Leon Springs pupfish
(*Cyprinodon bovinus*)
5-Year Status Review:
Summary and Evaluation

Image courtesy of John Karges, The Nature Conservancy, 2003.

U.S. Fish and Wildlife Service
Austin Ecological Services Field Office
Austin, Texas
20 December 2024

5-YEAR REVIEW

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

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1.0 GENERAL INFORMATION

1.1 Reviewers:

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Cooperating Regional Office(s):
Not applicable

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 ESA (ESA) 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 ESA. 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:

The U.S. Fish and Wildlife Service (Service) conducts status reviews of species on the List of Endangered and Threatened Wildlife and Plants (50 CFR 17.12) as required by section 4(c)(2)(A) of the Endangered Species Act (16 U.S.C. 1531 et seq.). The Service provides notice of status reviews via the Federal Register and requests new information on the status of the species (e.g., life history, habitat conditions, and threats). Data for this status review were solicited from interested parties through Federal Register notices announcing this review on May 5, 2021 (86 FR 23976) and January 25, 2024 (89 FR 4966). This review was conducted by the Austin Ecological Services Field Office and considered both new and previously existing information from federal and state agencies, non-governmental organizations, academia, and the public. The primary sources of information used in this analysis included information from Service-authored reports, peer-reviewed articles, geospatial data, and other files available in the Austin Ecological Services Field Office. We also engaged in discussions with staff from The Nature Conservancy, Texas Parks and Wildlife (TPWD), and Service employees affiliated with the Fisheries and Aquatic Conservation program, including the Texas Fish and Wildlife Conservation Office (FWCO) and the Southwestern Native Aquatic Resources and Recovery Center (SNARRC). Comments received were evaluated and incorporated as appropriate.

1.4 Background:

1.4.1 FR Notice citation announcing initiation of this review:

86 FR 23976: May 5, 2021
89 FR 4966: January 25, 2024

1.4.2 Listing history:

<u>Original Listing</u>
FR notice: 45 FR 54678
Date listed: August 15, 1980
Entity listed: Species, *Cyprinodon bovinus*
Classification: Endangered

1.4.3 Associated Rulemakings:

A final rule designating critical habitat for the Leon Springs pupfish was published in the Federal Register concurrent with final rule listing the species as Endangered, on August 15, 1980 (45 FR 54678).

1.4.4 Review History:

A five-year status review for the Leon Springs pupfish was approved in August 2013. This was the first five-year review completed for the species. The recommendation resulting from the review was that no change was warranted to the classification of the species as Endangered.

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

5

1.4.6 Recovery Plan or Outline

Name of plan or outline: Leon Springs Pupfish Recovery Plan
Date issued: August 14, 1985
Dates of previous plans/amendment or outline, if applicable: Not applicable

2.0 REVIEW ANALYSIS

Section 4 of the ESA (16 U.S.C. 1533) and its implementing regulations (50 CFR part 424) set forth the procedures for determining whether a species meets the definition of "endangered species" or "threatened species." The ESA defines an "endangered species" as a species that is "in danger of extinction throughout all or a significant portion of its range," and a "threatened species" as a species that is "likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range." The ESA requires that we determine whether a species meets the definition of "endangered species" or "threatened species" due to any of the five factors described below.

Section 4(a) of the Act describes five factors that may lead to endangered or threatened status for a species. These include: A) the present or threatened destruction, modification, or curtailment of its habitat or range; B) overutilization for commercial, recreational, scientific, or educational purposes; C) disease or predation; D) the inadequacy of existing regulatory mechanisms; or E) other natural or manmade factors affecting its continued existence.

The identification of any threat(s) does not necessarily mean that the species meets the statutory definition of an "endangered species" or a "threatened species." In assessing whether a species meets either definition, we must evaluate all identified threats by considering the expected response of the species, and the effects of the threats—in light of those actions and conditions that will ameliorate the threats—on an individual, population, and species level. We evaluate each threat and its expected effects on the species, then analyze the cumulative effect of all of the threats on the species as a whole. We also consider the cumulative effect of the threats in light of those actions and conditions that will have positive effects on the species—such as any existing regulatory mechanisms or conservation efforts. The Service recommends whether the species meets the definition of an "endangered species" or a "threatened species" only after conducting this cumulative analysis and describing the expected effect on the species now and in the foreseeable future.

2.1 Distinct Population Segment (DPS) policy (1996):

Not applicable; the Leon Springs pupfish is not listed as a DPS.

2.2 Updated Information and Current Species Status

2.2.1 Biology and Habitat

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

Behavioral Studies

Behavioral studies on Leon Springs pupfish by members of the Itzkowitz Lab from Lehigh University have continued since the previous five-year review. Several published papers have resulted from this work, which are summarized below in chronological order of when the research was conducted.

An investigation into the mating behavior of female Leon Springs pupfish was conducted in 2002 (Leiser et al. 2015, entire). The study looked into whether females were selective with respect to which males they selected for mating, and with how many males they mated (Leiser et al. 2015, pp. 605–606). The researchers observed that females first evaluated the territorial males present at a given time, and then mated with a subset of the available males (Leiser et al. 2015, p. 612). This behavior is called "choosy promiscuity" (Leiser et al. 2015, p. 612).

A study conducted in July and August of 2012 sought to identify the relationship between aggressive behaviors by territorial male Leon Springs pupfish and reproductive success (Snekser et al. 2017, pp. 86–87). A positive correlation was identified—greater territorial male aggression is associated with greater reproductive success, as measured by total number of eggs laid—but the mechanism behind the correlation was not able to be determined based on the study alone (Snekser et al. 2017, p. 91).

Habitat restoration in the form of the construction of two shallow pools on either side of Monsanto pool was completed in January 2013 (Al-Shaer et al. 2016, p. 3). Existing vegetation, including bulrush, was removed, and cement tiles were placed in the pool bottom to discourage vegetation regrowth and provide a hard substrate for egg laying (Al-Shaer et al. 2016, p. 3). The same year, Al-Shaer et al. (2016, p. 2) investigated the differences in site characteristics and use by Leon Springs pupfish between the wild population at the Diamond Y Spring pool and a recently introduced population at the Monsanto pool in June and July of 2013 (Al-Shaer et al. 2016, p. 2). Although differences in habitat use and the habitat itself were identified, there was insufficient data to draw conclusions about why this was the case or make inferences about its importance for the persistence of the population (Al-Shaer et al. 2016, pp. 6–7). For example, the study was conducted in the same year that the fish were introduced, so the population was not fully established, and the fish were in a new environment (Al-Shaer et al. 2016, p. 7). However, the study did find that Leon Springs pupfish will readily breed when introduced to a new environment, even when the conditions are not ideal (Al-Shaer et al. 2016, p. 7).

A second study occurred simultaneously (June and July of 2013) with the one described in Al-Shaer et al. (2016, entire). Paciorek et al. (2014, pp. 528–529) observed the behavior and movement of gambusia[1] in association with male Leon Springs pupfish territories in the wild population at the Diamond Y Spring pool and the introduced population at the Monsanto pool. The goal of this study was to better understand the potential threat to Leon Springs pupfish from egg predation by gambusia (Paciorek et al. 2014, p. 528). The study concluded that densities of gambusia were similar between unoccupied and occupied areas (territories) at both study sites, and that gambusia were more dense in the unoccupied areas of the Monsanto habitat (Paciorek et al. 2014, p. 531). The authors presented three main findings from the work. First, gambusia do not appear to preferentially invade or spend time in Leon Springs pupfish territories, and do not aggregate there, waiting for spawning to occur (Paciorek et al. 2014, p. 531). Second, multiple generations of captive breeding and isolation from other fish species did not eliminate the instinct of Leon Springs pupfish to chase off gambusia from territories (Paciorek et al. 2014, p. 532). Finally, expanding the total habitat available to the fishes seems to decrease negative interactions between Leon Springs pupfish and gambusia (Paciorek et al. 2014, p. 532).

Black, Snekser, et al. (2017, pp. 1520–1521) also compared the behavior of Leon Springs pupfish within the long-established Diamond Y Spring population to those introduced to the Monsanto pool from captivity at SNARRC in 2013. The variables evaluated were reproductive behavior, foraging, aggressive behavior (for territory defense), and territory size (Black, Snekser, et al. 2017, p. 1522). No significant difference between the two populations was found, indicating that behavior changes as a result of time in captivity are not the driving factor behind past extirpations at the Monsanto pool (Black, Snekser, et al. 2017, pp. 1523–1524). The 2013 introduction failed the same year, leading to

[1] A note about these publications is that they are based on observational studies that do not involve handling individual fish. For example, in studies that describe interactions of the Leon Springs pupfish with gambusia, the researchers could not distinguish between the native Pecos gambusia (*Gambusia nobilis*), the introduced western mosquitofish (*Gambusia affinis*), and Pecos gambusia x western mosquitofish hybrids. Thus, although the studies below describe the observed gambusia species as Pecos gambusia, we use the term “gambusia” to clarify that the precise species is not known. The gambusia genus contains more than the three species mentioned above, but here we use that term to refer to only those three fishes.

another reintroduction of Leon Springs pupfish from SNARRC into Monsanto pool in May 2015.

Black et al. (2016, p. 149) conducted an *in situ* experiment to evaluate the relationship between gambusia density and Leon Springs pupfish behavior and reproductive success. The study was motivated by previous research suggesting that gambusia may be a significant predator on Leon Springs pupfish eggs, and a subsequent observational study indicating that impact from such gambusia egg predation had decreased due to habitat modifications that increased the total habitat available to both species (Black et al. 2016, pp. 149–151). The experiment was conducted in June and July of 2014, and provided additional evidence that egg predation by gambusia is not an important driver of Leon Springs pupfish reproductive success except under extreme conditions (Black et al. 2016, p. 151).

A similar behavioral study was conducted in June and August of 2015 and 2016 on the new population (Al-Shaer et al. 2018, pp. 2–3). This two-year study again found that there were no significant differences in behavior between the two populations, confirming that despite the differences between the captive environment at SNARRC and the wild environment at Diamond Y, the captive population had not lost any important behaviors necessary for survival and reproductive success (Al-Shaer et al. 2018, pp. 6–7). This eliminates behavior as an explanation for the failure of repeated introductions of SNARRC-bred individuals into the Monsanto pool (Al-Shaer et al. 2018, pp. 6–7).

In June 2015, Bloch et al. (2018, pp. 467–468) completed a study investigating the extent to which female Leon Springs pupfish use social cues to inform spawning behavior, and how the presence of male Leon Springs pupfish, female Leon Springs pupfish, and gambusia within a breeding territory affects the territorial male's behavior. Based on the results, the authors concluded that females do observe the presence and behavior of other females, and spawn more times per visit when other females are present (Bloch et al. 2018, pp. 474–475). However, the presence of non-territorial male Leon Springs pupfish or gambusia did not influence female Leon Springs pupfish spawning behavior (Bloch et al. 2018, p. 475). Territorial male Leon Springs pupfish did not alter their territory defense behaviors due to the presence of female Leon Springs pupfish, non-territorial male Leon Springs pupfish, or gambusia (Bloch et al. 2018, p. 476).

