Title: Near shore ecology of Grand Canyon fish

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Section 1 Study motivation - The primary goal of this project is to understand how river flow, through its interaction with physical habitat structure, influences the survival rates of juvenile native and non-native fishes in the Colorado River in Grand Canyon. Nine research questions related to this goal have been identified in the RFP (RFP pages 27-28). These questions have a hierarchical structure and vary in scope. Some questions are fundamental and process oriented in nature (e.g., does river flow conditions alter juvenile native fish survival rates?) while others are focused on methodology (e.g., how to measure juvenile fish abundance, can small fish be marked?). Some questions, though related to the broader goals of the RFP, are quite specific (e.g., what is the Colorado River mainstem survival rate for humpback chub emigrating from the Little Colorado River (LCR) during freshets?). Some questions will be very difficult to answer within the time-frame of this project (e.g., how do biotic and abiotic factors influence individual fish growth and survival by habitat type?), or may be quite easy to answer with existing data (e.g., what is the feasibility of marking small humpback chub?).

We propose two key fundamental research questions (RQ) should guide the design of this project:

(RQ1) Do steadier flows during summer and/or fall increase survival rates of juvenile native and non-native fish?

(RQ2) To what extent does physical habitat structure (e.g., sand bars and backwaters), in conjunction with flows during these periods, influence survival rate?

We propose to address these research questions and link results to a proposed conceptual model for humpback chub *Gila cypha* (HBC) and other native and non-native fish (below) by first describing an approach to assess shifts in fish density by tracking habitat specific abundance and survival of native and non-native fish in response to changing nearshore habitat availability related to and created by the fall steady flow experiment. We then detail an approach to determine the source populations of juvenile native fish that populate nearshore habitats created by the proposed Fall Steady Experimental Flow (FSEF). Our intent is to link this new insight into juvenile fish ecology, with a focus on humpback chub, with the flow and habitat management capabilities of the Glen Canyon Dam (GCD) Adaptive Management Program (AMP), to create a better understanding of how flow and habitat management can be used to cultivate and enhance survival of juvenile native fish and, with time, adult native fish populations in Grand Canyon.

Section 1.1 Conceptual Model – The questions developed in the RFP (RFP pages 27-28) and RQ1 and RQ2 are part of a broader conceptual model of native and non-native fish population dynamics developed over the last 10-15 years in Grand Canyon. Such models have a long history of development in the Glen Canyon Dam Adaptive Management Program (e.g., Walters et al. 2000), and we summarize essential elements for juvenile humpback chub as they relate to this proposal:

A. Humpback chub juveniles recruit to the mainstem juvenile population from the Little Colorado River as very small juveniles during the spring, and larger juveniles recruit to the mainstem during monsoon-driven flood events in late-summer and fall, or from mainstem spawning events.

B. The quantity and quality of juvenile habitat in the mainstem is driven by variation in flow and temperature regimes and also channel morphology. Habitat characteristics are determined by the monthly average discharge and hourly variation in discharge from Glen Canyon Dam, as well as sediment supply in the mainstem, and the frequency and timing of flows from Glen Canyon Dam designed to create habitat believed to be important for native fish (e.g., backwaters).

C. Survival rates of HBC juveniles in the mainstem depend on the quantity and quality of physical habitat, food availability, and the intensity of competition and predation from both native and non-native fishes.

D. Abundance of HBC juveniles will increase with improvements in the quality and quantity of habitat because survival rates will be higher. Over the long-term, greater juvenile production will increase the abundance of the adult population.

Section 2 Experimental Design - To resolve uncertainties in the conceptual model above, survival rates, growth, and abundance of native and non-native juvenile fish must be quantified under contrasting conditions of flow, which, in turn, drives variation in habitat availability and quality. The experimental design for this project, which determines the extent and timing of contrasts, has already been defined, in large part, by the GCD AMP. Over the next five years, there will be hourly variation in flow rates (related to diel variations in energy demand) during summer months (Mean Low

Fluctuating Flows, MLFF, June-August), and lower, steady experimental flows during the fall (Fall Steady Experimental Flows, FSEF, September and October). We will quantify abundance, survival, habitat use, growth, and natal source of selected native and non-native juvenile fish over three flow periods (summer MLFF, MLFF-FSEF transition, FSEF) based on four sampling trips (trip launch month July, August, September, October, Table 1) given this experimental flow regime. Due to costs, natal source would be determined for only a sub-set of species (likely HBC only) after discussions with partner agencies.

Section 2.1 Sampling methods to estimate fish habitat use, growth, and survival – Estimation of juvenile abundance, survival rate, growth rate, and habitat use is fundamental to resolving uncertainties in the conceptual model and the two key research questions outlined and identified above. We propose sampling trips in late July and late August to characterize abundance, habitat use, growth, and survival rate of juvenile fish over the summer under MLFF flow fluctuations. These trips would be followed by sampling trips in early September and late October to characterize juvenile fish responses during the MLFF-FSEF transition, and FSEF period. Differences in abundance in each habitat type, between each sampling trips would be used to estimate habitat specific, reach-wide survival rates across flow events.

We propose two basic sampling approaches for estimating these characteristics: (1) reach-wide abundance estimation (RWAE); and (2) robust-design mark-recapture (RDMR) at replicate sites. The RWAE approach follows the two-stage design developed by Korman (2009) to track abundance, habitat use, growth and survival of age-0 rainbow trout in the Lee's Ferry reach of the Colorado River. Applying this approach to native and non-native juvenile fish in the lower Colorado River, we would:

(1) Select a relatively large study reach that contains multiple habitat types (e.g., Kwagunt rapid [River Mile 56] to Lava Chuar rapid [River Mile 65.5]) and stratify this reach into sites by habitat type (informed by, and integrated with, ongoing GCMRC project 2, Table 2);

(2) Sample fish at a large number of randomly selected sites within the reach using methods established in previous GCMRC efforts and Korman 2009 (e.g.,

single-pass electrofishing and mini-hoop nets) to quantify catch per effort. Site selection would be stratified by habitat type which, in turn, would likely require use of multiple gear types. Gear types and number of samples required will be informed by, and integrated with GCMRC ongoing projects 1, 3, 5, 6, 7, 11 and Trip 1 of this proposal, Table 2 ;

(3) Quantify capture probabilities by habitat/gear type, species, and fish size using short-term, closed, mark-recapture experiments (number of sampling events per trip ≥ 2 depending on recapture rate and number of sites from 2 above, e.g., Korman et al. 2009 [see Appendix]) at a smaller number of sites;

(4) Convert catch-rates at index sites (from 1 above) to population estimates based on capture probabilities estimated from (3 above); and

(5) Scale up site-specific estimates of population size to a reach-wide estimate based on the amount of habitat of each type in the reach.

