Section 6 (Texas Traditional) Report Review

Attachment to letter dated JAN 1 6 2008

TPWD signature date on report 5 Dec 2008

Project Title: <u>The Leon Springs Pupfish Recovery Project at Diamond Y Draw: further monitoring of</u> the population and the breeding habitat

Final or Interim Report? Final

Grant #: TX E-94-R

Reviewer Station: Austin ESFO

Lead station was contacted and concurs with the following comments:

Interim Report (check one):	Final Report (check one):
is acceptable as is	is acceptable as is
is acceptable as is, but comments below need to be addressed in the next report	is acceptable, but needs minor revision (see comments below)
needs revision (see comments below)	needs major revision (see comments below)

Comments:

Typographical errors need to be corrected throughout the final report (particularly in the Abstract: remove italics "of"; correct spelling of "bovinus"; spell out "Fort Stockton"; change "increased" to "increase"; delete repeated word "several").

Page 2, please re-phrase or define the phrase "on the verge of extinction."

Page 3, please define "DFFHTC."

Page 10, Results (Task 3). This paragraph needs to be expanded to include the extent of searches for pupfish in other habitats. Please provide a quantification of efforts to observe or capture fish and the results (were other species captured?). Also please provide a geographic extent of areas searched and sampled for pupfish. Did searches include the lower water course all the way down to near the Highway 18 bridge crossing (including other spring outlets and John's Pool) of Diamond Y Draw? Unless substantial collection efforts are made, it is likely premature (based on the results of past sampling efforts) to conclude that all the pupfish occur on the shelves in the headpool.

Page 11, Results (Task 4). You recommend continued use of tiles, based on the results observed so far. However, on Pages 9 and 13 you indicate that males preferred natural substrata for spawning.

Page 11, Results (Task 4). Please provide a quantitative summary of the results that support that you observed an increase in pupfish numbers from previous years.

Page 13. The paragraph describing behavioral observation in comparison to 2003 published results is confusing. Please review this text and revise to better explain the premises and how they lead to the conclusions. Also, if results from Figures 1 and 4 are to be compared, please put them on the same graph with the same scale or reference the numerical results in the text.

Page 14, regarding *Gambusia nobilis* predation. Was any *G. nobilis* predation observed in 2002 and 2003 data collection that could inform the discussion of the current observations? Also, it is not clear why "increasing the shallow areas would disperse the gambusia population." Does increased shallow areas make pupfish defenses more effective because there is less vegetative structure for gambusia to hide within?

Please provide a draft copy of the Gumm et al. (2008 In press) paper, as it is cited multiple times throughout the report. Also please provide a copy of the paper once it is published.

Figure 1. Please note that the data are from 2008. Also provide a time reference for the Y axis. These are the number of behaviors over what observation time frame?

Figures 3, 4, and 5. Please provide a time reference for the Y axis.

Figures 4 and 5. Please clarify that all of these data are from the 2003 paper. Please clarify that Figure 3 displays significant differences in results. The results and discussion of the report could better explain and emphasize the differences in spawning success between clustered territories and dispersed territories. It is not clear that the data support the conclusions in the text.

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-94-R

Endangered and Threatened Species Conservation

The Leon Springs Pupfish Recovery Project at Diamond Y Draw: further monitoring of the population and the breeding habitat

Prepared by:

Murray Itzkowitz



Carter Smith Executive Director

Clay Brewer, Acting Division Director, Wildlife

5 December 2008

FINAL REPORT

STATE: _____Texas_____ GRANT NUMBER: ____TX E-94-R____

GRANT TITLE: Endangered and Threatened Species Conservation

REPORTING PERIOD: <u>6 Sep 07 to 5 Sep 08</u>

PROJECT TITLE: The Leon Springs Pupfish Recovery Project at Diamond Y Draw: further monitoring of the population and the breeding habitat

OBJECTIVE(S):

To assess the status and to contribute to the recovery of the Leon Springs pupfish at Diamond Y Draw, Pecos County, Texas.

