Species Status Assessment Report

for the

Pecos Pupfish

(Cyprinodon pecosensis)



Pecos pupfish (Photo Credit: Kevin W. Conway, Texas A&M University)

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REPORT VERSIONS

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Version 1.2 is the final version of the SSA Report with all final edits and feedback incorporated.

Version 1.1 is the draft Species Status Assessment report that incorporates feedback from peer and technical reviews.

Version 1.0 was the draft Species Status Assessment report completed exclusively for peer and technical review.

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EXECUTIVE SUMMARY

The Pecos pupfish (*Cyprinodon pecosensis*) is a small fish that occurs in lentic and lotic aquatic environments in the Pecos River Basin of New Mexico and Texas. The individual needs of the Pecos pupfish vary somewhat by life stage (egg, hatchling, juvenile, adult); however, as an aquatic species, water quality and quantity are vital to Pecos pupfish habitat needs. The primary factors impacting the viability of Pecos pupfish are (1) introgression of the sheepshead minnow (*C. variegatus*), (2) the loss and decline of surface-waters, and (3) degradation of water quality.

The objective of this Species Status Assessment is to provide a synthesis of all the information that exists for Pecos pupfish to assess the current and future viability of the species. The Species Status Assessment is an integrated approach of describing the history of the species, and the needs of the species, given the past and present ecosystem dynamics (Chapters 2 and 3, respectively), current condition of the species (Chapter 4), and possible future scenarios through the end of the century (Chapter 5). Needs are described at the level of individuals, populations, and the species as a whole. This assessment is underpinned by the three principles of conservation biology: Resiliency, Redundancy, and Representation. Resiliency is the ability of a population or a species to endure after a disturbance, such as increasing in density after experiencing a decline because of a stochastic event. This is typically associated with population size, growth rate, or habitat quality, which may affect resiliency. The capacity of a population or species to persist after a large catastrophic event because of the number of populations across the landscape is called redundancy. Numerous groups across the landscape increases the probability that a single event will not cause catastrophic loss of the species. Representation is described as the diversity of the species and whether the species will allow it to adapt to changing environmental conditions. Representation is often referred to as the adaptive capacity of the species within and among populations.

The Pecos pupfish is currently distributed across seven of nine analysis units covering the historical range. Although there is uncertainty surround the demography of differing Pecos pupfish populations and their genetic relationships, data suggests that the Pecos pupfish still occurs in multiple populations representing the historical range of habitat variation for the species. Though declines in range extent and population size have likely occurred, monitoring data suggest that the Pecos pupfish continues to have multiple, long-term persistent populations throughout its range.

Under all three plausible future scenarios, species condition would be reduced by 2100. In the hottest and driest scenario (Scenario 1), shallow streams are likely to be lost leading to the extirpation of Pecos pupfish in Salt Creek (TX) and a reduction in redundancy and representation in the Salt Creek Wilderness and Bitter Creek Drainage units. Deeper sinkholes and wetlands are more stable and are expected to maintain suitable conditions for the Pecos pupfish under all scenarios. However, units such as Bitter Creek Drainage, Middle Tract, and Bureau of Land Management Overflow Wetlands are more vulnerable to losses in redundancy in Scenario 1 due to susceptibility to habitat losses from future drying climatic conditions; additionally, the Pecos River may be unable to sustain year-round flows under these predicted conditions. In the hot and

wettest scenario (Scenario 2), the increased temperature mirrors that in Scenario 1, but is coupled with increased stream flows from projected increase in monsoons that may help maintain sinkhole habitats throughout the range of the Pecos pupfish, and to a lesser extent, buffer wetland habitats from the most severe impacts of increased temperatures. However, small streams are likely still at elevated risk of being lost or experiencing long-term drying or mortality events. Finally, in the warm and dry (Scenario 3), the mildest future climate scenario, effects to most habitat (wetlands, sinkholes, and riverine) are anticipated to be minimal. However, like the other two scenarios, shallow streams likely will experience drying and mortality events. Although some additional changes to Pecos pupfish status are projected to occur by 2050, we anticipate that measurable changes to viability will be more apparent by 2100. This is largely due to the resilience of the aquifer and the adaptability of the Pecos pupfish to withstand variable habitat conditions. Under all scenarios, at least one analysis unit remains in high condition. Under both Scenarios 1 and 2 Pecos pupfish are projected to be extirpated from Salt Creek (TX) eliminating the only population outside of New Mexico that has been described as genetically different from the core populations in New Mexico. Pecos pupfish experience most losses of known occupied sites under Scenario 1, though losses would be likely to occur under scenarios 2 and 3 as well. Bottomless Lakes State Park remains the only analysis unit that would be in high condition under all 3 scenarios.

Concurrent with the effects of climate change is the risk of hybridization with sheepshead minnow. Salt Creek (TX) is already at a high risk of loss due to sheepshead minnow introgression. Only the Upper Pecos River is currently highly vulnerable to sheepshead minnow introduction via a bait bucket transfer. Should this occur, non-introgressed Pecos pupfish would likely be extirpated from this unit, and, as a consequence there would be no remaining Pecos pupfish in the Pecos River. This would also increase the potential for sheepshead minnow invasion into portions of the Salt Creek Wilderness, the Middle Tract Wetlands, and possibly the Overflow Wetlands units.

This report summarizes the results of a species status assessment (SSA) conducted for the Pecos pupfish (*Cyprinodon pecosensis*), which includes relevant information about the species' life history characteristics and how those characteristics are affected by stressors and conservation measures to address those stressors. This report is intended to provide the biological support for the decision on whether or not to propose to list the species as threatened or endangered under the Endangered Species Act of 1973, as amended (Act). The process and this SSA report do not represent a decision by the U.S. Fish and Wildlife Service (Service) whether or not to list a species under the Act. Instead, this SSA report provides a review of the best available scientific information strictly related to the biological status of the Pecos pupfish.

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CHAPTER 1 – INTRODUCTION

1.1 Background

This report summarizes the results of a species status assessment (SSA) conducted for the Pecos pupfish (*Cyprinodon pecosensis*). The Pecos pupfish was considered for possible addition to the List of Endangered and Threatened Wildlife under the Endangered Species Act of 1973, as amended (Act) on 30 December 1982 (54 FR 554), and was listed as a Candidate on 28 February 1996, but was precluded by other listing activities (61 FR 7596). The Pecos pupfish was proposed for listing as endangered without critical habitat on 30 January 1998 (63 FR 4608). Within the ensuing year between the proposal of the species for listing and the required final determination by the U.S. Fish and Wildlife Service (Service), a conservation agreement was developed. The Conservation Agreement was cited in the 2000 withdrawal of the proposed rule to list (65 FR 14513) as sufficient to assure the viability of the Pecos pupfish.