Population size by sampling trip would be estimated for each habitat type and will be used to examine seasonal and flow-driven changes in habitat use. Fish would most likely be marked with a gear, habitat, and sampling pass specific mark using unique location and color combinations of Visual-Implant-Elastomer (VIE) (Brennan et al. 2005; GCMRC NSE pilot sampling, fall 2008). Ideally, unique marks would be applied to each animal (Bailey et al. 2004 for an example with VIE marks). Growth during summer-unsteady and fall-steady periods could be quantified based on differences in length-frequency distributions over time, as well as by measurements of otolith increments on a small sub-sample of fish (as in Korman and Campana 2009 [see Appendix]; see also section 2.2.3 and 2.2.4 of this proposal). As in Korman (2009), we would integrate most information from the proposed program in a stock synthesis model to jointly estimate parameters of interest (including abundance and survival rate by habitat type, growth, recruitment to the juvenile population over the growing season) and to identify shifts in habitat use. This approach is based on standard closed population mark-recapture approaches to estimate abundance and capture probability (analogous to those outlined in Otis et al. (1978)) and allows for incorporation of size dependence in capture probability (to account for heterogeneity in capture probability by size). The critical assumption of the RWAE approach (and all closed population models) is that the population can be treated as effectively closed within trips and that differences in abundance across trips are caused by recruitment (LCR and mainstem) and mortality only. The approach is unable to separate emigration from mortality. Radio telemetry data, and movement rates of marked fish within sites across trips would help evaluate the extent of potential emigration from study sites (see section 2.3.2 of methods). Immigration would be estimated. Differences in abundance and distribution among habitat types across trips would be used to estimate survival and changes in habitat use during changing flow conditions from summer MLFF, the MLFF-FSEF transition period, and FSEF conditions. If significant changes in abundance are observed, an additional sampling trip, if supplemental funds were available, following the return to normal flow operations in November could be added to assess fish population responses to the transition from experimental steady flows to winter operation flows. Modal shifts in length-frequency and direct ageing would be used to evaluate growth.

The second approach to estimating growth, survival, and abundance by habitat type is based on a robust design mark-recapture approach (RDMR) that would estimate habitat specific abundance for each trip using closed models (RWAE), and relax the assumption of population closure between sampling trips (Pollock et al. 1982). Essentially, the RDMR provides a finer-scale assessment of abundance and survival, and is generally similar to RWAE with the following differences:

(1) Select a series of study sites within a broader reach (e.g., three to four 500-m sites between Kwagunt and Lava Chuar rapids). These sites would likely each contain a mix of all or most of the habitat types within the broader reach.

(2) Conduct multi-pass (3-5 pass events) mark-recapture assays in each of the study sites during each of the four trips.

(3) Estimate abundance at each study site on each trip.

As with the RWAE approach, we would employ a marking strategy to estimate abundance within habitat types at each site for each trip. Multiple gear types would likely be needed to sample the full range of habitat types and water depths. Under the RDMR approach, we would assume that populations within each site are effectively closed within trips, but not among trips. We would also assume that changes in abundance at the sites across trips represent changes at a reach-wide scale. Differences in abundance and distribution in each habitat type across trips will be assessed during summer-MLFF, MLFF-FSEF flow transition, and FSEF periods. Survival estimates for each habitat between sampling trips could be estimated using open population models (Pollock et al. 1990) following the robust design framework. If the recaptures between trips are too low to estimate survival, then the RDMR approach basically collapses to the RWAE approach for estimating survival – i.e., these approaches are not mutually exclusive in design or analyses employed. Shifts in length-frequency and direct ageing using otoliths will be used to evaluate growth (see section 2.2.3).

Section 2.2 Site Selection - We propose to use existing data and models from the GCMRC physical science program (Projects 1, 2, and 10, Table 2 to quantify habitat availability over the study reach that contains the RDMR sites, habitat availability within the sites, and how habitat changes with flow. Such an approach has already been used to quantify juvenile chub habitat use in the Colorado River near the LCR (Korman et al. 2003). The existing GCMRC shoreline GIS database and other surveys can be used to stratify habitat into classes such as talus slopes, open sand bars, vegetated sand bars, cobble bars, and backwaters. Existing bathymetry and hydrodynamic two-dimensional models that cover the entire study reach can be used to predict water temperature, depth and velocity in shoreline habitats at daily minimum and maximum flows during the summer-unsteady period, and at the average flow during the steady-fall period (Korman 2003; Projects 1 and 10, Table 2). Velocity and temperature criteria will be used to further classify habitat for various species and life stages (e.g., into usable and unusable categories, or some finer scale). Additionally, shoreline stability during the summer can be quantified based on daily flow variation. For example, in summer, backwaters that are barely flooded at the daily maximum flow may be far more stable, warmer environments than those subject to complete inundation on each diel flow cycle. Similarly, steeper shorelines with cover (e.g., vegetated cut banks) may be more stable than low angle, open sand bars or cobble bars, where low velocity littoral areas vary more. These characteristics of stability can be related to patterns of fish habitat use (density or occupancy) to help evaluate effects of fluctuating flows. A strong relationship between fluctuating flows, nearshore habitat use, and otolith growth has been observed for age-0 rainbow trout in the Lee's Ferry reach of the Colorado River (Korman and Campana, 2009). Similar relationships are expected for juvenile native and nonnative fish in the Grand Canyon and, in fact, is part of the motivation for the current fall steady flow test. We hypothesize that unstable habitat types will be used only minimally during the summer-unsteady flow period, but that use of these habitats will increase during the fall-steady period when flows are stabilized. If this difference in habitat use is ecologically important, we would also predict increase in growth and survival during the fall-steady flow period relative to the summer. Information we collect as part of Sections 2.1 and 2.3 can be used to test these ideas directly.