Summary Of Progress:

Please see Attachment A (see pdf file).

Significant Deviations:

None.

Location: Pecos County, Texas.

Cost: <u>available upon completion of grant.</u>

Prepared by: <u>Craig Farquhar</u>

Date: <u>5 December 2008</u>

Approved by: _

nijdorgulu

C. Craig Farquhar

Date: _____5 December 2008____

Final Report of the

ESA Section 6 Grant Proposal

To Texas Parks and Wildlife Department

The Leon Springs Pupfish Recovery Project at Diamond Y Draw:

Further Monitoring of the Population and the Breeding Habitat

Principle Investigator:

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Cooperating Partner

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Abstract

The intent of this work was to monitor the near extinct population *of Cyprinodon bovines* (the Leon Springs Pupfish) in Diamond Y Spring, Ft. Stock, Texas. To increased the breeding areas, in January 2007 we had removed emergent vegetation from several several square meters of shoreline and placed cement tiles on the substratum. Here we report on a modest increase in the number of territorial males and a similar number of satellite males. The newly available habitat did seem to cause a spreading of the territorial males which coincided with a dramatic decrease in the number of gambusia (*Gambusia nobilis*) egg predators around spawning fish. Given the amount of spawning we observed and the increased number of small pupfish, we anticipate larger numbers of territorial males in the succeeding years.

Introduction

The federally-endangered Leon Springs pupfish, *Cyprinodon bovinus*, is on the verge of extinction in its natural habitat in Diamond Y Draw. In May 2006 at Diamond Y Spring, we recorded approximately 10 adults (males and females) and no juveniles. In January-August 2007 with the support of an ESA Section 6 Grant from the Texas Parks and Wildlife Department (Itzkowitz 2006), we physically restored a small segment of the disappearing breeding habitat in Diamond Y Spring (see Itzkowitz 2007 for photograph). This is not the first restoration effort designed to protect the Leon Springs pupfish at Diamond Y Spring (see USFWS 1985). Echelle et al. (2001) replaced the resident hybridized pupfish population (see Echelle & Echelle 1997) with a pure strain from

DNFHTC, removed exotic species, and did a minor habitat restoration by removing some bulrush plants to increase the pupfish spawning area (see also Echelle et al 2004).

I have been observing this population every summer since May 2000, independent of Echelle et al. restoration. We have verified (Leiser & Itzkowitz 2003; Leiser et al. 2006) that in May 2001, a vibrant breeding population existed, with well over 25 territories established in the then newly-exposed habitat, and another 25 territories on a narrow shelf (Note: This shelf area has been one of the historical breeding localities for this species in Diamond Y Spring). Numerous adult females and nonterritorial males were also recorded. The success of the 2001 Echelle restoration was short-lived, perhaps because some of the recommendations offered at the conclusion of the Final Report (Echelle et al. 2001) (e.g., long-term monitoring of the population and continued control of the bulrush) were not followed.

This project was designed to ensure our restoration effort (Itzkowitz 2006) will be successful on a longer-term basis. This final report provides the results of the first year of a multi-year monitoring effort that was designed to investigate four important questions: (1) Are the historical and restored breeding sites at Diamond Y Spring being used at a maximal level by the pupfish? (2) Is the population sustaining itself and perhaps increasing? (3) Is there a migration of individuals from Diamond Y Spring to other parts of the Diamond Y Draw water system? (Note: Monitoring the effects of existing populations and habitats was suggested in the 1985 recovery plan; USFWS 1985, 1.38) (4) If the restored breeding site is not functioning as proposed, will further modifications be helpful? If the initial restoration is successful (see Itzkowitz 2006), then I propose (5) restoring more breeding habitat to other localities in Diamond Y Draw and then (6)

investigating whether the additional breeding sites provide spawning pupfish with relief from egg predation by the also endangered Pecos gambusia, *Gambusia nobilis* (Note: Studying the effects of such predators was suggested in the 1985 recovery plan; USFWS 1985, 1.13).

