summarized and discussed in Whitledge, G. W., B. M. Johnson, and P. J. Martinez. IN REVIEW. Stable hydrogen isotopic composition of fishes reflects that of their environment, submitted to Canadian Journal of Fisheries and Aquatic Sciences.

Identifying the origins of nonnative centrarchids based on δD were refined using a natural marker based on strontium (^{88}Sr) and calcium (^{44}Ca) ratios. δD distinguishes pond- from riverine-resident fish, whereas Sr:Ca differentiates between residence in high-salinity habitats (including some ponds) and low-salinity areas. A relationship between otolith Sr:Ca ratio and environmental salinity was developed from known provenance centrarchids from ponds our study area and the highest salinity value recorded in our water samples from riverine habitats (1.2 $^{o}/_{oo}$). The second otolith was embedded in epoxy, sectioned transversely and polished to reveal annuli. These thin sections were then mounted on acid-washed glass slides and ultrasonically cleaned in ultrapure water prior to laser ablating transects from the otolith nucleus to its edge for analyses using an inductively coupled plasma mass spectrometer (LA-ICPMS). Otolith Sr:Ca ratios complemented otolith δD analysis by identifying fish that resided in environments (some ponds, irrigation ditches) whose salinity exceeded that of riverine habitats. A threshold Sr:Ca ratio was used to distinguish periods of residence in high-salinity (salinity exceeding that of riverine habitats, high Sr:Ca) versus low-salinity (salinity not exceeding that of riverine habitats, low Sr:Ca) environments. Age at immigration was determined for individuals that showed evidence of movement from high-salinity to riverine environments by associating locations of abrupt declines in otolith Sr:Ca ratio along laser-ablated transects in relation to annuli.

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.

Of the 368 centrarchids collected in backwater and Colorado River main channel habitats, 82 (22%) possessed an otolith core δD signature characteristic of ponds, 218 (59%) exhibited a signature expected for riverine-resident fish, and 68 (19%) were of uncertain origin. For fishes collected in backwaters, presence or absence of direct inflowing ditches or tributary washes did not have a significant effect on the relative proportions of individuals with pond, uncertain, and riverine otolith core signatures. However, significant differences in relative proportions of centrarchids with pond, uncertain, and riverine otolith core δD signatures were present among species (Figure 1). Approximately 70% of largemouth bass and bluegill collected exhibited an otolith core δD signature expected for riverine-resident fish, with 19% possessing a pond δD signature in the otolith core, and 10-11% being of uncertain provenance. In contrast, 53% of black crappie collected had a pond otolith core δD signature, with 26% having a riverine otolith core δD signature and 21% of uncertain origin. Fifty-three percent of green sunfish examined displayed a riverine otolith core δD signature, with 23% showing evidence of emigration from ponds and 24% of unknown provenance.

The 1-2 mg sample size requirement for δD analysis of otoliths by bulk analysis using isotope-ratio mass spectrometry may limit the resolution of δD as a natural marker of fish's full environmental history. Resolution of the approach used for otolith δD analysis corresponded to approximately the first year of a fish's life based on otolith masses of known age centrarchids collected in our study area. However, a potential shortcoming is that the otoliths of recently hatched fish or the otolith core of a larger fish, representative of the fish's first months of life, may contain too little material for δD analyses. Thus, the possibility exists that individuals that emigrated from ponds very early during age-0 may have been misclassified as being of riverine origin, because material indicative of riverine residence could dominate the otolith core signature under such a scenario despite the fact that the fish originated in a pond. While other advancements in microsampling techniques may overcome this situation with δD, such as the use of an ion microprobe, some verification of otolith core δD signatures can be obtained by elemental ratio analyses (Sr:Ca).

