

1 Title: Near shore ecology of Grand Canyon fish

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

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

41

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

44

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

48

49 We propose to address these research questions and link results to a proposed  
50 conceptual model for humpback chub *Gila cypha* (HBC) and other native and non-  
51 native fish (below) by first describing an approach to assess shifts in fish density by  
52 tracking habitat specific abundance and survival of native and non-native fish in  
53 response to changing nearshore habitat availability related to and created by the fall  
54 steady flow experiment. We then detail an approach to determine the source  
55 populations of juvenile native fish that populate nearshore habitats created by the  
56 proposed Fall Steady Experimental Flow (FSEF). Our intent is to link this new insight  
57 into juvenile fish ecology, with a focus on humpback chub, with the flow and habitat

58 management capabilities of the Glen Canyon Dam (GCD) Adaptive Management  
59 Program (AMP), to create a better understanding of how flow and habitat management  
60 can be used to cultivate and enhance survival of juvenile native fish and, with time, adult  
61 native fish populations in Grand Canyon.

62

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

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

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

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

83 *D. Abundance of HBC juveniles will increase with improvements in the quality and*  
84 *quantity of habitat because survival rates will be higher. Over the long-term,*  
85 *greater juvenile production will increase the abundance of the adult population.*  
86

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

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

102  
103 *Section 2.1 Sampling methods to estimate fish habitat use, growth, and survival –*  
104 Estimation of juvenile abundance, survival rate, growth rate, and habitat use is  
105 fundamental to resolving uncertainties in the conceptual model and the two key  
106 research questions outlined and identified above. We propose sampling trips in late  
107 July and late August to characterize abundance, habitat use, growth, and survival rate  
108 of juvenile fish over the summer under MLFF flow fluctuations. These trips would be  
109 followed by sampling trips in early September and late October to characterize juvenile  
110 fish responses during the MLFF-FSEF transition, and FSEF period. Differences in  
111 abundance in each habitat type, between each sampling trips would be used to  
112 estimate habitat specific, reach-wide survival rates across flow events.

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

- 119
- 120 (1) Select a relatively large study reach that contains multiple habitat types (e.g.,  
121 Kwagunt rapid [River Mile 56] to Lava Chuar rapid [River Mile 65.5]) and stratify  
122 this reach into sites by habitat type (informed by, and integrated with, ongoing  
123 GCMRC project 2, Table 2);
  - 124  
125 (2) Sample fish at a large number of randomly selected sites within the reach  
126 using methods established in previous GCMRC efforts and Korman 2009 (e.g.,

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

132  
133 (3) Quantify capture probabilities by habitat/gear type, species, and fish size  
134 using short-term, closed, mark-recapture experiments (number of sampling  
135 events per trip  $\geq 2$  depending on recapture rate and number of sites from 2  
136 above, e.g., Korman et al. 2009 [see Appendix]) at a smaller number of sites;

137  
138 (4) Convert catch-rates at index sites (from 1 above) to population estimates  
139 based on capture probabilities estimated from (3 above); and

140  
141 (5) Scale up site-specific estimates of population size to a reach-wide estimate  
142 based on the amount of habitat of each type in the reach.

143  
144 Population size by sampling trip would be estimated for each habitat type and will  
145 be used to examine seasonal and flow-driven changes in habitat use. Fish would most  
146 likely be marked with a gear, habitat, and sampling pass specific mark using unique  
147 location and color combinations of Visual-Implant-Elastomer (VIE) (Brennan et al. 2005;  
148 GCMRC NSE pilot sampling, fall 2008). Ideally, unique marks would be applied to each  
149 animal (Bailey et al. 2004 for an example with VIE marks). Growth during summer-  
150 unsteady and fall-steady periods could be quantified based on differences in length-  
151 frequency distributions over time, as well as by measurements of otolith increments on  
152 a small sub-sample of fish (as in Korman and Campana 2009 [see Appendix]; see also  
153 section 2.2.3 and 2.2.4 of this proposal). As in Korman (2009), we would integrate most  
154 information from the proposed program in a stock synthesis model to jointly estimate  
155 parameters of interest (including abundance and survival rate by habitat type, growth,  
156 recruitment to the juvenile population over the growing season) and to identify shifts in  
157 habitat use. This approach is based on standard closed population mark-recapture  
158 approaches to estimate abundance and capture probability (analogous to those outlined  
159 in Otis et al. (1978)) and allows for incorporation of size dependence in capture  
160 probability (to account for heterogeneity in capture probability by size). The critical  
161 assumption of the RWAE approach (and all closed population models) is that the  
162 population can be treated as effectively closed within trips and that differences in

