Leon Springs pupfish
(Cyprinodon bovinus)

5-Year Review:
Summary and Evaluation

U.S. Fish and Wildlife Service
Austin Ecological Services Field Office
Austin, TX
August 2013

5-YEAR REVIEW

Species reviewed: Leon Springs pupfish (*Cyprinodon bovinus*)

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Cover photo: Male (above) and female (below) of Leon Springs pupfish, *Cyprinodon bovinus*. Courtesy of Braz Walker.

5-YEAR REVIEW
Leon Springs pupfish (*Cyprinodon bovinus)*

1.0 GENERAL INFORMATION

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1.2 Purpose of 5-Year Reviews:

The U.S. Fish and Wildlife Service (Service or USFWS) is required by section 4(c)(2) of the Endangered Species Act (Act) to conduct a status review of each listed species once every 5 years. The purpose of a 5-year review is to evaluate whether or not the species' status has changed since it was listed (or since the most recent 5-year review). Based on the 5-year review, we recommend whether the species should be removed from the list of endangered and threatened species, be changed in status from endangered to threatened, or be changed in status from threatened to endangered. Our original listing as endangered or threatened is based on the species' status considering the five threat factors described in section 4(a)(1) of the Act. These same five factors are considered in any subsequent reclassification or delisting decisions. In the 5-year review, we consider the best available scientific and commercial data on the species, and focus on new information available since the species was listed or last reviewed. If we recommend a change in listing status based on the results of the 5-year review, we must propose to do so through a separate rule-making process including public review and comment.

1.3 Methodology used to complete the review:

Public notice for this review was published in the Federal Register on March 20, 2008 (73 FR 14995). This review was conducted by Austin Ecological Services Field Office staff using information from the Leon Springs Pupfish Recovery Plan (USFWS 1985), peer-reviewed articles, agency reports, and other documents available in the Austin ES Field Office files.

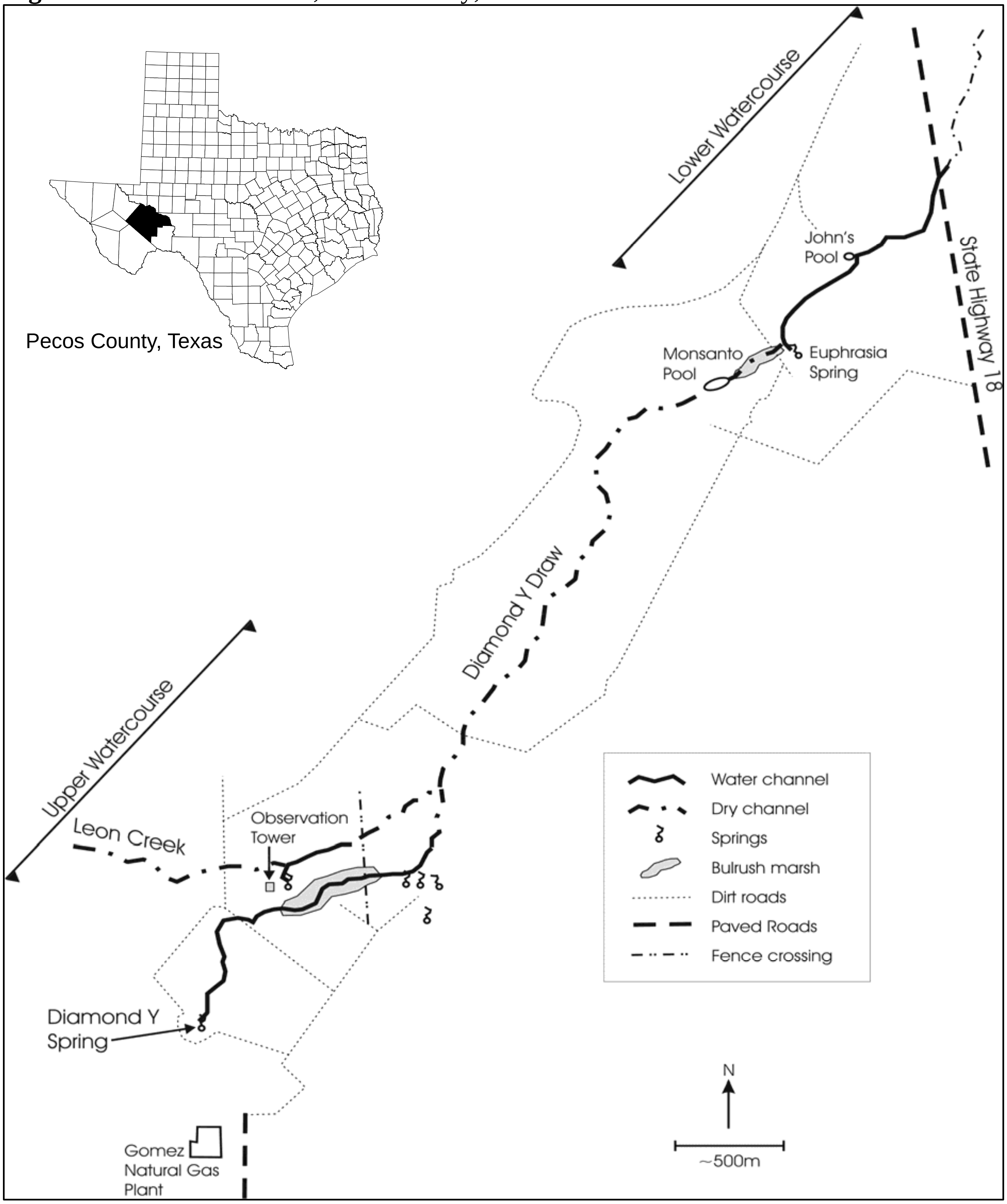
1.4 Background:

The purpose of this 5-year review is to examine new information and determine whether the Leon Springs pupfish (*Cyprinodon bovinus*) is appropriately listed as endangered and whether its listing classification should be changed (for example, de-listed, changed from endangered to threatened). The 5-year review examines new relevant information and documents a determination by the USFWS regarding whether the status of the pupfish has changed since the last status review, which in this case was conducted at the time of listing in 1980 (45 Federal Register 54678), and during development of the recovery plan (USFWS 1985). The review also provides updated information on the current threats to the species, ongoing conservation efforts, and the priority needs for future conservation actions.

The Leon Springs pupfish is a small, robust pupfish, up to 2 inches (in) [50 millimeters (mm)] long (Echelle and Miller 1974, p. 181). This species is a generalist in food habits, feeding predominantly on diatoms, marl, algae, amphipods and gastropods (Kennedy 1977, p. 99). Males exhibit “pit-digging” behavior, in which rounded pits are constructed in soft substrates, likely to locate buried food items (Kennedy 1977, p. 100). The Leon Springs pupfish is sexually dimorphic (males are larger and more brightly colored than females) (Echelle and Miller 1974, p. 186). The pupfish has an extended breeding season, with the greatest reproduction occurring in July (Kennedy 1977, p. 96). Pupfish reach sexual maturity at 1 in (29 mm) standard length (Kennedy 1977, p. 97); maximum lifespan is 20-23 months in the wild (Kennedy 1977, p. 96). Populations fluctuate based on seasonal conditions, peaking in September, October, and November (Kennedy 1977, p. 101). The pupfish has wide salinity and temperature tolerances (Kennedy 1977, p. 99). Kennedy (1977) provides additional life-history information and Echelle and Miller (1974) provide morphological details for this species.

