**Science- Genetics Sub-group pilot project 2**

**PIT tagging and genetic characterization of broodstock**

**Must be completed for broodstock that will be used in the paired spawning pilot study prior to undertaking Part II of that study.**

**Background**

The Middle Rio Grande Endangered Species Collaborative Program (Program) provides the framework for the protection and improvement of the status of listed species along the Middle Rio Grande (MRG) while protecting existing and future water rights under federal and state of New Mexico law. The Program is comprised of multiple stakeholders representing diverse interests on the Middle Rio Grande (MRG). The Science Workgroup of the Program was formed to oversee scientific studies and related investigative projects, including the Rio Grande Silvery Minnow (RGSM; *Hybognathus amarus*) Genetics Project. The Genetics Project is the Program’s ongoing genetic sampling and assessment program, which refers to the estimation of population genetic parameters, such as gene diversity, heterozygosity, allelic richness, and genetic effective size of RGSM (Osborne et al. 2012). The RGSM Genetics Project supports the implementation of conservation actions in the 2016 *Final Biological and Conference Opinion for Bureau of Reclamation, Bureau of Indian Affairs, and Non-Federal Water Management and Maintenance Activities on the Middle Rio Grande, New Mexico* (USFWS 2016). The Genetics Project is an essential component in tracking the genetic status of RGSM and assessing the effectiveness of the Program’s population augmentation program in the MRG, and the U.S. Fish and Wildlife Service’s RGSM Genetics Management and Propagation Plan (USFWS 2013), and RGSM Recovery Plan (USFWS 2010).

The Middle Rio Grande Collaborative program funded a Peer Review Panel of genetics experts to review the genetics work of the Rio Grande silvery minnow. Changes to the RGSM Genetics Management and Propagation plan recommended by these experts included:

* Assessing parental contributions and determining the number of breeders through parentage analysis;
* Equalizing family sizes before releasing fish to the river;
* Evaluating paired spawning versus the current protocol of group spawns;
* Calculating the relatedness of brood stock prior to artificially spawning to choose specific crosses that avoid inbreeding; and,
* If group spawning continues, using relatedness estimates to ensure that potential spawners in a group have low kinship.

Genetic characterization of broodstock is a necessary first step towards implementing these recommendations.

**Objectives**

* Characterize genotypes of identified RGSM individuals in the captive population using available markers and new markers as they become available.
* Maintain records to enable selection of unrelated fish for spawning.

**Tasks**

1. **Initiate PIT tagging and genetic characterization of broodstock** 
   1. Establish schedule to PIT tag and collect tissue samples for each cohort, starting with the oldest cohorts. Conduct genetic sample collection and analysis of hatchery stocks well in advance of RGSM spawning so that decisions can be made regarding which individuals to use in paired or communal spawns
      1. Tag and sample all 2018 broodstock with sufficient lead time to process tissue samples and report results NLT 1 Feb 2018
      2. Tag and samples all other age 1+ broodstock NLT 1 Sept 2018
   2. Use PIT tagging techniques developed by Archdeacon et al 2009 to tag all broodstock.
   3. Genetic material shall be collected from all broodstock using non-destructive fin clipping.
   4. Fin clips shall be preserved at the Museum of Southwest Biology.
   5. Initially, conduct genetic analysis as described in Osborne and Turner (2012). As additional techniques/markers are developed, tissue samples would be analyzed for these additional markers.
   6. Establish a database or “studbook” for maintaining genetic records by individual.

**Benefits**

1. This project would establish processes for characterizing broodstock to minimize relatedness of spawned fish and would reduce the need for genetic analysis of the progeny prior to release.
2. This project would address several recommendations from the peer review, including:

* To accurately estimate the effective number of breeders and to assess their respective contributions to the resulting offspring.
* To thoroughly assess potential domestication selection for all propagation activities and set up procedures to minimize such selection.
* To improve the genetic characterizations by (i) using large numbers of breeders that contribute equally to the augmentation population, and (ii) minimizing domestication selection during propagation. (Equalizing the family size solves many of these identified problems. Paired spawning accomplishes this without having to know anything else.)
* To calculate relatedness of brood stock prior to artificially spawning to choose specific crosses that avoid inbreeding. Alternatively, if group spawning continues, use relatedness estimates to ensure that potential spawners in a group have low kinship. See Project 5.
* To assess whether mortality causes a non-random shift in genotypic frequencies in the actual brood stock used in the hatcheries or in their progeny from hatching to release.
* To assess whether the composition of the gene pool shifts from generation to generation and to help establish whether there is “drift" in the gene pool due to sampling effects and population bottlenecks. (This is related to development of better markers and sampling effort. For future genetic monitoring it is important to ensure adequate sample sizes to detect change, and to have a “Plan B” for years with few wild samples.)
* Artificially spawn brood stock using a one female by one male RGSM mating scheme, while not reducing the total number of brood stock adults spawned.
* Equalize family sizes, Equalize contributions of different adults in the captive brood stock to new broods/lots as much as possible

**Cost Estimate**

**Dana will work on this**