Objective:

To assess the status and to contribute to the recovery of the Leon Springs pupfish at Diamond Y Draw, Pecos County, Texas

Expected Results or Benefits

This proposed project is designed to assess the effects of a habitat restoration at Diamond Y Spring. If the restoration is successful, the benefit will be the development of a sustainable population of the Leon Springs pupfish. The extent to which this restoration is successful will be easily quantified by: (1) observable crowding of the historical breeding shelf in Diamond Y Spring; i.e., about 30 large males defending small territories (approximately 30 cm in diameter), a similar number of smaller "satellite" males that are also reproductively active, at least 60 breeding females, and a host of juveniles; (2) a similar number of territorial males, satellite males, females, and juveniles on the newly developed 30 sq. ft. of breeding habitat that will be near to the shelf; (3) an increasing total number of nonbreeding fish in the Diamond Y Spring, as observed over the next two summers, (4) no evidence of hybridization with other pupfish species; (5) the addition of

two restored breeding habitat in other parts of Diamond Y Draw; (6) the utilization of these two restored breeding areas by breeding pupfish; and (7) increasing numbers of pupfish throughout the water system.

At the present time, I agree with Echelle et al. (2001) that the decline in the pupfish population was caused by the invasion of bulrush into the pupfish's shallow breeding areas (perhaps due to a minimal lowering of the water level). Echelle et al. (2001) strongly recommended some type of bulrush control system be developed in the future. Our use of submerged tiles proposed here, and in my previous proposal (Itzkowitz 2006), should be highly effective in this regard. A secondary contributor to this decline in pupfish numbers was the egg predation by the Pecos gambusia. This was observed clearly when pairs of pupfish that spawned attracted swarms of Pecos gambusia that appeared to eat the newly deposited eggs (Gumm et al. in press). I expect that a critical benefit of this project will be to determine whether the increased number of territorial clusters will deter the Pecos gambusia from swarming near breeding pairs of pupfish. Given the different ecological requirements of the pupfish and the Pecos gambusia, we believe that expanding the breeding habitat of the pupfish will have no detrimental influence on the numbers of Pecos gambusia. Thus, besides increasing the number of pupfish territorial clusters to deter the Pecos gambusia, an additional benefit from this project will be to spread out the pupfish breeding population and thereby dilute the effect of the predatory Pecos gambusia.

Location:

Diamond Y is located 30'30 N 102 degree 55'00. The study site is about 8 NNE of Fort Stockton, Pecos County, Texas, West of State Highway 18 crossing Diamond Y Draw.

Methods and Results

Text in quote and in italics are taken from the ESA Section 6 Grant proposal to the Texas Parks and Wildlife Department. The text in **bold** indicate the results of this work.

Approach: "The over-arching objective is straightforward: to support a naturally reproducing population of the Leon Springs pupfish in its natural habitat. The three underlying objectives for this proposed project are: (1) to determine whether the habitat restoration (and possible restocking program) performed during Spring '07 are successful at producing a sustainable population; (2) to increase the number of restored breeding sites in other parts of Diamond Y Draw; and (3) to increase the number of pupfish throughout the Diamond Y Draw water system."

Tasks Overview:

"There are two separate components to this proposed project: First, an assessment of the restorations completed during the summer of 2008 in Diamond Y Spring. Based on this assessment (summer of 2008) we may increase the number of breeding sites to other parts of this water system. Second, whether or not we increase the number of breeding sites, we will monitor the breeding success and population size of the pupfish during the summer of 2008."

"Task 1: Monitor breeding. On a biweekly basis, we will videotape all territories on the breeding shelf and the restored breeding area. Typically I can record approximately 3 to 5 neighboring territorial males at the same time. Each segment will be recorded for 15 minutes. Over the 12-week period, we will have many records of the same locality and the same males.