Historically, this species inhabited Leon Springs, a system that once flowed in the Leon Creek drainage about 6 miles (mi) [10 kilometers (km)] west of Fort Stockton, Pecos County, Texas (Baird and Girard 1853, p. 389-390; Hubbs et al. 2008, p. 41); however, this spring went dry by 1958 (Scudday 1977, p. 515). Subsequent attempts to collect this species were unsuccessful and it was believed to be extinct until it was discovered in Diamond Y Spring in 1965 (Echelle and Miller 1974, p. 179) (Figure 1). Currently, the pupfish only occurs in Diamond Y Draw drainage, a flood tributary of the Pecos River in western Texas, located 10 mi (16.1 km) north of Fort Stockton, owned and acquired by The Nature Conservancy in 1990 (Hubbs et al. 2008, p. 41). Diamond Y Draw consists of an upper and lower watercourse separated by about 0.6 mi (1 km) of dry stream channel. The upper watercourse consists of Diamond Y Spring and its outflow, which holds the most stable pupfish population (Echelle et al. 2004, p. 131). The lower watercourse has a small headpool spring (Euphrasia Spring) and outflow stream, as well as several isolated pools including Monsanto Pool and Lower Monsanto Pool.

Figure 1: Diamond Y Draw, Pecos County, Texas.



In 1974, non-native sheepshead minnows (*Cyprinodon variegatus*) were discovered in the lower watercourse (Echelle and Echelle 1997, pp. 154-155; Garrett 1979, p. 55), likely introduced by the transport of live bait from the Gulf Coast to the Pacific Coast (Hubbs 1980, p. 5). By 1974, an extensive hybrid population had established in the lower watercourse (Hubbs 1980, p. 5), but hybrids were absent from the upper watercourse (Echelle and Echelle 1997, p. 154). As a precaution to protect the native Leon Springs pupfish genome, individuals were collected in 1976 from the upper watercourse and a population was established at the Southwestern Native Aquatic Resources and Recovery Center (SNARRC), formerly known as Dexter National Fish Hatchery and Technology Center (Garrett 1979, p. 55; Hubbs 1980, p. 6). Subsequent genetic testing showed that the captive population was free of non-native genes; however, the wild population in the lower watercourse had experienced complete genetic introgression from the invasive sheepshead minnows (Echelle and Echelle 1997, pp. 154,160). Extensive efforts were employed to eradicate the hybrid genome and the efforts were reported as successful (Hubbs 1980, p. 6-8). However, sheepshead minnows were again introduced into Diamond Y Draw apparently in the late 1980s or early 1990s, and the populations in both the upper and the lower watercourse were genetically introgressed by 1994 (Echelle and Echelle 1997, p. 154; Echelle et al. 2004, p.131). This precipitated a second effort to eradicate the invasive genome in 1998-2000 (Echelle et al. 2001, p. 4) and implement small-scale habitat improvements by manually removing bulrush, (*Scirpus americanus*) an aquatic plant that encroaches into, and eliminates, the shallow, open-water, breeding habitat of the pupfish (Echelle et al. 2004, p. 132).

1.4.1 FR Notice citation announcing initiation of this review: 73 FR 14995

1.4.2 Listing history

Original Listing
FR notice: 45 FR 54678
Date listed: 8/15/1980
Entity listed: Leon Springs pupfish (*Cyprinodon bovinus*)
Classification: Endangered.

1.4.3 Associated rulemakings: 8/15/1980, 45 FR 54678-54681: Designation of critical habitat

1.4.4 Review History: No other 5-year reviews have been prepared for this species.

1.4.5 Species' Recovery Priority Number at start of 5-year review: 2

1.4.6 Recovery Plan or Outline

Name of plan or outline: Leon Springs Pupfish Recovery Plan
Date issued: 08/14/1985
Dates of previous revisions, if applicable: N/A

2.0 REVIEW ANALYSIS

2.1 Application of the 1996 Distinct Population Segment (DPS) policy

2.1.1 Is the species under review a vertebrate? Yes

2.1.2 Is the species under review listed as a DPS? No

2.1.3 Is there relevant new information for this species regarding the application of the DPS policy? No

2.2 Recovery Criteria

2.2.1 Does the species have a final, approved recovery plan? Yes

2.2.1.1 Does the recovery plan contain objective, measurable criteria? No

The recovery plan does not contain measurable criteria. The goal and prime objective of the plan is to improve the status of the Leon Springs pupfish to the point that survival is secured and viable populations of all morphotypes are maintained in the wild (USFWS 1985, p. 11). This goal and objective has not been met. The plan states that while it may be possible to down list the species, it may not be possible to delist it because of extreme limited habitat.

The recovery plan lists four main recovery actions for the Leon Springs pupfish: 1) maintain and enhance existing Leon Springs pupfish population and habitats, 2) maintain genetic reserves of Leon Springs pupfish in captivity, 3) disseminate information about Leon Springs pupfish, and 4) enforce State and Federal laws protecting the Leon Springs pupfish and its habitats (USFWS 1985, pp. 9-17).

The survival of the species has not been secured. The species has experienced two known genetic introgression events in the Diamond Y Spring system with the non-native sheepshead minnow, the most recent event occurring in the late 1980s or early 1990s, followed by native genome restoration efforts from 1998-2000. These efforts were mostly successful, and required reintroduction of genetically pure fish held in refugia at SNARRC (Echelle et al. 2001, p. 4). Additionally, this species experienced a dramatic decline in numbers between 2000 and 2006, presumably due to breeding habitat loss and predation of eggs by Pecos gambusia (*Gambusia nobilis)*(Gumm et al. 2008, p. 656; Itzkowitz 2010, p. 9). Maintaning suitable habitat for this species has proven difficult, as bulrush encroachment has resulted in a substantial loss of suitable spawning areas (Itzkowitz 2010, pp. 13-14).

2.3 Updated Information and Current Species Status

2.3.1 Biology and Habitat

2.3.1.1 New information on the species' biology and life history:

The pupfish requires hard substrate in shallow water (2-6 in [5-15 cm] deep) for spawning (Leiser and Itzkowitz 2003, p. 101). Males defend territories, driving away other males and potential egg predators (Leiser et al. 2006, pp. 419-420). Territory size can range from 4-27 sq ft (130-820 sq cm) (Leiser et al. 2006, p. 419). Spawning events are brief, with the female laying one egg; many spawning events may occur in succession (Leiser et al. 2006, p. 420). About one quarter of spawning events may be interrupted by a conspecific male, which is attacked by the resident male (Leiser et al. 2006, p. 420).