On 18 June 2007, the Service was petitioned to list the Pecos pupfish as endangered as part of a multi-species petition to list 475 species in the Service's Southwest Region. On 16 December 2009, the Service issued a positive 90-day finding that the petition presented information indicating that the listing of the Pecos Pupfish may be warranted (74 FR 66866). Per a court approved settlement agreement, we agreed to send a 12-month petition finding for the Pecos pupfish to the Federal Register by December 1, 2024. Thus, we conducted a SSA to compile the best scientific and commercial data available regarding the species' biology and factors that influence the species' viability.

1.2 Analytical Framework

This Species Status Assessment (SSA) is a concise review of the species' biology and factors influencing the species, an evaluation of its biological status, and an assessment of the resources and conditions needed to maintain long-term viability (Smith et al. 2018, entire). The intent is for the SSA report to be easily updated as new information becomes available, and to support all functions of the Endangered Species Program. As such, the SSA report will be a living document upon which other documents, such as listing rules, recovery plans, and 5-year reviews, would be based if the species warrants listing under the Act. This SSA report for the Pecos pupfish is intended to provide the biological support for the decision on whether or not to propose to list the species as threatened or endangered and if so, whether or not to propose designating critical habitat. This species is unique in that the pupfish has an existing Conservation Agreement that will be considered during the analyses of this report, along with what role, it contributes to the effective conservation of the species. The process and this SSA report do not represent a decision by the Service whether or not to list a species under the Act. Instead, this SSA report provides a review of the best available information strictly related to the biological status of the Pecos pupfish. The listing decision will be made by the Service after reviewing this document and all relevant laws, regulations, and policies, and a decision will be announced in the Federal Register. Using the SSA framework (*Figure 1*), we consider what a species needs to maintain viability by characterizing the biological status of the species in terms of its resiliency, redundancy, and

representation (Smith et al. 2018, p. 306). For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in natural stream ecosystems within a timeframe for which effects to Pecos pupfish can be reasonably evaluated; in this case, to 2100. We chose this time frame because the available data on climate impacts allow us to reasonably predict the potential significant effects of stressors within the range of the Pecos pupfish.

Resiliency, redundancy, and representation are defined as follows:

Resiliency means having sufficiently large populations for the species to withstand stochastic events (arising from random factors). We can measure resiliency based on metrics of population health; for example, birth versus death rates and population size, if that information exists. Resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of human activities.

Redundancy means having a sufficient number of populations for the species to withstand catastrophic events (such as a rare destructive natural event or episode involving many populations). Redundancy is about spreading the risk and can be measured through the duplication and distribution of populations across the range of the species. Generally, the greater the number of populations a species has distributed over a larger landscape, the better it can withstand catastrophic events.

Representation means having the breadth of genetic makeup of the species to adapt to changing environmental conditions. Representation can be measured through the genetic diversity within and among populations and the ecological diversity (also called environmental variation or diversity) of populations across the species' range. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or anthropogenic) in its environment. In the absence of species-specific genetic and ecological diversity information, we evaluate representation based on the extent and variability of habitat characteristics within the geographical range.

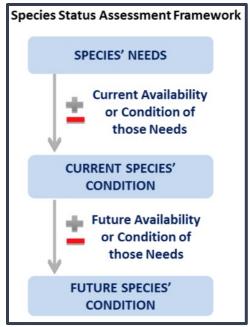


Figure 1. Overview of the Species Status Assessment (SSA) Framework.

The decision whether to list a species is based on an assessment of the species' risk of extinction. Therefore, to inform this assessment of extinction risk, we describe the species' current biological status and assess how this status may change in the future under a range of scenarios to account for the uncertainty of the species' future. We evaluate the current biological status of the Pecos pupfish by assessing the primary factors negatively and positively affecting the species to describe its current condition in terms of resiliency, redundancy, and representation (together, the 3Rs). We then evaluate the future biological status of the Pecos pupfish by describing a range of plausible future scenarios representing a range of conditions for the primary factors affecting the species and forecasting the most likely future condition for each scenario in terms of the 3Rs. As a matter of practicality, the full range of potential future scenarios and the range of potential future conditions for each potential scenario are too large to individually describe and analyze. These scenarios do not include all possible futures, but rather include specific plausible scenarios that represent examples from the continuous spectrum of possible futures. Consequently, the results of this SSA do not describe the overall risk to the species. Recognizing these limitations, the results of this SSA nevertheless provide a framework for considering the overall risk to the species in listing decisions. The Pecos Pupfish Conservation Agreement does provide some detail to address the reactive threat of introgression by the installation of fish barriers; however, future scenarios/conditions are outside of the scope of the agreement and are not considered in future scenarios.

CHAPTER 2 – SPECIES NEEDS, LIFE HISTORY, AND BIOLOGY

In this chapter, we provide biological information about the Pecos pupfish, including its taxonomic history and relationships, morphological description, historical and current distribution throughout its range, and known life history.

2.1 Taxonomy and Genetics

The Pecos pupfish (*Cyprinodon pecosensis*) is a member of the Cyprinodontidae family (pupfish and killifish), a group that includes 9 genera, 115 species, and 8 subspecies (ITIS 2023, entire). It is recognized as a valid taxon by the American Fisheries Society (Page et al. 2013, p. 107), and the currently accepted classification is:

Kingdom: Animalia Phylum: Chordata Class: Actinopterygii Order: Cyprinodontiformes Family: Cyprinodontidae Genus: *Cyprinodon* Species: *pecosensis*

The Pecos pupfish was first described from a specimen collected in 1972 from an oxbow of the Pecos River at Bitter Lake National Wildlife Refuge (Bitter Lake NWR; BLNWR), near Roswell, Chaves County, New Mexico (NM) (Echelle and Echelle 1978, entire). Other specimens used to describe the species were collected in 1972 from the following locations (Echelle and Echelle 1978, p. 575):

- Comanche Draw, Chaves County, NM
- gypsum springs and sinkholes at Bitter Lake NWR, Chaves County, NM
- sinkholes at Bottomless Lakes State Park, Chaves County, NM
- various sites along the Pecos River in Eddy County, NM and Reeves, Ward, Pecos, and Upton Counties, TX
- Salt Creek, Reeves County, TX
- a small creek near Orla, Reeves County, TX
- a ditch in Pecos County, TX

The Pecos pupfish is closely related to the Leon Springs pupfish (*C. bovinus*) and the Brazos River and the Red River pupfish (*C. rubrofluviatilis*) (Echelle and Echelle 1992, p. 691) (*Figure 2*). The Pecos pupfish and Leon Springs pupfish are both found in the Pecos Basin and are believed to be sister species, sharing *C. rubrofluviatilis* (both the Red River and Brazos River forms) as a common ancestor (Echelle and Echelle 1992, pp. 701, 703; Echelle et al. 2005, p. 329; Hoagstrom and Osborne 2021, pp. 38, 46–47).