163 abundance across trips are caused by recruitment (LCR and mainstem) and mortality  
164 only. The approach is unable to separate emigration from mortality. Radio telemetry  
165 data, and movement rates of marked fish within sites across trips would help evaluate  
166 the extent of potential emigration from study sites (see section 2.3.2 of methods).  
167 Immigration would be estimated. Differences in abundance and distribution among  
168 habitat types across trips would be used to estimate survival and changes in habitat use  
169 during changing flow conditions from summer MLFF, the MLFF-FSEF transition period,  
170 and FSEF conditions. If significant changes in abundance are observed, an additional  
171 sampling trip, if supplemental funds were available, following the return to normal flow  
172 operations in November could be added to assess fish population responses to the  
173 transition from experimental steady flows to winter operation flows. Modal shifts in  
174 length-frequency and direct ageing would be used to evaluate growth.

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

- 181 (1) Select a series of study sites within a broader reach (e.g., three to four 500-m  
182 sites between Kwagunt and Lava Chuar rapids). These sites would likely each  
183 contain a mix of all or most of the habitat types within the broader reach.
- 184
- 185 (2) Conduct multi-pass (3-5 pass events) mark-recapture assays in each of the  
186 study sites during each of the four trips.
- 187
- 188 (3) Estimate abundance at each study site on each trip.
- 189

190 As with the RWAE approach, we would employ a marking strategy to estimate  
191 abundance within habitat types at each site for each trip. Multiple gear types would  
192 likely be needed to sample the full range of habitat types and water depths. Under the  
193 RDMR approach, we would assume that populations within each site are effectively  
194 closed within trips, but not among trips. We would also assume that changes in  
195 abundance at the sites across trips represent changes at a reach-wide scale.  
196 Differences in abundance and distribution in each habitat type across trips will be

197 assessed during summer-MLFF, MLFF-FSEF flow transition, and FSEF periods.  
198 Survival estimates for each habitat between sampling trips could be estimated using  
199 open population models (Pollock et al. 1990) following the robust design framework. If  
200 the recaptures between trips are too low to estimate survival, then the RDMR approach  
201 basically collapses to the RWAE approach for estimating survival – i.e., these  
202 approaches are not mutually exclusive in design or analyses employed. Shifts in  
203 length-frequency and direct ageing using otoliths will be used to evaluate growth (see  
204 section 2.2.3).

205  
206 *Section 2.2 Site Selection* - We propose to use existing data and models from the  
207 GCMRC physical science program (Projects 1, 2, and 10, Table 2 to quantify habitat  
208 availability over the study reach that contains the RDMR sites, habitat availability within  
209 the sites, and how habitat changes with flow. Such an approach has already been used  
210 to quantify juvenile chub habitat use in the Colorado River near the LCR (Korman et al.  
211 2003). The existing GCMRC shoreline GIS database and other surveys can be used to  
212 stratify habitat into classes such as talus slopes, open sand bars, vegetated sand bars,  
213 cobble bars, and backwaters. Existing bathymetry and hydrodynamic two-dimensional  
214 models that cover the entire study reach can be used to predict water temperature,  
215 depth and velocity in shoreline habitats at daily minimum and maximum flows during the  
216 summer-unsteady period, and at the average flow during the steady-fall period (Korman  
217 2003; Projects 1 and 10, Table 2). Velocity and temperature criteria will be used to  
218 further classify habitat for various species and life stages (e.g., into usable and  
219 unusable categories, or some finer scale). Additionally, shoreline stability during the  
220 summer can be quantified based on daily flow variation. For example, in summer,  
221 backwaters that are barely flooded at the daily maximum flow may be far more stable,  
222 warmer environments than those subject to complete inundation on each diel flow cycle.  
223 Similarly, steeper shorelines with cover (e.g., vegetated cut banks) may be more stable  
224 than low angle, open sand bars or cobble bars, where low velocity littoral areas vary  
225 more. These characteristics of stability can be related to patterns of fish habitat use  
226 (density or occupancy) to help evaluate effects of fluctuating flows. A strong  
227 relationship between fluctuating flows, nearshore habitat use, and otolith growth has