Section 2.3 Assumption evaluation and alternative approaches to assess native fish habitat use and response to FSEF - Any mark-recapture approach to estimating abundance and density depends on recapturing sufficient numbers of marked individuals to draw inferences on the parameters of interest. In many fisheries mark-recapture studies, capture probabilities are low (often < 10%, Pine et al. 2003) and heterogeneity in capture probability across fish size is often observed (Pine et al. 2003; Korman et al. 2009; Coggins 2008). Closed population models generally have fewer parameters (and assumptions) than open models and are thus better able to estimate parameters of interest (capture probability and abundance) when recaptures are low. By design, some closed models can account for heterogeneity in capture probability directly (i.e., M_h type models, Otis et al. 1978) or captures and recaptures can be stratified by length group and capture probability estimated for each length stratum. These are two key reasons why closed population models have been recommended for use in fisheries studies to estimate abundance (Pine et al. 2003). One concern is the closed model assumption of no emigration from the sites of interest (i.e., habitat specific sites) both for the RDMR and RWAE approaches to estimate site specific abundance. Korman et al. 2009 successfully used closed model techniques in the Lee's Ferry reach of the Colorado River to estimate capture probabilities of juvenile rainbow trout across spatially discrete sites. These authors evaluated closure assumptions by employing a site specific marking technique and then sampling up and down-stream of their study reach to estimate emigration from each site. Emigration rates were low, ranging from

2.2-2.6%, leading these investigators to conclude that rainbow trout populations within discrete sites effectively can be considered closed. We would evaluate the closure assumption in our mark-recapture experiments using methods similar to Korman et al. 2009. Additionally, recaptures of fish marked on previous trips will provide useful information on growth and movement (e.g., movement into backwaters during periods of steady flow) between sampling trips and associated flow conditions. The NSE pilot sampling data from 2008 should provide some information on closure and also provide information on capture probability which is necessary to fully assess how violation of the closure assumption biases abundance estimates. Zehfuss et al. (1999) demonstrate declining bias in abundance estimates with increasing capture probability and emigration from the study site for Jolly-Seber and robust design models.

Section 2.3.1 Estimating site occupancy - If catches and recapture rates within trips are low, abundance estimates within sites will be highly uncertain. However, as pointed out in the RFP, in this case it will be possible to retreat from estimating abundance to estimating the probability of site occupancy (by habitat type and over time; MacKenzie et al. 2006). Site occupancy, although not as useful as direct estimates of abundance, can be used to index habitat use and changes in habitat use over time and with associated changes in flow. In all likelihood, the mark-recapture program will be able to estimate habitat-specific abundances for some species and size classes, and occupancy would be estimated for species and sizes with lower abundance and/or capture probability. Total number of site visits required would be based on trip 1 and information from ongoing GCMRC projects 3, 6, 7, 11 (Table 2).

Section 2.3.2 Incorporating telemetry information - The potential exists for radical changes in capture probability or behavioral shifts by fishes across sampling events within a season (~July-October) due to fish growth and possible ontogenetic habitat shifts such as fish moving into deeper, less accessible portions of the river. These behavioral shifts could also be caused by the designed flow experiments and associated changes in available habitat, or biotic interactions (changes in food availability or predation risk). To examine these behavioral responses, directly assess habitat use, and test capture-recapture model assumptions such as closure, a small sub-set of native and non-native fish (5-10 individuals of 3-4 key species, including humpback chubs, > 150-mm TL) will be tagged with telemetry tags and their movements, habitat use, and fates assessed directly.

Implantable, compact telemetry tags coupled with autonomous receivers (which monitor a given area continuously for extended time periods for the presence of tagged animals) and deployed as a fine scale array within a sample reach could allow the telemetered animals to be "virtually" captured by detecting their tags as they move through the environment via either the autonomous receiver array, or from boat-based receiver units when field crews were onsite. This technology is mature, and thousands of telemetered fish and autonomous receivers are currently deployed globally (Simpfendorfer et al. 2008; Hedger et al. 2008). However, the use of telemetry techniques in Grand Canyon is technologically challenging, given the large amount of ambient noise from rapids, moving rocks, and coarse sediments, all of which greatly impair the ability to detect acoustic tags. Radio tags have been used successfully in Grand Canyon to track movements of adult humpback chubs (Valdez and Hoffnagle 1999) in response to flood events, but the attenuation distance of radio tags is greatly impaired by high conductivity in areas of Grand Canyon near the Little Colorado River. Current GCMRC projects 3 and 7 have been involved in several pilot projects exploring the utility of these techniques with non-native fishes to assess movement and capture probability. Industry cooperator Marlin Gregor, President of Sonotronics Inc. (http://sonotronics.com) has been involved in the above projects and has developed specific tag and receiver design combinations to maximize the potential of this technology in the challenging conditions presented by the Colorado River in Grand Canyon. Marlin Gregor has also agreed to accompany on our first sampling trip as we test a variety of receiver and tag types. If this pilot sampling during trip 1 reveals poor performance of telemetry equipment due to high levels of ambient noise in Grand Canyon, we will abandon the telemetry component of this project. We will work with cooperators to then identify alternative research efforts such as experiments to assess DNR:RNA ratios as a measure of short-term growth as recommended by NSE review panel.

The incorporation of telemetry information with the mark-recapture sampling is experimentally risky, but has huge potential to boost our ability to draw inferences about juvenile fish abundance and habitat use. Pollock et al. (2004) showed an example of using both tag types to improve estimation of fishing and natural mortality rates using a complementary, multinomial approach to the tagging component of the stock synthesis model used in Korman et al. in-press and this proposal. We have extensive previous experience with these types of telemetry systems and similar applications (Bennett 2005; Marcinkiewicz 2007; Tetzlaff 2008; Burgess 2008). Coupled with ongoing GCMRC projects 3 and 7, and industry cooperator commitment, it is likely that technological hurdles could be overcome. Exact tag types, receiver deployment strategies, numbers of tags and receivers required would be determined based on in-situ range testing of tag and receiver combinations. An array network would not guarantee perfect, continuous detection because behaviors of individual animals such as occupying habitat types similar to interstitial spaces between rocks, would attenuate the signal from the sonic tags. Conceptually, an autonomous receiver array could be deployed during a July sampling trip (first trip of sampling season) and a small batch of fish tagged and released with telemetry tags. The receivers would then remain deployed, monitoring these fish until the next sampling trip, at which time the data would be retrieved and an additional batch of fish tagged. Similar procedures would be followed for each additional trip, until the last trip of the year when no additional fish would be tagged and the receivers would be removed from the study site. Multiple batches of fish would be used (a “staggered entry” design, Pollock et al. 1989) because of limitations in tag battery life. The staggered entry design would allow specific size cohorts of fish to be tracked from the first through the fourth study period coincident with the same flow transition periods discussed in Section 2.0.

