Work Task C40: Genetic and Demographic Studies to Inform Conservation Management of Razorback Suckers and Bonytail in Off-Channel Habitats

FY16 Estimate	FY16 Actual Obligations	Cumulative Expenditures Through FY16	FY17 Approved Estimate	FY18 Proposed Estimate	FY19 Proposed Estimate	FY20 Proposed Estimate
$275,000	$274,332.93	$928,617.03	$300,000	$ 415,000	$0	$0

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Start Date: FY10

Expected Duration: FY18

Long-Term Goal: Effective fishery management of backwater habitats developed by the LCR MSCP

Conservation Measures: RASU2, RASU6, BONY2, and BONY5

Location: Backwater habitats (Reaches 2–5)

Purpose: To quantify genetic and demographic parameters that are necessary for informed, long-term management of razorback suckers (*Xyrauchen texanus*) and bonytail (*Gila elegans*) in off-channel habitats

Connections with Other Work Tasks (Past and Future): This work task is related to Work Tasks B7, C25, C31, C56 (closed), F5, and G3.

Project Description: In Lake Mohave and elsewhere, razorback suckers and bonytail demonstrate a group spawning behavior whereby a female will spawn with multiple partners many times over a period of a few weeks. These observations led biologists to believe that all possible genetic crosses were being made during the spawn. However, analyses of adult razorback suckers placed into the Yuma Cove backwater in 1991 and 1992, along with analyses of the larval razorback suckers produced each year, showed that not all of the adults contributed genetic material to the next generation. It is possible that individual adults do not spawn every year, or that even if they do, they do not always contribute genetic material to the next generation. This hypothesis needs to be tested in order to model a population structure within these isolated habitats over subsequent generations and to predict at what frequency genetic material needs to be exchanged between habitats to maintain the diversity of the overall razorback sucker and bonytail populations within the LCR MSCP planning area.

Demographic and genetic information will be collected that will lead to recommendations to optimize long-term management of off-channel habitats for these two critically endangered fishes. Genetic data will be captured from larval, juvenile, and adult razorback suckers and bonytail from at least two replicate groups from off-channel habitats. Characterization of deoxyribonucleic acid (DNA) variation will be used to assign the parentage of individual larvae to specific adults. The data can then be compared and contrasted to (1) determine the actual number of individuals that participate in annual spawning activities, (2) census the populations, and (3) quantify patterns of survivorship.

Previous Activities: Adults, larvae, and juveniles razorback suckers have been genotyped, and multiple iterations of in situ spawning have been completed in the Arizona Juvenile (AJ), Dandy, and Yuma Cove backwaters along Lake Mohave. Collections from FY10 to FY15 were analyzed, identifying considerable variability in individual reproductive success within and especially among different lakeside ponds.

In FY14, three Lake Mohave backwater ponds were no longer being used for razorback sucker production, so they were dedicated to bonytail genetic experiments. The North Nine Mile, Nevada Egg, and Nevada Larvae backwaters were all stocked with 80 male and female adult bonytail. The Nevada Larvae backwater experienced a fishkill shortly after stocking, and it was removed from the study. However, spawning was successful in the North Nine Mile and Nevada Egg backwaters. From these backwaters, 397 and 593 genetic samples of larvae and age-0 fish samples were collected from the North Nine Mile and Nevada Egg backwaters, respectively. Parentage was determined for almost all larvae and age-0 fish samples produced within these two backwaters. Variance in reproductive success differed dramatically among backwaters. Specifically, in Nevada Egg, certain males and females contributed disproportionate numbers of progeny. High variance in reproductive success acts to reduce the genetic effective size of the population, which in turn can increase the rate at which genetic diversity is lost from the population.

In FY15, adult bonytail were again stocked into the Nevada Egg and North Nine Mile backwaters with an equal sex ratio comprising 100 males and 100 females. A total of 1,542 larvae and age-0 fish were produced in the backwaters. Parentage could be determined for 95% of the progeny; this was slightly lower than in FY14 due to the reduced genetic diversity of the broodstock used in FY15. The FY15 results indicate that genetic diversity was maintained between parents and offspring. Reproductive success was similar between the two backwaters.

FY16 Accomplishments: The number of adults stocked into the AJ and Dandy backwaters was reduced from 100 individuals of each sex to 50 for FY16. There were a number of challenges associated with the backwaters used in these studies for FY16. In the summer of FY16, a flash flood compromised the berm that separates the AJ backwater from Lake Mohave and allowed razorback

suckers to escape. Because of this breach, only 14 larvae were collected from the AJ backwater. Reproduction was limited in the Dandy backwater (six juveniles). The samples were too small to generate meaningful results. Genetic samples were also obtained from the Yuma Cove backwater: 291 larvae, 125 juveniles, and 56 previously untagged individuals. These individuals were fin clipped, and the DNA was extracted and is in the process of being genotyped.