Otolith thin sections from 210 centrarchids collected from Colorado River backwaters were analyzed for Sr:Ca ratio using LA-ICPMS. All 79 individuals with riverine otolith core δD signatures exhibited a riverine otolith core Sr:Ca ratio consistent with that expected for riverine-resident fish. Eight fish whose origins were uncertain based on δD analysis exhibited elevated otolith core Sr:Ca ratios characteristic of residence in high-salinity ponds, resolving uncertainty regarding provenance of these individuals. The 50 centrarchids with pond δD signatures in their otolith cores exhibited a wide range of otolith core Sr:Ca ratios. Median otolith core Sr:Ca ratios were significantly higher for fish with pond and uncertain (59 fish) otolith core δD signatures compared to fish with riverine otolith core δD signatures. Maximum estimated salinity corresponding to otolith core Sr:Ca ratios was highest for black crappie, intermediate for green sunfish and bluegill, and lowest for largemouth bass (Table 1).

The relative abundance of fish with riverine otolith core δD and Sr:Ca signatures indicates that low-velocity backwater and beaver pond habitats are likely the primary source of most centrarchids (recently invading smallmouth bass *Micropterus dolomiei* were not part of this investigation) inhabiting the Colorado River in our study area. All four species analyzed in this study are associated with low-velocity, river margin habitats in rivers and construct nests in these areas. Black crappie was the only species for which the majority of individuals collected showed evidence of having emigrated from ponds, which may be due to their tendency to spawn in or near vegetation. Macrophytes are common in Grand Valley ponds but are rare or absent in backwaters (Martinez et al. 2001).

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).

Relative proportions of fish with pond, uncertain, and riverine otolith core δD signatures were not significantly different above versus within the Grand Valley or among fishes collected in river main channel versus backwater habitats. However, 60 of the 82 fish (73%) with pond δD signatures in their otolith cores were collected below the Gunnison River confluence. Relative proportions of fish with pond, uncertain, and riverine otolith core δD signatures were significantly different above versus below the Gunnison River confluence, with the proportions of pond and uncertain provenance individuals higher below the Gunnison River confluence than above (Figure 2). Twenty-two fish exhibited evidence of emigration from high-salinity habitats to the Colorado River based on changes in otolith Sr:Ca ratios along laser-ablated transects. Seventeen (77%) of these individuals were collected below the Gunnison River confluence. At least four individuals were determined to have immigrated to riverine habitats at each age from 0 to 3 years. Of these, all five fish that showed evidence of immigration to riverine habitats at age 3 were black crappie.

Pinpointing locations within the study area that were contributing large numbers of nonnatives was deemed important for focusing control efforts to problem areas. The greater proportion of fishes with pond otolith core δD signatures collected below in comparison to above the Gunnison River confluence is not likely the result of the Gunnison River contributing substantial numbers of pond-origin fish to the Colorado River, as relatively few ponds are present along the Gunnison River (Martinez and Nibbelink 2004). Rather, the higher incidence of centrarchids emigrating from ponds to the Colorado River below the Gunnison River confluence is likely related to the greater number of ponds and the comparatively high number of irrigation ditches and washes that enter the Colorado River downstream from where the Gunnison River enters (Martinez 2004). Another possible explanation is that the generally larger, more structurally complex backwaters found below the Gunnison River confluence may be more attractive to centrarchids or more conducive to their growth or survival than the generally smaller, and structurally simpler backwaters found above the Gunnison confluence. The centrarchid species collected in this study are typically associated with structure. Lack of a significant association between relative frequencies of individuals with pond, riverine, and uncertain otolith core δ^2H signatures and presence or absence of direct inflowing ditches or washes to backwaters suggests that centrarchids that immigrate to riverine habitats may be selecting the best available habitats rather than simply occupying those closest to their point of entry to the river.

The high proportion (83%) of pond emigrants that left ponds with low δD water values likely reflects a higher probability of immigration to riverine habitats from ponds that are closely associated hydrologically with the Colorado River.

