# Final Report

# Spawning and Recruitment Study at the New Mexico Interstate Stream Commission Los Lunas Silvery Minnow Refugium

## Contract No. R09PC40009

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Prepared by

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## Period of Investigation

This federal contract is funded as a one-year contract with two option years for the Middle Rio Grande Endangered Species Collaborative Program project.

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## Introduction

Federal contract R09PC40009, issued by the U.S. Bureau of Reclamation (Reclamation), funds a research project on spawning preference of Rio Grande silvery minnow (RGSM; *Hybognathus amarus*) at the Los Lunas Silvery Minnow Refugium (Refugium), which is owned and operated by the New Mexico Interstate Stream Commission (NMISC), a member of the Middle Rio Grande Endangered Species Collaborative Program. This final report presents activities that have been performed or occurred in the calendar year 2011 and 2012.

The Refugium was designed and built by the NMISC to partially satisfy the Reasonable and Prudent Alternative (RPA) of the U.S. Fish and Wildlife Service (USFWS) 2003 Biological Opinion. The facility is located on the State of New Mexico's Los Lunas Program Campus. At the Refugium, there is an outdoor stream system, an indoor hatchery, an outdoor tank system, a quarantine building, and a storage building. Descriptions of the facility and its operations are in the Reclamation grant for its operations and maintenance and its U.S Fish and Wildlife Service endangered species research permit (USFWS TE Permit TE169770), and the facility and its operation are described in a peer-reviewed paper:

Tave, D., G. Haggerty, C.N. Medley, A.M. Hutson, and K.P. Ferjancic. 2011. Los Lunas silvery minnow refugium: a conservation hatchery. *World Aquaculture* 42(2):28-34, 67.

## Refugium personnel

The NMISC currently employs three full-time personnel to operate and manage the facility. Dr. Douglas Tave was hired in November 2007 as the Refugium Manager. Dr. Alison Hutson was hired in January 2009 as the Assistant Refugium Manager. Louie Toya was hired full-time in June 2008 as an engineering technician.

## USFWS TE Permit

At the beginning of 2011 we operated under TE Permit 169770-3.

We submitted TE Permit amendments to USFWS on February 18, 2011, to be able to conduct the following activities:

- Stock 750 Rio Grande silvery minnow (RGSM) for spawning in the outdoor refugium.
- Tag 100 RGSM with PIT tags and VIE tags to monitor fish movement throughout the spawning study.

We received a new TE Permit on April 27, 2011 that enabled us to conduct the project listed above and we currently operate under ESA permit TE169770-4.

## 2010 Spawning and Recruitment Study

2010 marked the first year of the spawning study. However, the Los Lunas Silvery Minnow Refugium was not permitted by US Fish and Wildlife Service to perform the study. Equipment was ordered and components of the study were set up to ensure our ability to perform the study the following year.

## 2011 Spawning and Recruitment Study

### Introduction

The proposal called for 750 adult RGSM. On March 29, 2011, 450 fish from Dexter National Fish Hatchery were received. One hundred forty fish remained in the indoor hatchery from earlier studies. Of these 580 fish, 89 were given a PIT tag and a VIE tag as a secondary marker on April 7, 2011. On April 28, all fish were stocked into the outdoor refugium. Fifty-three of these fish were PIT tagged.

#### *Outdoor refugium reconfiguration*

A major goal of the spawning study was to determine if fish would spawn in the stream or the overbank (or both). To do this, the outdoor refugium was reconfigured by building sand bag levees around the edges of the ponds, shelves, and overbank habitats. In the lower overbank levee, three 110-cm-wide gaps were made (Fig. 1). This configuration meant that fish could only use the stream and the lower overbank. The outdoor refugium is a unique system and replication of the study is not possible; this configuration meant we could compare the use of the stream to a single overbank.

#### *Velocity study*

Velocity profiles of the system were conducted from March 2 to April 11. Velocity profiles were conducted at various depths and pumping rates and during a flood to determine what the velocities and depths would be during the study. Velocities and depths were determined prior to stocking so that fish would not be disturbed during the study.