228 been observed for age-0 rainbow trout in the Lee's Ferry reach of the Colorado River  
229 (Korman and Campana, 2009). Similar relationships are expected for juvenile native  
230 and nonnative fish in the Grand Canyon and, in fact, is part of the motivation for the  
231 current fall steady flow test. We hypothesize that unstable habitat types will be used  
232 only minimally during the summer-unsteady flow period, but that use of these habitats  
233 will increase during the fall-steady period when flows are stabilized. If this difference in  
234 habitat use is ecologically important, we would also predict increase in growth and  
235 survival during the fall-steady flow period relative to the summer. Information we collect  
236 as part of Sections 2.1 and 2.3 can be used to test these ideas directly.

237

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



259 2.2-2.6%, leading these investigators to conclude that rainbow trout populations within  
260 discrete sites effectively can be considered closed. We would evaluate the closure  
261 assumption in our mark-recapture experiments using methods similar to Korman et al.  
262 2009. Additionally, recaptures of fish marked on previous trips will provide useful  
263 information on growth and movement (e.g., movement into backwaters during periods of  
264 steady flow) between sampling trips and associated flow conditions. The NSE pilot  
265 sampling data from 2008 should provide some information on closure and also provide  
266 information on capture probability which is necessary to fully assess how violation of the  
267 closure assumption biases abundance estimates. Zehfuss et al. (1999) demonstrate  
268 declining bias in abundance estimates with increasing capture probability and  
269 emigration from the study site for Jolly-Seber and robust design models.

270

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

282

283 *Section 2.3.2 Incorporating telemetry information* - The potential exists for radical  
284 changes in capture probability or behavioral shifts by fishes across sampling events  
285 within a season (~July-October) due to fish growth and possible ontogenetic habitat  
286 shifts such as fish moving into deeper, less accessible portions of the river. These  
287 behavioral shifts could also be caused by the designed flow experiments and  
288 associated changes in available habitat, or biotic interactions (changes in food  
289 availability or predation risk). To examine these behavioral responses, directly assess

290 habitat use, and test capture-recapture model assumptions such as closure, a small  
291 sub-set of native and non-native fish (5-10 individuals of 3-4 key species, including  
292 humpback chubs, > 150-mm TL) will be tagged with telemetry tags and their  
293 movements, habitat use, and fates assessed directly.

294         Implantable, compact telemetry tags coupled with autonomous receivers (which  
295 monitor a given area continuously for extended time periods for the presence of tagged  
296 animals) and deployed as a fine scale array within a sample reach could allow the  
297 telemetered animals to be “virtually” captured by detecting their tags as they move  
298 through the environment via either the autonomous receiver array, or from boat-based  
299 receiver units when field crews were onsite. This technology is mature, and thousands  
300 of telemetered fish and autonomous receivers are currently deployed globally  
301 (Simpfendorfer et al. 2008; Hedger et al. 2008). However, the use of telemetry  
302 techniques in Grand Canyon is technologically challenging, given the large amount of  
303 ambient noise from rapids, moving rocks, and coarse sediments, all of which greatly  
304 impair the ability to detect acoustic tags. Radio tags have been used successfully in  
305 Grand Canyon to track movements of adult humpback chubs (Valdez and Hoffnagle  
306 1999) in response to flood events, but the attenuation distance of radio tags is greatly  
307 impaired by high conductivity in areas of Grand Canyon near the Little Colorado River.  
308 Current GCMRC projects 3 and 7 have been involved in several pilot projects exploring  
309 the utility of these techniques with non-native fishes to assess movement and capture  
310 probability. Industry cooperator Marlin Gregor, President of Sonotronics Inc.  
311 (<http://sonotronics.com>) has been involved in the above projects and has developed  
312 specific tag and receiver design combinations to maximize the potential of this  
313 technology in the challenging conditions presented by the Colorado River in Grand  
314 Canyon. Marlin Gregor has also agreed to accompany on our first sampling trip as we  
315 test a variety of receiver and tag types. If this pilot sampling during trip 1 reveals poor  
316 performance of telemetry equipment due to high levels of ambient noise in Grand  
317 Canyon, we will abandon the telemetry component of this project. We will work with  
318 cooperators to then identify alternative research efforts such as experiments to assess  
319 DNR:RNA ratios as a measure of short-term growth as recommended by NSE review  
320 panel.