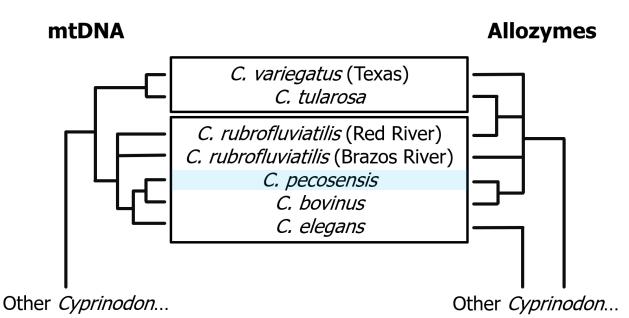


Figure 2. Simplified synthesis of relationships of Pecos pupfish based on mtDNA and allozyme variation. Adapted from Echelle et al. (2005, p. 329).

Genetic relationships within Pecos pupfish have historically been investigated under the purview of understanding the spread of sheepshead minnow (Cyprinodon variegatus) introgression in the Pecos pupfish (Connor 1987, entire; Echelle and Connor 1989, entire; Childs et al. 1996, entire; Echelle et al. 1997, entire; A. F. Echelle et al. 2003, entire; Echelle and Echelle 2007, entire). However, work by Echelle et al. (2003, entire) in Texas, and Whiteley (2023, entire) in New Mexico, has helped illustrate some of the genetic relationships among Pecos pupfish populations. The population of Pecos pupfish in the upper reaches of Salt Creek (TX) appears to be genetically distinct from other Pecos pupfish sampled elsewhere in its range. A specific allele, Gpi-A¹⁵⁷ is found only in this population (Echelle et al. 2003, p. 6). Work by Whiteley (2023, entire) in New Mexico examined the genetic relationships among Pecos pupfish sampled from Bitter Lake NWR, Bottomless Lakes State Park, and the BLM Overflow Wetlands. While Principal Component Analysis values did not indicate strong variation among the sampled populations, Pecos pupfish in the BLM Overflow Wetlands and Lower Figure 8 Lake (Bottomless Lakes State Park) were the most genetically differentiated (Whiteley 2023, p. 18). Sampled Pecos pupfish at these sites also showed low within-sample genetic variation and high inbreeding coefficients suggesting a potential population bottleneck at some point in the more recent (60 generations) past (Whiteley 2023, pp. 7-8). Principal Component Analysis of the remaining samples from Bottomless Lakes State Park showed a clustering of Mirror Lake and Lazy Lagoon samples. All of the sampled sites at Bitter Lake NWR clustered together as well (Whiteley 2023, p. 18). On Bitter Lake NWR two distinct clusters were observed that likely reflects restricted gene flow, (1) the sampled sites in the Middle Tract Wetlands and Bitter Creek, and (2) the four sampled sinkholes (Whiteley 2023, p. 8).

2.2 Physical Description

The Pecos pupfish is a small, deep-bodied (28 to 46 millimeter (mm) (1.1 to 1.8 inch (in.)) (standard length) fish varying in body color from gray to-brown to iridescent blue (Echelle and Echelle 1978, pp. 574, 577). Compared to females, males have deeper bodies, a more pronounced arch to their nape, and blunter snouts (Kodric-Brown 1975, p. 8). Juveniles, females, and some non-territorial males are gray to brown in coloration, and breeding males exhibit a dull blue iridescence on their nape (Echelle and Echelle 1978, pp. 573–574, 577). Male dorsal and anal fins are black almost to the margin with no yellow on the dorsal, anal, or caudal fins (*Figure 3*).

Female lateral bars are typically broken into blotches ventrolaterally. The abdomen is generally naked (i.e., without scales) except for a few scales in front of the pelvic fins and a patch just behind the gill membrane isthmus (*Figure 3*). Pecos pupfish have 20 to 21 gill rakers, and usually 3 or 4 preorbital pores on each side of the head (Echelle and Echelle 1978, p. 573).

Pecos pupfish vary phenotypically amongst isolated habitat types, which may be advantageous for adapting to different food availability, dissolved oxygen availability, and salinity levels (Collyer et al. 2015, entire; Xu 2017, p. 22). For example, Pecos pupfish in sinkholes were found to have shallower caudal peduncles and larger heads compared to Pecos pupfish in marshland habitats; these morphological characteristics may provide a better opportunity for these individuals to respire at the water surface (Collyer et al. 2015, p. 189). Pecos pupfish mouth position also varies between habitat types with Pecos pupfish in sinkhole habitats having terminal to sub-terminal mouths, allowing for benthic feeding rather than surface feeding (Xu 2017, pp. 26–27).



Figure 3. Male Pecos pupfish (top) and female Pecos pupfish (bottom). (Photo credit: Service)

2.3 Life Cycle and Longevity

Adult Pecos pupfish lay eggs that hatch into fry, grow into juvenile fish, and eventually mature into adults (Figure 4). Adult Pecos pupfish breed in the late spring through early fall (May to September), however breeding peaks when water temperatures are warm and food is abundant (late-June to July) (Farrington and Brandenburg 2003, p. 426; Farrington et al. 2010, p. 80; Delaune et al. 2017, p. 5). Larvae are collected in shallow areas between June through October, suggesting eggs hatch approximately one month after fertilization (Brandenburg and Farrington 2003, p. 35; Farrington and Brandenburg 2003, p. 45; Farrington et al. 2010, p. 45). Pecos pupfish sexually mature at 20 mm standard length, within a few months of hatching (Kodric-Brown 1983, p. 128). One study, conducted at Mirror Lake (Bottomless Lake State Park, New Mexico), found that between summer and winter surveys, only a small proportion of adult Pecos pupfish were collected during winter surveys, suggesting that few individuals lived more than one year (Kodric-Brown 1977, pp. 752, 756). This research suggested that wild populations of Pecos pupfish in seasonal environments with natural fluctuations in temperatures would have a single generation each year (Kodric-Brown 1977, p. 752). Conversely, in captivity where conditions are more consistent, Pecos pupfish have an average lifespan of 2.5 years (Doege 2023, entire). The Leon Springs pupfish, sister species of the Pecos pupfish, has a maximum lifespan of 20-23 months in the wild (Kennedy 1977, p. 96).