A three-year study conducted during June and July of 2017, 2018, and 2019 evaluated two separate behavioral questions (Snekser and Itzkowitz 2022, p. 145). First, the behavior of territorial male Leon Springs pupfish was evaluated to determine whether aggressive behavior was directed to gambusia, an apparent egg predator (Snekser and Itzkowitz 2022, p. 145). The research results showed that while territorial male Leon Springs pupfish chase 100% of conspecific males from their territory, they inconsistently chased gambusia, and often did not chase them completely from a territory (Snekser and Itzkowitz 2022, p. 147). Thus, it appears that territorial male Leon Springs pupfish do not have an effective behavior response to egg predation by gambusia (Snekser and Itzkowitz 2022, p. 147). The second part of the study examined the trigger for gambusia to forage for newly laid Leon Springs pupfish eggs (Snekser and Itzkowitz 2022, p. 144). The researchers hypothesized that gambusia are triggered by any form of disturbance, but the study results showed that they specifically respond to the act of spawning by Leon Springs pupfish (Snekser and Itzkowitz 2022, p. 147).

In June 2022, Snekser et al. (2024, p. 2) evaluated the relationship between patrolling and chase behaviors in territorial male Leon Springs pupfish in the Diamond Y Spring pool and the number of eggs deposited in a single male's territory. This study found that patrolling did not affect the number of intrusions by conspecific males or by gambusia, but did find a positive correlation between the amount of time spent patrolling and the total number of eggs laid in the territory (Snekser et al. 2024, p. 4).

Other Research

Incidental to the behavior studies, territory sizes were noted in some of the literature. Paciorek et al. (2014, p. 528) reported territories 0.5 meters ([m], 1.64 feet [ft]) in diameter. Snekser et al. (2024, p. 2) reported territories 0.25–1.0 m (0.82–3.28 ft) in diameter.

Also recorded incidentally to the behavior studies is the observation that Leon Springs pupfish breeding in the Diamond Y Spring pool ceases when the water level of that pool drops sufficiently far to expose the shallow breeding shelf where most territories are established (Itzkowitz 2021, pp. 4–5). This was confirmed through the use of underwater video to search for breeding Leon Springs pupfish at lower depths (Itzkowitz 2021, pp. 4–5).

In comparing breeding activity in the Diamond Y Spring pool, the Monsanto pool, and constructed pools, researchers found that Leon Springs pupfish prefer compact substrates over silty substrates, and that they prefer natural compact substrates over cement blocks (Al-Shaer et al. 2016, p. 7).

An egg predation study was initiated in 2021. Findings have not yet been published in peer-reviewed publications, but some information is available from permittee annual reports. Preliminary results from that effort are that egg predation is significant within the Diamond Y Spring pool (Itzkowitz 2021, p. 4). In 2022, the researchers found that 70% of eggs without predator exclusion materials were consumed at night (Itzkowitz 2022, pp. 3–4). They originally hypothesized that predacious beetles might be consuming Leon Springs pupfish eggs (Itzkowitz 2023, pp. 1–2). Infrared video recorded during the night failed to find any evidence of a diving beetle or similar insect predator, but did record numerous instances of nocturnal crayfish foraging on the eggs (Itzkowitz 2023, pp. 1–2).

Finally, observations on habitat conditions are available from SNARRC in Dexter, New Mexico. The well water that supplies both the outdoor ponds and the indoor tanks is a constant 17.8° Celsius (° C) (64° Fahrenheit [° F]), with a pH of 7.5-8.5, total hardness of 2,100 parts per million (ppm), and total dissolved solids of 3,500 ppm (U.S. Fish and Wildlife Service 2020, p. 7). The water temperature range tolerated by Leon Springs pupfish held in refuge ponds at SNARRC is wide and includes lows of approximately 2–3° C (35.6–37.4° F) in winter and highs of approximately 33–34° C (91.4–93.2° F) in the summer (Wilson 2024, p. 1). At SNARRC, rooted vegetation (primarily *Potamogeton, Najas, Scirpus*, and *Typha* [pondweed, naiad, bulrush, and cattail]) typically covers 30% of the pond bottom, banks, and edges (Wilson 2024, p. 1). The captive population is observed to establish territories and breed from early April through late June in the sandy areas between plants and near the concrete catch basin (U.S. Fish and Wildlife Service 2020, p. 9). Water temperatures during this period range from 15–24° C (59-75° F) (U.S. Fish and Wildlife Service 2020, p. 9).

2.2.1.2 Abundance, population trends (e.g. increasing, decreasing, stable), demographic features (e.g., age structure, sex ratio, birth rate, seed set, germination rate, age at mortality, mortality rate, etc.), or demographic trends:

<u>Diamond Y Spring Pool</u>

This location has the most consistent monitoring for the species due to the long-term monitoring of territorial male abundance and behavior studies conducted by the Itzkowitz Lab. Since the last five-year review was completed in 2013, TPWD and The Nature Conservancy have also conducted Leon Springs pupfish monitoring surveys. Table 1 shows the historical count of territorial males by month and year for the period 2000–2015. No surveys were conducted in 2011, 2016–2018, or 2020. Surveys resumed for 2021–2023 (Table 2). A recent report from The Nature Conservancy combines these two datasets into a single figure and is reproduced in Figure 1 below.

Table 1. Count of maximum number of territorial males in Diamond Y Spring pool for the period 2000--2020. Counts were completed in May or June. No counts were conducted in 2011, 2016, 2017, 2018, or 2020. Data from personal notes of Dr. Jen Snekser (2024, pp. 1–2).

Year	Count of Territorial Males
2000	16
2001	23
2002	12
2003	12
2004	14
2005	8
2006	1
2007	5
2008	11
2009	10
2010	13
2011	—
2012	17
2013	16
2014	19
2015	13
2016	—
2017	—
2018	—
2019	187
2020	—
2021	250
2022	119
2023	83
2024	75

Table 2. Count of territorial males in Diamond Y Spring pool for in summer 2021, 2022, and 2023. Data from Table 3 in The Nature Conservancy (2023, p. 9).

Month	Date	Year	Count of Territorial Males
June	1	2021	250
June	21	2021	77
July	1	2021	Not visible
July	4	2021	122
July	7	2021	120
July	14	2021	Water level too low
July	22	2021	65
August	3	2021	72
June	2	2022	119
June	5	2022	118
June	9	2022	9
May	23	2023	85
May	31	2023	84
June	16	2023	33
June	21	2023	22
June	29	2023	0

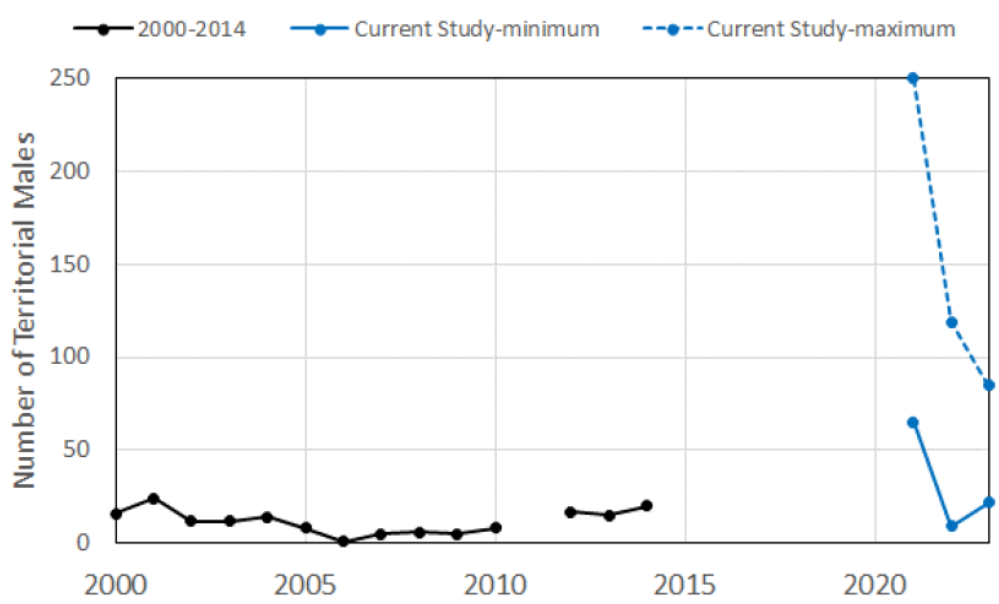

Figure 1. Number of territorial male pupfish in Diamond Y Spring from two periods, 2000-2015 and 2021-2023. Both minimum and maximum counts are shown for the current study years of 2021-2023. Figure from (The Nature Conservancy 2023, p. 9).

For the period 2021–2023, minnow trapping was conducted at Diamond Y Spring. Table 3 shows the results from that effort. Two other fishes live in the Diamond Y Spring pool: Pecos gambusia and western mosquitofish; some hybrids of these two are also likely present (U.S. Fish and Wildlife Service 1985, p. 5; The Nature Conservancy 2023, p. 11). The analysis of that data also evaluated the relative abundance of these three species (The Nature Conservancy 2023, p. 11). Leon Springs pupfish comprised over 80% of the trapped fishes in fall 2021 and spring 2022, but for the remainder of the sampling period (summer 2022 through spring 2023), they comprised less than 40% of the trapped fishes (The Nature Conservancy 2023, p. 11).

Table 3. Results from minnow trap sampling at Diamond Y Spring from 2021 to 2023. Data from The Nature Conservancy (2023, p. 11). The sampling effort on 7/25/2022 had lower trap hours because low flows in Diamond Y Spring necessitated modifications to the survey protocol. Fewer traps were set, and locations were adjusted.

Date	Trap Hours	Number of fish captured	Catch per Unit Effort: Fish per Hour
11/11/2021	45.77	17	0.37
4/27/2022	60.57	187	3.09
7/25/2022	20.53	2	0.10
11/2/2022	48.68	2	0.04
2/27/2023	57.17	22	0.38
5/2/2023	62.33	11	0.18
11/12/2021	34.92	60	1.72
4/27/2022	55.92	42	0.75
7/26/2022	63.62	36	0.57
11/3/2022	59.58	32	0.54
2/28/2023	57.23	23	0.40

Karges Spring

Minnow trapping also took place in Karges Spring pool from 2021 to 2023 (The Nature Conservancy 2023, p. 11). Table 4 shows the results from that effort. The same relative abundance analysis was conducted for this locality. There, Leon Springs pupfish relative abundance hovered around 10% for four of the five sampling periods, with a spike to just above 40% for the spring 2022 sampling period (The Nature Conservancy 2023, p. 11). Because the topography of the Karges Spring pool is more complex, the straightforward counts of fish and territorial males could not be replicated at this location (The Nature Conservancy 2023, p. 9). However, The Nature Conservancy (2023, p. 9) reports that the population in this locality is approximately 100 individuals, and that the presence of breeding males and overall abundance was stable during the 2021 and 2022 observation windows.