321           The incorporation of telemetry information with the mark-recapture sampling is  
322 experimentally risky, but has huge potential to boost our ability to draw inferences about  
323 juvenile fish abundance and habitat use. Pollock et al. (2004) showed an example of  
324 using both tag types to improve estimation of fishing and natural mortality rates using a  
325 complementary, multinomial approach to the tagging component of the stock synthesis  
326 model used in Korman et al. in-press and this proposal. We have extensive previous  
327 experience with these types of telemetry systems and similar applications (Bennett  
328 2005; Marcinkiewicz 2007; Tetzlaff 2008; Burgess 2008). Coupled with ongoing  
329 GCMRC projects 3 and 7, and industry cooperator commitment, it is likely that  
330 technological hurdles could be overcome. Exact tag types, receiver deployment  
331 strategies, numbers of tags and receivers required would be determined based on in-  
332 situ range testing of tag and receiver combinations. An array network would not  
333 guarantee perfect, continuous detection because behaviors of individual animals such  
334 as occupying habitat types similar to interstitial spaces between rocks, would attenuate  
335 the signal from the sonic tags. Conceptually, an autonomous receiver array could be  
336 deployed during a July sampling trip (first trip of sampling season) and a small batch of  
337 fish tagged and released with telemetry tags. The receivers would then remain  
338 deployed, monitoring these fish until the next sampling trip, at which time the data would  
339 be retrieved and an additional batch of fish tagged. Similar procedures would be  
340 followed for each additional trip, until the last trip of the year when no additional fish  
341 would be tagged and the receivers would be removed from the study site. Multiple  
342 batches of fish would be used (a “staggered entry” design, Pollock et al. 1989) because  
343 of limitations in tag battery life. The staggered entry design would allow specific size  
344 cohorts of fish to be tracked from the first through the fourth study period coincident with  
345 the same flow transition periods discussed in Section 2.0.

346  
347 *Section 2.3.3 Determining origin of recruits* – The FSFE will likely almost immediately  
348 create thermally favorable microhabitats for juvenile HBC and other native fish (RFP,  
349 page 27) which motivates Question 6 of the RFP (page 27), “*Are replicated flows*  
350 *September-October steady flows (2008 through 2012) likely to improve the survival and*  
351 *recruitment of young humpback chub in the Colorado River ecosystem?*” Additionally,

352 while management actions such as FSFE are certainly likely to modify and create  
353 habitat types, some of which may be favorable to juvenile HBC, where do the juvenile  
354 HBC come from to colonize these habitats and if they colonize these habitats, how well  
355 do they survive? As outlined in Sections 2-2.2, we describe approaches to assess  
356 survival of young HBC (and other species) in response to the FSFE. However,  
357 recruitment of young HBC and other juvenile fish from the LCR to the mainstem study  
358 site during July-September (Conceptual Model point A) could confound information on  
359 patterns in juvenile fish density in different habitats and their survival, unrelated to flow  
360 events. For example, in the absence of any direct measure of age-0 fish recruitment  
361 from the LCR to the mainstem population, observed patterns in density, growth, and  
362 survival of juvenile fish from our mark-recapture study could be attributed to the flow  
363 experiment, when, in fact, these density changes were actually independent of the  
364 experiment, and instead related to natural emigration from the LCR to the mainstem.  
365 Without an understanding of this recruitment from the LCR to the mainstem, the  
366 estimated growth, survival, and movement among habitat types in the mainstem from  
367 the tagging data (Sections 2-2.2) could be confounded with new recruits.