Differences in water δ^2D among ponds reflected varying degrees of hydrologic isolation from the Colorado River. Centrarchids with pond δD signatures in their otolith cores exhibited a wide range of otolith core Sr:Ca ratios, reflecting emigration from ponds with differing salinities. Most individuals that exhibited evidence of emigration from high-salinity habitats were collected below the Gunnison River confluence, reflecting the relative abundance of high-salinity ponds and washes in that area. Significantly higher mean otolith core Sr:Ca ratio for black crappie compared to the other three species indicates a greater tendency for black crappie to originate in high-salinity ponds. Although results of δD analyses indicate that any effort to control centrarchid escapement from ponds should be directed primarily toward locations closely associated with the river, our findings do not provide any more specific evidence that particular ponds or groups of ponds are disproportionately contributing to centrarchid abundance in riverine habitats. While largemouth bass displayed predominately pond δD signatures in their otolith cores, they were also associated with the lowest Sr:Ca ratios and salinities. This suggests that if additional control measures were deemed necessary to control movement of this species from ponds, such efforts could be applied on ponds with a salinity < 1.8 $^{o}/_{oo}$, the maximum salinity associated with largemouth bass (Table 1).

No clear pattern with respect to age at immigration was evident from Sr:Ca data, although results indicate that centrarchids have the capacity to move into critical habitat from age-0 to at least age-3. However, the increasing proportion of centrarchids with pond otolith core δD signatures with increasing fish age (Figure 3) and the significantly greater median lengths of individuals with pond otolith core δD signatures compared to those with riverine otolith core δD signatures for three of the four species may be a consequence inter-annual variation in river hydrology and its potential effects on centrarchid reproduction, larval nursery, and immigration to the river. The upper Colorado River basin experienced below average precipitation and mean annual discharge in the upper Colorado River was below average from 2000-2004. During dry years, decreased river-pond connectivity and increased temporal and spatial extent of low-velocity habitat in the river would be expected. Such conditions could be more favorable for centrarchid reproduction and recruitment in riverine habitats due to decreased probability of scouring flows and flushing of larvae from nesting sites while simultaneously limiting access to the river for pond-dwelling fish. Thus, the recent drought may explain why the majority of the smallest, youngest fish carried a riverine δD signature in the otolith core.

The fact that age-4 and older fish had the highest proportion of individuals with pond otolith core signatures and that the largest individuals of three species (particularly largemouth bass and black crappie) almost always carried a pond otolith core δD signature suggests that although the percentage of pond-origin fish in riverine habitats was relatively low at the time of our collections, it may have been higher prior to the current drought and could increase again during years with normal or above average precipitation and river discharge. During wetter years, increased river-pond connectivity and a reduction in temporal and spatial extent of low-velocity habitat in the river would be expected. These conditions would be anticipated to be detrimental to centrarchid reproduction and recruitment in riverine habitats while enhancing access to the river for pond-dwelling fish.

VII. Recommendations:

1. Complete and submit draft manuscript, Whitledge, G. W., B. M. Johnson, P. J. Martinez, and A. Martinez. Provenance of non-native fishes in the upper Colorado River revealed by stable isotope and microchemical analyses of otoliths, for peer-review publication.

2.. Efforts to control abundance of centrarchids (except black crappie and smallmouth bass) in critical habitat for native threatened and endangered fishes should emphasize backwaters and beaver ponds that contain abundant structure irrespective of presence or absence of direct tributaries rather than focusing on those with inflowing washes or ditches.

3. Any efforts to control centrarchid escapement from ponds to the Colorado River should focus on the reach below the Gunnison River confluence, although such actions should be secondary to management activities in riverine habitats given that the majority of centrarchids examined in this study exhibited riverine otolith core δD signatures.

4. If additional control measures were deemed necessary to control movement of largemouth bass from ponds, such efforts could be applied on ponds with a salinity $< 1.8\ ^{o}/_{oo}$, thus narrowing the number of candidate ponds for treatment.

5. Management of black crappie abundance, in particular, within critical habitat would require an emphasis on restricting escapement from ponds; however, black crappie are the least numerous of the five centrarchids present in our study area.