Task 2: Analyze the videotapes. I have used such recordings with considerable success (e.g., Leiser & Itzkowitz 2003, Leiser et al. 2006) to get an estimate of breeding activities on both the micro (i.e., each male) and macro levels (i.e., community of males). Over the course of several days, nearly all breeding males will be recorded. At a later time, the following quantitative data will be taken from the video records: (a) the number of territorial males; (b) the size of their territories; (c) the number of times a female entered a territory and either spawned or did not spawn (if the female did spawn, where in the territory were the eggs deposited?); (d) the number of reproductively active nonterritorial males within the territory (note: these are called sneaker or satellite males and they are a critical component in the maintenance of genetic diversity); (e) the number of successful and unsuccessful spawnings of these nonterritorial males; (f) the egg predation by the Pecos gambusia."

Results (Tasks 1 & 2)

Although I proposed monitoring the Diamond Y Spring community during the 2008 summer, I made my initial visits during the second week of March 2008. Not unexpectedly, no male was territorial so early in the breeding season. However, I did observe approximately 8 adult-sized males (4 -5 cm) moving over the recently exposed areas and the natural spawning shelf.

Observations were continued in May, July and August of 2008. Two or three times each week, areas of the shelf were video recorded for 20 minutes each day. The behaviors were recorded of all territorial males present on both the historical breeding shelf as well as the newly restored breeding area. Videotapes were analyzed using methods similar to those in previous studies of these pupfish (e.g., Leiser & Itzkowitz 2003, Leiser et al. 2006) and will thus be directly comparable. Data collected was used to get an estimate of breeding activities on both the micro (i.e., each male) and macro levels (i.e., community of males).

Figure 1 summarizes the behavioral data collected during the 2008 summer. Across the 2008 summer we observed between 8 – 10 territorial males. Individual markings suggested that they remained consistent to a specific area, although some individuals wandered long distances. We observed an increasing number of chases against gambusia and conspecific individuals throughout the summer. This most certainly reflected a general increase in population size for both species. Interestingly, the number of females entering territories, females attempting to spawn, and the number of successful spawns remained consistent across the summer. The gradual increase in the number of chases against other pupfish was a reflection of the increase in the number of smaller, but adult-like conspecifics such

nonterritorial satellite and sneaker males, as the number of territorial males in each breeding area remained relatively constant. The satellite and sneaker males drift around the study site attempting to mate with females that had entered the territorial male's territory for spawning (Figure 2C).

Approximately 4 of the males consistently observed defended territories in the newly exposed areas but only a portion of their territories extended over the cement tiles. Instead their territorial behavior revolved around natural substrata in which the emergent weeds were removed (Fig 2 A-C). However, while territorial males seemed to prefer to defend territories on the natural substrata, females would frequently leave these areas and draw territorial males to spawn directly on the cement tiles (Fig 2D).

"Task 3: Estimate population sizes. There are many ways to assess population sizes but often they involve manipulations of the fish (e.g., fin clips, tags) that are probably inappropriate for such an endangered species. Instead, I propose placing funnel fish traps in the water for 24 hours, counting and measuring all trapped fish, and then releasing them. This will be done once per month in each of the three summer months. Over a 3-month period, I should be able to assess the status of the population adequately. I propose using 12 such traps in Diamond Y Spring. I also propose that such a sampling be done throughout the Diamond Y Draw watercourse. The intent is to determine whether an increasing Diamond Y Spring pupfish population will migrate into new areas of the Diamond Y Draw water system."

Results (Task 3)

We made frequent observations around the Diamond Y Spring and found no other pupfish either swimming in the open water or near the pond edge nor did we see any other territorial males in other areas of the pool. We also walked along the lower water course to determine if pupfish were found amongst the emergent vegetation. We found none. Our preliminary attempts to capture pupfish using funnel traps were all unsuccessful. Because of these observations, we believe nearly all pupfish in Diamond Y Spring occur on or near the natural and exposed shelves.