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

380 Otoliths are paired structures that form part of the inner ear of teleost fish and are  
381 commonly used by fisheries researchers to estimate fish age, and increasingly, their  
382 chemical constituents are used to determine fish movements (Campana 2005). Otoliths

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

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

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

436 As an example of this approach Limburg (1995, 1998, 2001) used ratios of  
437 strontium (Sr) relative to calcium (Ca) from American shad (*Alosa sapidissima*) otoliths  
438 along a transect in the Hudson River, linked with otolith derived age estimates to identify  
439 the age and size when American shad were transitioning from the Hudson River to the  
440 Atlantic ocean. Limburg (2001) compared Sr:Ca ratios and size and age structure in  
441 young-of-year American shad with the Sr:Ca ratios of adult fish from these same  
442 cohorts migrating back to spawn in the Hudson River in subsequent years. From these  
443 comparisons Limburg (2001) found that in three of the four year classes analyzed,  
444 differential mortality had occurred in juveniles, and was related to time of emigration

445 from the Hudson River. A similar approach with humpback chubs over multiple years,  
446 coupled with Sections 2-2.2 of this proposal, could help us to identify the linkages  
447 between the timing of out migrating juveniles from the LCR, available rearing habitat in  
448 the mainstem as determined by flow conditions, and the interaction between available  
449 habitat and juvenile fish survival.

450

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

466 We propose to assess growth in three ways: (1) by tracking modal progression of  
467 native and non-native fish size in during each sampling trip, (2) if unique VIE marks are  
468 possible through recaptures of marked fish, and (3) direct estimation using otoliths  
469 collected for the microchemistry analyses of HBC described above. The first approach  
470 is straight forward and simply requires plotting the size frequency of individuals from  
471 each species and sample trip as a function of length and identifying distinct peaks in the  
472 distribution. These peaks are then followed in subsequent trips and growth estimated  
473 by tracking the progression in the modal peaks of each size frequency distribution. This  
474 approach generally works best if sample sizes for each cohort are large and growth is  
475 fairly uniform among cohorts such that the size modes for each cohort can be

476 distinguished. If sample sizes are small or growth within a cohort is inconsistent, then  
477 cohort modes become indistinguishable in the length frequency distribution and  
478 estimating growth is not possible. This approach is low cost, non-lethal, and would be  
479 used to estimate growth for all possible fish species.

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

489 The third approach is based on the standard technique of visually counting daily  
490 growth rings on otoliths to estimate age (Pannella 1971; Stevenson and Campana  
491 1992; Pine and Allen 2001). Following this approach, the second HBC otolith from  
492 whichever otolith pair is used for the microchemistry analyses (most likely the lapillar  
493 pair; Hendrickson 1997) would be mounted individually on microscope slide, polished to  
494 the mid-plane following standard otolith preparation procedures, examined at 400-1250x  
495 using a compound microscope, and then the daily increment marks on the otoliths from  
496 the origin to the edge counted 2-4 times. Daily otolith increments have not been  
497 validated in humpback chubs, but preliminary work by Hendrickson (1997) provides  
498 strong circumstantial evidence to suggest that the lapillar increments do form with daily  
499 periodicity. If recapture rates between trips are sufficiently high as evident from trips 1  
500 and 2 in field season 1, a validation study could be designed by immersing juvenile HBC  
501 in an alizarin solution to “mark” the otolith, then releasing the fish with the expectation to  
502 recapture that fish on a future trip. Recaptured fish would then be sacrificed, otoliths  
503 removed and prepared as described in section 2.3.4, and the otolith rings since the  
504 immersion in alizarin counted and compared to the actual days elapsed (Devries and  
505 Frie 1996). Average daily growth rates can then be determined from dividing the fish TL  
506 when captured by the age.