Pecos pupfish become inactive over winter, when water temperatures drop below 10 °C (50 °F), and can be found in areas with dense vegetation and flocculent material (such as fine detritus or non-living organic matter) present in the substrate (*Table 1*) (Kodric-Brown 1977, p. 752; Hoagstrom et al. 2015, p. 17).

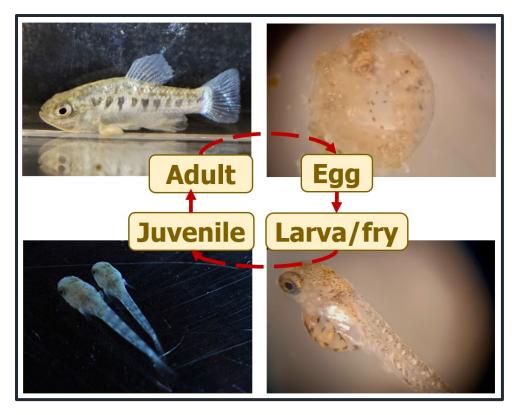


Figure 4. Pecos pupfish life cycle diagram. (Photo credit: R. Doege, Fort Worth Zoo).

Table 1. Environmental needs (breeding, feeding, and sheltering) for each life stage of the Pecos pupfish.

Life stage	Resources and/or circumstances needed for individuals to complete each life stage
Spawning adult	 Warm water temperatures between May and September Suitable oviposition sites (such as crevices, boulders, pebbles, scattered rocks, and subsurface vegetation mats)
	• Shallow water less than 2 meters (m) deep
Egg	• Salinities less than 35,000 milligrams/liter (mg/L)
Juvenile/non-breeding	• Adequate abundance of food (algae, insect, vegetation,
adults	etc.)
Overwintering adults and	Dense vegetation
juveniles	• Deeper water
All	• Hydrologic conditions conducive to survival (sufficient water levels, sufficient water temperature, etc.)

2.4 Reproduction

Pecos pupfish spawning occurs May through September; however, as mentioned previously, spawning peaks in late June through July when water temperatures consistently exceed 30 °C (86 °F) (Table 1) (Garrett et al. 2002, p. 366; Kodric-Brown 1986, p. 426). As with most pupfish, the Pecos pupfish often occurs in quiet water less than 2 meters (m) (6.56 feet (ft)) deep, and in areas with topographic diversity for spawning (Kodric-Brown 1977, pp. 750–751). A variety of underwater features such as crevices, boulders, large rocks, scattered pebbles, and aquatic plants provide topographic diversity throughout the range of the Pecos pupfish (Kodric-Brown 1975, p. 35; 1977, pp. 750–751, 753–756, and 761–762). Rocky embankments appear to be the most desirable breeding substrate, as the most aggressive and largest males occupy these areas at Mirror Lake, Bottomless Lakes State Park, Chaves County, NM (Kodric-Brown 1975, pp. 34–35). The percentage of males holding territory can vary year to year and is influenced by the amount of breeding and foraging habitat available (dependent on water levels) and that density of territorial males was highest in dense patches of aquatic vegetation, and lowest in flat silty areas with isolated rocks (Kodric-Brown 1975, pp. 20, 34–35).

Female Pecos pupfish lay individual eggs that adhere to spawning substrate, such as vegetation or rocks (Kodric-Brown 1977, pp. 751, 761–762, 764). Female Pecos pupfish lay an average of 10 eggs per day, with females in populations with higher densities having smaller ovaries and laying fewer eggs than those in populations with lower densities (Garrett 1982, pp. 360, 363; Farrington and Brandenburg 2003, p. 1). Egg size varies across populations and may be tied to genetic differences (Garrett 1982, pp. 360, 363). Both males and females may mate multiple times each reproductive period (Kodric-Brown 1975, p. 7).

Reproductive males exhibit three types of breeding behavior including territorial, satellite, and sneaker males (Kodric-Brown 1986, p. 425). Most reproductive males are territorial (Kodric-Brown 1986, p. 426) and defend a small area of substrate as well as a few centimeters of the water column above (Kodric-Brown 1975, p. 36). Territorial defense includes driving away other Pecos pupfish males as well as potential egg predators (Kodric-Brown 1977, p. 754). Once a female enters the territorial male's territory, the male approaches her and they spiral to the substrate where they quickly spawn (either once or multiple times) (Kodric-Brown 1977, p. 754). Satellite males are smaller than territorial males and do not defend a territory; however, they generally occupy the undefended areas above territories, and will opportunistically intrude on territories to disrupt spawning of territorial males (Kodric-Brown 1977, p. 754, 1986, p. 429). Sneaker males are the only reproductive males to not possess the bright blue nuptial male coloration (Kodric-Brown 1986, p. 426). Sneaker males reproduce with females either on a territory by joining a pair in the act of spawning, or by spawning with a female away from a defended territory (Kodric-Brown 1986, p. 426).

2.5 Diet

Pecos pupfish are opportunistic omnivores and vary their diet depending on available food sources; however, diet can be reflective of habitat, sex, and gut length (Davis 1981, entire). An examination of diets from 117 individuals collected from Salt Creek (TX) found that the Pecos

pupfish diet is primarily composed of a diatom-detritus mixture, but may also include animal material, filamentous algae, macrophytes, sand, and seeds (Davis 1981, p. 536). Males have been observed consuming a higher amount of animal materials than females (Davis 1981, p. 536). Individuals with shorter gut lengths (less than 50 millimeters (mm) (less than 2 inches)) have also been observed consuming a higher amount of animal materials than those with longer gut lengths (Davis 1981, p. 537). Habitat conditions, such as chloride concentration, has also been found to influence the diversity of animal taxa consumed and percent volume of animal material ingested, with an inverse relationship between chloride concentration and ingested animal material (Davis 1981, p. 537–538). Additionally, data suggest that as more animal prey are available, Pecos pupfish preferentially switch to a more carnivorous diet (Davis 1981, p. 539).

As mentioned previously, Pecos pupfish feeding behavior and diet varies between different habitat types. For example, Pecos pupfish in some sinkhole habitats are adapted for benthic feeding to avoid surface level feeding competition (Xu 2017, pp. 26–27).