Table 4. Results from minnow trap sampling at Karges Spring from 2021 to 2023. Data from The Nature Conservancy (2023, p. 11).

Date	Trap Hours	Number of fish captured	Catch per Unit Effort: Fish per Hour
11/12/2021	34.92	60	1.72
4/27/2022	55.92	42	0.75
7/26/2022	63.62	36	0.57
11/3/2022	59.58	32	0.54
2/28/2023	57.23	23	0.40

Monsanto Ponds

In May 2013, 400 Leon Springs pupfish from SNARRC were introduced into the main Monsanto pool (Black, Snekser, et al. 2017, p. 1520; Al-Shaer et al. 2018, p. 2). This population was found to be extirpated by early autumn 2013 (Al-Shaer et al. 2018, p. 2). In May 2015, 500 Leon Springs pupfish from SNARCC were introduced into the main Monsanto pool (Al-Shaer et al. 2018, p. 2). Monitoring of this population took place in June and August of 2015 and 2016 (Al-Shaer et al. 2018, p. 3). Over the study period, the introduction appeared successful: territory establishment, territorial disputes, and spawning was observed, as were juveniles, indicating recruitment (Al-Shaer et al. 2018, p. 6). However, this population was again extirpated by 2019, and no additional stocking efforts at this site have occurred (Bretzing-Tungate 2023, p. 10). This site no longer has consistent water or flow and is unable to support Leon Springs pupfish (The Nature Conservancy 2023, pp. 10, 12, 16).

Captive Population at SNARRC

Currently, the captive population at SNARRC averages 5,000 individuals, with numbers lowest at the end of winter and highest at the end of summer (U.S. Fish and Wildlife Service 2020, p. 8; Wilson 2024, p. 1). These populations are stable and no demographic concerns were reported (Wilson 2024, p. 1). The establishment of a second captive population at the San Marcos Aquatic Resources Center was attempted in April 2018, with 200 fish transferred as the founding population (U.S. Fish and Wildlife Service 2020, p. 5). These fish were kept indoors and did not survive in captivity at the station (Britton 2024, pp. 1–2). After the transfer, the captive fish steadily died off; all were deceased by the end of June (Britton 2024, pp. 1–2). No further attempts to transfer Leon Springs pupfish to other USFWS facilities have been made.

Summary

Population trends for species such as Leon Springs pupfish are inherently highly dynamic, which fluctuations of an order of magnitude or more possible on a year-over-year basis. This makes it challenging to reach conclusions about population trends. The population in the Diamond Y Spring pool illustrates this, with the number of territorial males shifting from one in 2006 to 250 in 2021. There is an apparent declining trend in the count of territorial males during the past three years, but since the reason for either the increase to 250 in June 2021 or the decrease to 70 in May 2024 is not clear, we are unable to predict what these numbers will be in the future. The population at Karges Spring appears to be small but stable, based on two years of observations (The Nature Conservancy 2023, p. 9). The population at the Monsanto pool has been extirpated, which means that the species is now completely absent from the lower watercourse. We infer from the continued loss of perennial waters within the species' range that the abundance of the species as a whole has also continued to decline. The captive population is significantly larger than the wild population and has been stable for decades.

2.2.1.3 Genetics, genetic variation, or trends in genetic variation (e.g., loss of genetic variation, genetic drift, inbreeding, etc.):

In May 2013, 72 individuals from the refuge population at SNARRC and 66 individuals from the Diamond Y Spring were fin-clipped and the material used for genetic analysis (Black, Seears, et al. 2017, p. 2240). The study demonstrated that moderate genetic divergence had occurred between the 1998–2001 founding period and 2013 (Black, Seears, et al. 2017, p. 2251). The wild population had lower genetic variation, which is probably related to the bottleneck that occurred when the Diamond Y population declined to very low levels from 2000 to 2006 (Black, Seears, et al. 2017, p. 2251). Effective population size was calculated as 28 in the wild population and 231 in the captive population (Black, Seears, et al. 2017, p. 2251). The authors additionally report morphological differences between the wild and refuge populations (Black, Seears, et al. 2017, p. 2252). Specifically, the pectoral fin attachment points were different, and the slope of the snout of the captive fish was upward, something also found in captive Devils Hole pupfish (Black, Seears, et al. 2017, p. 2252).

More recently, Bretzing-Tungate (2023, pp. 7–38) completed a genomic assessment of the Leon Springs pupfish at Diamond Y Spring, Karges Spring, and SNARRC. Fin clips for this effort were collected from the wild (23 from Diamond Y Spring, 20 from Karges Spring) in 2020 and from SNARRC (33) in 2021 (Bretzing-Tungate 2023, pp. 11, 38). One whole Leon Springs pupfish was taken from each location (Bretzing-Tungate 2023, p. 12). As with the study by

Black, Seears, et al. (2017, entire), there were significant differences between the wild and refuge populations (Bretzing-Tungate 2023, pp. 19–20). In addition, the study by Bretzing-Tungate (2023, pp. 19–20) detected population structuring between the two wild populations. Diamond Y Spring and Karges Spring are approximately 285 m (935 ft) apart, but due to drought, the surface flows that connected them even in the past few decades have disappeared for most or all of the year, allowing genetic differentiation to occur (Bretzing-Tungate 2023, pp. 19–20). Similarly to the study by Black, Seears, et al. (2017, entire), the captive population continues to hold more genetic diversity and has a higher effective population size (Bretzing-Tungate 2023, p. 20). Comparing the two wild populations to one another, they had similar expected heterozygosity (Bretzing-Tungate 2023, p. 20). Diamond Y Spring had higher allelic richness, and Karges Spring had a larger effective population size estimate (Bretzing-Tungate 2023, p. 20). The author suggests that the most likely explanation for the divergence in the genetics between the refuge and wild populations is ongoing genetic drift within the wild populations due to small population size (Bretzing-Tungate 2023, p. 21). They additionally warn that the genetic data indicate that the species is at risk of entering (or potentially may be in) an extinction vortex (Bretzing-Tungate 2023, p. 21).

Table 5. Effective population size estimates with lower and upper 95% confidence intervals done using a jackknife method, as well as point estimates for all three contemporary Leon Springs pupfish populations. Data reproduced from Bretzing-Tungate (2023, p. 35).

Locality	**Lower**	**Point**	**Upper**
Diamond Y Spring	62	126	1701
Karges Spring	160	252	559
SNARRC	387	601	1325

The effective population size estimates from Bretzing-Tungate (2023, p. 35) were much higher than from Black, Seears, et al. (2017, p. 2251). We reached out to Dr. David Portnoy, who was involved in the Bretzing-Tungate-authored study, to gain insight as to the reason behind the difference in these estimates. Dr. Portnoy suggested that the difference may be due to an admixture disequilibrium in the data used by Black, Seears, et al. (2017, entire; Bean 2024, p. 1). This can happen if there is an assumption that everything at Diamond Y Preserve is one population (Bean 2024, p. 1). Admixture disequilibrium creates an artificial downward bias (Bean 2024, p. 1). Other potential causes of the differences in effective population size estimates are including non-random kin in the analysis or failing to apply a Robin Waples correction for physical linkage (Bean 2024, p. 1). It is possible that the effective population size has increased since the previous study, but the most likely explanation is the downward bias (Bean 2024, p. 1).

Both of these studies also examined whether there was any evidence of introgression from the sheepshead minnow genome. Based on the samples that were run, neither study found any evidence that Leon Springs pupfish x sheepshead minnow hybrids are present, nor any evidence of introgression by sheepshead minnow, across all three analyzed populations (Black, Seears, et al. 2017, p. 2253; Bretzing-Tungate 2023, p. 19).

In summary, there has been no change in the hybridization status of Leon Springs pupfish either in the wild or in captivity. The fact that the two populations studied in the most recent genetic study (Diamond Y Spring and Karges Spring) are showing divergence shows that the loss of perennial water connecting these two habitats is affecting the two populations through genetic drift, and in turn reducing the overall genetic health of the species in the wild.

2.2.1.4 Taxonomic classification or changes in nomenclature:

There have not been any questions or concerns regarding the taxonomic classification or nomenclature of the Leon Springs pupfish since the previous five-year review was approved in 2013.

2.2.1.5 Spatial distribution, trends in spatial distribution (e.g. increasingly fragmented, increased numbers of corridors, pollinator availability, etc.), or historic range (e.g. corrections to the historical range, change in distribution of the species' within its historic range, etc.):

Upper Watercourse

The 2013 five-year status review stated that Leon Springs pupfish were found only in the Diamond Y Draw drainage, and were not found in the Leon Creek outside of the Diamond Y Draw (U.S. Fish and Wildlife Service 2013, p. 3). That document reports that Leon Springs pupfish are documented "near an observation platform" and within a "section of the upper watercourse which receives flow from the marsh fed by Diamond Y Spring" (U.S. Fish and Wildlife Service 2013, p. 10). The area "near an observation platform" refers to the confluence between Diamond Y Draw and Leon Creek. The "section of the upper watercourse" appears to refer to the entire stretch from the outflow from the Diamond Y Spring pool to the confluence of that drainage channel with Leon Creek (Echelle et al. 1987, p. 670).

As of 2024, the confluence of Diamond Y Draw and Leon Creek and the upper watercourse past a private road downstream from Karges Spring do not hold enough water to support Leon Springs pupfish (Smith 2024b, p. 1; Figure 2).

The “section of the upper watercourse” previously described has been fragmented as a result of declining spring flows and the subsequent drying of aquatic habitat. In 2018, the Diamond Y Spring pool dropped sufficiently low to cease discharging into its outlet channel for the first time (Smith 2024b, p. 2). This phenomenon has occurred every summer since, and the outflow is dry for long portions of the summer each year (The Nature Conservancy 2023, p. 16). Leon Springs pupfish use the outflow channel habitat when it exists, but must retreat to the spring pools when it dries (The Nature Conservancy 2023, p. 16).