Section 2.3.3 Determining origin of recruits – The FSFE will likely almost immediately create thermally favorable microhabitats for juvenile HBC and other native fish (RFP, page 27) which motivates Question 6 of the RFP (page 27), “*Are replicated flows September-October steady flows (2008 through 2012) likely to improve the survival and recruitment of young humpback chub in the Colorado River ecosystem?”* Additionally, while management actions such as FSFE are certainly likely to modify and create habitat types, some of which may be favorable to juvenile HBC, where do the juvenile HBC come from to colonize these habitats and if they colonize these habitats, how well do they survive? As outlined in Sections 2-2.2, we describe approaches to assess survival of young HBC (and other species) in response to the FSFE. However, recruitment of young HBC and other juvenile fish from the LCR to the mainstem study site during July-September (Conceptual Model point A) could confound information on patterns in juvenile fish density in different habitats and their survival, unrelated to flow events. For example, in the absence of any direct measure of age-0 fish recruitment from the LCR to the mainstem population, observed patterns in density, growth, and survival of juvenile fish from our mark-recapture study could be attributed to the flow experiment, when, in fact, these density changes were actually independent of the experiment, and instead related to natural emigration from the LCR to the mainstem. Without an understanding of this recruitment from the LCR to the mainstem, the estimated growth, survival, and movement among habitat types in the mainstem from the tagging data (Sections 2-2.2) could be confounded with new recruits.

We propose to use otolith microchemistry to determine the origin of juvenile fish (mainstem vs. LCR), most likely only HBC because of cost concerns, utilizing available habitats across the different flow regimes from July-October. As pointed out in the RFP (page 27) there are at least two potential sources of juvenile HBC that use habitats available during MLFF and migrate to habitats created by FSFE: (1) LCR spawned juveniles that enter the mainstem river in May-June outmigration and (2) LCR spawned juveniles that remain in the LCR until they enter the mainstem following July-September freshets. A third possible source of HBC juveniles would be mainstem spawned individuals who could also be identified through otolith microchemistry. This information will be used to compliment and strengthen inference from estimates of habitat specific patterns in abundance, movement patterns of telemetered fish, and fish size distributions (Sections 2.1-2.3).

Otoliths are paired structures that form part of the inner ear of teleost fish and are commonly used by fisheries researchers to estimate fish age, and increasingly, their chemical constituents are used to determine fish movements (Campana 2005). Otoliths are composed of biogenic calcium carbonate that accretes new crystalline and protein material daily. Within these accumulating layers, trace elements are incorporated into the otolith from the fishes environment, creating a temporal sequence of accumulated elements. This represents a chronology of the environment the fish has occupied over its life (Elsdon et al. 2008). Within the otolith, the different accumulated elements and chemical signatures can be used as a natural tag. These tags can then be linked to groups of fish that have similar chemical signatures to show associations of fish with their environment in both time and space (reviewed in Elsdon et al. 2008). Chemical signatures in an otolith can be examined, for example across a transect, to assess patterns in chemical profiles at different ages of the fish. When these chemical signatures are paired with information on the chemical profiles of the water in the spatial locations where the fish could have lived, movement patterns of individuals and groups of fish can be determined. A key assumption is that the chemical signatures from the different environments are separable, and that these signatures can be matched on the correct temporal scale. To test these assumptions, during 2009 we will test our proposed methods on flannelmouth sucker (*Catostomus latipinnis*) a more common native species thought to have similar life history characteristics as humpback chub. Samples of flannel mouth sucker are also available from the 2008 NSE pilot sampling (M. Yard, USGS-GCMRC, personal communication). We will attempt to analyze at least a sample of these otoliths from 2008 to inform 2009 field collection efforts. Additionally, we will also examine a sample of previously collected adult humpback chubs used in earlier analyses (Hendrickson 1997).

Recent advances in otolith microchemistry will enable us to assay for a suite of major, minor, and trace elements through the use of laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS; Figure 1). SUNY-ESF has just acquired a state-of-the-art laser ablation system (New Wave Research UP-193 nm solid state) and has connected it to an also new Perkin-Elmer DRC-e ICPMS. This setup, with analytical resolution to low ppb and spatial resolution to approx. 10 microns, will be used to collect the bulk elemental data, using one of each pair of lapillar otoliths. Other data, potentially including strontium and other stable isotopic ratios will be taken on the corresponding otolith at a partner institution such as University of Arizona Lunar and

Planetary Science Laboratory or the Department of Nuclear Physics at Lund University in Sweden (where KL has ongoing collaborations since 1997). In addition, we will explore the utility of trace elemental mapping by means of synchrotron-based X-ray fluorescence (S-XRF) at the Cornell High Energy Synchrotron Source (CHESS). The S-XRF will allow us to explore which elements might be of interest to trace using the LA-ICPMS. This approach of using the S-XRF to first map multiple trace elements in otoliths and the coupling of this information with high-resolution LA-ICPMS work has provided new insights into the environmental history of a variety of fish (marine and freshwater) and their lifetime movement patterns (see Limburg et al. 2007). Given that run times per otolith are long with S-XRF, and time available is limited at the CHESS facility, we anticipate that bulk of the work will be accomplished using LA-ICPMS once multiple otoliths have been mapped at CHESS. In addition to addressing questions related to the nearshore ecology of Grand Canyon fishes proposed here, there are obviously additional research questions that these approaches could be applied to using otoliths from existing collections. For example, older fish from archival collections (HBC otoliths used in Hendrickson 1997) are prime candidates for S-XRF mapping coupled with LA-ICPMS analysis to assess movement patterns and provide alternative age assessments of larger, older, fish collected at earlier time periods during different river conditions. If suitable archived samples were available, such as from HBC spawned prior to the Glen Canyon Dam, it would be very interesting to assess the life time movement patterns (spawning, rearing, and migratory) or pre- vs. post-dam HBC or other native fish.

As an example of this approach Limburg (1995, 1998, 2001) used ratios of strontium (Sr) relative to calcium (Ca) from American shad (*Alosa sapidissima)* otoliths along a transect in the Hudson River, linked with otolith derived age estimates to identify the age and size when American shad were transitioning from the Hudson River to the Atlantic ocean. Limburg (2001) compared Sr:Ca ratios and size and age structure in young-of-year American shad with the Sr:Ca ratios of adult fish from these same cohorts migrating back to spawn in the Hudson River in subsequent years. From these comparisons Limburg (2001) found that in three of the four year classes analyzed, differential mortality had occurred in juveniles, and was related to time of emigration from the Hudson River. A similar approach with humpback chubs over multiple years, coupled with Sections 2-2.2 of this proposal, could help us to identify the linkages between the timing of out migrating juveniles from the LCR, available rearing habitat in the mainstem as determined by flow conditions, and the interaction between available habitat and juvenile fish survival.