At flood stage, velocity in the stream center in front of the overbank area was 7.62 cm/s; that in the overbank ranged from -1.22 cm/s to 0.61 cm/s.

***Movement of artificial eggs***

To determine if eggs would drift between the two habitats, artificial semi-buoyant beads were placed in the stream and on the overbank areas during the pre-stocking flood on April 13. Movement of the beads was observed, and no bead placed in the stream moved onto the overbank area, and no bead placed in the overbank area moved to the stream. This meant that eggs observed in one of these habitats would be there because that is where the fish spawned.

***Outdoor refugium prepared for the study***

Following the velocity study, the outdoor refugium was lowered to a gate height of 34.3 cm. Pumping rate was set at 1,514 L/min.

PIT tag readers were placed over the three gaps in the lower overbank levee that separated the lower overbank from the stream. Two PIT tag readers were placed in the stream (Fig. 1).

A DIDSON sonar camera was placed in the stream, with the lens facing upstream to the overbank entrance (Fig. 1).

HOBO temperature loggers were placed in two locations in the stream and on the overbank to provide temperature profiles of the two habitats throughout the study (Fig. 1).

Three Moore egg collectors were set in the channel in front of the rotating barriers so that we could determine if spawning occurred in the stream (Fig. 1).

***Flood***

On May 11, a flood was created. The gate started at 34.3 cm and pumping rate at 1,514 L/min. Water was added and pumping rate and gate height were increased to induce a flood. On May 11 at 1535, the gate height was set at 63.7 cm, the pumping rate was 5,942 L/min, and the overbank area was inundated. On May 12 at 0630, the gate height was raised to final flood height at 66.2 cm with a pumping rate of 5,905 L.min. Flood conditions were maintained until May 24, when water level was dropped over a 50-hour period to a gate height of 41.6 cm. Pumping rate was not changed. At this point, there was no water on the overbank.

Two stream pulses were produced to see if fish would spawn in the channel with no water on the overbank. On May 27, gate height was brought up from 41.6 cm to 46.6 cm over 2 hours with a constant pumping rate of 5,961 L/min. Gate height was lowered on June 1 to 46.6 cm within 2 hours, with a constant pumping rate of 5,961 L/min. Because no spawning was detected during the two stream pulses, the study was ended on June 5.

***Monitoring fish movement***

PIT tag data was downloaded every 12 hours. DIDSON camera video was continuously stored on a computer.

Moore egg collectors were examined daily to determine if spawning occurred in the stream.

***Harvest***

The outdoor refugium was drained and fish harvested on June 5.

## Results

No spawns were observed during the study.

We captured over 52,000 pit-tag hits. The fish moved on and off the overbank during the duration of the flood. Fish appeared to move throughout the day and night with no discernible periodic movement.

Video of schools of fish was recorded with the DIDSON camera. Fish moved in and out of the field of vision throughout the day and night. Temperature profiles during the study are shown in Figs. 2-4.

Four hundred one fish were harvested (survival was 68%). Thirty-one harvested fish had pit-tags, giving a survival rate of 58.5% for the pit-tagged fish stocked in the outdoor refugium.

## 2012 Spawning and Recruitment Study

### Methods

Seven hundred (700) fish averaging 74 mm TL were received from Dexter National Fish hatchery on December 13,2011 and were brought into the indoor hatchery. Of the 700 fish, 100 fish received pit tags on January 9, 2012, with assistance from US Fish and Wildlife Service. Fish were stocked into the outdoor refugium on February 15. Of the 100 fish, 91 pit tagged fish were stocked from the group tagged on January 9, 2. Additionally, 10 fish used in the 2011 spawning study that had been pit tagged the previous year were stocked, along with 44 untagged survivors from the 2011 spawning study. All fish received a secondary VIE tag so that the pit tagged fish could be identified as such. 745 fish total were stocked. Fish were stocked early to ensure that they could acclimate to the system and allowed for natural seasonal changes from winter to spring. Following what would have been the 2012 runoff in the Middle Rio Grande, the first flood took place on April 24,.