2.6 Habitat

Pupfish are a euryhaline group of fish and are able to withstand conditions such as elevated salinity, higher water temperatures, and lower dissolved oxygen, that many other fish cannot tolerate (Kodric-Brown 1975, pp. 3, 6). The Pecos pupfish occurs in a variety of aquatic environments including wetlands, sinkholes, waterfowl impoundments, streams, springs, and the Pecos River mainstem (*Figure 5* and *Figure 6*) (Hoagstrom and Brooks 1999a, pp. 14 - 16; Collyer et al. 2015, p. 182).



Figure 5. Photos of Pecos pupfish habitat at Bitter Lake NWR.Top: Hunter Marsh; Bottom: Sinkhole 11. (Photo credits: Timothy Ludwick and Carl Jacobsen, Service).

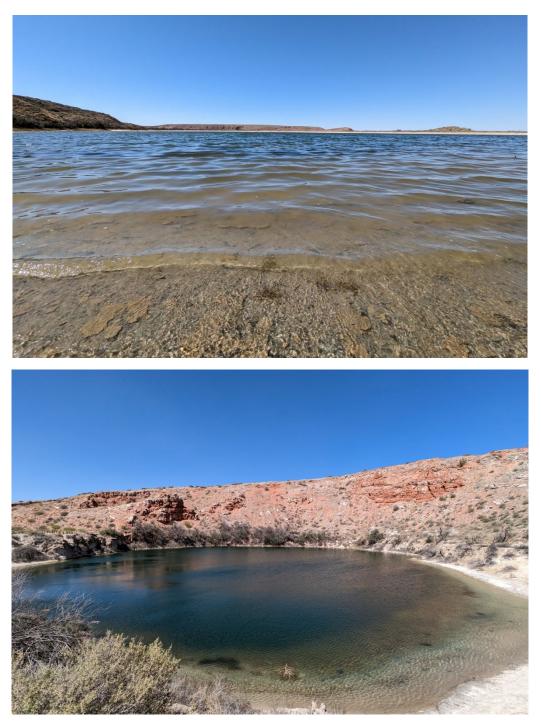


Figure 6. Photos of Pecos pupfish habitat at Bottomless Lakes State Park.Top: Lazy Lagoon; Bottom: Mirror Lake. (Photo credit: Timothy Ludwick, Service)

Pecos pupfish prefer environments with little to no water flow, and, in areas with flows, they typically occupy pools and shallow runs and riffles (Hoagstrom and Brooks 1999, pp. 36, 45). Pecos pupfish habitat can contain a diverse variety of substrates such as detritus, silt, sand, cobble, and gravel (Hoagstrom and Brooks 1999, pp. 28–52; Farrington et al. 2010, pp. 4–5). Vegetation found in Pecos pupfish habitat is similarly diverse with a variety of algae, aquatic,

semi-aquatic, and halophytic (salt tolerant) vegetation (Hoagstrom and Brooks 1999, pp. 28–52; Farrington et al. 2010, pp. 4–5), although not all of these types are present or required for an environment to provide suitable habitat for the Pecos pupfish.

Pecos pupfish tolerate high salinity and are often found in areas with salinities ranging from 3,000 to 50,000 milligrams per liter (mg/L); however, salinities higher than 35,000 mg/L may retard egg and larval development (Table 1) (Hoagstrom and Brooks 1999, p. 21; Propst 1999, p. 67). Pecos pupfish can also tolerate low dissolved oxygen, with measurements of dissolved oxygen levels as low as 2.5 mg/L during Pecos pupfish sampling (Hoagstrom and Brooks, 1999, p. 31; Propst 1999, pp. 67–68). Pecos pupfish tolerance to high salinities and low dissolved oxygen allow them to do well in extreme conditions that other fish may not be able to tolerate. While the specific thermal tolerance of Pecos pupfish is unknown, studies examining thermal tolerance of other pupfish found tolerance to range from below 0 °C to 45 °C (32 °F to 113 °F) (Bennett and Beitinger 1997, pp. 81-85; Hoagstrom and Brooks 1999a, pp. 21, 31; Propst 1999, p. 67-68). However, data collected in studies of desert pupfish (*C. macularius*) found that temperatures above 42.7 °C (108.9 °F) may be lethal (Schoenherr and Feldmeth 1992, p. 50; BEEC 2010, p. 8). These physical conditions (dissolved oxygen, salinity, and temperature) can be greatly affected by spring discharge and other flow parameters (BEEC 2010, p. 8).

Little is known regarding the minimal habitat patch size or degree of habitat connectivity necessary to support persistent Pecos pupfish populations or subpopulations. However, it is generally understood in the field of conservation biology that larger and more connected populations contribute to the long-term viability of a species and that smaller, isolated populations are more at risk of decline or extirpation as a result of genetic drift, demographic or environmental stochasticity, and catastrophic events (Fagan 2002, pp. 3243, 3248; Wiegand et al. 2005, pp. 109, 117–119; Letcher et al. 2007, pp. 5–6; Peterson et al. 2014, pp. 556–557, 564– 565). Historical occurrence data show Pecos pupfish inhabited a variety of aquatic environments throughout their range, and that Pecos pupfish are able to move into previously unoccupied environments where conditions are suitable (Hoagstrom and Brooks 1999b, p. 21; NHNM 2021, entire; GBIF 2022, entire). It is reasonable to conclude that the Pecos pupfish colonized suitable uninhabited areas, possibly during periods of high-water flow when previously isolated areas become connected, allowing for expansion and contraction of ranges and abandonment and recolonization of streams as environmental and/or demographic conditions changed (as is the case with a sinkhole that formed in 2013 and subsequently became inhabited by Pecos pupfish) (Hoagstrom and Brooks 1999b, pp. 21–22; BEEC 2010, p. 13; Caldwell 2014, p. 12; Hatt 2019, p. 7). Some areas, such as Lea Lake and the BLM Overflow Wetlands are currently connected via an outflow stream, allowing for seasonal movement (Hoagstrom and Brooks 1999, p. 22). Pecos pupfish likely experience periodic cycles of connectivity and isolation and thus experience a limited level of genetic flow between populations. However, in most cases, at any given time, there is limited to no connectivity between occupied sites with many inhabited areas being isolated sinkholes and oxbows (Hoagstrom and Brooks 1999b, pp. 21-22, 33, 41-42, 49; BEEC 2010, pp. 9, 20). Connectivity between different sites is further discussed in Section 2.9.3 below.