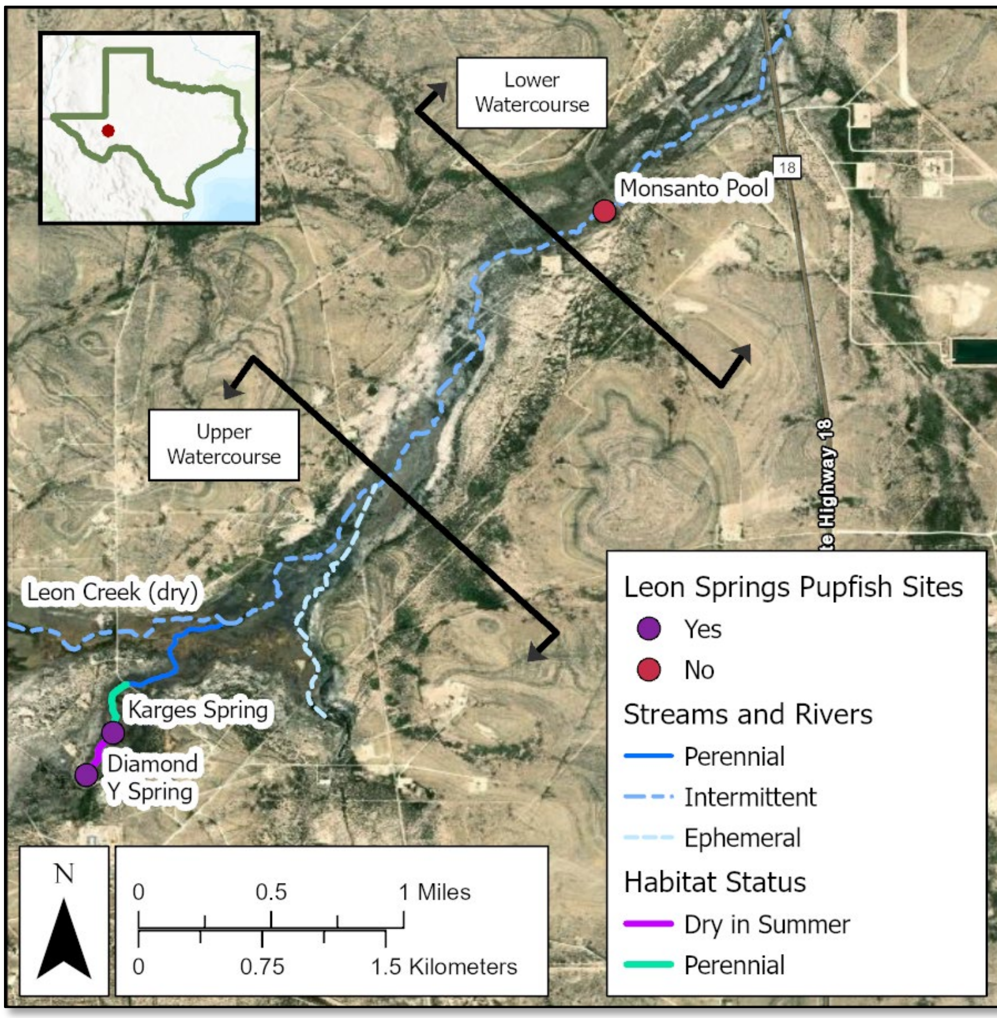

Figure 2. Overview map of the portion of the Diamond Y Preserve where Leon Springs pupfish were present within the past ten years. The presence of Leon Springs pupfish at each location as of 2024 is depicted by color: purple indicates that Leon Springs pupfish are present and red indicates that Leon Springs pupfish are not present. The linear feature between Diamond Y Spring and Karges Spring is colored pink to denote that it becomes dry in summer. The linear feature below Karges Spring is colored teal to denote that it is perennial. Other linear features within the Diamond Y Draw and Leon Creek are not given a habitat status because the Leon Springs pupfish is not present in them.

After the section between the Diamond Y Spring and Karges Spring dried in 2018, The Nature Conservancy staff walked the habitat in the upper watercourse and found that the spring now called Karges Spring contained numerous Leon Springs pupfish and provided outflow to a channel approximately 300 m (984 ft) in length (Smith 2024b, p. 2). This channel ceases to flow when it intersects a private road, where there is a collapsed culvert (Smith 2024b, p. 1). This section of the upper watercourse is still reliably perennial, and represents the stronghold for the species and its habitat within the Diamond Y Preserve—and, therefore, the species' range (Smith 2024b, p. 2). The remainder of the draw to the confluence with Leon Creek is dry and does not support Leon Springs pupfish (Smith 2024b, p. 1).

Lower Watercourse

At the time of the previous review, reintroduction efforts were underway at Monsanto pool, located in the lower watercourse (U.S. Fish and Wildlife Service 2013, pp. 7–15). The Leon Springs pupfish population in the lower watercourse, centered around Monsanto pool was extirpated by 2019 (Bretzing-Tungate 2023, p. 10). This section of the drainage only holds water after rain, and that flow comes from overland flow or runoff, rather than from a spring input (Mallek 2023a, p. 1; The Nature Conservancy 2023, p. 15). Leon Springs pupfish are no longer present in the lower watercourse.

Summary

Figure 2 shows the current Leon Springs pupfish range and denotes the status of the species at each known spring pool locality. The species remains extant in the upper watercourse, but has been extirpated from the lower watercourse. Within the upper watercourse, Leon Springs pupfish are only found from Diamond Y Spring pool to the collapsed culvert, and portions of the flowing water in this section are intermittently dry. Overall, the drying observed across the Diamond Y Preserve represents a contraction in perennial habitat for the species and thus an increase in extinction risk for the species.

2.2.1.6 Habitat or ecosystem conditions (e.g., amount, distribution, and suitability of the habitat or ecosystem):

Spring Flows

Declining spring flows are a major threat to the Leon Springs pupfish (U.S. Fish and Wildlife Service 2013, pp. 11–13). Reductions in spring flow have led to very low water levels and frequent drying in the lower watercourse, significantly reducing the total available habitat for the species (see Section 2.2.1.2). The main Diamond Y headwater spring pool stopped discharging into its outlet channel in summer 2018, and in recent years has flowed at very low discharge volumes (Freese and Nichols, Inc. 2020a, pp. 1–36; Smith 2024b, p. 2). Each year in the late spring or early summer, the water level begins to drop, exposing the area used by Leon Springs pupfish for breeding territories, and disconnecting that pool from Karges Pool by drying up the channel connecting the two pools (Mallek 2023b, pp. 1, 6–8; McKinney et al. 2024, pp. 30–35). A plausible secondary effect of low flows is increased siltation of the Diamond Y Spring pool substrate because flows are not sufficiently strong to push out sediment and maintain a cleaner gravel bottom (Brune 1981, p. 37; Dewson et al. 2007, pp. 404–406). In addition, when large amounts of sediment are present on the substrate or as part of it, then if they are stirred up due to high intensity rain events or hog activity, this can cause a temporary degradation of water quality (e.g., increased turbidity, decreased dissolved oxygen) that can harm Leon Springs pupfish (U.S. Environmental Protection Agency 2015a, unpaginated; 2015b, unpaginated; Helcel et al. 2018, p. 4).

The Nature Conservancy contracted with professional geologists to study the faulting of the geology in the vicinity (five-mile radius) of the Diamond Y Spring Preserve and construct a 3D geological model of the area (Standen and Sutherland 2021, p. 1). The authors used 137 well control points to build the model and map the faults (Standen and Sutherland 2021, pp. 6–9). A total of nine faults were mapped, generally oriented either west-east or northwest-southeast (Standen and Sutherland 2021, p. 9). A large fault crossing the Leon Creek drainage provides support for previous work from Veni (1991, entire) and Boghici (1997, entire) in the 1990s that described an upper and lower watercourse with distinct water sources (Standen and Sutherland 2021, p. 10). Other springs and seeps within the Diamond Y Preserve are also located along or very near faults, and this faulting structure appears to provide a path for pressurized Rustler Aquifer water to reach the surface (Standen and Sutherland 2021, p. 10). The 3D model revealed patterns suggesting that a shallow groundwater flow system associated with Cretaceous outcrops may exist, which could explain changes in flow and water chemistry after precipitation events (Standen and Sutherland 2021, p. 15).

Beginning in 2017, The Nature Conservancy has also funded efforts to obtain continuous, long-term measurements of spring pool level, discharge, and water quality for Diamond Y and Karges Springs (McKinney et al. 2024, p. 3). This was in part a response to declining water levels in the Diamond Y Spring pool and decreasing stability in the surface water connection between the Diamond Y and Karges Spring pools (McKinney et al. 2024, pp. 9–10). Although there have been challenges in setting up all of the instruments and obtaining accurate data despite decreasing water levels, several preliminary observations were reported (McKinney et al. 2024, pp. 29–37). In an initial draft report, the authors share that the depth of the Diamond Y Spring pool varies within each year by up to several hundred millimeters (mm), generally having the greatest depth in late spring, and the lowest depth in late summer (McKinney et al. 2024, p. 30). In contrast, the depth of the Karges Spring pool is very consistent, varying by less than 100 mm (3.9 inches [in]) (McKinney et al. 2024, p. 35). Figure 3 reproduces the depth plot from this work (McKinney et al. 2024, p. 31).

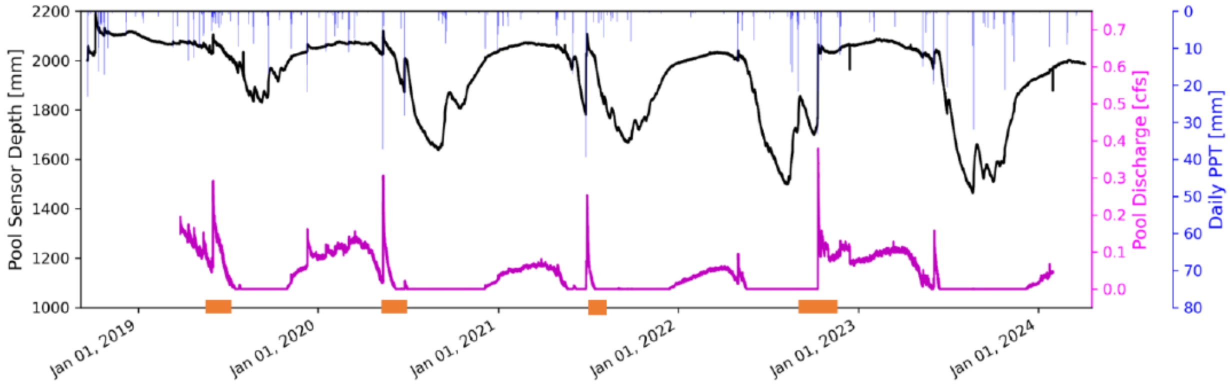

Figure 3. Monitoring data from the Diamond Y Spring pool station covering the period from late 2018 to early 2024. This figure is reproduced from McKinney et al. (2024, p. 31, Figure 3.9).

The fact that the Diamond Y Spring pool's decrease in spring discharge and water levels occurs regularly each year in late spring suggests that groundwater pumping for irrigation may be affecting spring flow, since groundwater pumping for oil and gas production is not as seasonal in nature (McKinney et al. 2024, p. 30). In recent years, the spring pool has reached its lowest pool depth values in August, declining to approximately 1,500 mm in 2022, 2023, and 2024 (McKinney et al. 2024, p. 32; Smith 2024a).

A flume in the outlet channel of the Diamond Y spring pool measures flow rates during periods when there is sufficient spring flow to fill the pool and sustain an outlet flow (McKinney et al. 2024, pp. 32–35). That is, the pool level must be over 2,000 mm deep or discharge cannot be measured via the flume (McKinney et al. 2024, pp. 32–34). Based on the data from late 2018 to early 2024, discharge from the pool varies seasonally and is impacted by precipitation events (McKinney et al. 2024, p. 32). The lowest values are recorded in summer, when the outflow channel has dried every year since 2018, and the highest values are recorded as brief pulses of 0.3–0.4 cubic feet per second (cfs) following significant precipitation (McKinney et al. 2024, p. 32). In late spring, recorded discharge averages about 0.1–0.2 cfs (McKinney et al. 2024, p. 32).