The pit-tag MUX unit was not functioning because the polarities on the power source were reversed and an internal fuse was blown and the unit was sent in for repairs. All equipment remained in the outdoor refugium until repairs were made on the MUX box.

Three Moore egg collectors were set up at then bottom of the system to catch egg drifting in the current.

***Outdoor refugium reconfiguration***

A major goal of the spawning study was to determine if fish would spawn in the stream or the overbank (or both). To do this, the outdoor refugium was reconfigured by building sand bag levees around the edges of the ponds, shelves, and overbank habitats. In the lower overbank levee, three 110-cm-wide gaps were made (Fig. 1). This configuration meant that fish could only use the stream and the lower overbank. The outdoor refugium is a unique system and replication of the study is not possible; this configuration meant we could compare the use of the stream to a single overbank area.

***Outdoor refugium prepared for the study***

Following the velocity study, the outdoor refugium was lowered to a gate height of 34.3 cm. Pumping rate was set at 1,514 L/min.

PIT tag readers were placed over the three gaps in the lower overbank levee that separated the lower overbank from the stream. Two PIT tag readers were placed in the stream (Fig. 1).

A DIDSON sonar camera was placed in the stream, with the lens facing upstream to the overbank entrance (Fig. 1).

HOBO temperature loggers were placed in two locations in the stream and on the overbank to provide temperature profiles of the two habitats throughout the study (Fig. 1).

Three Moore egg collectors were set in the channel in front of the rotating barriers so that we could determine if spawning occurred in the stream (Fig. 1).

***Flood***

On April 24, a flood was created. The gate started at 35.6 cm and pumping rate at 1,893 L/min. Water was added and pumping rate and gate height were increased to induce a flood. At 1456, the gate height was set at 68.6 cm, the pumping rate was 6,284 L/min, and the overbank area was inundated. Flood conditions were maintained until April 29, when water level was dropped over a 30.5-hour period to a gate height 61.0 cm. The pumping rate was lowered to 3,406 L/min. At this time, the overbank area was no longer inundated. On May 7, a second flood was created. The gate height was set at 61.0 cm and by 1319, the gate height was raised to 68.6 cm. Pumping rate began at 3,142 L/min at 0710 and was increased to 5,678 L/min. This flood was held until May 15, when the gate height decreased to 63.5 cm. The flood was brought down to culture level on May 17, 2012. A third flood was initiated on May 22 with all equipment including PIT

tag readers (MUX box) were functional. The final flood was brought down on May 29 to culture level (23.5").

***Monitoring fish movement***

PIT tag data was downloaded every 24 hours. The DIDSON was monitored daily to ensure the information was being properly recorded and saved. The pit tag readers were relocated from the 2011 experiment when all of the larval fish were found in ponds. On May 21, readers were moved to the inlets of ponds 2 and 4. The pit tag readers were up and running for the 3rd and final flood, occurring on May 22nd.

Moore egg collectors were examined daily to determine if spawning occurred in the stream.

***Harvest***

Fish will be harvested in October.

***Fish spawned***

RGSM spawned in response to the flood induced on April 24 (first flood). The fish spawned in response to the change in water depth and velocity. Turbidity was essentially zero thought the study. These results show that turbidity is not a needed environmental cue for spawning. However, it is not known if more fish would have spawned had turbidly been greater.

Larval fish were found on May 10 in Ponds 3, 4, and 5. It is likely that this is where spawning occurred. Eggs were never found in the Moore egg collectors, suggesting that no spawning occurred in the stream. No eggs were detected in the overbank area, suggesting that spawning did not occur there.

Brood fish entered Ponds 3, 4, and 5 during the first flood. The sand bags were installed in January, and many of the bags were frozen and had minor curves that could not be straightened. When the weather warmed, the sand bags thawed, and there was minor settling. The difference in sand bag levee height was not detectable until the first flood when small sections were 1-3 cm below water level. When the flood occurred, the brood fish entered Ponds 3, 4, and 5 via these 1-3 cm entrances and used the ponds as flood-produced overbank areas and spawned here. Brood fish were observed entering the ponds at these shallow entrances. It is interesting that they chose these low-velocity (basically 0 m/sec) deep-water off-channel areas to spawn instead of the shallow low-velocity (basically 0 m/sec) off-channel overbank area to spawn.