2.7 Pecos River Basin Hydrology/Hydrogeology

The hydrology of the Pecos River Basin and the underlying geology supports permanent water for the Pecos pupfish. Management of the Pecos River, surface waters, and the groundwater supply are critical for maintaining perennial water in the Pecos River Basin. Surface water within the Pecos River Basin is supplied by snowmelt (from the Sangre de Cristo and Sacramento Mountains), rainfall, and groundwater (Llewellyn et al. 2021, p. 7). The Pecos River drainage area encompasses approximately 113,959 square kilometers (km) (44,000 square miles (mi)) and receives input from springs, streams, and groundwater inflows (*Figure 7*) (Llewellyn et al 2021, p. 10).

2.7.1 Pecos River

Within our interest area (southeastern New Mexico to the confluence with the Rio Grande in Val Verde County, TX), the Pecos River is sometimes fairly shallow and meandering (especially near Roswell, Chaves County, NM) though it typically is narrower and deeper (near Artesia, Eddy County, NM) (Llewellyn et al 2021, p. 11). Many of the Pecos River tributaries are ephemeral (Llewellyn et al 2021, pp. 10–11).

Historically, the Pecos River above the current location of Brantley Reservoir, Eddy County, NM, had much higher flows and erosive banks with a potentially shifting channel, and it received sediment inputs from tributaries during flash flood events associated with the summer monsoon (Follansbee et al. 1915, pp. 475–488). Below Brantley Reservoir, Eddy County, NM, the Pecos River was historically influenced by spring flow, had a riffle-and-pool morphology, and could be deep and fast-moving (Hoagstrom 2003, pp. 93–94).

Today, the Pecos River is more homogenous than it was before dams and diversions in terms of water depth, substrate, flow, and channel morphology (Blann et al. 2022, pp. 17–20). Springflow and streamflow into the Pecos River has declined or ceased due to water extraction from the artesian and shallow aquifers in the basin (Havenor 1968, p. 3).

2.7.2 Groundwater

All of the non-riverine habitats for the Pecos pupfish are either completely or partially supported by springs sustained by aquifers that underlay the range of the Pecos pupfish in both Texas and New Mexico (*Figure 8*). In New Mexico, these springs originate from shallow and deep aquifers (San Andres Artesian Aquifer) in the Roswell Artesian Basin (Land and Huff 2009, p. 2) (*Figure 8* and *Figure 9*). The Roswell Artesian Basin spans the area 10 to 15 mi west of the Pecos River from north of Roswell, Chaves County, NM to Brantley Reservoir, Eddy County, NM (*Figure 8*) (Land and Huff 2009, p. 2; Houston et al. 2019, p. 13; Llewellyn et al. 2021, p. 14). Chemical investigations of water in sinkholes on Bitter Lake NWR show a mixture of both recent and pre-modern water indicating a complicated recharge system composed of multiple flows and pathways (Land and Huff 2009, p. 20). The artesian carbonate aquifer is recharged by surface waters flowing over permeable surfaces such as the Pecos Buckles (*Figure 9* and *Figure 10*). The shallow alluvial aquifer is recharged by the artesian carbonate aquifers below, precipitation, and irrigation return flow (Llewellyn et al 2021, p. 14). Aquifer levels in the Pecos Basin are

influenced by precipitation and groundwater pumping, which have led to reduced spring flows, decreasing water connectivity across the landscape (Hoagstrom and Brooks 1999b, p. 14; Land and Newton 2008a, p. 189). The picture in Texas is less clear, but surface waters in Salt Creek (TX) are likely at least partially supported by springs from the Rustler Aquifer (*Figure 8*) (Boghici and Broekhoven 2001, pp. 209, 225).

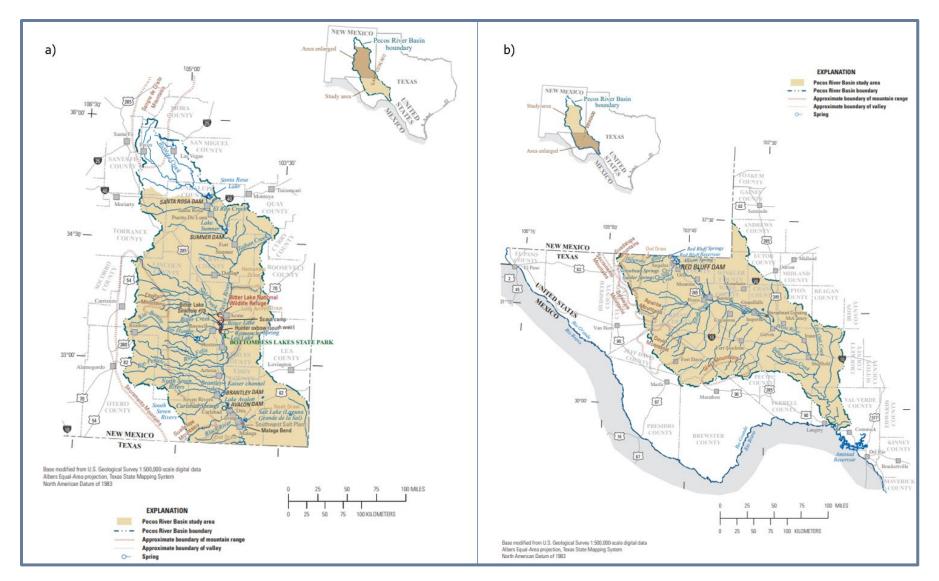


Figure 7. Aquatic Habitats and Surface Waters within the Pecos River Basin in (a) New Mexico, and (b) Texas (Houston et al. 2019, p. 4).

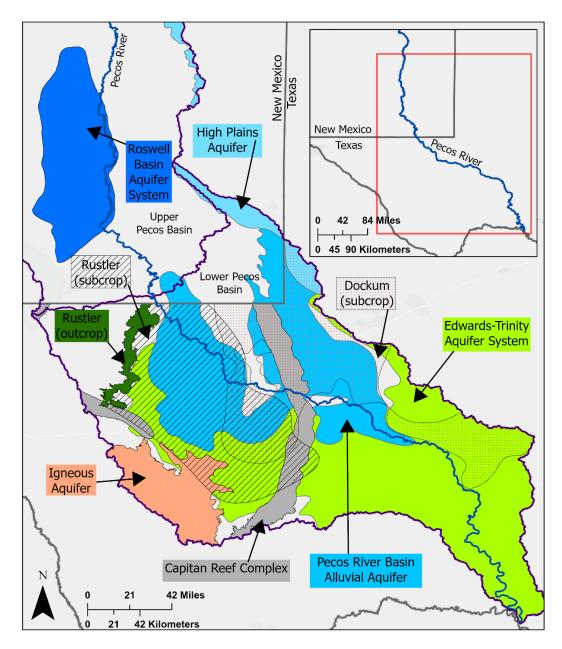


Figure 8. Aquifers throughout the Upper Pecos Basin and Lower Pecos Basin in New Mexico and Texas.