The simultaneous study of Karges Spring pool has revealed that its depth is consistent, generally staying within a 50 mm (2 in) range, regardless of the depth of the Diamond Y Spring pool, discharge through the outlet channel, or precipitation (McKinney et al. 2024, pp. 35–37). One potential explanation for the contrast is that Karges Spring flow comes from a different source than the Diamond Y Spring flow (McKinney et al. 2024, p. 35). Another is that the Karges Spring, being somewhat lower in elevation than the Diamond Y Spring, has not (yet) been affected by the same factor that is lowering the Diamond Y Spring flow (McKinney et al. 2024, p. 35).

Water Quality

As discussed in the previous five-year status review, water quality in the Diamond Y Spring Preserve is at risk of degradation due to activities associated with oil and gas development (U.S. Fish and Wildlife Service 2013, pp. 13–15). Intense oil and natural gas activity occurs within and adjacent to the preserve. Problems with illegal dumping of materials such as produced water are an issue in oil and gas producing areas (Mulder 2015, unpaginated; newswest9.com 2015, unpaginated). The injection of brines into wells for brine disposal or secondary recovery presents an opportunity for water contamination (Ashworth 1990, p. 31; Scanlon et al. 2020, p. 3515; Karanam et al. 2024, pp. 7–8). Such wells can introduce chemical contaminants to surface or groundwater, and the high salinity of the brines can increase aquatic salinity (Houston et al. 2019, pp. 30–33). Improperly or inadequately cased oil and gas wells are another source of potential contamination to both surface and groundwater; older wells are riskier because, as they age, they may fail and contaminate groundwater (Ashworth 1990, pp. 30–31). Contaminants found in water wells include brine, dissolved hydrocarbons, oil on water, and dissolved natural gas (Boyer 1986, p. 308). Pipelines present another potential route of contamination; they may contain oil, gas, or brines; leaks or ruptures to pipelines can allow these materials to enter underground aquifers (Ashworth 1990, p. 31). In addition, surface spills of oil, brines, or other materials such as drilling mud have contaminated waterways in the past (U.S. Fish and Wildlife Service 2013, pp. 13–15).

We used data from S&P Global's Enerdeq Browser to identify wells and pipelines within one, three, and five kilometers (km) (0.6, 1.8, and 3.1 miles [mi]) of the two extant sites (Diamond Y Spring; Karges Spring) (S&P Global 2024c; 2024a). We summarize the results of all wells, regardless of status, in Table 6. Table 7 shows these values for the wells, classified into active, inactive, and unknown status, within the five km (3.1 mi) buffer. Eleven new wells have been drilled within five km (3.1 mi) of the Diamond Y and Karges Springs since the last five-year status review was prepared in 2013 (S&P Global 2024b). Table 8 shows the total calculated length of all natural gas pipelines within these buffer distances. The very large pipeline sums are due to the presence of a natural gas refinery within one km (0.6 mi) of the springs.

Table 6. Wells associated with oil and gas production in the vicinity of the Diamond Y and Karges spring pools by type of well. The number of wells within 1 km, 3 km, and 5 km of those two springs is shown. These are cumulative values. That is, the single gas well within 1 km is included in the 18 gas wells within 3 km, and so on. Data obtained from S&P Global's Enerdeq Browser (S&P Global 2024c; 2024a).

Well Classification	1 km	3 km	5 km
gas well	1	18	35
oil well	17	66	121
injection	6	27	59
suspended	0	0	1
plugged gas well	0	12	31
plugged oil well	13	93	165
dry hole	4	26	48
abandoned	3	25	45
dry w/gas shows	0	2	6
dry w/oil shows	1	11	25
dry w/oil and gas shows	0	1	3
Total	**45**	**281**	**539**

Table 7. Wells associated with oil and gas production within 5 km of the Diamond Y and Karges spring pools by well status. Wells with an attribute of "<Null>" in the geospatial layer are labeled "Undefined" in this table.

Well Classification	Active	Inactive	Undefined
gas well	28	5	2
oil well	82	26	13
injection	23	18	18
suspended	0	0	1
plugged gas well	0	29	2
plugged oil well	3	102	60
dry hole	2	9	37
abandoned	0	0	45
dry w/gas shows	0	5	1
dry w/oil shows	0	18	7
dry w/oil and gas shows	0	0	3
Total	**138**	**212**	**189**

Table 8. Total length of pipelines in the vicinity of the Diamond Y and Karges spring pools, calculated using the GCS North American 1983 coordinate system. The length of pipelines within 1 km, 3 km, and 5 km of those two springs is shown. These are cumulative values.

Well Classification	1 km	3 km	5 km
Natural Gas	22.6	137.1	275.7

We used Railroad Commission of Texas loss report data to evaluate spills from oil and gas production operation in Pecos County for the period 2013–2023 (Railroad Commission of Texas 2013; 2014; 2015; 2016; 2017; 2018; 2019; 2020; 2021; 2022; 2023). We evaluated the entire county because the spills data was not consistently available at more precise locations. Spills are reported to the Railroad Commission of Texas each year; since 2013, this value has ranged from 0 to 22 spill events per year. The pattern over time and the type of liquid spills (typically crude oil) is shown in Figure 4.

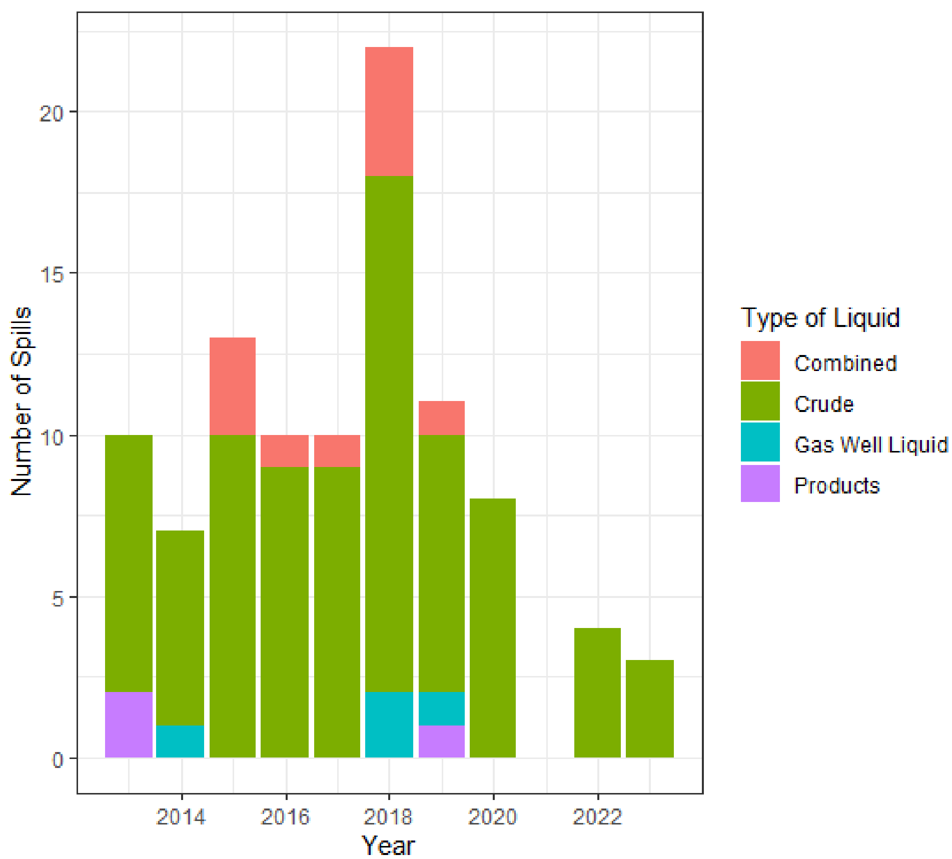

Figure 4. Number of spills per year in Pecos County, Texas, based on information reported to and made available by the Texas Railroad Commission.

Some water quality data is available from the draft report commissioned by The Nature Conservancy (McKinney et al. 2024, pp. 27–37). Within the Diamond Y Spring pool, conductivity is typically about 7,000 microsiemens per centimeter (µS/cm), but this increases to about 10,000 µS/cm when the pool level is at the lower part of its range (McKinney et al. 2024, p. 30). Within the Karges Spring pool, conductivity has ranged from about 7,000–7,400 µS /cm (McKinney et al. 2024, p. 36). Water temperature at that location ranges from a low of about 14° C (57° F) in winter to a high of about 20–22° C (68–71.6° F) in summer (McKinney et al. 2024, p. 35).

Summary

In summary, concerns about the threat to water quality are unchanged from the previous five-year review, completed in 2013. In contrast, the threat from declining spring flows has increased. The effect on the species and its habitat from the loss of spring flows is increased habitat quality impacts and degradation and a reduction in the available habitat for the species.

2.2.1.7 Other:

Climate Change

New climate projections have been developed since the previous five-year review was conducted in 2013. Downscaled climate projections for Pecos County were obtained from the U.S. Climate Resilience Toolkit and are presented in Table 9, Table 10, and Table 11 (U.S. Federal Government 2023). Details about the models and projections are available from the Climate Explorer website (National Oceanic and Atmospheric Administration 2020). Across both RCP 4.5 and RCP 8.5 scenarios, temperatures and the number of dry days are projected to increase (U.S. Federal Government 2023). The projections for total annual precipitation include projections for both increases and decreases, indicating uncertainty, particularly at the end of the century (U.S. Federal Government 2023).

Table 9. Future climate projections from the U.S. Climate Toolkit (U.S. Federal Government 2023). Baseline data for the projections is from 2005. Future projections are based on RCP 4.5 and RCP 8.5. The table enumerates the average number of days projected to have high temperatures above 105° F (40.6° C), low temperatures above 80° F (26.7°C), or no precipitation. Fractional days are shown because they are computed averages.

Year	**2005**	**2050**		**2099**	
Projection	**Baseline**	**RCP 4.5**	**RCP 8.5**	**RCP 4.5**	**RCP 8.5**
Days with a high temperature > 105°F	3.6	9.9	15.5	19.8	63.4
Days with a low temperature > 80°F	0.1	1.7	3.4	4.6	31.7
Days without precipitation	279.3	281.9	279.5	278.4	289.3

Table 10. Future climate projections from the U.S. Climate Toolkit (U.S. Federal Government 2023). Baseline data for the projections is from 2005. Future projections are based on RCP 4.5 and RCP 8.5. The table enumerates the average daily minimum temperature in degrees Fahrenheit (see Table 11 for Celsius conversions), the average daily maximum temperature in degrees Fahrenheit, and the average total annual precipitation in inches (see Table 11 for metric conversions).