A second group of larval fish was observed on May 23. This suggests that a spawn occurred in response to the second flood. However, it was impossible to differentiate fish spawned from the two flood events.

The third flood induced no additional spawning.

It is unknown how many fish spawned or how many offspring were produced.

*Growth of YOY*

Lengths and weights of YOY are shown in Table 1. Growth was exceptional. Adjusted mean harvest length (TL) was 75.98 mm and 3.86 g. Growth at all sample periods and at harvest increased significantly ($P = 0.05$). Fish grew so well, that it was impossible to identify adults from YOY (except for the VIE-tagged fish). We did not tag all brood fish for two reasons. The initial experimental design was to drain the outdoor refugium after spawning, and to then refill it and stock it with YOY produced at Dexter. US Fish and Wildlife Service kindly allowed us to alter the experiment and to raise the naturally-produced YOY. Secondly, all brood fish were not pit tagged because we never thought that YOY could grow to the size they did in a single summer. The fact that YOY grew so large demonstrated the growth potential of this species.

*Survival*

Twenty-three pit tagged fish were harvested, so survival of pit tagged brood fish was 23%, and it was estimated that overall brood fish survival was the same; based on this assumption 172 of 745 brood fish survived.

It is impossible to determine survival of the YOY, because we do not know how many were produced.

Eight hundred twenty five fish were harvested (an estimated 172 of these were adults). The following number of YOY were removed prior to harvest for various projects: 100 for the fatty acid study (see Project 6 below); 8 for observation in an aquarium (see Project 9 below); 15 for HAI; 77 for the gut-study (see Project 5 below). Total number removed prior to harvest was 170. Consequently, we know that at minimum of 823 YOY were produced.

*Water temperature during spawning experiment*

Figure 1 profiles temperature during the spawning study.

*Behavioral observations*

For the first few days after stocking, brood fish were observed in all parts of the stream, but after that, they had coalesced into a single school near the base of the stream. When disturbed, they would move a few meters up or downstream, but would always return to that area. Prior to the spawning study in 2011, water velocities were determined throughout the system at the various water depths and pumping rates that would be used during the spawning study. This was done to enable us to know the velocity at a given location without disturbing the fish. The location preferred by the brood fish prior to the flood had a water depth of 35.6 cm and a velocity of 0.12 m/s.

The brood fish responded to the first flood by leaving the stream and moved into off-channel area. Some brood fish were observed on the overbank, and movement onto and off of the overbank was observed. Most brood fish moved into Ponds 3, 4, and 5, and remained there until the spawning part of the study was over and the sand bag levee was removed. Brood fish were observed entering the ponds over the shallow opening caused by the settling of the sand bags.

When the water from the second flood was brought down on May 15, YOY respond to the change in water level and swam actively from the ponds into the channel. The fish were not measured, but were 8-10 mm TL. The fish remained in the channel and stayed in the slow-moving water along the 2 to 4-cm deep edge of the channel for the remainder of the spawning part of the study. These fish were observed feed on the bottom.

Schooling behavior started shortly after the fish were free swimming. Small schools of 10-mm fish were observed. Fish schooled by size.

Three "egg bound" females were found dead on May 21. This suggests that even though a spawn was produced in response to a flood, the environmental cues were not sufficient to induce all of the fish that were ready to spawn to spawn.

Fish of all ages and sizes were most frequently observed grazing along the bottom. Fish were seldom observed between mid-water and the surface.

After the sand bag levees were removed and the outdoor refugium was changed to normal culture condition, brood fish continued to remain in the ponds most of the time. They normally inhabited the deepest areas of Ponds 2 and 4. A temperature/DO depth profile of Ponds 2 and 4 on August 27 and 28 showed little change for either parameter from top to bottom, so it is unlike that these were the reasons the fish chose this habitat. For example, on August 14 at 1420, temperature and DO 15 cm below the surface were 27.9°C and 7.77 mg/L, respectively, and readings at the bottom (59 cm below the surface) were 26.9°C and 11.09 mg/L.