6. Although results of this project indicate that centrarchid control efforts in the upper Colorado River should focus on riverine habitats when hydrologic conditions are similar to those during this study, reevaluation of relative proportions of riverine-dwelling centrarchids with pond and riverine otolith core signatures is recommended during and immediately following years of above average precipitation and river discharge. Such a follow-up study would be useful for assessing whether management of centrarchid abundance in critical habitat should always be focused within riverine habitats themselves or if additional emphasis should be placed on controlling centrarchid escapement from ponds to curtail immigration to riverine habitats during high-water years.

VIII. Project Status:

This project should be considered "scheduled for completion". Results address project objectives, provide a basis for management recommendations and funding is sufficient to complete remaining project tasks. A presentation of project findings will be given by P. Martinez or Dr. Johnson at the Upper Basin Researcher's Meeting in Moab, UT in January 2005. A draft final report for this project will be submitted by 15 December 2005, but may be delayed as previously described.

IX. FY2006 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 have been expended as outlined in Scope of Work.

B. Funds Expended: $154,774.61 by CSU

C. Difference: $30,993.39 (CSU)

Dr. Whitledge departed in mid-August and is presently an assistant professor in the Fisheries and Illinois Aquaculture Center, Department of Zoology, at Southern Illinois University, in Carbondale, IL. Dr. Johnson has been temporarily sidelined due to injuries sustained in his accident. The remaining funds will be spent for salaries required by Dr.'s Whitledge and Johnson to participate in finalizing manuscripts, conclusions and recommendations which comprise the final report. Some funds are reserved for travel by these professors to attend and participate in Recovery Program workshops and meetings to discuss project findings and management recommendations. Given the implications of low- vs. high-flow scenarios as they relate to the findings of this report, technicians will archive the remaining otoliths samples used in our analyses for potential future comparisons of samples analyzed under normal to high-flow conditions. It is anticipated that up to $3,000 will be consumed in page charges to publish the findings of this research in peer-reviewed journals.

D. Percent of FY2006 Work Completed and Projected Costs to Complete: 90%.
Projected costs to finalize project: $30,993.39 (CSU)

E. Recovery Program Funds Spent for Publication Charges: None to date, anticipate $3,000 for peer-reviewed publication of manuscripts.

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

XI. Signed: *Patrick J. Martinez, Anita M. Martinez* **11-16-05**

Literature Cited:

Martinez, A. M. 2004. An evaluation of nonnative fish control treatments in ponds along the Colorado and Gunnison Rivers, 1996-2002. Upper Colorado River Endangered Fish Recovery Program Project No. 18/19, Final Report. Colorado Division of Wildlife, Grand Junction. 60 pp.

Martinez , P. J. 2003. Westslope warmwater fisheries. Federal Aid in Fish and Wildlife Restoration Progress Report. Colorado Division of Wildlife, Grand Junction. 106 pp.

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.

Martinez, P. J., and N. P. Nibbelink. 2004. Colorado nonnative fish stocking regulation evaluation. Upper Colorado River Endangered Fish Recovery Program Project No. 106, Final Report. Colorado Division of Wildlife, Grand Junction. 84 pp.

Table 1. Mean (± SE) and maximum otolith core Sr:Ca ratios (mmol/mol) for black crappie (BCR, n=11), bluegill (BGL, n=23), green sunfish (GSF, n=104), and largemouth bass (LMB, n=74). Estimated salinity ($^{o}/_{oo}$) associated with each Sr:Ca ratio is also shown.

Species	Mean Sr:Ca (SE)	Mean salinity	Maximum Sr:Ca	Maximum salinity
BCR	3.11 (0.65)	2.2	7.95	5.0
BGL	1.50 (0.13)	0.8	3.60	3.0
GSF	1.42 (0.06)	0.7	3.70	3.1
LMB	1.28 (0.04)	0.5	2.15	1.8