For more data regarding reproductive behavior, see Leiser and Itzkowitz 2003; Leiser et al. 2006; Gumm et al. 2008.

2.3.1.2 Abundance, population trends (for example increasing, decreasing, stable), demographic features (for example, age structure, sex ratio, family size, birth rate, age at mortality, mortality rate, etc.), or demographic trends:

Between 2000 and 2006, the Leon Springs pupfish population experienced a dramatic decline in numbers in the upper watercourse of Diamond Y Draw (Allan and Gumm 2007, p. 13; Itzkowitz 2010, p. 25). This decline was likely due to the loss of breeding habitat from the encroachment of bulrush; this loss of breeding habitat also created an increase in the number of sympatric Pecos gambusia associated with pupfish spawning events (Itzkowitz 2007, p. 2; Itzkowitz 2010, p. 25; Itzkowitz 2011, p. 1). The Pecos gambusia, an endangered species, preys on the eggs of the pupfish (Itzkowitz 2011, p. 1). In 2007, an ESA Section 6 Grant from the USFWS through the Texas Parks and Wildlife Department was provided to restore a small segment of pupfish breeding habitat (by physically removing bulrush and enhancing the habitat with submerged tiles) and to implement a three-year monitoring program to assess whether these efforts had any effect on population size (Itzkowitz 2007, pp. 1-2). By the spring of 2007, the population had responded positively; researchers observed an increase in the number of territorial and satellite males and a dispersion of gambusia (for example, fewer gambusia present at spawning events) (Allan and Gumm 2007, p. 13; Itzkowitz 2010, pp. 9, 25). The sex ratio of the species is presumed to be 1:1 (Itzkowitz 2011, p. 1).

In the upper watercourse area, the headwaters of Diamond Y Spring hold the most stable population of Leon Springs pupfish (Echelle et al. 2004, p. 13; Figure 1). Population approximations in May 2011 found roughly 40 males in the spring (Itzkowitz 2011, p. 1). The lower watercourse area consists of the Lower Monsanto Pool and several shallow runoff pools (Itzkowitz 2010, pp. 11-12). Population approximations in 2009 and 2010 estimated roughly 80 adult fish; however, no evidence of reproduction was observed during this time period in the lower pool (Itzkowitz 2010, p. 12). Observations showed that most pupfish resided in the southern edge of the pool within 6 in (15 cm) of the surface and were not utilizing the deeper areas (Itzkowitz 2010, pp. 11-12). Prior to 2009, no pupfish had been reported in the shallow runoff pools (Itzkowitz 2010, p. 12). In 2009, however, 25 male and 19 female pupfish were observed in one such pool (82 sq ft [25 sq m], with an average depth of 8 in [20 cm]), that was connected to the Lower Monsanto Pool by an overflow (for example, water bridge that had overflow from Monsanto Pool going into the smaller pool) (Itzkowitz 2010, p. 12-13). This pool possessed little hard substrate, the preferred substrate for spawning of pupfish; however, there was evidence of a small amount of reproductive activity (Itzkowitz 2010, pp. 12-13). After a vegetation burn by The Nature Conservancy, a subsequent growth of bulrush decreased available habitat and blocked the water bridge by the spring of 2010 (Itzkowitz 2010, pp. 13-14). In May, 2011, Itzkowitz (2011, p. 1-2) observed a “substantial” pupfish community in Lower Monsanto Pool; however, the Lower Monsanto Pool population has experienced an extensive loss of breeding habitat due to the encroachment of bulrush (Itzkowitz 2011, p. 1-2). Current population numbers remain relatively low, putting the species at considerable risk of extinction from subtle habitat changes and inbreeding events (Itzkowitz 2010, p. 22).

Between 1998 and 2000, which was the most recent attempt to eradicate the non-native genome from the Leon Springs pupfish population, Diamond Y Spring and its outflow were treated with the ichthyocide Antimycin A. The Antimycin A treatment also eliminated two non-native fishes from the system, the largespring gambusia (*Gambusia geiseri*) and the common carp (*Cyprinus carpio*)(Echelle et al. 2001, p. 2). It was noted that pupfish were significantly more abundant post-treatment in the headpool of Diamond Y Spring than in the past. This coincided with the disappearance of dense growths of filamentous algae and pond weed, an effect that increased the amount of open, shallow-water habitat preferred by the pupfish. This was presumed due to the removal of the carp, which may have maintained the vegetation as a result of their feeding activities, which are known to move nutrients from the sediment back into the water column (Echelle et al. 2001, p. 2).

2.3.1.3 Genetics, genetic variation, or trends in genetic variation (for example, loss of genetic variation, genetic drift, inbreeding, etc.):

The Leon Springs pupfish has experienced two occurrences of introgressive hybridization with the non-native sheepshead minnow. Both occurrences were followed by extensive efforts to remove the invasive genes from the population (Allan and Gumm 2007, pp. 12-13). The first eradication effort proved successful, with subsequent genetic testing of pupfish showing no non-native genes remaining in the population (Hubbs 1980, p. 6-8).

During the second restoration effort, the Diamond Y Spring headpool and approximately 1640 ft (500 m) of its outflow channel were treated with the ichthytoxin Antimycin A, followed by the release of genetically pure pupfish from SNARRC (Echelle et al. 2001, p. 8). Restoration in other downstream areas of Diamond Y Draw did not include Antimycin A treatment but did include physically removing fish (for example, seining, dipnetting) and stocking the habitat with large numbers of fish from SNARRC (Echelle et al. 2001, p. 9; Echelle et al. 2004, p. 132). The last genetic analysis revealed that the upper watercourse appeared to have no non-native genetic introgression and there was less than 1 percent genetic introgression in the lower watercourse. However, a small population near the observation tower had higher levels of genetic introgression (4.2 percent) that may not be acceptable, meaning that it could endanger the genome. Echelle et al. (2004, p. 135) suggested that present levels of genetic introgression in both watercourses were acceptable post-treatment with the exception of the population near the observation tower. The level of genetic introgression that is considered acceptable is debated among geneticists. Some geneticists contend that genetic introgression at less than 1 percent is acceptable while others accept a higher level depending on site-specific conditions (Echelle et al. 2004, p. 135; Echelle et al 2001, p. 22). Echelle et al. (2004, p. 135) suggested additional releases of the pupfish stock from SNARRC near the observation tower to dilute the non-native genes in this population. This population threatens the rest of the Diamond Y system because it is in the mainstream of the Diamond Y draw and could expand if water levels increased and connected this normally isolated location to other reaches (Echelle et al. 2004, p. 135).

In addition to having the highest level of non-native genes, the population near the observation tower appears to have undergone a severe bottleneck, which reduced variability (Echelle et al. 2001, p. 21). Genetic analysis revealed that the captive population is free of non-native genes (Echelle and Echelle 1997, pp. 154,160) and demonstrated levels of genetic variability similar to the wild population, although some rare alleles might have been lost in the captive stock (Edds and Echelle 1989, p. 443).