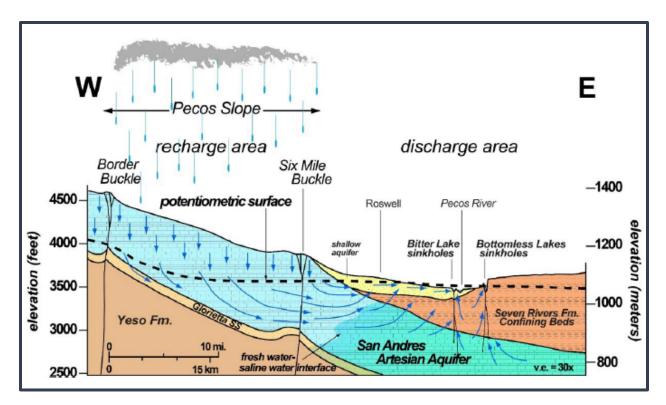


Figure 9. Schematic of the groundwater movement from the San Andres Formation to the Pecos River.Note the Pecos Buckle, Bitter Lake sinkholes, the Pecos River, and Bottomless Lakes State Park (Llewellyn et al. 2021, p. 16, based on Land and Huff, 2010).

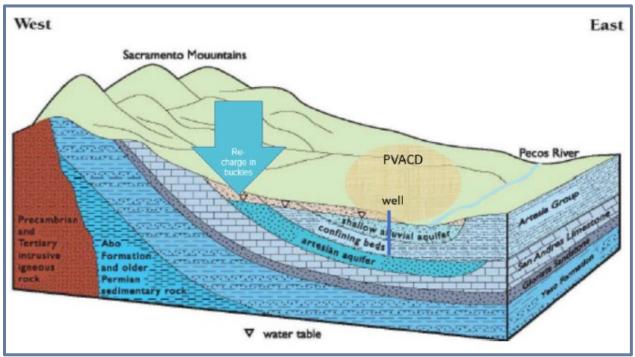


Figure 10. Schematic of the Roswell Artesian Basin aquifer (Llewellyn et al. 2021, p. 14).

Within the range of the Pecos pupfish, water in the Pecos River is managed by the Bureau of Reclamation to support deliveries to irrigation districts, fulfill obligations under the Pecos River Compact, and maintain environmental flows for endangered species. Within the Pecos Basin, groundwater is managed only within the Roswell-Artesian Basin in Chaves and Eddy Counties, NM, by the Pecos Valley Artesian Conservancy District (PVACD).

2.8 Summary of Individual, Population, and Species Needs

Each population of the Pecos pupfish needs to be able to withstand, or be resilient to, stochastic events or disturbances. These events occur frequently enough that they can drastically alter local ecosystems. Examples of stochastic events include golden algae blooms, drought, and fire (Sabo and Post 2008, pp. 19–20). To be resilient to stochastic events, populations of Pecos pupfish need to have enough individuals (abundance) and occupy multiple types of habitats, such as sinkholes, streams, and wetlands to withstand stochastic events. Additionally, populations need to exist in locations where environmental conditions provide suitable habitat and water quality such that adequate numbers of individuals can be supported. Without all these factors, a population has an increased likelihood for localized extirpation.

For a species to persist over time, it must exhibit attributes across its range that relate to either representation or redundancy. Representation describes the ability of a species to adapt to changing environmental conditions over time (Shaffer and Stein 2000, p. 308). Representation is characterized by the breadth of genetic and environmental diversity within and among populations. For the Pecos pupfish to exhibit adequate representation, resilient populations should occur within the Pecos River Basin to which it is native. The breadth of morphological, genetic, and behavioral variation should be preserved to maintain the evolutionary variation of the species.

Redundancy describes the ability of a species to withstand catastrophic events (Tear et al. 2005, p. 841; Redford et al. 2011, p. 42). Adequate redundancy minimizes the effect of localized extirpation on the range-wide persistence of a species (Shaffer and Stein 2000, p. 308). Redundancy for the Pecos pupfish is characterized by having multiple, resilient, and representative populations across the range of the species. Also important for measuring redundancy is the connectivity among discrete populations that allows for immigration and emigration between populations and increases the likelihood of recolonization, should a population become extirpated. In the case of the Pecos pupfish, however, increasing connectivity among populations can present a hybridization risk.

2.9 Range-wide Distribution

In this section we first provide an overview of hydrology and then discuss the historical and current distribution. To determine the historical and current range of the Pecos pupfish we reviewed all available state agency survey reports from New Mexico Department of Game and Fish (NMDFG) and Texas Parks and Wildlife Department (TPWD), occurrence data obtained from Natural Heritage New Mexico and Global Biodiversity Information Facility (GBIF), and

literature. Occurrence data from GBIF includes iNaturalist observations, entries in the Fishes of Texas Project Database, and other museum records.

2.9.1 Historical Range and Distribution

The Pecos pupfish is endemic to the Pecos River and adjacent waters in New Mexico and Texas (*Figure 11*). For this SSA, we split the Pecos River into three segments: (1) the Upper Pecos River, north of Brantley Reservoir, (2) the Middle Pecos River, from Brantley Reservoir to Red Bluff Reservoir, and (3) the lower Pecos River, south of Red Bluff Reservoir. The species historically inhabited the Upper, Middle, and lower Pecos River from just above Bitter Lake NWR, Chaves County, NM in the north, to south of the mouth of Independence Creek, Crockett and Terrell Counties, TX in the south (Echelle et al. 1972, p. 578; GBIF 2022, entire; Montagne 2023, entire). One museum record exists near the confluence of the Pecos River and the Rio Grande in Val Verde County, TX, however, there is uncertainty in the location of collection (Hendrickson and Cohen 2022, entire). Within the historical distribution, the Pecos pupfish inhabited a variety of habitats including the mainstem river, sinkholes, streams, and likely more abundant wetlands (Brooks 1992, entire; Hoagstrom and Brooks 1999b, p. 16). Pecos pupfish habitat was likely more abundant and more connected than today due to higher water tables from both a lack of dams and groundwater pumping (Hoagstrom and Brooks 1999a, pp. 11, 21).