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

528           A major drawback of using otoliths to estimate age and growth is that the fish  
529 must be sacrificed to extract the otoliths. Given the listing status of most native fish in  
530 Grand Canyon, including HBC, we share concerns that any research associated  
531 mortality could be deleterious to the population overall, and we acknowledge that  
532 permits to collect and sacrifice these fish may be difficult to receive. Recruitment trends  
533 for humpback chubs and other native fish have been increasing over the last 4-5 years  
534 (ongoing GCMRC projects 5, 6, Table 2; Coggins 2008) reducing the potential for  
535 population impact from removing a small number of native fish from the population for  
536 research needs. Exact numbers of fish required would be determined by examining  
537 otoliths from juvenile humpback chubs incidentally taken as part of routine sampling in

538 prior research efforts (i.e., Near Shore Ecology pilot sampling in August and September  
539 2008 and backwater sampling efforts in summer 2005). We propose to collect a small  
540 number of juvenile fish, most likely 5-10 age-0, age-1, age-2, and age-3 humpback  
541 chubs and also flannel mouth suckers each trip (no more than 50 fish per age class and  
542 species per year). In addition to providing information from the microchemistry and age-  
543 growth analyses in response to the FSEF, there are at least two other major areas that  
544 could be assessed by using tissues from these animals. First, age information on these  
545 small fish would be very useful in informing the age-length key used to assign age-at-  
546 first capture in the standard age-structured mark-recapture program (ASMR, Coggins et  
547 al. 2006a; GCMRC projects 4 and 6, Table 2) used to assess trends in HBC growth,  
548 recruitment, and survival (Coggins et al. 2006b; Coggins 2008). This age-length key is  
549 currently based on a limited set of approximately 60 estimates of HBC length-age  
550 (Coggins 2008) and this small growth sample, particularly for very small and young fish,  
551 could be improved with additional growth information possibly leading to reduced bias in  
552 the recruitment estimates from the ASMR model. Additionally, the previous growth data  
553 were not collected during the recent time period of warmer water temperatures, which  
554 may have led to increased HBC growth. Thus, the currently used growth information  
555 may be negatively biased due to slow growth of humpback chubs in cold-water  
556 conditions vs. the current warmer-water conditions.

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

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

574  
575 *Section 3.1 Contingency plans to alternate flow scenarios, expanded spatial replication,*  
576 *or inability to secure variance during no-motor season* – The proposed sampling  
577 framework and experimental design (i.e., timing of trips) can easily be modified to  
578 address alternate flow-related hypotheses. For example, question (9) in the RFP (page  
579 28), “*What happens to juveniles that used the warmed areas when fluctuating flows are*  
580 *resumed?*”, could be addressed by adding one or two trips (and additional costs) to the  
581 proposed four-trip schedule. In this case, a trip following the resumption of fluctuating  
582 flows, (mid-November) and an additional trip one month later, would quantify immediate  
583 changes in habitat use as well as survival rates under fluctuating flows during early  
584 winter. A similar strategy, where sampling is conducted before and after a beach  
585 habitat building flow, could be used to evaluate the effects of flows above power plant  
586 capacity or normal daily maxima (item 6, RFP page 31). Additional spatial sampling, at  
587 other known HBC aggregation sites (“Randy’s Rock”, RM 126; “30-mile”, RM 29-32)  
588 could also be implemented based on results from Section 2 above. The key point is  
589 that our approach will provide estimates of habitat use, survival, growth, and origin of  
590 juvenile fish during summer and fall, and that it is relatively easy to expand sampling  
591 into other times of year to look at other flow-related hypotheses. That said, the  
592 objectives and research questions that are the focus of the RFP are very challenging.  
593 Our philosophy is to do a few things well, rather than many things poorly. Given  
594 available resources, we will focus on population dynamics during summer and fall only  
595 as outlined above.

596 We expect that some elements of the proposed sampling framework would  
597 become part of the GCD AMP’s long-term monitoring protocol. For example, methods  
598 developed in this project could be used to estimate juvenile HBC abundance in the

599 mainstem during late summer and late fall. Over many years, these estimates could be  
600 related to recruitment estimated from the Age-Structure Mark-Recapture (ASMR) model  
601 (Coggins et al. 2006 a, b). Ultimately, a stock-recruitment relationship between age-0  
602 chub in the mainstem during both fall and summer, and recruitment determined from the  
603 ASMR model, would be very helpful in the interpretation of the juvenile data. For  
604 example, if there is strong density dependence in survival between summer and fall,  
605 and little density dependence for later life stages, we would expect an asymptotic  
606 relationship (e.g., Beverton-Holt shaped relationship) between summer and fall juvenile  
607 abundance, and between summer juvenile abundance and ASMR-based recruitment  
608 estimates. In this case, there would also be a strong linear relationship between fall  
609 juvenile abundance and ASMR recruitment. Such analyses are very relevant to current  
610 policy debates. For example, strong density dependence between summer and fall  
611 would indicate that it is unlikely that improving habitat conditions during the summer will  
612 result in substantive changes in juvenile abundance during the fall, and consequently to  
613 improvements in recruitment to the adult population. It will take many years to establish  
614 reliable relationships (which will likely be noisy), but we see no alternative for the GCD  
615 AMP. Ultimately, life-stage specific stock-recruitment functions need to be established  
616 to fully interpret results from the proposed study in the context of GCD AMP  
617 management objective for adult HBC.