"Task 4: Additional Restoration. If the restorations at Diamond y Spring are successful, I propose establishing two additional breeding sites in other parts of the water course. Their design will be similar to that used in Diamond Y Spring, e.g., shoreline areas will receive 30 sq. ft. of roughened ceramic tiles approximately 15 cm below the water line (Itzkowitz 2006). These tiles will prevent the regrowth of bulrushes and will provide the hard substrata that are required for pupfish spawning. Specific locations in downstream areas will be chosen based on suitable habitat conditions (e.g., water depth, flow, and substrate). Along with these habitat modifications, I also propose adding between 200 – 400 adult pupfish from the stock held at DNFHTC, if earlier work shows this augmentation is warranted. This effort will be closely coordinated with the U.S. Fish and Wildlife Service and Texas Parks and Wildlife Department."

Results (Task 4)

We observed that the cement tiles were highly successful in keeping the newly exposed areas free from vegetation regrowth. This was not the case in areas where we simply cut the vegetation. Thus, it appears that simple removal of the bulrush is not sufficient to provide suitable habitat for pupfish breeding. Additional restoration should include the use of cement tiles or other substrate that will prevent the regrowth of vegetation around the exposed habitat.

In 2008, we did observe an increase in pupfish numbers from recent previous years (Gumm et al. in press), however, considerable available space for additional territories remained. As the population continues to grow and territorial males begin to utilize all available breeding areas, additional restoration areas will need to be considered and realized.

"Task 5: Gambusia Egg Predation. Evaluate the extent to which Pecos gambusia preys on pupfish eggs. The videotape record of pupfish territorial males will indicate the density of Pecos gambusia within the territories and the swarming of the Pecos gambusia around spawning pupfish pairs is an obvious indicator of their egg predation."

Results (Task 5)

Pre-restoration in 2006, it was observed that *G. nobilis* would swarm at the spawnings of the Leon Springs pupfish. It appeared that *G. nobilis* would respond to female Leon Springs pupfish that approached the substrate in order to spawn. On average, 19 ± 1 *G. nobilis* were present within one-fish body length of each

pupfish spawning. Significantly fewer *G. nobilis* were found randomly on the shelf at numbered tags (only 3 ± 0.2) (Gumm et al. in press).

As in the previous study in 2006, we used digital photographs to estimate the number of *G. nobilis* present at spawnings and arbitrarily throughout the shelf near numbered tags. The number of *G. nobilis* present at spawnings was significantly lower post-restoration in 2008. Numbers of *G. nobilis* found at numbered tags (2 ± 0.2) (Figure 2B) was similar to 2006 levels. However, just 4.8 ± 0.4 *G. nobilis* were present within one-fish body length of Leon Springs pupfish spawnings (Figure 2C). Comparison of the number of *G. nobilis* present at Leon Springs pupfish spawnings and at numbered tags pre- and post–restoration revealed significantly more *G. nobilis* at Leons Springs pupfish spawnings in 2006 as compared to numbered tags in either year or at spawnings in 2008 (ANOVA: $F_{3,254}$ 110.46, p < 0.001; Tukey HSD p < 0.001) (Fig 3).

Discussion

It is clear that the pupfish population during the 2008 summer is larger than during 2006 (in which there was only one territorial male, see Gumm et al. in press) and nearly double since 2007 (5 territorial males to 10 territorial males; see Itzkowitz 2007). We also observed a large number of smaller adult males exhibiting the satellite and sneaker alternative reproductive behavioral tactic while very few were observed in 2006. These smaller males have the potential to grow into larger territorial males. While the number of territorial males greatly increased over the past two years, it represents only about 30% of the size observed in 2002. However, we believe that the recent steady increase indicates that there are sufficient numbers

to 'seed' the next reproductive event in Spring 2009. We are especially encouraged by the presence of smaller individuals that were probably hatched in Spring 2008. We suspect these small individuals will become large territorial males and females in the early Spring 2009. For this reason I do not currently recommend any restocking with additional pupfish fish from the Dexter National Fish Hatchery and Technology Center.