2.3.1.4 Taxonomic classification or changes in nomenclature:

The Leon Springs pupfish was first described as *Cyprinodon bovinus* in 1853 (Baird and Girard 1853, pp. 389-390). No changes in classification or nomenclature have occurred since that time (Nelson et al., 2004).

2.3.1.5 Spatial distribution, trends in spatial distribution (for example, increasingly fragmented, increased numbers of corridors, etc.), or historic range (for example, corrections to the historical range, change in distribution of the species' within its historic range, etc.):

Please see the historical distribution information in the background section (section 1.3) and Figure 1.

No significant changes in spatial distribution or trends in spatial distribution have been documented since the listing of the species in 1980. In the upper watercourse, Diamond Y Spring typically sustains the most stable population of Leon Springs pupfish (Echelle et al. 2004, p. 131). Pupfish have also been documented near the observation platform in shallow pools 1.6 mi (2.5 km) upstream from the confluence of Leon Creek and a marsh fed by Diamond Y Spring, and within a 1.2 mi (2 km) long section in the upper watercourse which receives flow from the marsh fed by Diamond Y Spring. Pupfish have also been observed within the lower watercourse of Diamond Y Draw, which includes the Lower Monsanto Area (Echelle et al. 1987, p. 670).

2.3.1.6 Habitat or ecosystem conditions (for example, amount, distribution, and suitability of the habitat or ecosystem):

Diamond Y Draw is located within a preserve purchased by The Nature Conservancy in 1990 (Karges 2003, p. 144). The Nature Conservancy actively manages for invasive plant species along the watercourse, including mesquite (*Prosopis glandulosa*) and salt cedar (*Tamarix ramosissima*), using a combination of mechanical removal, herbicide application, and prescribed fire (Karges 2003, p. 145).

Diamond Y Draw is composed of unconnected upper and lower watercourses, separated by dry stream channel. The deep and shallow areas are contiguous in Diamond Y Spring, whereas shallow pools of the lower watercourse are dependent on overflows (for example, water bridges) from the deep pools (Itzkowitz 2010, p. 14). Researchers believe that it is important that these corridors remain open so that the pupfish can travel to the deep pool to escape low temperatures in the winter months and return to the shallow pools in warmer months to breed (Itzkowitz 2010, p. 14). Observations in May, 2011 in the lower watercourse revealed that encroachment of bulrush had filled the shallow pool and completely eliminated the water bridge (Itzkowitz 2011, p. 2). It appears that the Lower Monsanto Area population may be trapped in the deep pool with no access to spawning areas (Itzkowitz 2011, p. 2).

In 2007, bulrush was removed in a portion of the shoreline in Diamond Y Springs and the substrate was covered with cement tiles to prevent bulrush growth (Itzkowitz 2010, p. 8). The substrate alteration reduced the number of Pecos gambusia that clustered around pupfish spawning territories (for example, the Pecos gambusia became more dispersed), and thus reduced predation of the gambusia on pupfish eggs (Itzkowitz 2010, pp. 9, 11). Additionally, the pupfish used the cement tiles for spawning (Itzkowitz 2010, p. 20).

2.3.2 Five-Factor Analysis (threats, conservation measures, and regulatory mechanisms)

2.3.2.1 Present or threatened destruction, modification or curtailment of its habitat or range:

Spring flow loss

A major threat to this species is the potential failure of spring flow due to groundwater pumping or drought, which would result in total habitat loss for the species (USFWS 1985, p. 6, USFWS 1980, p. 54678). Diamond Y Spring is the last major spring still flowing in Pecos County, Texas. Pumping of the regional aquifer system for agricultural production of crops has resulted in the drying of most other springs in this region (Brune 1981, p. 356). Other springs that have already failed include nearby Comanche Springs and Leon Springs (Brune 1981, p. 358; Scudday 1977, p. 515). Comanche Springs was once a large surface spring in Fort Stockton, Texas, about 8 mi (13 km) from Diamond Y Draw that flowed at more than 42 cubic feet per second (cfs) [1200 liters per second (lps)] (Brune 1981, p. 358). Comanche Springs ceased flowing by 1956, resulting in the extirpation of many species of fish, amphibians, and other fauna (Scudday 1977, p. 516). Leon Springs, located upstream of Diamond Y in the Leon Creek watershed, was measured at 18 cfs (500 lps) in the 1930s and was also known to contain rare fish, but ceased flowing in the 1950s following significant irrigation pumping (Brune 1981, pp. 358-359).

The Diamond Y upper watercourse was estimated by Veni (1991, p. 86) to have a total discharge of 0.05 to .08 cubic meters per second (cms) (2 to 3 cfs), and total discharge from the lower watercourse of 0.04 to 0.05 cms (1 to 2 cfs). The nature of the system with many diffuse and unconfined small springs and seeps makes the estimates of water quantity discharging from the spring system difficult to attain. Additionally, there have been no continuous records of spring flow discharge at Diamond Y Spring to determine any trends in spring flow. However, many authors (Veni 1991, p. 86; Echelle et al. 2001, p. 27; Karges 2003, pp. 144–145) have described the reductions in available surface waters observed compared to older descriptions of the area (Kennedy 1977, p. 93; Hubbs et al. 1978, p. 489; Taylor 1985, pp. 4, 15, 21). The amount of aquatic habitat at Diamond Y may vary to some degree based on annual and seasonal conditions, but the overall trend in the reduction in the amount of surface water over the last several decades is apparent. The precise reason for the declining spring flows remains uncertain, but it is presumed to be related to a combination of groundwater pumping, mainly for agricultural irrigation, and a lack of natural recharge to the supporting aquifers. In addition, future changes in the regional climate are expected to exacerbate declining flows.

Studies by Veni (1991, p. 77) and Boghici (1997, p. *v*) indicate that the spring flow at Diamond Y Spring comes from the local aquifers located west of the spring outlets. Initial studies of the Diamond Y Spring system suggested that the Edwards-Trinity aquifer was the primary source of flows (Veni 1991, p. 86). However, later studies seem to confirm that the Rustler aquifer is instead more likely the chief source of water (Boghici 1997, p. 107). The Rustler aquifer is one of the less-studied aquifers in Texas and encompasses most of Reeves County and parts of Culberson, Pecos, Loving, and Ward Counties in the Delaware Basin of west Texas (Boghici and Van Broekhoven 2001, pp. 209–210). The Rustler strata are thought to be between about 75 and 200 m (250 and 670 ft) thick (Boghici and Van Broekhoven 2001, p. 207). Very little recharge to the aquifer likely comes from precipitation in the Rustler Hills in Culberson County, but most of it may be contributed by cross-formational flows from old water from deeper aquifer formations (Boghici and Van Broekhoven 2001, pp. 218–219). Groundwater planning for the Rustler aquifer anticipates that recharge, defined as the addition of water from precipitation or runoff by seepage or infiltration to the aquifer from the land surface, streams, or lakes directly into the formation or indirectly by way of leakage from another formation, on an annual basis is effectively zero (Middle Pecos Groundwater Conservation District 2010b, p. 18).