Year	**2005**	**2050**		**2099**	
Projection	**Baseline**	**RCP 4.5**	**RCP 8.5**	**RCP 4.5**	**RCP 8.5**
Minimum temperature (°F)	50.5	53.1	54.5	54.9	59.8
Maximum temperature (°F)	80.3	82.9	83.9	84.4	89.7
Annual precipitation (inches)	12.63	12.99	13.62	13.18	12.29

Table 11. Future climate projections from the U.S. Climate Toolkit (U.S. Federal Government 2023). Baseline data for the projections is from 2005. Future projections are based on RCP 4.5 and RCP 8.5. The table enumerates the average daily minimum temperature in degrees Celsius (see Table 10 for Fahrenheit conversions), the average daily maximum temperature in degrees Celsius, and the average total annual precipitation in millimeters (see Table 10 for imperial conversions).

Year	**2005**	**2050**		**2099**	
Projection	**Baseline**	**RCP 4.5**	**RCP 8.5**	**RCP 4.5**	**RCP 8.5**
Minimum temperature (°C)	10.3	11.7	12.5	12.7	15.4
Maximum temperature (°C)	26.8	28.3	28.8	29.1	32.1
Annual precipitation (millimeters)	320.8	329.9	345.9	334.8	312.2

Increased variability in precipitation patterns and the concentration of precipitation events into fewer days is likely to lead to more and longer drought events in the future (Nielsen-Gammon et al. 2021, p. 15). Higher temperatures and increased aridity are likely to result in increased evaporation of lentic habitats (Nielsen-Gammon et al. 2021, p. 17), potentially compromising water quality and/or water quantity within Leon Springs pupfish surface water habitats, especially if the inflows from springs decline simultaneously. Although groundwater is generally considered less directly pressured by climate change than surface water, groundwater pumping is related to water demand (Nielsen-Gammon et al. 2020, p. 9). Hotter and drier weather could lead to increased groundwater use due to increased water needs by users or due to a shift in water sourcing from surface water to groundwater as surface water supplies become unavailable or unreliable (Nielsen-Gammon et al. 2020, p. 9).

Regional Water Planning

Planning for future water needs in Texas is guided by projections developed by 16 regional water planning groups (Freese and Nichols, Inc. 2020a, p. 1-1). The Texas Water Development Board generated water demand projections for the water planning groups covering the period 2020 to 2070, which were used to develop the 2020 Regional Water Plans (Freese and Nichols, Inc. 2020a, p. 2-1). The Diamond Y Preserve lies within water planning Region F (Figure 5).

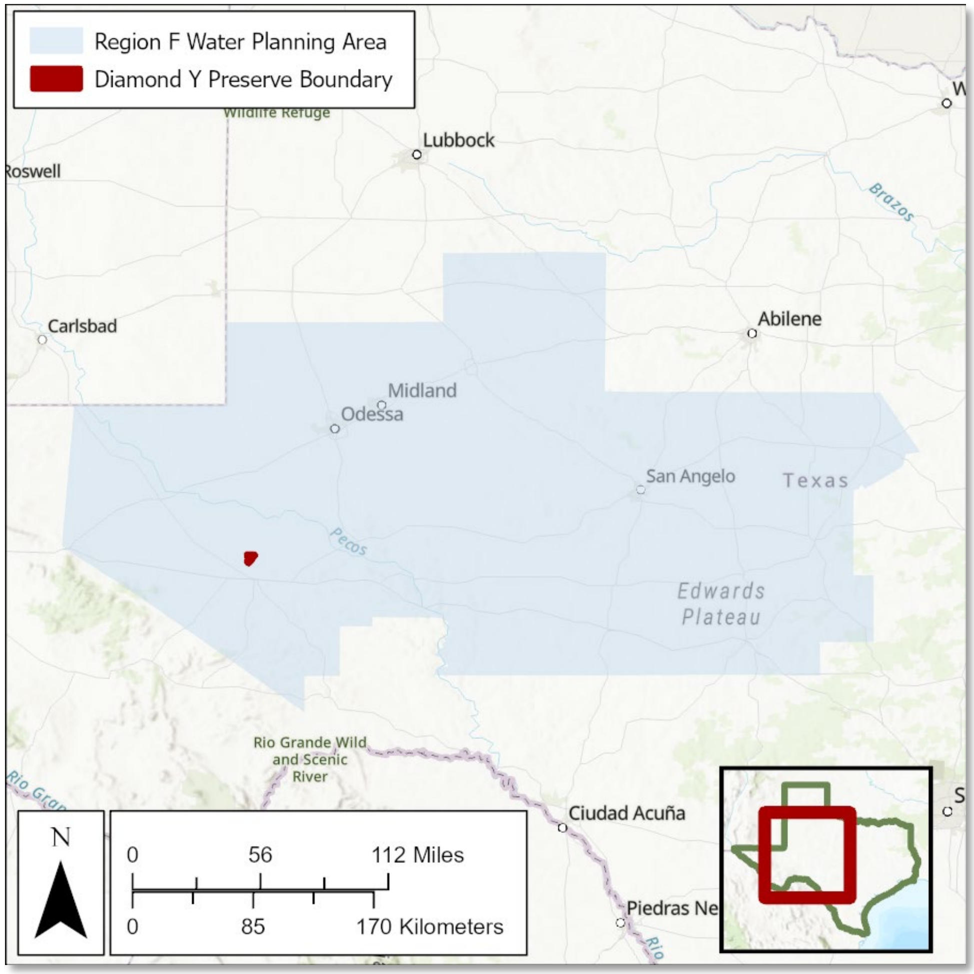

Figure 5. Map of west Texas highlighting the boundary of Water Planning Region F, which includes Pecos County and the Diamond Y Preserve.

Groundwater is the dominant water source for Water Planning Region F (Freese and Nichols, Inc. 2020a, p.3-1). The primary groundwater use is irrigation; 92% of groundwater supplies in Pecos County are used for irrigation (Freese and Nichols, Inc. 2020b, pp. 355–356). Water demand in Pecos County, which had the highest water demand by far in the planning region (132,030 acre-feet per year; the next highest demand is from Tom Green County, with a demand of 67,915 acre-feet per year), is projected to increase substantially compared to 2010 levels (Freese and Nichols, Inc. 2020a, p. 2-8). This is due both to increases in demand for water for irrigation and in demand for water for "mining" (i.e. hydraulic fracturing) (Freese and Nichols, Inc. 2020a, pp. 2-17, 2-20). Water demand to support hydraulic fracturing associated with oil and gas production in the water planning region is projected to increase through 2040 and then decline (Freese and Nichols, Inc. 2020a, p. 2-19). Although water demand for irrigation is projected to increase from 126,033 acre-feet per year in 2010 to 143,345 in 2020, the plan indicates there will be no additional increases beyond the 2020 values and therefore no increase in demand for groundwater (Freese and Nichols, Inc. 2020a pp. 2-15–2-20, 5E-51). The planning process identified a deficit between existing water supplies and projected demand for the mining industry throughout the planning horizon (Freese and Nichols, Inc. 2020a p. 5E-51). The extraction of additional groundwater from the Edwards-Trinity Plateau Aquifer is the method recommended by the planning team to address this deficit (Freese and Nichols, Inc. 2020a p. 5E-51).

The plan recognizes drought as a persistent challenge to meeting future water needs but leaves the specifics of drought planning to a future step-down plan (Freese and Nichols, Inc. 2020a, pp. 7-1–7-20). The plan does not discuss or analyze the effects of climate change on groundwater supplies or demand. Continued use of groundwater at current levels is likely to continue the ongoing reduction in groundwater flow to the Diamond Y Spring System. An increase in use will exacerbate or accelerate the trend.

The Texas Water Development Board website includes a dashboard with information on water use by region and county. Bar graphs of water use by both category and source are reproduced in Figure 6. From 2011 to 2021, water use exceeded the 2020 projections in four years: 2011, 2014, 2015, and 2016 (Texas Water Development Board 2024). During the same timeframe, water use was below the 2010 levels in five years: 2012, 2018, 2019, 2020, and 2021 (Texas Water Development Board 2024). More years of data are needed to ascertain whether the recent lower levels of use represent a sustained trend, or a temporary anomaly. In addition, these data highlight the need to analyze specific wells with the flow path leading to the Diamond Y Spring System, as the seasonal drying believed to be associated with summer irrigation has occurred annually from 2018 to 2024, despite pumping at the county level being lower in those years compared to earlier in the decade.

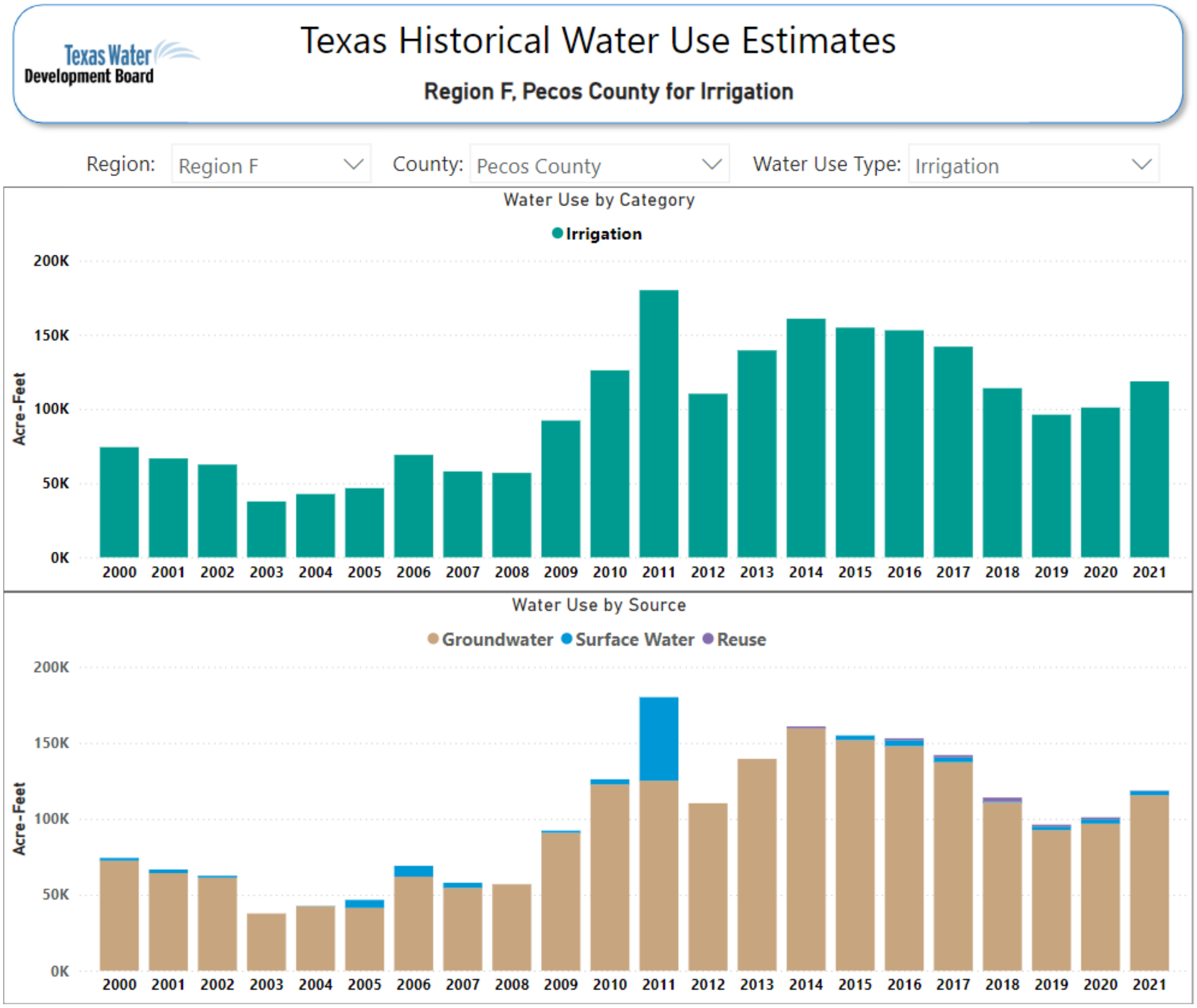

Figure 6. Texas Historical Water Use Estimates for irrigation in Pecos County from 2000–2021. Figure generated on Texas Water Development Board website (Texas Water Development Board 2024).