Our behavioral observations from the Summer 2008 were reminiscent of those for dispersed territories in 2003 (Leiser & Itzkowitz 2003, Figures 4 & 5). That is, territories that are not clustered (i.e., have no territorial neighbors) in 2003 show similar numbers of females approaching males and similar numbers of females that did spawn to those observed in 2008 (comparison of Figures 1 & 4). Territories observed in clusters in 2003 appeared to be in higher quality habitat and that may explain the larger numbers of females that entered and spawned. Dispersed territories in 2003 received far more spawns per female than those observed for 2008 suggesting that females stayed longer and released more eggs. We cannot account for this decline in fecundity per female and we will carefully consider this aspect in subsequent years.

The pupfish did use the cement tiles in the restored habitat (Figure 2D). While this artificial substratum was sometimes used by females for spawning, the natural substratum was clearly more desirable to territorial males. More territorial males were found on the historic natural breeding shelf and those territorial males near the restored breeding shelf generally defended territories over natural substratum that had been cleared of vegetation with only occasional travel over the

cement tiles. We believe that as the population size increases, competition for space will cause males to defend these cement tiled areas as well.

In 2006, there was obvious egg predation by *Gambusia nobilis* (see Gumm et al. in press). This predation was problematic because both the gambusia and the pupfish are endangered. Our hypothesis that increasing the shallow areas would disperse the gambusia population and thus reduce their egg predation on pupfish was supported. With the dispersing of the gambusia population, territorial male pupfish seemed be more effective at chasing them from their territories and this also would seemed to reduce egg predation. It is our hope that a further increase in the numbers of side-by-side territorial males in the succeeding years would further increase their effectiveness at repelling gambusia.

In summary, our observations indicate that the restoration of some breeding habitat using cement tiles was successful as indicated by the reduction in the numbers of *G. nobilis* egg predators near spawning pairs and the increase in the overall number Leon Springs Pupfish.

Literature Cited

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- Leiser, J. K., Bryan, C. M., & M. Itzkowitz. 2006. Disruption of dear enemy recognition among neighboring males by female Leon Springs pupfish, *Cyprinodon bovinus*. *Ethology*. 112: 1-7.

United States Fish and Wildlife Service. 1985. Leon Springs pupfish recovery plan.



Figure 1. Mean + SE number of territorial male Leon Springs Pupfish and number of behaviors of territorial male Leon Springs Pupfish in May (black), July (white), and August (gray) of 2008. Behaviors occurred within territories throughout the breeding shelf at Diamond Y Spring and included: chases toward G. nobilis; chases toward other male Leon Springs Pupfish (territorial or satellite); individual spawnings; approaches from female Leon Springs Pupfish; and attempted (but failed) spawnings.



Figure 2. Leon Springs Pupfish at Diamond Y Springs 2008. (A) A large male Leon Springs Pupfish defends his territory. (B) Numbered tag used to delineate breeding shelf areas and estimate the relative distribution of *G. nobilis*. (C) Spawning of Leon Springs Pupfish surrounded by *G. nobilis*. A small sneaker or satellite male Leon Springs Pupfish approaches the spawning to attempt fertilization (yellow arrow). (D) Territorial male Leon Springs Pupfish spawning on the edge of one of the cement tiles in the restoration area.



Figure 3. Mean + SE *G. nobilis* on the Diamond Y breeding shelf arbitrarily distributed at numbered tags (white) and at Leon Springs Pupfish spawnings (black) pre-restoration (2006) and post-restoration (2008). After restoration, significantly fewer *G. nobilis* are found at Leon Springs Pupfish spawnings (* p < 0.001).



Figure 4. Mean + SE number of females that spawned or approached territorial males that were in clustered (white) and dispersed territories (black) (Taken from Leiser & Itzkowitz 2003).



Figure 5. Mean + SE number of total spawns received by clustered (white) and dispersed (black) territory residents. There was no significant difference in the total number of spawns between the two types of territories. (Taken from Leiser & Itzkowitz 2003)