

Interim Fiscal Year Report 2008 (version for web posting)

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

Funding Period: FY08 (July 1, 2007 through June 30, 2007)

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We will address the work completed and ongoing for this project, specifically this fiscal year's grant period. We would like to point out that the project is still ongoing due to two reasons:

1. The major reason for the short nature of this interim report is that, although the contract was granted in February of 2008, the index number assigned by the University under which expenditures could be made was not assigned until 2 weeks before the end of the fiscal year and one week before this report was due. Therefore, although supplies have been purchased to carry out the work outlined in the contract, that work is only just now started with the arrival of the supplies.
2. 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 interim report once the results of these molecular analyses and other experiments are obtained. These results are expected by the end of 2008 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, 2008 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.

(NOTE: All activities are performed according to the University of New Mexico Institutional Animal Care and Use Committee protocol #04MCC006).

Contractor will, after anesthetizing the live, captive fish, collect fin tissue samples, which will be used later in genetic tests of hybridization, as well as for stable isotopic analysis, which will determine foraging differences between the two species.

Currently, we are measuring the influence of behavioral isolation via mate choice in laboratory experiments done in our previous contract for Share with Wildlife. In order to determine if their ecology/environment can isolate the two *Gambusia* populations of interest (*Gambusia nobilis* and *G. affinis*) two populations we are quantifying the nature of their habitats at Bitter Lake NWR (BLNWR) in our field study. We cannot tease apart the role of ecology, however, from behavior in the field without comparing the degree of hybridization in the field versus that in the laboratory studies. If ecology is critical in isolating these two species, regardless of the role of behavioral isolation, than we would expect a low degree of hybridization in the field relative to the lab experiments in which ecological differences are controlled (Table 1). Therefore, in order to determine if ecology plays a role in the isolation of *Gambusia affinis* and *G. nobilis* we must compare the degree of hybridization in the field to that of the laboratory experiments along with any ecological differences we might find in our field study. In order to further quantify the role of ecology we must determine any dietary differences between the two species.

We will gather these data with a stable isotope study that will not only determine any differences in prey source, but also in trophic level feeding differences.

The main objective of the genetic study is to measure the presence and degree of hybridization in our semi-natural populations and our field populations. We are determining this using a microsatellite analysis in collaboration with Dr. Thomas Turner, a molecular ecologist at the University of New Mexico. We collected fin clip tissue from 10 female, 10 male, and 10 juvenile individuals from each sympatric field site as well as two each of the allopatric sites for both species. This span of collection represents the range of these fishes at BLNWR. We will also test the individuals from the semi-natural populations. At the end of the experiment this fall these individuals will be euthanized and we will extract whole DNA from fin tissue. The individuals will then be deposited in the Museum of Southwest Biology.

We have already isolated all fin tissue samples. With the arrival of the contract budgeted molecular supplies last week, we have started amplifying the DNA tissue using a polymerase chain reaction (PCR). We are using 7 microsatellite primers developed by Spencer et al. (1998) for *G. affinis* that are well known to work across Poeciliid species, especially other *Gambusia* species. We can also use several *Poeciliopsis* primers that work across Poeciliid species (Parker et al. 1997). Using the amplified DNA we are running a microsatellite analysis and we will use alleles at 7 nuclear loci and one mtDNA loci to determine the degree of genetic introgression between these two species. All analyses are performed at the University of New Mexico molecular facilities. Our molecular data are very preliminary as we had to wait for supplies to continue working. At this point we have started to identify distinguishing alleles between the two species

that will be used to identify hybrid individuals. As indicated in the beginning of this report, we will submit an appendix to this report once a few months worth of active molecular work have been performed.

Table 1. Expected patterns of isolation in *G. affinis* and *G. nobilis*. The role of ecology in isolating *G. affinis* and *G. nobilis* can only be determined with a genetic analysis of natural populations. The role of behavior in isolating the two species can be determined in the semi-natural populations only with a genetic analysis.

Data Source	Mechanism of Isolation		
	Ecology Only	Sexual Selection Only	Ecology and Sexual Selection
Mate choice experiments	High Hybridization	Low Hybridization	Low Hybridization
Semi-natural populations	High Hybridization	Low Hybridization	Low Hybridization
Field data: stable isotope analysis and microhabitat use	Differences Exist	No Differences	Differences Exist
Field data: natural hybridization	Low Hybridization	Low Hybridization	Low Hybridization

In the summer of 2008, we will use carbon and nitrogen stable isotope data to measure: 1) the differences, if any, in prey items between *Gambusia affinis* and *G. nobilis* using carbon isotope differences and 2) to determine differences in trophic feeding levels using nitrogen isotope differences. We will collect tissue from 15 fish from each site as

well as approximately 20 prey items including plants and animals, as these are omnivorous fish. The tissue from the fish will be a dorsal fin clip that will grow back.