

End-of-Contract/End-of-Year Report

Share with Wildlife Grant # 08-516-0000-00049

Project Title: Molecular Analysis of Hybridization Between Two Species of *Gambusia*

Funding Period: May 22, 2008—June 30, 2009

Project Principal Investigators: Daniella Swenton and Astrid Kodric-Brown;

University of New Mexico, Department of Biology

As stated in the contract we, the contractors, performed and are currently performing the following services outlined in small print in the following sections for the grant period of 5/22/08 - 6/30/09. We will address the work completed and ongoing for this project. We would like to point out that the final phase of this project is still ongoing because although supply purchase has been completed, the use of these physical supplies continues in a molecular examination of the degree of hybridization in the laboratory. We will submit a supplement to this End-of-Contract report once the results of these molecular analyses and other experiments are obtained. These results are expected by the end of 2009 and can therefore be included with the End-of-Calendar Year Report due to the Assistant Chief of Endangered Species and Nongame Wildlife by Dec. 31, 2009 in addition to the supplement. We will also submit the finalized data at this time, if not before, to NM Department of Game and Fish.

1. Non-destructively collect fin clips from a minimum of 30 the state Threatened and federally Endangered Pecos Gambusia (*Gambusia nobilis*) and western mosquitofish (*Gambusia affinis*) per population, from 4 sympatric populations and 4 allopatric populations of each species at Bitter Lake National Wildlife Refuge.

These tissue samples have been collected and we may collect additional samples if the final analyses deem it necessary or if BLNWR has other populations of interest.

2. Extract DNA from fin clips and perform polymerase chain reaction amplifications and automated DNA sequencing at a minimum of 1 mitochondrial DNA and 6 nuclear microsatellite loci.

We have currently extracted the DNA and are analyzing them for hybridization and other pertinent genetic analyses using 7 nuclear microsatellite markers as well as one mtDNA marker.

3. Analyze the results of DNA sequencing to quantify the extent of hybridization between the 2 species of *Gambusia*. Based on these results, evaluate conservation implications relating to the co-occurrence of these 2 *Gambusia* species.

We are currently continuing collection of these genetic data in the molecular ecology lab of Thomas Turner. Ms. Swenton-Olson will complete the work this Fall 2009 as she is graduating and this work is an integral part of her dissertation project.

4. Establish methods and a protocol for distinguishing hybrids from pure *Gambusia nobilis*.

Again, this work is ongoing but to date we have found some segregating alleles that seem to characterize each species and individuals in hybrid zones are heterozygous for said alleles.

PLEASE NOTE: This project is part of a greater Scope of Work from a previous Share with Wildlife Grant awarded to Kodric-Brown and Swenton-Olson. When it is complete it will comprise four manuscripts (and associated data sets) authored by Swenton-Olson, Kodric-Brown and others and will form Swenton-Olson's Dissertation Committee. The first manuscript concerns the behavioral and reproductive isolation between these two species. The second covers the life history differences these two species have evolved and how that ties into their current co-occurrence. The third chapter is an analysis of BLNWR sinkhole foodwebs and dietary differences between the two species. And finally the fourth chapter will detail the hybridization between these two species- the scope of work for this particular grant. These manuscripts as well as the final molecular data on hybridization will be submitted to Share with Wildlife this fall as an addendum to complete this End of Contract Report.