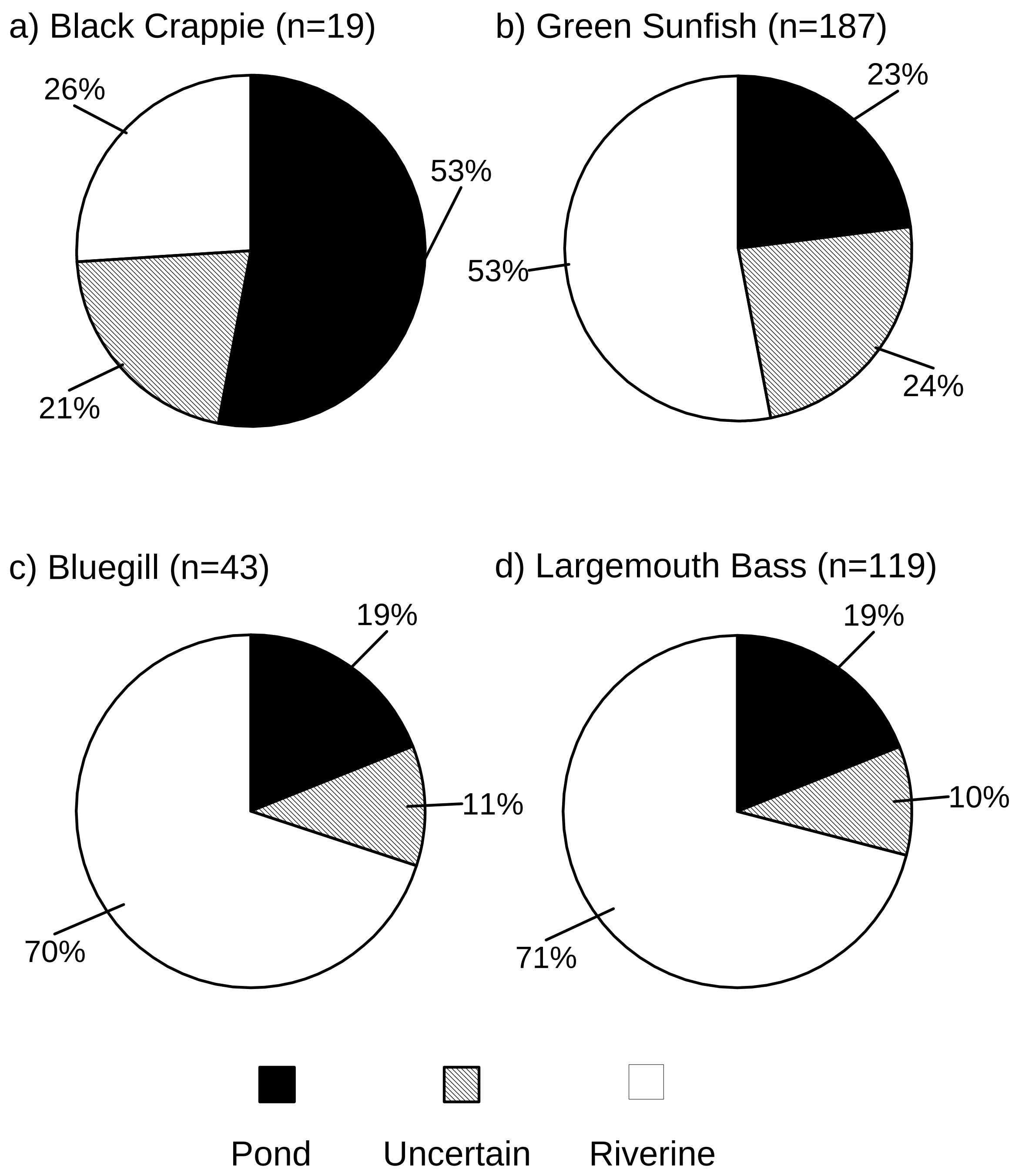

Figure 1. Relative proportions of black crappie (a), green sunfish (b), bluegill (c), and largemouth bass (d) collected in Colorado River backwater and main channel habitats with pond, uncertain, and riverine otolith core signatures. Number of individuals analyzed (n) is indicated for each species as are percentages contained within each slice.