Historical pumping from the Rustler aquifer in Pecos County may have contributed to declining spring flows, as withdrawals of up to 9 million cubic meters (cm) (7,500 acre-feet (af)) in 1958 were recorded, with estimates from 1970 to 1997 suggesting groundwater use averaged between 430,000 cm (350 af) to 2 million cm (1,550 af) per year (Boghici and Van Broekhoven 2001, p. 218). As a result, declines in water levels in Pecos County wells in the Rustler aquifer from the mid-1960s through the late 1970s of up to 30 m (100 ft) have been recorded (Boghici and Van Broekhoven 2001, p. 213). We assume that groundwater pumping has had some impacts on spring flows of the Diamond Y Spring system in the past; however, they have not yet caused the main springs to cease flowing.

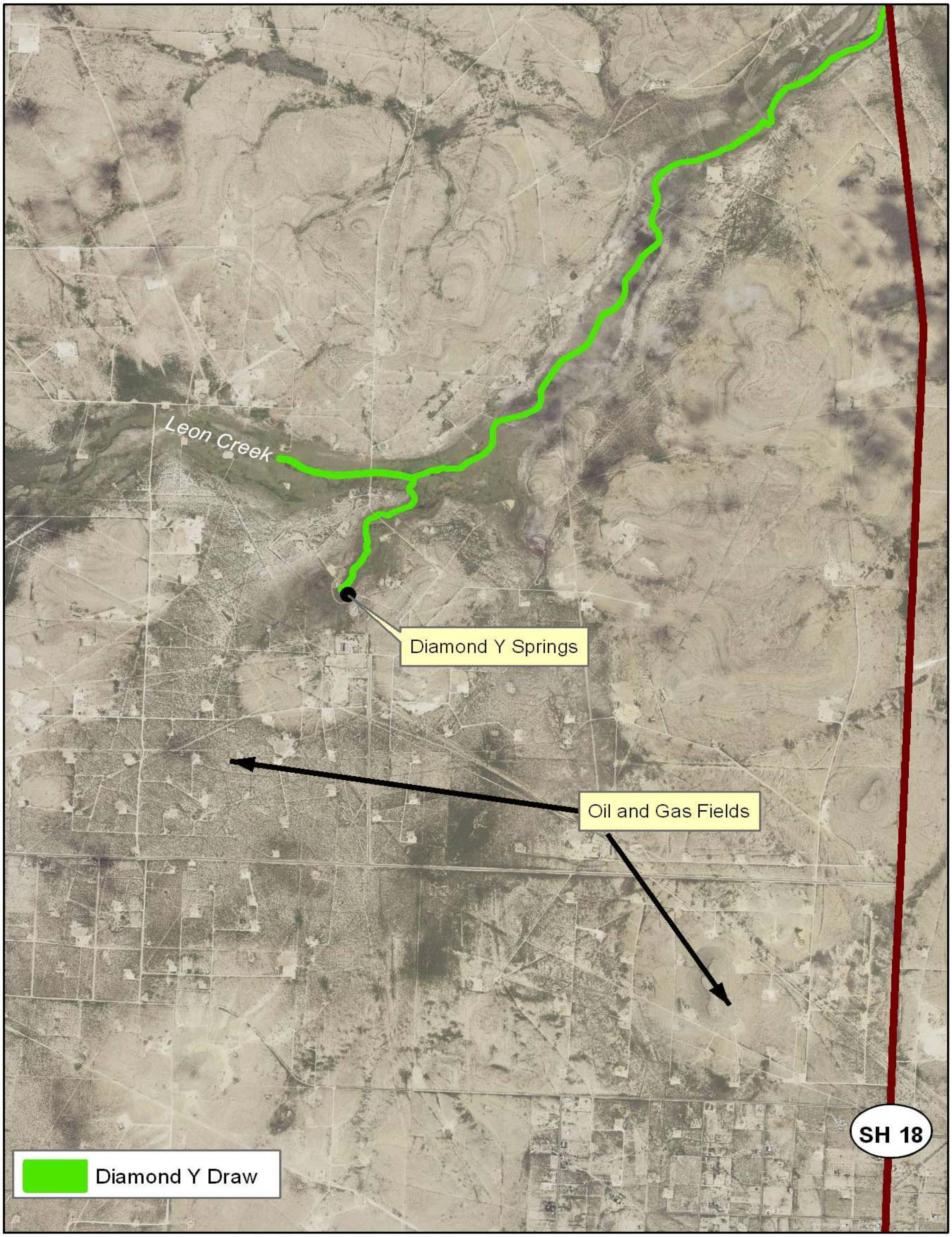
Future groundwater withdrawals may further impact spring flow rates if they occur in areas of the Rustler Aquifer that affect the spring source areas. Groundwater pumping withdrawals in Pecos County are expected to continue in the future mainly to support irrigated agriculture (Far West Texas Water Planning Group 2011, pp. 2-16–2-19) and will result in continued lowering of the groundwater levels in the Rustler aquifer. The latest plans from Groundwater Management Area 3 (the planning group covering the relevant portion of the Rustler Aquifer) allows for a groundwater withdrawal in the Rustler Aquifer not to exceed 90 m (300 ft) in the year 2060 (Middle Pecos Groundwater Conservation District 2010a, p. 2). This level of draw down will accommodate 12.9 million cm (10,508 af) of annual withdrawals by pumping (Middle Pecos Groundwater Conservation District 2010b, p. 15). This level of pumping would be 30 times more than the long-term average and could result in an extensive reduction in the available groundwater in the aquifer based on the total thickness of the Rustler strata. Therefore, it is anticipated that this level of groundwater draw down may contribute to continued declines in spring flow rates in the Diamond Y Spring system.

Although The Nature Conservancy owns and manages the property surrounding the Diamond Y Spring system, it has no control over groundwater use that affects spring flow (Karges 2003, p. 144). The spring flow is very small (1-2 cfs [28-57 lps] for Diamond Y Spring, the largest spring in the system), so any measurable decrease could prove substantial for the downstream ecosystem. Additionally, comparative analysis of maps and descriptions of the region show that the extent of surface waters in both the upper and lower watercourses of Diamond Y Draw have been reduced over the years (Echelle et al. 2001, p. 27).

<u>Pollution from oil and gas activities</u>
The Diamond Y Spring system is within active oil and gas extraction fields (Echelle et al. 2001, p. 26; Karges 2003, p. 144). These activities threaten the Leon Springs pupfish because of the potential groundwater or surface water contamination from pollutants (Veni 1991, p. 83; Fullington 1991, p. 6). There are still many active wells located within about 328 ft (100 m) of surface waters. In addition, a natural gas refinery is located within 0.5 mi (0.8 km) upstream of Diamond Y Spring. Oil and gas pipelines traverse the habitat, and many oil extraction wells are located near the occupied habitat (Figure 2) (Echelle et al. 2001, p. 26). A catastrophic spill event is possible at any time. Additionally, there are old brine pits from previous drilling within feet of surface waters, which could contaminate the habitat if they were to leak. Oil and gas pipelines traverse under the spring outflow channels and marshes where the species occurs, creating a constant potential for contamination from pollutants from leaks or spills. These activities pose a threat to the habitat by creating the potential for pollutants to enter underground aquifers that contribute to spring flow or by spills and leaks of petroleum products directly into surface waters. Presently, there is no evidence of habitat destruction or modification due to groundwater or surface water contamination from leaks or spills; however, an event catastrophic to the Diamond Y Spring species from a contaminant spill or leak is possible at any time (Veni 1991, p. 83).