Section 2.3.4 Determining growth and survival using otoliths - Measurements of fish growth integrates information from a variety of environmental (e.g., temperature) and biotic (e.g., food availability) sources into a useful single metric. Growth can be used, for example, as a response metric to a variety of management options (DeVries and Frie 1996) and also used to provide insight into survival patterns (Lorenzen 1996). For larval and juvenile fish, growth information is commonly measured by analyzing size frequency distributions or using fish hard parts such as otoliths (DeVries and Frie 1996). The use of otoliths to determine juvenile fish growth has been used to address a variety of questions for Colorado River native (Bestgen et al. 2006) and nonnative fish (Korman and Campana 2009). For example, Korman and Campana (2009) documented a strong relationship between fluctuating flows, nearshore habitat use, and rainbow trout otolith growth rates in the Lee’s Ferry reach of the Colorado River. As proposed in section 2.2, we hypothesize that fish growth may change as a function of shifts in habitat utilization driven by flow related changes in habitat availability – thus differences in growth may be evident between MLFF, MLFF-FESF transition, and FESF period.

We propose to assess growth in three ways: (1) by tracking modal progression of native and non-native fish size in during each sampling trip, (2) if unique VIE marks are possible through recaptures of marked fish, and (3) direct estimation using otoliths collected for the microchemistry analyses of HBC described above. The first approach is straight forward and simply requires plotting the size frequency of individuals from each species and sample trip as a function of length and identifying distinct peaks in the distribution. These peaks are then followed in subsequent trips and growth estimated by tracking the progression in the modal peaks of each size frequency distribution. This approach generally works best if sample sizes for each cohort are large and growth is fairly uniform among cohorts such that the size modes for each cohort can be distinguished. If sample sizes are small or growth within a cohort is inconsistent, then cohort modes become indistinguishable in the length frequency distribution and estimating growth is not possible. This approach is low cost, non-lethal, and would be used to estimate growth for all possible fish species.

The second approach is dependent on being able to mark fish for the capture-recapture experiment using unique marks. The VIE marks we propose to use come are available in a variety of colors and there are numerous body locations in which fish can potentially be marked. However, it is not until live fish are examined and the color patterns and marking locations assessed to determine whether VIE colors can contrast sufficiently with natural fish color patterns to offer reliable marking locations. We acknowledge the difficulty in creating a unique marking scheme and the potential bias induced in the analyses from mistakes made in the marking program thus necessitating careful consideration to the design of the fish marking program.

The third approach is based on the standard technique of visually counting daily growth rings on otoliths to estimate age (Pannella 1971; Stevenson and Campana 1992; Pine and Allen 2001). Following this approach, the second HBC otolith from whichever otolith pair is used for the microchemistry analyses (most likely the lapillar pair; Hendrickson 1997) would be mounted individually on microscope slide, polished to the mid-plane following standard otolith preparation procedures, examined at 400-1250x using a compound microscope, and then the daily increment marks on the otoliths from the origin to the edge counted 2-4 times. Daily otolith increments have not been validated in humpback chubs, but preliminary work by Hendrickson (1997) provides strong circumstantial evidence to suggest that the lapillar increments do form with daily periodicity. If recapture rates between trips are sufficiently high as evident from trips 1 and 2 in field season 1, a validation study could be designed by immersing juvenile HBC in an alizarin solution to “mark” the otolith, then releasing the fish with the expectation to recapture that fish on a future trip. Recaptured fish would then be sacrificed, otoliths removed and prepared as described in section 2.3.4, and the otolith rings since the immersion in alizarin counted and compared to the actual days elapsed (Devries and Frie 1996). Average daily growth rates can then be determined from dividing the fish TL when captured by the age.

Recent juvenile HBC growth over short time periods, such as just prior to and during the transition from MLFF to FSFE and during the FSFE, could be estimated by measuring otolith growth increments from the nucleus to a distance along the growth axis that would correspond to a point in time (and the fish's life) during MLFF, and then measure from this point to a second point that would represent the time period including the MLFF-FSEF transition, and then a third measurement from the MLFF period to a time period near the end of the FSEF. The approach outlined would obviously be dependent on collecting juvenile HBC near the end of the FSEF such that the growth transition could be measured across each of the three flow periods described. Obviously these candidate fish would have to survive any changes created by the flow events – and it would be impossible to collect growth information on any fish that died during the flow events. Alternatively, juvenile HBC could be sampled on each of the four sampling events and incremental growth measured from these fish using methods described above. However, given the short time period between the flow treatment and the timing of the sampling trip, contrasting growth information may be not detectable along the post-rostral edge of the otolith because not enough time may have elapsed for sufficient otolith deposition along the edge to see or measure. This growth information would be linked to the otolith microchemistry results related to natal origin of HBC. For example, this linkage could be useful in assessing growth patterns of HBC that were early vs. late season emigrants from the LCR to mainstem Colorado River or growth patterns of mainstem origin juvenile fish.

A major drawback of using otoliths to estimate age and growth is that the fish must be sacrificed to extract the otoliths. Given the listing status of most native fish in Grand Canyon, including HBC, we share concerns that any research associated mortality could be deleterious to the population overall, and we acknowledge that permits to collect and sacrifice these fish may be difficult to receive. Recruitment trends for humpback chubs and other native fish have been increasing over the last 4-5 years (ongoing GCMRC projects 5, 6, Table 2; Coggins 2008) reducing the potential for population impact from removing a small number of native fish from the population for research needs. Exact numbers of fish required would be determined by examining otoliths from juvenile humpback chubs incidentally taken as part of routine sampling in prior research efforts (i.e., Near Shore Ecology pilot sampling in August and September 2008 and backwater sampling efforts in summer 2005). We propose to collect a small number of juvenile fish, most likely 5-10 age-0, age-1, age-2, and age-3 humpback chubs and also flannel mouth suckers each trip (no more than 50 fish per age class and species per year). In addition to providing information from the microchemistry and age-growth analyses in response to the FSEF, there are at least two other major areas that could be assessed by using tissues from these animals. First, age information on these small fish would be very useful in informing the age-length key used to assign age-at-first capture in the standard age-structured mark-recapture program (ASMR, Coggins et al. 2006a; GCMRC projects 4 and 6, Table 2) used to assess trends in HBC growth, recruitment, and survival (Coggins et al. 2006b; Coggins 2008). This age-length key is currently based on a limited set of approximately 60 estimates of HBC length-age (Coggins 2008) and this small growth sample, particularly for very small and young fish, could be improved with additional growth information possibly leading to reduced bias in the recruitment estimates from the ASMR model. Additionally, the previous growth data were not collected during the recent time period of warmer water temperatures, which may have led to increased HBC growth. Thus, the currently used growth information may be negatively biased due to slow growth of humpback chubs in cold-water conditions vs. the current warmer-water conditions.