Groundwater Conservation Districts

There is one Groundwater Conservation District (GCD) whose surface boundaries overlap the Diamond Y Preserve: the Middle Pecos Groundwater Conservation District. According to the Middle Pecos GCD's 2020 management plan, the Diamond Y Preserve area is not a focal management zone (Middle Pecos Groundwater Water Conservation District 2020, p. 5). The plan does not mention the Leon Springs pupfish or any of the other federally threatened or endangered species that are present at the Diamond Y Preserve (Middle Pecos Groundwater Water Conservation District 2020, entire). We infer from this that the Middle Pecos GCD is not managing groundwater with the goal of ensuring the persistence of springs in the Diamond Y Spring Complex.

Texas Administrative Code (Title 31, Part 10, Chapter 36) defines desired future conditions as "the desired, quantified condition of groundwater resources (such as water levels, spring flows, or volumes) within a management area at one or more specified future times as defined by participating groundwater conservation districts within a groundwater management area as part of the joint planning process." Under Texas Water Code Chapter 36, groundwater conservation district can consider a set of nine factors when developing desired future conditions, including aquifer uses, water supply needs, hydrological conditions (e.g., average annual recharge), environmental impacts (e.g., spring flow), and impacts on private property rights. As a result, developed conditions can be a complicated amalgam of economic, scientific, and political considerations.

A joint planning process occurred in 2021 under the umbrella of Groundwater Management Areas, which cover the spatial extent of multiple GCDs. Changes in groundwater flows in Groundwater Management Area 7 may impact Leon Springs pupfish habitat. Through the joint planning process, the GCDs agreed to desired future conditions resulting in drawdowns for aquifers that contribute to springs within the Leon Springs pupfish range (Groundwater Management Area 7 2021, pp. 1–5; see Table 12).

Table 12. Groundwater conservation district desired future conditions for managed aquifers as a result of the 2021 joint planning process. The drawdown shown in the table is the total maximum net drawdown for 2070 as compared to 2010 aquifer levels (Groundwater Management Area 7 2021, pp. 1–5)

Aquifer	Drawdown in meters	Drawdown in feet
Capitan Reef Complex	17	56
Dockum	16	52
Edwards-Trinity (Plateau), Pecos Valley, and Trinity	4	14
Rustler	29	94

2.2.1.8 Conservation Measures:

The Nature Conservancy

The Nature Conservancy has owned and managed the Diamond Y Preserve since 1990 (Garrett et al. 2002, p. 442). During this time they have developed relationships with the Middle Pecos GCD and local oil and gas developers, and, as a result, various conservation efforts have occurred over the years (Karges

2003, p. 144). The organization has also taken a leading role in coordinating on-site research into water quality, spring flow, and monitoring of not only Leon Springs pupfish, but also the other endangered and threatened species present within the preserve (Smith 2024b, p. 2). Since the last five-year review, The Nature Conservancy has worked with the Middle Pecos GCD to fund updates to the Pecos County groundwater availability model and the purchase of equipment enabling the expansion of the well monitoring network in the county (Smith 2024b, p. 2). They have also secured external funding to conduct invasive feral hog control, remove salt cedar (*Tamarix* spp.), install long-term hydrology monitoring equipment at four sites on the preserve, update the existing vegetation map for the preserve, and implement a long-term monitoring protocol for the fishes present on the preserve (The Nature Conservancy 2023, pp. 1–4).

Middle Pecos Groundwater Conservation District

The Middle Pecos GCD has funded research to better understand the hydrogeology of the Diamond Y Spring System and the aquifers that supply it; recent finished studies are described in this report, but the effort is generally ongoing (Middle Pecos Groundwater Conservation District 2022, p. 3). In addition, the Middle Pecos GCD is developing a Rustler Aquifer well monitoring network that complements the existing well monitoring network, which may yield aquifer management recommendations in the future (Middle Pecos Groundwater Conservation District 2022, p. 5). The Middle Pecos GCD also requires registration of all wells in the area under its jurisdiction, notice of intent for any new wells filed in advance of drilling, and annual production reports for most wells (Middle Pecos Groundwater Water Conservation District 2020, pp. 21, 31–32).

Captive Population

According to SNARRC's draft Refuge Population and Genetics Management Plan (U.S. Fish and Wildlife Service 2020, pp. 4–6), some relatively minor discrepancies exist between records from SNARRC and what has been reported in the scientific literature, with respect to the captive population. We summarize the information from SNARRC here. Briefly, broodstock collection events occurred in 1974 (30-35 individuals brought to SNARRC), 1976 (80), 1986 (61), and 1989 (31) (U.S. Fish and Wildlife Service 2020, pp. 4–5). Some of the fish died before they could be added to the captive population, and the 1976 cohort represented the highest proportion of the individuals that made up this founding population (U.S. Fish and Wildlife Service 2020, pp. 4–5). No wild fish have been added to the captive population since 1989.

There have been two multi-year stocking events in which Leon Springs pupfish from SNARRC were introduced into the wild at the Diamond Y Preserve (U.S.

Fish and Wildlife Service 2020, pp. 4–6). The first took place each year from 1998 to 2001, following removal of all fishes, including Leon Springs pupfish x sheepshead minnow hybrids, from the lower watercourse (U.S. Fish and Wildlife Service 2020, p. 4). Thousands of fish were stocked over those five years into both the upper and lower watercourse, with the lower watercourse receiving the majority of the fish (U.S. Fish and Wildlife Service 2020, pp. 4–5). The wild population declined after these introductions, and Leon Springs pupfish were extirpated from the lower watercourse by 2013 (U.S. Fish and Wildlife Service 2020, p. 6). Habitat restoration was conducted in the lower watercourse, focusing on Monsanto pool, and Leon Springs pupfish from SNARRC were introduced again in 2013 and 2015 (U.S. Fish and Wildlife Service 2020, pp. 5–6). No negative effects to the captive population from the removal of part of the population have been recorded (Wilson 2024, p. 1).

The captive population is kept year-round in a single 0.04 hectare (ha) (0.1-acre [ac]) unlined pond (U.S. Fish and Wildlife Service 2020, p. 8; Wilson 2024, p. 1). In late fall of each year, 500–1,000 individuals are captured and brought indoors, where they are kept in circular fiberglass tanks containing flow-through water (U.S. Fish and Wildlife Service 2020, p. 8). This water is maintained at 18.5° C (65.3° F) (U.S. Fish and Wildlife Service 2020, p. 8). The purpose of moving fish indoors is to hedge against loss of the wild population due to low temperatures or predation; each spring these fish are returned to their outdoor pond (U.S. Fish and Wildlife Service 2020, p. 8). Bird netting is used in the winter months to deter predators (U.S. Fish and Wildlife Service 2020, p. 9). Every year, 60 individuals are sacrificed for the purpose of monitoring fish health (U.S. Fish and Wildlife Service 2020, pp. 8–9). Approximately every two years, Leon Springs pupfish are moved to a "fresh" pond that has been drained, disced, cleaned, releveled, and refilled (U.S. Fish and Wildlife Service 2020, p. 8).

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

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

Habitat loss, due to declining spring flow and reduced surface water habitat, was identified as a major threat to Leon Springs pupfish in the 1985 Recovery Plan (U.S. Fish and Wildlife Service 1985, p. 3). The five-year status review completed in 2013 also discusses how groundwater pumping that, exceeds aquifer recharge, can lead to diminished spring flows and loss of Leon Springs pupfish habitat (U.S. Fish and Wildlife Service 2013, pp. 11–13). Reductions in spring flow and aquatic habitat have only accelerated since 2013, with greater declines and/or cessations in flow in the 2020s to present.

Leon Springs pupfish remain extant at the pools associated with Diamond Y Spring and Karges Spring, and in the Karges Spring outflow channel for a length of approximately 300 m (984 ft) (Section 2.2.1.5). The flow of the Diamond Y Spring is declining with no indication that this decline will slow or stop, much less reverse and produce an increase in spring flow (Section 2.2.1.6). Increasing temperatures and aridity will continue due to climate change and will likely lead to increased demand for groundwater in the region that influences spring flow in the Leon Springs pupfish range (Section 2.2.1.7). If declines in spring flows are not abated before the aquifer is so drawn down that Diamond Y and Karges Springs cease to flow, the surface habitat the remaining populations depend on will cease to exist. The population in the Diamond Y Spring pool is the most vulnerable to extirpation, given the current seasonal drop in pool volume.

Separately from the stressor of habitat loss, potential contamination of the species' aquatic habitat is also important. Given the location of the species' range within the Permian Basin, a major oil and gas-producing region, the risk of contamination is ever-present. A surface spill is a potential risk, but more important and likely more catastrophic would be contamination of the aquifers or conduits that supply the Diamond Y Spring System, which could occur due to a pipeline break, leaking well, illegal dumping, or injected wastewater (Section 2.2.1.6).

Consequently, the risk of extinction for this species has increased since the last five-year status review. The risk is less from a potential catastrophic event that damages habitat, and more from the relatively slow but monotonic decline in spring flow. The loss of habitat resulting in the loss of wild populations is the most important threat to the species at this time.

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

There is no evidence at this time 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 TPWD (Title 31, Part 2, Chapter 69, subchapter J).

2.2.2.3 Disease or predation:

There are no documented diseases impacting the Leon Springs pupfish wild or captive populations. No known predators of adult Leon Springs pupfish are present in their habitat. Predation of Leon Springs pupfish eggs has been observed to occur by gambusia and nocturnal crayfish. More research is needed to determine whether the impacts from this predation are significant enough to impact Leon Springs pupfish at the population and species level, but given the low abundance of the species, it is a concern, and a potential area where future action might be taken.