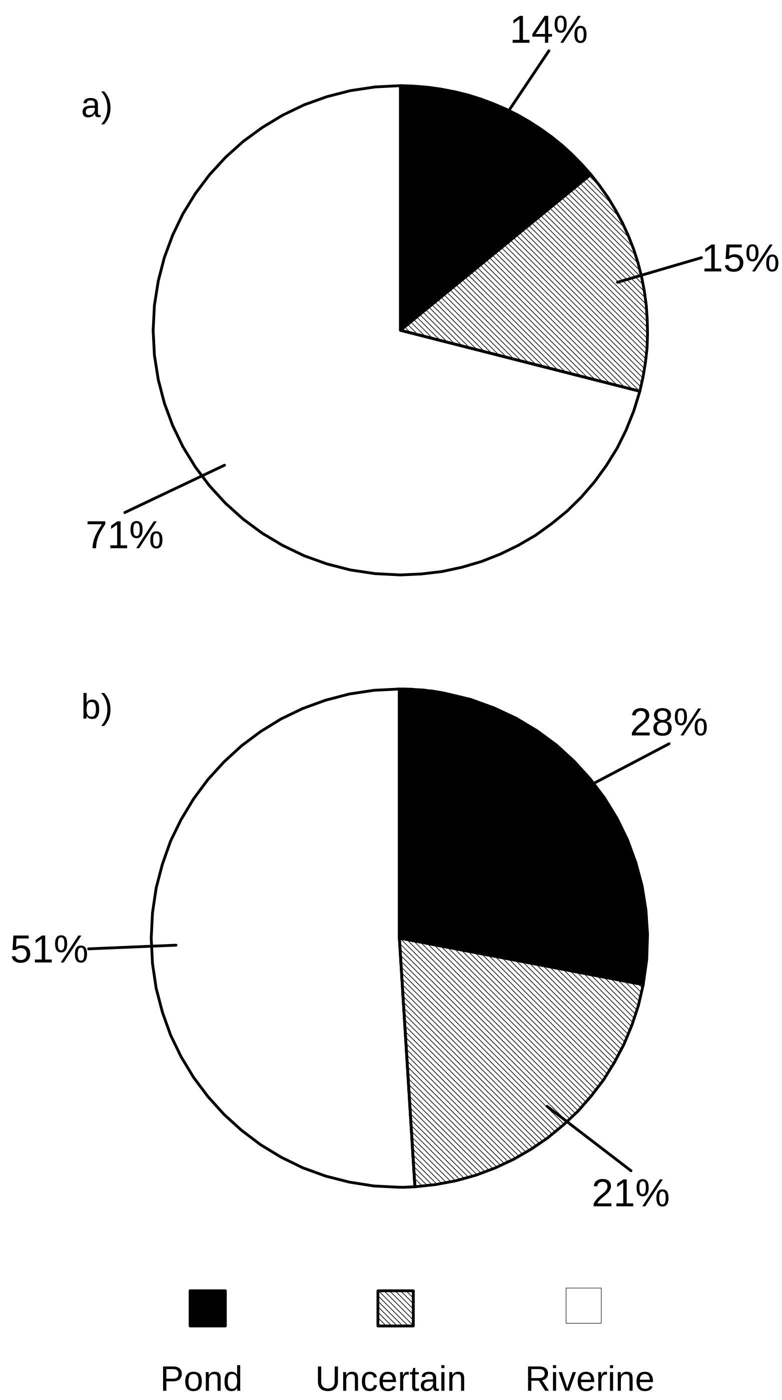

Figure 2. Otolith core environmental signatures (pond, uncertain, or riverine) for centrarchids collected in Colorado River backwater and main channel habitats above (a) and below (b) the Gunnison River confluence. Numerical values indicate percentages contained within each slice. n=154 and n=214 fish collected above and below the Gunnison River confluence, respectively.