Table 1. Mean±SD for length and weight at the four samples and at harvest. Means for the harvest data were adjusted, as described in the text. Means followed by different letters were significant at P = 0.05.

| Sample | Length (mm) | Weight (g) |
|---|---|---|
| July 3 | 34.70±3.79[a] | 0.38±0.12[a] |
| July 27 | 47.88±9.81[b] | 1.22±0.27[b] |
| August 17 | 59.40±2.86[c] | 2.14±0.41[c] |
| September 19 | 69.42±4.78[d] | 3.00±0.71[d] |
| Harvest (October 22-24) | 75.98±4.45[e] | 3.86±0.80[e] |

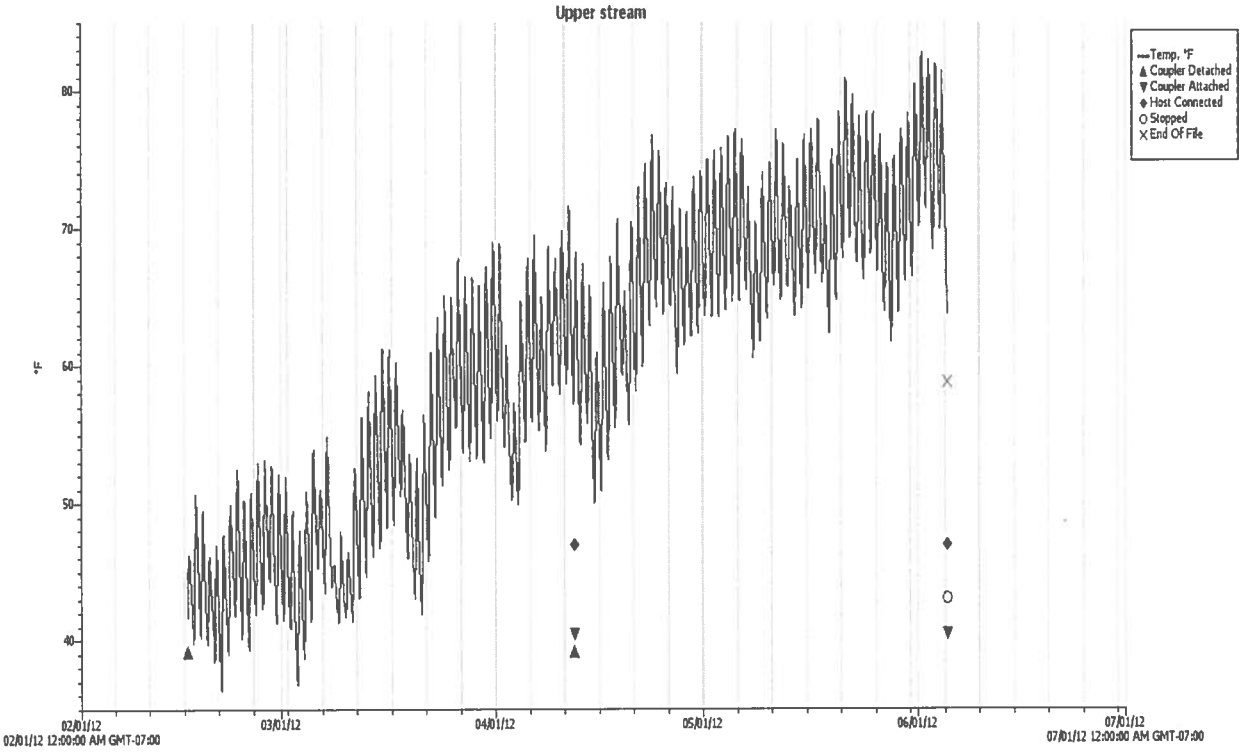


**Figure 1. Temperature of the channel in the outdoor refugium during the duration of the spawning study.**

**Plans for Next Quarter:** This serves as the final report for the study.

**Project state:** The project is ending in February.

**Changes affecting program elements:** None

**Financials**

The project is currently within its budget. Financial reimbursements are up to date.