2.2.2.4 Inadequacy of existing regulatory mechanisms:

The inadequacy of existing regulatory mechanisms to ensure continued spring flows for the springs that are the source of Leon Springs pupfish surface water habitat represents an ongoing threat to the species in the wild. Groundwater management in Texas is regulated via GCDs, but the GCDs that regulate the groundwater in the aquifers that are the source for Diamond Y Preserve springs do not appear to have thus far prioritized maintenance of spring flows as a goal or desired future condition for those groundwater resources (Section 2.2.1.7). They do not currently serve as a regulatory mechanism that protects Leon Springs pupfish or their habitat.

The Railroad Commission of Texas regulates many activities of the oil and natural gas industry to minimize the opportunity for the release of contaminants into groundwater or surface water in Texas (Texas Administrative Code, Title 16. Economic Regulation, Part 1). While these regulations may potentially reduce the risk of contaminant releases, they cannot remove the threat of a catastrophic event that could lead to the extinction of Leon Springs pupfish in the wild. Given the inherent risks associated with oil and natural gas activities in proximity to Leon Springs pupfish habitat, Railroad Commission of Texas regulations cannot currently remove or adequately alleviate threats associated with water contamination from a petroleum, produced water, and/or wastewater leak, spill, or release.

The inadequacy of existing regulatory mechanisms resulted in additional impacts to the species (through habitat loss) since the previous five-year review was completed, but there have not been any substantive changes in the regulatory environment itself that could shift the current outlook for the species in terms of its risk of extinction.

2.2.2.5 Other natural or manmade factors affecting its continued existence:

Competition and hybridization with the introduced sheepshead minnow (*C. variegatus*) was identified as a threat to Leon Springs pupfish in the previous five-year review and in the species' recovery plan (U.S. Fish and Wildlife Service 1985, pp. 6–7; 2013, p. 17). Hybridization between Leon Springs pupfish and sheepshead minnow has twice resulted in the removal of all fishes from the lower watercourse and subsequent reintroduction of Leon Springs pupfish from captive stocks (U.S. Fish and Wildlife Service 2013, pp. 5, 8, 20). The threat of competition or hybridization with sheepshead minnow may be reduced by the extensive drying of Leon Creek and the Diamond Y Draw, which limits the opportunity for that species to reach the remaining population of Leon Springs pupfish. Because of this, the scope and severity of this threat is essentially the same as when the previous five-year review was completed in 2013.

2.3 Synthesis

The best available scientific information indicates that the primary threats to the Leon Springs pupfish are: 1) habitat loss associated with declining spring flows, 2) potential contamination of aquatic habitat as a result of local oil and gas development, and 3) hybridization or competition with sheepshead minnow due to future unintended introductions into Leon Springs pupfish populations. The threat of habitat loss is the most immediate and pressing of the threats.

The Leon Springs pupfish no longer has disjunct, self-sustaining populations, which means it no longer has redundancy (i.e., ability to withstand catastrophic events) at the species or population level. The genetic diversity of this species in the wild is declining, perhaps rapidly. What was once—as recently as the 1990s—a diversity of habitat types (springs, ciénegas, wetlands, creeks) is now limited to two spring pools, one perennial channel, and one seasonally dry channel. These two factors together indicate an ongoing decline in representation (i.e., the ability to adapt to near-term and long-term changes in the physical and biological environment). Finally, the total amount of habitat available for Leon Springs pupfish has continued to decline, and remains at risk of contamination, which negatively affects the resiliency (i.e., ability to withstand environmental and demographic stochasticity) of the species. The status of the species with respect to resiliency, redundancy, and representation has declined since the previous five-year status review was completed in 2013. We note that the previous review also demonstrated a decline since the recovery plan was signed in 1985.

Although Karges Spring and its 300 m (984 ft) outflow are today considered the species' "stronghold," this habitat is small in total area and its water source is not secured into the future. The Diamond Y Spring pool's seasonal declines are on a trajectory that will lead to the drying of this pool and the elimination of this habitat for Leon Springs pupfish, and an accompanying loss of the individual fish that inhabit it. The reduction in habitat and issues with genetic drift in the wild population is partially ameliorated by the fact that the captive population at SNARRC is healthy, stable, and retains genetic diversity. However, at this time there are no additional prospective locations where Leon Springs pupfish could be introduced into the wild, and no near-term strategy to restore water to the dried habitat on the Diamond Y Preserve.

Important conservation work has been conducted in order to advance recovery of Leon Springs pupfish. The Texas FWCO, TPWD, the Itzkowitz lab from Lehigh University, and The Nature Conservancy have engaged in major efforts to protect, restore and enhance Leon Springs pupfish habitat, and study and monitor the species itself. SNARRC maintains a captive population. However, the threats to the species discussed above continue to exert pressure on Leon Springs pupfish in its only remaining wild population, despite ongoing recovery efforts. Overall, the viability of Leon Springs pupfish has declined since the previous five-year review. Consequently, the species continues to be in danger of extinction, and we recommend that it remain classified as Endangered.

3.0 RESULTS

3.1 Recommended Classification:

No change is needed

3.2 New Recovery Priority Number (indicate if no change; see 48 FR 43098):

5C

Brief Rationale:

We base our rationale on the Service's ranking system for determining Recovery Priority Numbers per the 1983 policy (48 FR 43098, September 21, 1983, as corrected in 48 FR 51985, November 15, 1983). The degree of threat to the species is high because of the dramatic reduction in surface aquatic habitat that has occurred over the past 30 years, with no evidence of an abatement in the loss, and the measured impact to the species revealed by genetic analysis. The threat of habitat loss is enhanced by the potential for water quality contamination due to the location of the remaining habitat within a region of intense oil and gas development. The recovery potential is low due to the challenges associated with restoring adequate spring flows to the system and lack of potential introduction sites in the wild. The taxonomy of Leon Springs pupfish reflects a species, one of several in its genus. This gives a priority of 5. For the determination of conflict, we note that the primary threat to the species is habitat loss, the primary mechanism for the loss of habitat is declining spring flow, and the primary cause of declining spring flow is the extraction of groundwater for use in economic activities. The risk of contamination is also associated with economic development, specifically the oil and gas industry. Therefore, there is significant competition for the use of the spring flows and that provide Leon Springs pupfish habitat, as well as conflicting interest by industry groups with respect to the regulation of water use and quality. It is appropriate to acknowledge this with the use of the conflict code C.

4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

The Leon Springs pupfish recovery plan was signed in 1985, and its associated recovery team last met in 2010. We recommend developing a Recovery Implementation Strategy to complement the existing plan and provide more specific guidance on the activities that will achieve the recovery actions from the plan. We also recommend developing or enhancing relationships with appropriate partners, including governmental agencies and stakeholders, in order to carry out actions that will prevent the extinction of the species and promote its recovery. This will be a multi-year process. In the meantime, we recommend individual actions to be taken in coordination with The Nature Conservancy, TPWD, the Middle Pecos GCD, and other partners.

We recommend that several currently occurring actions should continue. For example, the Nature Conservancy should continue their important role in managing the Diamond Y Preserve and promoting conservation of that landscape and of Leon Springs pupfish. Similarly, we recommend that SNARRC continue to manage their Leon Springs pupfish population and monitor it for fish health and genetic concerns. These recommendations align with actions 1.1, 1.2, 1.3, and 2.1 from the 1985 recovery plan, and are still relevant for the species and its current status.

In addition to the ongoing actions, we also recommend revitalizing or initiating other actions to support the Leon Springs pupfish and strive to recover the species to the point where it could be downlisted to *Threatened*, including but not limited to those listed below.

1) Work with The Nature Conservancy and TPWD to continue to implement the long-term monitoring protocol for Leon Springs pupfish within the preserve. Identify entities who can reliably conduct monitoring over the long-term. Expand the current protocol into a formal plan that will guide monitoring into the future. These recommendations align with actions 1.1 and 1.2, including sub-actions, from the 1981 recovery plan.
2) Restore and enhance Leon Springs pupfish habitat. Evaluate options for habitat restoration or enhancement in both the upper and lower watercourse. Conduct research to understand the full range of microhabitat needs for Leon Springs pupfish to increase in abundance. These recommendations align with actions 1.1, 1.2, and 1.3, including sub-actions, from the 1985 recovery plan.
3) Evaluate the possibility of restoring Leon Springs pupfish to the lower watercourse. These recommendations align with actions 1.3 and 2.2, including sub-actions, from the 1985 recovery plan.

4) Determine if and when translocation of wild Leon Springs pupfish within the Diamond Y Preserve is warranted. If implemented, monitor affected populations. These recommendations align with action 1 from the 1985 recovery plan, “Maintain and enhance the existing Leon Springs pupfish populations and habitats.”
5) Collaborate internally to maintain and advance captive stock management at SNARRC. Develop management plans that include target population abundance, clearly identify the purpose of the captive populations, and include specific genetic management recommendations, such as the type of analyses to conduct and at what interval. These plans should also identify conditions for supplementation of wild or captive stocks. These recommendations align with action 2.1 from the 1985 recovery plan.
6) Develop a catastrophic response plan for the Diamond Y Preserve, in collaboration with The Nature Conservancy, that addresses both contamination and drying events. This recommendation aligns with action 1.3, including sub-actions, from the 1985 recovery plan.
7) Develop a genetic management plan for the species as a whole, inclusive of both wild and captive populations. The plan should include a consideration of biobanking options. This recommendation aligns with actions 1.1 and 2.1, including sub-actions, from the 1985 recovery plan.
8) Conduct experiments and monitoring to understand what management actions would maximize Leon Springs pupfish abundance, in both the wild and in captivity. This recommendation aligns with actions 1.1, 1.2, 1.3, and 2.1, including sub-actions, from the 1985 recovery plan.
9) Complete hydrogeological studies to better understand groundwater/surface water interactions. For example, improve understanding of where groundwater development within the flowpath supplying the Diamond Y Spring System would be likely to deplete flows at those springs and where such development would be unlikely to affect spring flow. This recommendation aligns with action 1.3, including sub-actions, from the 1985 recovery plan.
10) Continue research into the habitat needs of the co-occurring Pecos gambusia and develop habitat management strategies that promote both species, to ensure that actions taken for the benefit of Leon Springs pupfish do not harm Pecos gambusia. This recommendation aligns with actions 1.1, 1.2, and 1.3, including sub-actions, from the 1985 recovery plan.
11) Conduct research into the viability of underwater video for monitoring Leon Springs pupfish populations across the range of the species. This recommendation aligns with actions 1.1 and 1.2, including sub-actions, from the 1985 recovery plan.

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U.S. FISH AND WILDLIFE SERVICE

5-YEAR REVIEW of LEON SPRINGS PUPFISH (CYPRINODON BOVINUS)

Current Classification: Endangered

Recommendation resulting from the 5-Year Review:

No change needed

Appropriate Listing/Reclassification Priority Number, if applicable: Not applicable

FIELD OFFICE APPROVAL:

Field Supervisor, Fish and Wildlife Service, Austin Ecological Services Field Office

Approve KAREN MYERS Digitally signed by KAREN MYERS Date: 2024.12.20 09:54:17 -06'00' ____