As an example of the possibility for spills, in 1992 approximately 10,600 barrels of crude oil were released from a 15-cm (6-in) pipeline that traverses Leon Creek above its confluence with Diamond Y Draw. The pipeline ruptured at a point several hundred feet away from the Leon Creek channel. The spill site itself is about 1.6 km (1 mi) overland from Diamond Y Spring. The pipeline was operated at the time of the spill by the Texas–New Mexico Pipeline Company, but ownership has since been transferred to several other companies. The Texas Railroad Commission has been responsible for overseeing cleanup of the spill site. Remediation of the site initially involved aboveground land farming of contaminated soil and rock strata to allow microbial degradation. In later years, remediation efforts focused on vacuuming oil residues from the surface of groundwater exposed by trenches dug at the spill site. No impacts on the rare fauna of Diamond Y Springs have been observed, but no specific monitoring of the effects of the spill was undertaken (Industrial Economics, Inc. 2005, p. 4-12).

Figure 2: Oil and gas development around Diamond Y Draw, Pecos County, Texas



Another example of possible contaminants affecting the Diamond Y Spring system occurred in early June 2013, when an unknown whitish substance described as a surface scum and clouding of the water was reported in the lower watercourse area of the upper Monsanto Pool. A large number of Pecos gambusia were reported dead and collected in the pool area, though no mortality of Leon Springs pupfish was documented (Itzkowitz 2013b, pers. comm.) It is presumed that the most likely cause of the contaminant, until a biological source can be ruled out, is related to an oil and gas pad site that is approximately 328 ft (100 m) south of the upper Monsanto pool. The incident continues to be investigated, with lab results of water samples expected to establish whether or not the contaminant is associated with surrounding oil and gas activity and is potentially affecting groundwater resources tied to the pool (Orsak 2013, pers. comm.).

Habitat loss from bulrush

Native bulrush is an imminent threat, as it has encroached upon and thus reduced pupfish spawning habitat (Echelle et al. 2004, p. 132; Itzkowitz 2007, p. 2; Itzkowitz 2010, p. 25). The shallow pools of the Lower Monsanto area are dependent on overflows (for example, water bridges) from the deep pools (Itzkowitz 2010, p. 14). Researchers believe that it is important that these corridors remain open so that the pupfish can travel to the deep pool to escape low temperatures in the winter months and return to the shallow pools in warmer months to breed (Itzkowitz 2010, p. 14). Observations in May 2011 revealed that encroachment of bulrush had completely eliminated the water bridge and filled the shallow pool with vegetation (Itzkowitz 2011, p. 2).

Due to the encroachment of bulrush, the elimination of the water bridge, and other detrimental changes to suitable habitat within the Lower Monsanto area, pupfish had disappeared from this locale by 2012 (Itzkowitz 2013a, pers. comm.). Under an awarded section 6 grant, Dr. Itzkowitz and his graduate students began removal of bulrush and restoration of the Monsanto pool area in 2013 (Itzkowitz 2013a, pers. comm.). By May, restoration of the habitat had progressed sufficiently to warrant the reintroduction of pupfish to the Monsanto pool area. On May 15, SNARRC provided 500 juvenile and adult pupfish which were subsequently stocked into the pools (Ulibarri 2013, pers. comm.; Itzkowitz 2013a, pers. comm.). After reintroduction, pupfish breeding behavior was seen almost immediately. As of August 2013, the reintroduction appears to have been successful; however, longer term monitoring is necessary to determine if the population will remain stable over time.

2.3.2.2 Overutilization for commercial, recreational, scientific, or educational purposes:

There is no indication that the Leon Springs pupfish is threatened by overutilization. The only collections of the fish occur rarely for scientific purposes and are regulated by the Service pursuant to section 10(a)(1)(A) of the

Act, and Texas Parks and Wildlife Department (TPWD, Title 31, Part 2, Chapter 69, subchapter J).

2.3.2.3 Disease or predation

Except for a survey for gill flukes (McDermott 2000; McDermott et al. 2012), no data are available on the diseases or parasites of the Leon Springs pupfish. There was no evidence that the pupfish was infected with the gill fluke in the aforementioned study (McDermott 2000, p. 15; McDermott et al. 2012).

The endangered Pecos gambusia occurs sympatrically with the Leon Springs pupfish. The overgrowth of bulrush has altered the dynamics between these two species. Gambusia will feed on the eggs of pupfish (Itzkowitz 2011, p.1). Male pupfish are territorial and actively defend spawning territories and chase away gambusia as they approach to prey upon eggs. When there is an overgrowth of bulrush in pupfish spawning habitat, the cluttered habitat allows large congregations of gambusia to approach pupfish during spawning events (Gumm et al. 2008, p. 655). If a spawning event is interrupted by gambusia, the eggs may not be laid (Leiser and Itzkowitz 2003, p. 107). Also, the increased number of gambusia at pupfish spawning events may have resulted in changes in the breeding system such that smaller males remain satellites instead of defending available territories. This causes a decrease in the overall number of territorial males, which increases egg predation by Pecos gambusia (Gumm et al. 2008, p. 655, 657). However, Itzkowitz (2010, p. 8, 20) found that creating a more open habitat, by removing bulrush and installing cement tiles, increases the number of territorial pupfish males and disperses the gambusia, thus reducing egg predation.

2.3.2.4 Inadequacy of existing regulatory mechanisms:

The State of Texas lists the Leon Springs pupfish as endangered under Title 31 Part 2 of Texas Administration Code. TPWD regulations prohibit the taking, possession, transportation, or sale of any animal species designated by state law as endangered or threatened without the issuance of a permit.

There is no protection by state law for habitat or minimum stream or spring flows for state-listed species. Therefore, only minimal protections are afforded the Leon Springs pupfish by the State of Texas and these protections do not address the most significant threats to the species associated with habitat loss.

Some protection for the habitat of this species is provided with the land ownership of the springs by The Nature Conservancy. However, this land ownership only provides protection to the spring outflow channels only and provides no protection for maintaining groundwater levels to ensure continuous spring flows.

2.3.2.5 Other natural or manmade factors affecting its continued existence:

Hybridization

Leon Springs pupfish readily interbreed with pupfish of different species. Garrett (1979, pp. 56-57) demonstrated that female Leon Springs pupfish do not discriminate between males of their own species and male sheepshead minnows (*Cyprinodon variegatus*). Diamond Y Draw is in a remote location owned by The Nature Conservancy. This affords some protection from anthropogenic introductions of non-native fishes; however, the past two introductions of sheephead minnows demonstrate that hybridization events remain a threat of high magnitude for the pupfish (Echelle and Echelle 1997, p. 160; USFWS 1985, p. 6).