Fin rays also offer an alternative approach for aging fish to otoliths with the major advantage of not having to sacrifice the fish (DeVries and Frie 1996). Using a sub-set of fish sacrificed for otolith analyses, we will conduct an exploratory analysis to assess the feasibility of the same age and microchemistry assessment on the fin ray sections as on the otoliths. This will provide information on the possibly utility of using fin rays as a non-lethal technique to determine age of young fish to help inform the HBC stock assessment and monitoring program (ongoing GCMRC projects 3 and 4). If successful, and necessary, this approach could provide information useful to assessing recruitment responses of HBC to planned and unplanned experiments at younger ages (and less time lag) than the current 3-4 year response time required before fish recruit to the tagging program and recruitment trends assessed as part of ASMR.

A final alternative for both the microchemistry and otolith based age and growth estimation would be to use a surrogate species for humpback chubs that was thought to have a similar life history. Alternative species to humpback chubs could include native fish such as bluehead suckers *Catostomus discobolus*, flannelmouth *Catostomus latipinnis* suckers, speckled dace *Rhinichthys osculus* or nonnative species such as fathead minnows *Pimephales promelas*.

Section 3.1 Contingency plans to alternate flow scenarios, expanded spatial replication, or inability to secure variance during no-motor season – The proposed sampling framework and experimental design (i.e., timing of trips) can easily be modified to address alternate flow-related hypotheses. For example, question (9) in the RFP (page 28), "*What happens to juveniles that used the warmed areas when fluctuating flows are resumed?*", could be addressed by adding one or two trips (and additional costs) to the proposed four-trip schedule. In this case, a trip following the resumption of fluctuating flows, (mid-November) and an additional trip one month later, would quantify immediate changes in habitat use as well as survival rates under fluctuating flows during early winter. A similar strategy, where sampling is conducted before and after a beach habitat building flow, could be used to evaluate the effects of flows above power plant capacity or normal daily maxima (item 6, RFP page 31). Additional spatial sampling, at other known HBC aggregation sites ("Randy's Rock", RM 126; "30-mile", RM 29-32) could also be implemented based on results from Section 2 above. The key point is that our approach will provide estimates of habitat use, survival, growth, and origin of juvenile fish during summer and fall, and that it is relatively easy to expand sampling into other times of year to look at other flow-related hypotheses. That said, the objectives and research questions that are the focus of the RFP are very challenging. Our philosophy is to do a few things well, rather than many things poorly. Given available resources, we will focus on population dynamics during summer and fall only as outlined above.

We expect that some elements of the proposed sampling framework would become part of the GCD AMP's long-term monitoring protocol. For example, methods developed in this project could be used to estimate juvenile HBC abundance in the mainstem during late summer and late fall. Over many years, these estimates could be related to recruitment estimated from the Age-Structure Mark-Recapture (ASMR) model (Coggins et al. 2006 a, b). Ultimately, a stock-recruitment relationship between age-0 chub in the mainstem during both fall and summer, and recruitment determined from the ASMR model, would be very helpful in the interpretation of the juvenile data. For example, if there is strong density dependence in survival between summer and fall, and little density dependence for later life stages, we would expect an asymptotic relationship (e.g., Beverton-Holt shaped relationship) between summer and fall juvenile abundance, and between summer juvenile abundance and ASMR-based recruitment estimates. In this case, there would also be a strong linear relationship between fall juvenile abundance and ASMR recruitment. Such analyses are very relevant to current policy debates. For example, strong density dependence between summer and fall would indicate that it is unlikely that improving habitat conditions during the summer will result in substantive changes in juvenile abundance during the fall, and consequently to improvements in recruitment to the adult population. It will take many years to establish reliable relationships (which will likely be noisy), but we see no alternative for the GCD AMP. Ultimately, life-stage specific stock-recruitment functions need to be established to fully interpret results from the proposed study in the context of GCD AMP management objective for adult HBC.

Depending on the start of the FSFE, it is likely that one or two of our proposed sampling trips would launch after the start of the no-motor season (September 15). In this case, we would require a variance from the Park Service for operating motorized equipment (boat motors and generators) required for our electrofishing sampling. While we intend to use multiple gear types (most likely electrofishing and hoopnets) during each of our sampling events, we have high expectations for successful electrofishing sampling for use in mark-recapture (Section 2.1) based on results from Korman et al. (2009) and fall 2008 Near Shore Ecology pilot sampling. Failure to secure a variance allowing us to use electrofishing would increase our dependence on hoopnet sampling to track patterns in habitat specific abundances, estimate survival, and track growth via changes in length frequency. Gear selectivity patterns between electrofishing and hoopnets likely differ, and by deploying both gears on each sampling trip we intend to minimize the confounding that can occur in the data due to changes in gear between trips and associated selectivity patterns (i.e., responses could be due to the flow experiment or simply to changes in the gear type). Compromises to help meet the Park Service's no-motor requirements are possible. One option would be to create a stash of 1-2 electrofishing boats in the study reach just prior to the end of no-motor season. Any trips that occurred during the no-motor season would then be supported by rowing trips out to the sampling sites, where electrofishing boats would be used to complete the required sampling effort. At the end of sampling, these boats would be floated or flown out of Grand Canyon in cooperation with other sampling efforts with USFWS and AZGF. Additional options would be to increase the reliance on telemetered fish and to follow the movements and habitat use of telemetered fish using rafts and kayaks during no motor season. A final option would be to not grant the variance; thus, electrofishing would not be possible. Our proposed closed population models (both mark-recapture and occupancy) estimates capture probability with each sampling trip, so if hoopnets were the only sampling gear available during the last trip, then our capture probabilities would be based on marks and recaptures from hoopnet gear only. Hoopnet catches alone would likely result in very low capture probabilities, limiting the use of some mark-recapture models. However, habitat occupancy using methods outlined in section 2.3.1 may be used to estimate occupancy even with low capture probabilities. If no variance is granted, extensive comparisons between electrofishing and hoopnet catches based on pilot sampling during motor season and previous fish sampling efforts in Grand Canyon would be done to develop a better understanding of the selective properties of each gear. The style of electrofishing developed and used by Korman et al. (2009) was first used in the Lee's Ferry reach, but was also used as part of the pilot NSE sampling during fall 2008 thus limiting the number of comparisons that can be made between gear types using historical data.