618         Depending on the start of the FSFE, it is likely that one or two of our proposed  
619 sampling trips would launch after the start of the no-motor season (September 15). In  
620 this case, we would require a variance from the Park Service for operating motorized  
621 equipment (boat motors and generators) required for our electrofishing sampling. While  
622 we intend to use multiple gear types (most likely electrofishing and hoopnets) during  
623 each of our sampling events, we have high expectations for successful electrofishing  
624 sampling for use in mark-recapture (Section 2.1) based on results from Korman et al.  
625 (2009) and fall 2008 Near Shore Ecology pilot sampling. Failure to secure a variance  
626 allowing us to use electrofishing would increase our dependence on hoopnet sampling  
627 to track patterns in habitat specific abundances, estimate survival, and track growth via  
628 changes in length frequency. Gear selectivity patterns between electrofishing and  
629 hoopnets likely differ, and by deploying both gears on each sampling trip we intend to

630 minimize the confounding that can occur in the data due to changes in gear between  
631 trips and associated selectivity patterns (i.e., responses could be due to the flow  
632 experiment or simply to changes in the gear type). Compromises to help meet the Park  
633 Service's no-motor requirements are possible. One option would be to create a stash of  
634 1-2 electrofishing boats in the study reach just prior to the end of no-motor season. Any  
635 trips that occurred during the no-motor season would then be supported by rowing trips  
636 out to the sampling sites, where electrofishing boats would be used to complete the  
637 required sampling effort. At the end of sampling, these boats would be floated or flown  
638 out of Grand Canyon in cooperation with other sampling efforts with USFWS and AZGF.  
639 Additional options would be to increase the reliance on telemetered fish and to follow  
640 the movements and habitat use of telemetered fish using rafts and kayaks during no  
641 motor season. A final option would be to not grant the variance; thus, electrofishing  
642 would not be possible. Our proposed closed population models (both mark-recapture  
643 and occupancy) estimates capture probability with each sampling trip, so if hoopnets  
644 were the only sampling gear available during the last trip, then our capture probabilities  
645 would be based on marks and recaptures from hoopnet gear only. Hoopnet catches  
646 alone would likely result in very low capture probabilities, limiting the use of some mark-  
647 recapture models. However, habitat occupancy using methods outlined in section 2.3.1  
648 may be used to estimate occupancy even with low capture probabilities. If no variance  
649 is granted, extensive comparisons between electrofishing and hoopnet catches based  
650 on pilot sampling during motor season and previous fish sampling efforts in Grand  
651 Canyon would be done to develop a better understanding of the selective properties of  
652 each gear. The style of electrofishing developed and used by Korman et al. (2009) was  
653 first used in the Lee's Ferry reach, but was also used as part of the pilot NSE sampling  
654 during fall 2008 thus limiting the number of comparisons that can be made between  
655 gear types using historical data.

656

657 *Section 4 Conclusions* - Certain aspects of the proposed experimental design are  
658 beyond our control, and will limit the inferences from the resulting data. We propose to  
659 compare juvenile habitat use, survival, and growth during summer-unsteady and fall-  
660 steady flow periods to evaluate the potential benefits of steady flows. However, such an

661 assessment assumes there is no seasonality in the parameters of interest, which we  
662 know is not likely to be the case. For example, growth rates will likely decline as fish  
663 increase in size, and as water temperature declines due to reduced solar irradiance.  
664 Habitat use may also have a flow-independent component that depends on fish size.  
665 Ultimately, the proposed work, or key elements of the proposed work, will need to be  
666 repeated under an alternate flow regime. The two logical alternatives are to extend the  
667 period of steady flows (e.g. July-Oct), or to revert to unsteady flows (MLFFA or greater  
668 fluctuations) during the fall. Results from the proposed work will be helpful in choosing  
669 among these alternatives because: (a) we will have a much better sense of our ability to  
670 detect changes in juvenile survival rates and other life history characteristics; and (b)  
671 expected and serendipitous insights from the proposed work will provide for more  
672 refined hypotheses and a more fully articulated conceptual model.