For the razorback sucker portion of this project, much of the effort was spent analyzing data from 2010--15 and developing a comprehensive report that will be available on the LCR MSCP Web site in FY17.

The third year for bonytail backwater research was conducted in FY16. Two backwaters (North Nine Mile and Nevada Egg) were each stocked with 100 males and females. Genetic data were collected from all adults that were stocked into the backwaters. No larvae were able to be collected from North Nine Mile, but 64 young-of-year were collected in October. Larval and young-of-year samples were collected from the Nevada Egg backwater (275 and 286, respectively). Lack of contacts from the remote scanning data in the North Nine Mile backwater suggests that all adults died shortly after they were stocked. However, some of the adults had spawned before they died. Genetic analyses suggested that 23–30% of females and males contributed offspring. Consequently, genetic effective population size and genetic diversity were less in progeny from this backwater. In the Nevada Egg backwater, more than 90% of males and females contributed to offspring production. Genetic diversity was maintained between parents and progeny, and genetic effective size was high in this backwater. The results from data obtained from 2014–16 indicate that both bonytail males and females are capable of high parental contribution to resulting progeny.

FY17 Activities: The relative survival of the newly stocked razorback suckers versus the surviving razorback suckers from previous years will be monitored in the Yuma Cove backwater; in previous years, high mortality was observed in newly stocked razorback suckers, compared to "resident" razorback suckers (mostly comprised of fish from the initial stocking), which had very low mortality. Additional manipulations of this population may be required to maintain a genetically stable population. The number of individuals stocked into the AJ and Dandy backwaters will again be used to examine the impacts of density on reproductive success. FY17 is the first year this study will transition to the Imperial Ponds Conservation Area. These populations will be monitored and allowed to reproduce over multiple years with little to no interference.

The study of bonytail genetics and demographics will transition to the Imperial Ponds Conservation Area in FY17. Protocols for collections and analyses will be similar to those used at the North Nine Mile and Nevada Egg backwaters. However, the number of stocked bonytail and razorback suckers will be 150 males and 150 females for each pond. This number of stocked individuals was increased due to the ponds' larger sizes.

Work initiated under Work Task G3 during FY16 titled "Development of SNP Markers for Gender Determination, Parentage Assessment, and Population Genetics of Razorback Sucker" will be moved to Work Task C40 in FY17. Costs associated with this study were not accounted for in the FY17 estimate, so it is anticipated that the budget will exceed estimates. In FY16, potential single nucleotide polymorphism (SNP) markers for sex determination and identification of parentage in razorback suckers have been generated. Re-extraction of DNA from known males and females and their progeny will be completed using individuals previously characterized in the razorback sucker genetics backwater project (C40). This process is expected to yield 600 million sequences and more than 5,000 SNPs. Once sequences have been obtained, bioinformatic analyses will be used to identify SNPs specifically associated with sex determination and SNPs that are of sufficient variability for use in parentage analysis.

Proposed FY18 Activities: Efforts in FY18 will be similar to those in FY17. Protocols for collections and analyses will continue similar to previous years. Additional genetic tools will be used to assess the differences in parental contributions among backwaters and in an attempt to identify the factors contributing to these differences.

The draft razorback sucker genome will be assembled, and markers identified in year 1 will be mapped onto the genome. For sex determination markers, this will allow for identification of the genes linked to the sex-specific SNPs. Identifying these genes will allow the LCR MSCP to redesign more effective markers in the genes themselves. Mapping of SNP loci for parentage analyses will allow the LCR MSCP to select independent markers necessary for parentage determination. Once identified by bioinformatic analyses, SNPs will be further validated by characterizing individuals of known sex and parentage. This will require the development of a platform for screening large numbers of individuals for large numbers of loci in a cost-effective and efficient manner. Once validated, these markers will be ready for general use and may be applied to all genetic samples.

This work task will be closed in FY18. Any future genetic monitoring will be conducted under system-wide monitoring (Section D).

Pertinent Reports: A comprehensive report (2010 – 2015) covering the backwater work for razorback suckers is in draft and will be posted on the LCR MSCP Web site upon completion. The 2014 and 2015 bonytail reports titled *Genetic and Demographic Studies to Guide Conservation Management of Bonytail in Off-Channel Habitats* are complete and will be also posted.