Section 4 Conclusions - Certain aspects of the proposed experimental design are beyond our control, and will limit the inferences from the resulting data. We propose to compare juvenile habitat use, survival, and growth during summer-unsteady and fall-steady flow periods to evaluate the potential benefits of steady flows. However, such an assessment assumes there is no seasonality in the parameters of interest, which we know is not likely to be the case. For example, growth rates will likely decline as fish increase in size, and as water temperature declines due to reduced solar irradiance. Habitat use may also have a flow-independent component that depends on fish size. Ultimately, the proposed work, or key elements of the proposed work, will need to be repeated under an alternate flow regime. The two logical alternatives are to extend the period of steady flows (e.g. July-Oct), or to revert to unsteady flows (MLFFA or greater fluctuations) during the fall. Results from the proposed work will be helpful in choosing among these alternatives because: (a) we will have a much better sense of our ability to detect changes in juvenile survival rates and other life history characteristics; and (b) expected and serendipitous insights from the proposed work will provide for more refined hypotheses and a more fully articulated conceptual model.

References

Bailey, L. L., T. R. Simons, and K. H. Pollock.(2004). Estimating detectability parameters for plethodon salamanders using the robust capture-recapture design. Journal of Wildlife Management 68: 1-13.

Bennett, J. P. 2006. Using acoustic telemetry to estimate natural and fishing mortality of common snook in Sarasota Bay, Florida. Master's Thesis, Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, Florida.

Bestgen, K. R., D. W. Beyers, J. A. Rice, and G. B. Haines. 2006. Factors affecting recruitment of young Colorado pikeminnow: Synthesis of predation experiments, field studies, and individual-based modelling. 2006. Transactions of the American Fisheries Society 135:1722-1742.

Brennan, N. P., K. M. Leber, H. L. Blankenship, J. M. Ransier, and R. DeBruler, Jr.. 2005. An evaluation of coded-wire and elastomer tag performance in juvenile common snook under field and laboratory conditions. North American Journal of Fisheries Management 25:437-445.

Burgess, O. T. 2008. Importance of floodplain connectivity to fish populations in the Apalachicola River, Florida. Master's Thesis, Department of Fisheries and Aquatic Sciences, University of Florida.

Campana, S. E. 2005. Otolith science entering the 21st century. Marine and Freshwater Research 56:485-495.

Coggins, L. G., Jr. 2008. Active adaptive management for native fish conservation in the Grand Canyon: Implementation and evaluation. Doctoral Dissertation. University of Florida, Gainesville, Florida.

Coggins, L. G., Jr., W. E. Pine, III, C. J. Walters, and S. J. D. Martell. 2006a. Age Structured Mark Recapture Analysis (ASMR): A VPA Based Model for Analyzing Age Structured Capture-Recapture Data. North American Journal of Fisheries Management 26:201-205.

Coggins, L. G.,Jr., W. E. Pine, III, C. J. Walters, D. R. Van Haverbeke, D. Ward, and L. Johnstone. 2006b. Abundance trends and status of the Little Colorado River population of Humpback Chub *Gila cypha*. North American Journal of Fisheries Management 26:233-245.

DeVries, D. R. and R. V. Frie. 1996. Determination of age and growth. Pages 483-512 in B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Elsdon, T. S., B. K. Wells, S. E. Campana, B. M. Gillanders, C. M. Jones, K. E. Limburg, D. H. Secor, S. R. Thorrold, and B. D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations, and inferences. Oceanography and Marine Biology: an Annual Review 46: 297-330.

Hedger, R. D., F. Martin, J. J. Dodson, D. Hatin, F. Caron, and F. G. Whoriskey. 2008. The optimized interpolation of fish positions and speeds in an array of fixed acoustic receivers. ICES Journal of Marine Science 65:1248-1259.

Hendrickson, D. A. 1997. A preliminary study of utility of data obtainable from otliths to management of humpback chub in the Grand Canyon. Final Project Report to Arizona Game and Fish Department KR92-00310-LNR G20019-A

Korman, J. 2009. Early life history dynamics of rainbow trout in a large regulated river. PhD dissertation. University of British Columbia, Department of Zoology.

Korman, J., Wiele, S.M., and M. Torizzo. 2003. Modelling effects of discharge on habitat quality and dispersal of juvenile humpback chub (*Gila cypha*) in the Colorado River, Grand Canyon. Riverine Research and Applications 12: 1-23.

Korman, J., and S.E. Campana. 2009. Effects of hydropeaking on nearshore habitat use and growth of age-0 rainbow trout in a large regulated river. Transactions of the American Fisheries Society. 138:76-87.

Korman, J., Yard, M., Walters, C., and L.G. Coggins. 2009. Effects of fish size, habitat, flow, and density on capture probabilities of age-0 rainbow trout estimated from electrofishing at discrete sites in a large river. Transactions of the American Fisheries Society. 128:58-75.

Limburg, K. E. 1995. Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. Marine Ecology Progress Series 119:25-35.

Limburg, K. E. 1998. Anomalous migrations of anadromous herrings revealed with natural tracers. Canadian Journal of Fisheries and Aquatic Sciences 5:431-437.

Limburg, K. E. 2001. Through the gauntlet again: Demographic restructuring of American shad by migration. Ecology 82:1584-1596.

Limburg, K. E., R. Huang, and D. H. Bilderback. 2007. Fish otolith trace element maps: new approaches with synchrotron microbeam x-ray fluorescence. X-Ray Spectrometry 36:336-342.

Lorenzen, K. 1996. The relationship between body weight and natural mortality in fish: a comparison of natural ecosystems and aquaculture. Journal of Fish Biology 49:627-647.

MacKenzie, D. I., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey, L. L., and Hines, J. E. 2006. Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence. Elsevier. San Diego, California.

Marcinkiewicz, L. L. 2007. Examining seasonal movement and habitat use patterns of adult common snook. Master's Thesis, Department of Fisheries and Aquatic Sciences, University of Florida.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed populations. Wildlife Monograph 62.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodic patterns. Science 173:1124-1127.

Pine, W. E., III and M. S. Allen. 2001. Differential growth and survival of weekly age-0 black crappie cohorts in a Florida lake. Transactions of the American Fisheries Society. 130:80-91.