Climate change

Future climate change may also impact water quantity for the Leon Springs pupfish. According to the Intergovernmental Panel on Climate Change (IPCC) (2007, p. 1) "Warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level." Average Northern Hemisphere temperatures during the second half of the 20th century were very likely higher than during any other 50-year period in the last 500 years and likely the highest in at least the past 1,300 years (IPCC 2007, p. 1). It is highly likely that, over the past 50 years, cold days, cold nights and frosts have become less frequent over most land areas, and hot days and hot nights have become more frequent (IPCC 2007, p. 1). It is likely that heat waves have become more frequent over most land areas, and the frequency of heavy precipitation events has increased over most areas (IPCC 2007, p. 1).

The IPCC (2007, p. 6) predicts that changes in the global climate system during the 21st century will very likely be larger than those observed during the 20th century. For the next two decades a warming of about 0.4°F (0.2°C) per decade is projected (IPCC 2007, p. 6). Afterwards, temperature projections increasingly depend on specific emission scenarios (IPCC 2007, p. 6). Various emissions scenarios suggest that by the end of the 21st century, average global temperatures are expected to increase 1.1°F to 7.2°F (0.6°C to 4.0°C) with the greatest warming expected over land (IPCC 2007, p. 6-8). Localized projections suggest the southwest may experience the greatest temperature increase of any area in the lower 48 States (IPCC 2007, p. 8). The IPCC also predicts an increase in hot extremes and heat waves, and a decrease in water resources (IPCC 2007, p. 8).

There is also high confidence that many semi-arid areas like the western United States will suffer a decrease in water resources due to climate change (IPCC 2007, p. 7; Karl et al. 2009, pp. 129-131), as a result of less annual mean precipitation and reduced length of snow season and snow depth. Milly et al. (2005, p. 347) also project a 10 to 30 percent decrease in precipitation in mid-latitude western North America by the year 2050 based on an ensemble of 12 climate models. Even under lower emission scenarios, recent projections forecast a 4 to 6 ºF (2 to

3 ºC) increase in temperature and a 10 percent decline in precipitation in western Texas by 2080-2099 (Karl et al. 2009, pp. 129-130). Assessments of climate change in west Texas suggest that the area is likely to become warmer and drier (TWDB 2008, pp. 22-25).

The potential effects of future climate change could reduce overall water availability in this region of western Texas. If this were to occur, spring flows could decline directly because of decreases in recharge from declining precipitation or indirectly as a result of increased pumping of groundwater to accommodate human needs for additional water supplies (Mace and Wade 2008, p. 664). Other effects of climate change include, but are not limited to, alteration of water quality, accelerated invasion of non-native species, and increased disease susceptibility. Because of the extremely small range of the Leon Spring pupfish, any potential changes to this species' environment could result in the extinction in the wild. For instance, increase in temperatures may affect fecundity, as spawning behavior begins to decline in this species at temperatures of 84°F (29°C) and above (Kennedy 1977, p. 98).

<u>Small Population Size and Stochastic Events</u>

The Leon Springs pupfish is susceptible to threats associated with small population size and impacts from stochastic events. The risk of extinction for any species is known to be highly inversely correlated with population size (O'Grady et al. 2004, pp. 516, 518; Pimm et al. 1988, pp. 774-775). In other words, the smaller the population the greater the overall risk of extinction. Accurate population size estimates have not been generated for this species, but the small area of suitable habitat severely limits the number of individuals. Small population sizes can also act synergistically with other traits (for example, habitat specialization or limited distribution) to greatly increase risk of extinction (Davies et al. 2004, p. 270). Stochastic events from either environmental factors (for example, severe weather) or demographic factors (for example, random birth and death rates) are also heightened threats to species with small population sizes (Melbourne and Hastings 2008, p. 100).

Accurate population numbers of Leon Springs pupfish at Diamond Y Spring are not currently known; however, Itzkowitz (2010, p. 19) indicated that although populations of pupfish during 2008, 2009, and 2010 were larger than in 2006 and nearly double that of 2007, they were a mere fraction of the numbers observed in 2001. Itzkowitz (2010, p19) estimated the total population (upper and lower watercourse) of the Diamond Y Spring system in 2010 to be less than 50 individuals, and noted that irrespective of which year's population numbers were considered, they are all too small to sufficiently maintain the species. The small population size of the pupfish appears to also play a role in number of males that are able to establish breeding territories and their ability to defend against egg predation from the Pecos gambusia. It is possible that this overall reduction in fecundity may be the loss of critical adaptations from the captive stock at SNARRC due to their many generations in captivity (Itzkowitz 2010, p. 19, 20).

As of August 2013, the current captive stock of pupfish at SNARRC consists of approximately 4000 juveniles and 2000 adult pupfish, totaling around 6000 individuals. The population at SNARRC is considered stable and healthy, with approximately 60-150 individuals tested annually for any diseases or pathogens of concern (Ulibarri 2013, pers. comm.) Due to previous stochastic events affecting the small population size of the pupfish in the Diamond Y system, the refugia population at SNARRC has and will continue to be used as a safeguard to repopulate or supplement the existing population when the need arises.

Non-native Species Interactions

An exotic snail, *Melanoides tuberculata*, has become established in Diamond Y Spring (Echelle et al. 2001, p. 14; McDermott 2000, p. 15). The exotic snail is the most abundant large snail in the upper watercourse of the Diamond Y Spring system. Currently, it has not been detected in the lower watercourse (Echelle 2001, p. 26). In many locations, the exotic snail is so numerous that it is, in essence, the substrate in the small stream channel. The effects of this invasive snail on the Leon Springs pupfish are not yet known. It is known that *Melanoides* can be used as an intermediate host for the parasitic Asian gill fluke *Centrocestus formosanus* (McDermott 2000, p. 1-2). *Centrocestus formosanus* has been shown to infect native fish species occurring in other central and west Texas spring systems (McDermott 2000, p. 4; McDermott et al. 2012), and has the potential to negatively impact the Leon Springs pupfish at some point in the future. The gill parasite is highly pathogenic to piscine hosts because their encystment in the gills causes respiratory problems and decreased ability to obtain dissolved oxygen from the water (McDermott 2000, p. 4). However, the gill parasite is not currently known to occur in the Diamond Y Spring system despite the presence of the exotic *Melanoides* snail (McDermott 2000, p. 19; McDermott et al. 2012). Other introduced non-native species could potentially compete for food or resources (USFWS 1985, p. 7) or transfer pathogens that infect the pupfish.

2.4 Synthesis

The best available information indicates that the primary threats to the Leon Springs pupfish are: 1) habitat loss from the potential loss of spring flow due to a decline in groundwater levels, 2) egg predation by the Pecos gambusia, 3) habitat loss due to the encroachment of bulrush into the species habitat, 4) hybridization with introduced species, primarily the sheepshead minnow, and 5) potential contamination of habitat from local oil and gas activities, all of which are compounded by the small size of the pupfish population in the wild. Loss of suitable habitat due to bulrush encroachment is a relatively new threat to the species not originally addressed in the 1985 recovery plan.