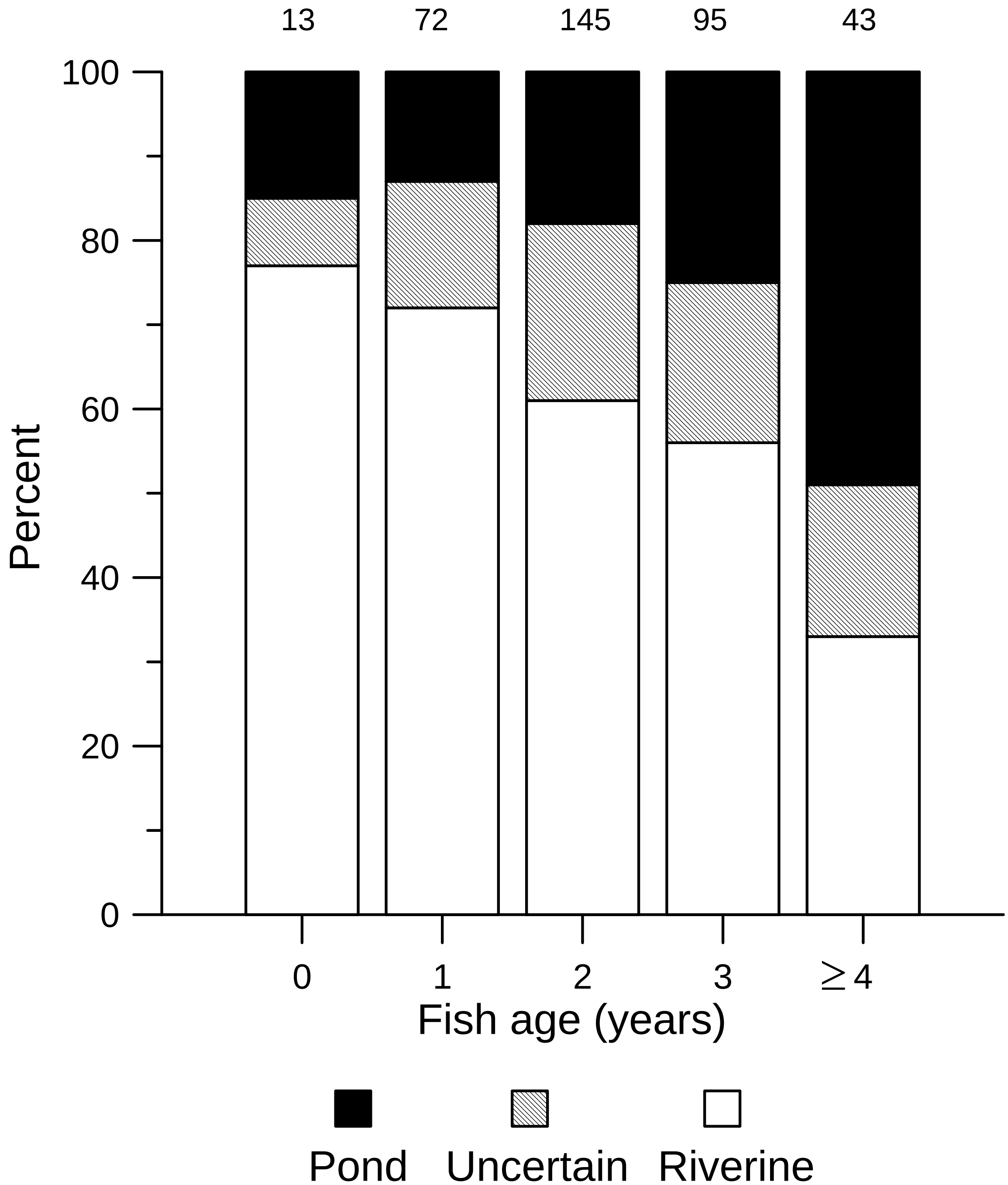

Figure 3. Relative proportions of centrarchids collected in Colorado River backwater and main channel habitats with pond, uncertain, and riverine otolith core signatures within fish age classes from age-0 to age ≥ 4 years. Values above bars indicate number of fish analyzed for each age class.