Pine, W. E., III, K. H. Pollock, J. E. Hightower, T. J. Kwak, J. A. Rice. 2003. A review of tagging methods for estimating fish population size and components of mortality. Fisheries 28:10-23.

Pollock, K. H. 1982. A capture-recapture design robust to unequal probability of capture. Journal of Wildlife Management 46:757-760.

Pollock, K. H. S. R. Winterstein C. M. Bunck, P. D. Curtis. 1989. Survival analysis in telemetry studies: the staggered entry design. Journal of Wildlife Management 53:7-15.

Pollock K. H., J. D. Nichols, C. Brownie, and J. E. Hines. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs 107:1-97.

Pollock, K. H., H. Jiang, H. and J. E. Hightower. 2004. Combining radio-telemetry and fisheries tagging models to estimate fishing and natural mortality rates. Transactions of the American Fisheries Society, 133:639-648.

Simpfendorfer, C. A., M. R. Heupel, and A. B Collins. 2008. Variation in the performance of acoustic receivers and its implication for positioning algorithms in a riverine setting. Canadian Journal of Fisheries and Aquatic Sciences 65:482-492.

Stevenson, D.K. and Campana, S.E. (Editors). 1992. Otolith microstructure examination and analysis. Canadian Special Publication of Fisheries and Aquatic Sciences 117.

Tetzlaff, J. C. 2008. Energetic consequences of habitat loss: Trade-offs in energy acquisition and energy expenditure by *Micropterus salmoides*. Master's Thesis, Department of Fisheries and Aquatic Sciences, University of Florida.

Valdez, R.A., and T.L. Hoffnagle. 1999. Movement, habitat use, and diet of adult humpback chub. Pages 297-307 in R.H. Webb, J.C. Schmidt, G.R. Marzolf, and R.A. Valdez eds.,The controlled flood in Grand Canyon. Geophysical Monograph 110. American Geophysical Union, San Francisco, California.

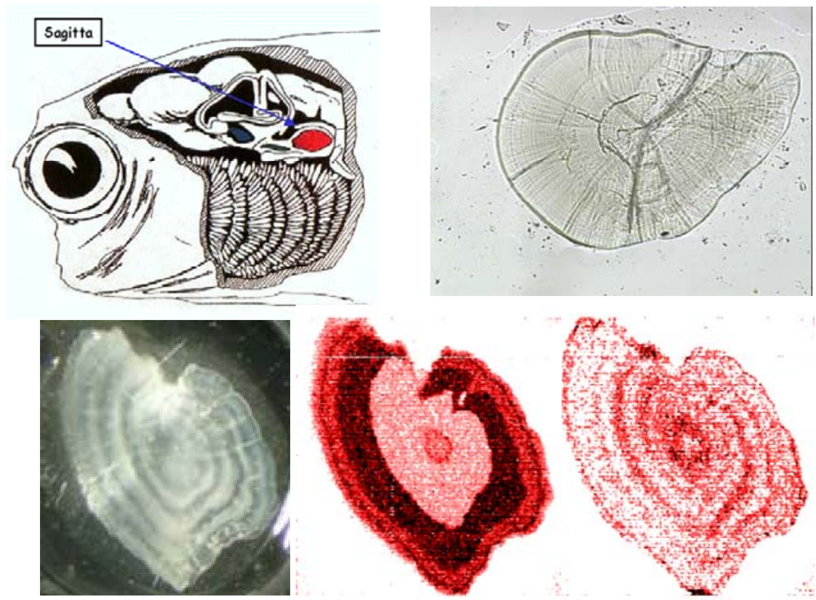
Walters, C., J. Korman, L. E. Stevens, and B. Gold. 2000. Ecosystem modelling for evaluation of adaptive management policies in the Grand Canyon. Conservation Ecology 4: 1.

Table 1. Proposed sampling schedule and key tasks for Near Shore Ecology of Grand Canyon Fish project. MLFF = Mean Low Fluctuating Flows, FSEF = Fall Steady Experimental Flows.

Year	Activity	Month	Trip Number	Likely river flow	Key tasks or project milestones
2009-2011	Field sampling	Late July	1	MLFF	Mark-recapture, deploy receiver array, implant telemetry tags, manual tracking of telemetered fish, collect fish for otolith analyses
2009-2011	Field sampling	Late August	2	MLFF	Mark-recapture, implant telemetry tags, manual tracking of telemetered fish, collect fish for otolith analyses
2009-2011	Field sampling	Early September	3	MLFF-FSEF	Mark-recapture, implant telemetry tags, manual tracking of telemetered fish, collect fish for otolith analyses, possible otolith validation experiment
2009-2011	Field sampling	Late October	4	FSEF	Mark-recapture, retrieve receiver array, implant telemetry tags, manual tracking of telemetered fish, collect fish for otolith analyses, possible otolith validation experiment
2009-2011	Sample processing	October - March			Estimate growth from size distributions, estimate abundance and survival from mark recapture, assess movement and habitat use from telemetry information, and conduct otolith analyses
2009-2011	Annual Report	April			Present annual project updates, submit reports, meet with all cooperators to revise sampling plans for upcoming field season
2011-2012	Analyze data and write report	May 2011-Aug 2012			2012 will include additional isotopic analyses of otoliths and funds for mapping chemical signature of water
2012	Final Report	September			Submit final report and presentation to GCMRC staff

Table 2. Existing monitoring and research studies in Grand Canyon as listed in the RFP. Numbers in this table correspond to referenced cooperative and complimentary research described in this proposal.

Project Number	Project title
1	Monitoring of biological and physical aspects of backwater habitats
2	Integrated analyses and modeling: mapping shoreline habitat changes
3	Monitoring mainstem fishes
4	Stock assessment of native fish in Grand Canyon
5	Investigate factors affecting the survival rate of juvenile native fishes in the mainstem Colorado River
6	Native fishes habitat data analysis
7	Nonnative control planning and pilot testing
8	Status and trends of Lees Ferry trout
9	Monitoring rainbow trout redds and larvae
10	Thermal modeling of near shore habitat
11	Aquatic food base program



Optical image Sr map Zn map

Fig. 1. Fish otoliths are located in the semi-circular canals underneath the brain (top left); daily growth increments seen in 0.5 mm otolith of young fish (top right); annual rings (bottom left) can be used to age adult fish, and elements such as strontium are deposited in proportion to ambient concentrations (bottom, middle) whereas zinc (bottom right), in this case, is deposited in a seasonal pattern. The analyses shown in the bottom two panels were conducted with a nuclear microprobe. This kind of analysis will continue to complement the work we are proposing to do with LA-ICPMS.