The information reviewed does not indicate that impacts to spring flows from a significant increase in groundwater use or declines in recharge are imminent (defined here as likely to occur in the next 15 years). However, diminished spring flows could occur over the foreseeable future of 50 to 100 years as a result of climate change or to meet increased human needs for more water resources. The magnitude of impact on the Leon Springs pupfish if this threat were realized is extremely high. Because the range of the species is limited to a small, isolated location, habitat modification due to a decline in spring flows could result in its extinction in the wild.

The threat of egg predation during breeding events from the Pecos gambusia is high and ongoing, and the magnitude of the impact of this threat on the species is also high. Additional threats include habitat modification from water quality degradation, local habitat changes, and the introduction of a disease, parasite, or non-native species.

Climate change is another source of potential threats to the species. All possible impacts associated with future climate change cannot presently be reliably assessed. However, accelerating climate change could exacerbate any of the threats already considered or could result in entirely new threats that are not conceived at this time. Either way, subtle but significant changes in the ecosystem of the Leon Springs pupfish resulting from climate change in the foreseeable future of 50 to 100 years could cause the species' extinction in the wild and present a high magnitude threat.

All of these threats must be considered in the context of a fish with an extremely small range, no opportunity for natural movement (relocation), a small population size, and a short life span. Therefore, the magnitude of impact of any potential threat or future stochastic event is exceptionally high. Any events negatively affecting the species or its habitat could result in extinction of the Leon Springs pupfish in the wild. The species has been considered completely extirpated from the wild once since the original 1980 listing due to introgression with sheepshead minnows in the late 1980s and early 1990s, requiring eradication efforts and restocking with genetically pure individuals from the refugia population at SNARRC. Therefore, we recommend that the Leon Springs pupfish retain its endangered status.

3.0 RESULTS

3.1 Recommended Classification:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist
__X__ **No change is needed**

3.2 New Recovery Priority Number: We propose that the Leon Springs pupfish, currently with a Recovery Priority Number of 2, be assigned a new Recovery Priority Number of 5.

Brief Rationale: The Leon Springs pupfish faces multiple imminent, high magnitude threats, and its entire range is limited to one small spring system. Any decreases in spring flow rates or any future events that negatively impact the pupfish could easily result in the complete loss of the species in the wild. Additionally, there are no currently known suitable areas available where the pupfish can likely be established because it is a very narrow habitat specialist. Its potential for recovery (de-listing) is low. Therefore, per the Service's recovery priority guidance, the Leon Springs pupfish more appropriately fits in a category 5 than 2.

3.3 Listing and Reclassification Priority Number: N/A

4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

Control Bulrush

A management program should be implemented immediately to control bulrush in the Leon Springs pupfish habitat. The pupfish population residing in Lower Monsanto Pool has experienced a dramatic loss of spawning habitat from bulrush encroachment (Itzkowitz 2011, p. 2). Removal of bulrush would reestablish the water bridge between the Lower Monsanto Pool and the shallow spawning pools (Itzkowitz 2011, p. 2). Additionally, artificially increasing spawning areas (by installing cement tiles) will prevent bulrush growth (Itzkowitz 2010, p. 5) and may aid pupfish recovery by increasing the number of territorial males and thus decreasing high aggregations of Pecos gambusia at spawning events (Gumm et al. 2008, p. 657).

Bulrush encroachment has replaced shallow, open-water habitats with dense vegetation and seems to have contributed both directly and, by altering interspecific interactions, indirectly to a marked decline of Leon Springs Pupfish in Diamond Y Spring (Gumm et al. 2008, p. 656-657). However, any habitat restoration, enhancement, and/or creation efforts in the Diamond Y Spring system benefitting the Leon Springs pupfish should be carefully considered and balanced with the potential negative impacts that may occur to other species in the system, such as the endangered Pecos gambusia and three species of invertebrates that were listed under the Act on August 8, 2013, the Diamond tryonia (*Pseudotryonia adamantina*), Gonzales tryonia (*Tryonia circumstriata*), and Pecos amphipod (*Gammarus pecos*).

Routine Population and Habitat Monitoring

Currently, there is no regular program in place to monitor population levels and conditions in the Leon Springs pupfish. A program should be employed to regularly monitor population numbers and potential contamination from local oil and gas activities and other threats.

Genetic Monitoring

A regular program of genetic monitoring of wild populations should be implemented to assess any changes in genetic structure, such as hybridization with non-native fishes (Echelle and Echelle 1997, p. 160; Garrett 2002, p. 442).

Maintenance of Captive Population

The hybridization events between the Leon Springs pupfish and the non-native sheepshead minnow demonstrate the importance of maintaining this species at SNARRC. A regular program of genetic monitoring should be implemented, as special care should be taken to maintain the genetic diversity of this captive population (Echelle et al. 2001, p. 27). To maintain genetic variability and rare alleles, the captive populations ideally should be supplemented with genetically pure individuals from natural populations (Edds and Echelle 1989, p. 444); however, the population at SNARRC appears to be the only population of Leon Spring pupfish that has not been introgressed with sheepshead minnow genes (Echelle and Echelle 1997, p. 159-160), so this must be done with the utmost discretion.

Additional Stockings

Echelle et al. (2001, p. 22) recommended stocking the area near the observation tower in Diamond Y Draw with genetically pure fish from SNARRC. The population near the tower showed the highest level of non-native genes (4.2 percent) and appears to have undergone a severe bottleneck, which reduced variability (Echelle et al. 2001, p. 22). To prevent the founder effect, a minimum of 200 adult fish should be introduced in the initial stocking (Edds and Echelle 1989, p. 444). Stockings should consist of small pupfish (<20 mm), added in early spring to achieve the greatest results (Echelle et al. 2001, p. 27). Unless future information suggests otherwise, current information by Itzkowitz (2010, p. 19) did not recommend stocking the Diamond Y headpool area of the upper watercourse, and Echelle et al. (2001, p. 28) indicated that the lower watercourse area probably should not be stocked with pupfish from SNARRC without justification based on future genetic monitoring. The population in the lower watercourse potentially carries rare alleles that were lost from the SNARRC stock and are, therefore, absent from the upper watercourse as well.

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6.0 SIGNATURE PAGE

U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW of The Leon Springs pupfish, *Cyprinodon bovinus*

Current Classification: Endangered

Recommendation resulting from the 5-Year Review:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist
_X_ No change needed

Appropriate Listing/Reclassification Priority Number, if applicable: N/A

Review Conducted By: Clayton Napier, Austin Ecological Services Field Office, Austin, Texas

FIELD OFFICE APPROVAL:

Lead Field Supervisor, U.S. Fish and Wildlife Service, Austin Ecological Services Field Office, Austin, Texas

Approve ______________________ Date Aug. 28, 2013

REGIONAL OFFICE APPROVAL:

Assistant Regional Director, Ecological Services, U.S. Fish and Wildlife Service, Region 2

Approve ______________________ Date 8/28/13