673

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

823  
 824

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

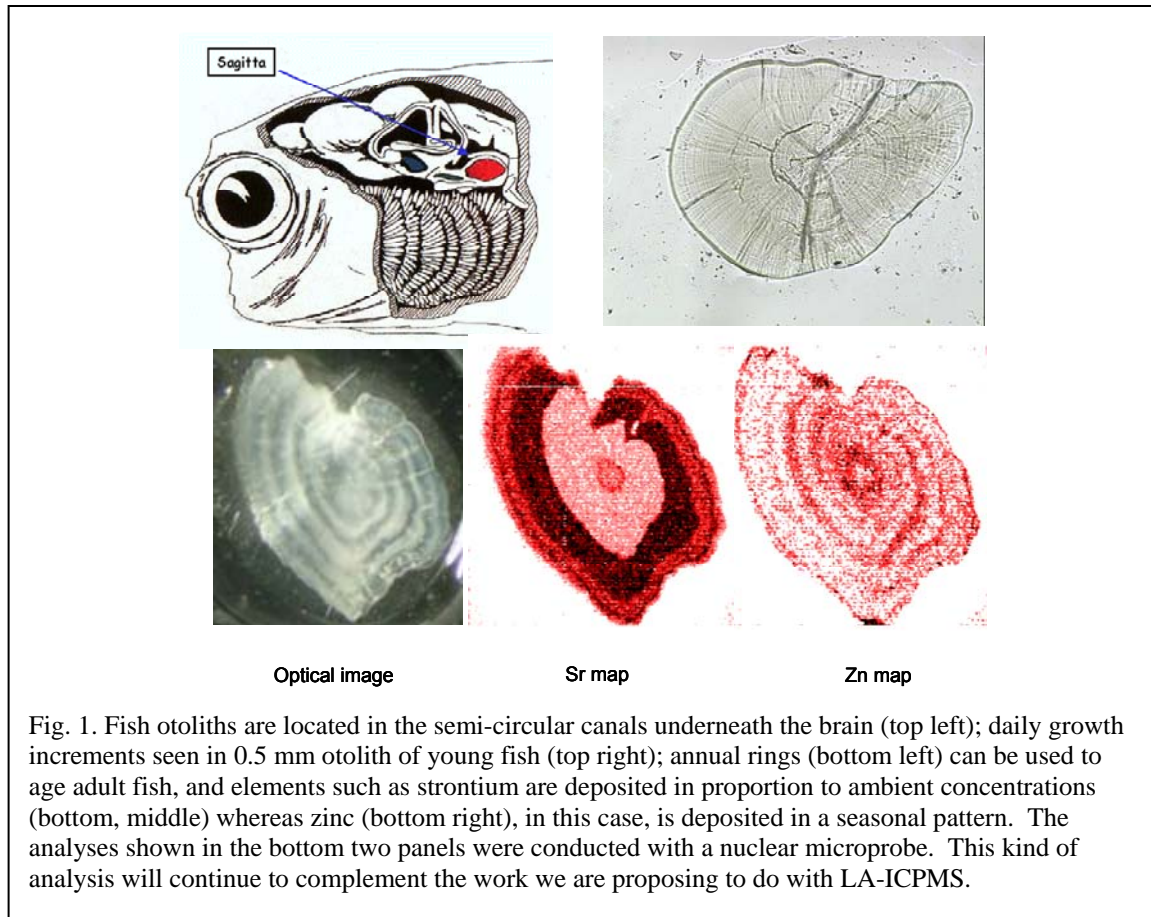
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826 Table 2. Existing monitoring and research studies in Grand Canyon as listed in the  
827 RFP. Numbers in this table correspond to referenced cooperative and complimentary  
828 research described in this proposal.  
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Project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

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