2.9.2 Current Range and Distribution

All currently known extant populations of the Pecos pupfish exist within or near Bitter Lake NWR and Bottomless Lakes State Park, the Bureau of Land Management's (BLM) Overflow Wetlands, the Pecos River between Bitter Lake NWR and north of Brantley Reservoir, and Salt Creek (TX) (*Figure 12*). The species has been extirpated from the entirety of the Pecos River south of Brantley Reservoir, and several historically occupied off-channel habitats throughout New Mexico and Texas, excluding those mentioned below (Hoagstrom and Brooks 1999b, p. 13; BEEC 2010, p. 9). Extirpation of the Pecos pupfish from the Middle and lower Pecos River in New Mexico and Texas is attributed to introgression with sheepshead minnow (Hoagstrom and Brooks 1999b, p. 13; BEEC 2010, p. 9).

Land management where the Pecos pupfish is found is a mix of Federal, State, and private property (*Figure 12*). Known occupied areas include Bitter Lake NWR, managed by the Service, Bottomless Lakes State Park, managed by the NM Energy, Minerals and Natural Resources Department, the BLM Overflow Wetlands Area of Critical Environmental Concern (ACEC), managed by the BLM's Roswell Field Office, and the mainstem Pecos River and Salt Creek (TX), managed by various landowners, including private landowners (*Figure 12*). A full list of all sites where Pecos pupfish have been documented is found in Appendix A.

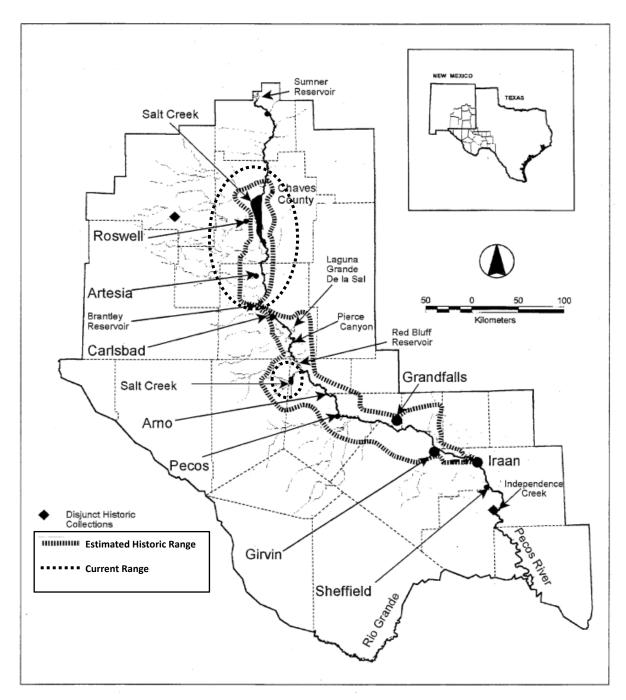


Figure 11. Historical and Current Range of the Pecos Pupfish. Modified from (Hoagstrom and Brooks 1999b, p. 15).

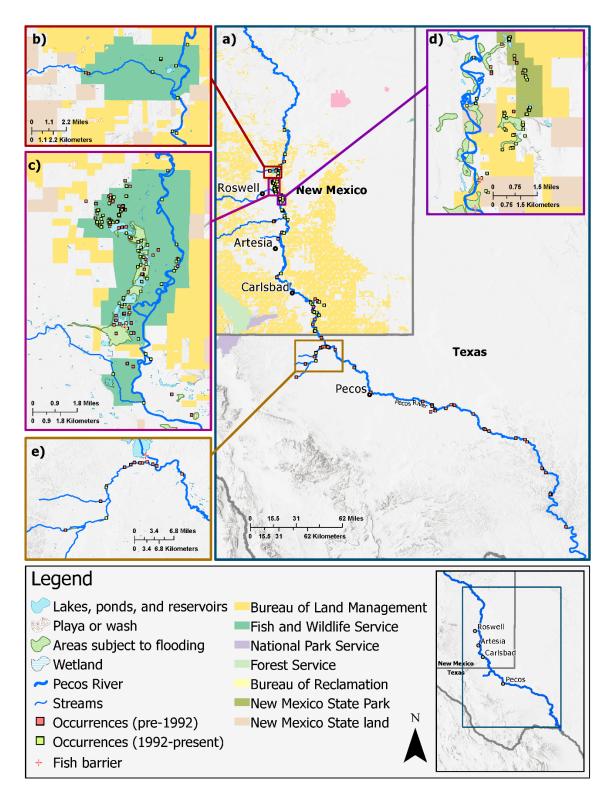


Figure 12. Range and distribution of Pecos pupfish occurrence in Pecos River Basin (a). Inset maps show areas off the mainstem Pecos River where Pecos pupfish are extant, including Bitter Lake NWR (b-c), Bottomless Lakes State Park and BLM Overflow Wetlands (d), and Salt Creek (TX) (e).

Pecos River Mainstem

The Pecos River originates in the Sangre de Cristo Mountains and spans 1,561 km (970 mi) until its confluence with the Rio Grande northwest of Del Rio, Val Verde County, TX (Llewellyn et al. 2021, p. 7). The Pecos River is among the most saline river systems in the southwestern United States (Israël et al. 2014, p. 89). Pecos pupfish can currently be found in the Pecos River mainstem from north of Bitter Lake NWR to just upstream of Brantley Reservoir (BEEC 2010, p. 11). Pecos pupfish observations in the mainstem Pecos River are infrequent, but they are consistently collected and typically inhabit ephemeral pools and shallow areas within the river (Hoagstrom and Brooks 1999b, p. 16).

Bitter Lake National Wildlife Refuge

Bitter Lake NWR is broken into two units, (1) the Salt Creek Wilderness, or the Refuge North Tract, and (2) the Middle Tract (BEEC 2010, p. 13). Within Bitter Lake NWR, Pecos pupfish inhabit isolated sinkholes, creeks (Bitter Creek, Salt Creek (NM)), springs (Sago Spring, Dragonfly Spring), and waterfowl impoundments (Hoagstrom and Brooks 1999b, p. 16). Pecos pupfish are often the only fish that occupy sinkholes where salinity exceeds 20,000 mg/L, however in sinkholes with salinities less than 20,000 mg/L they co-occur with plains killifish (*Fundulus zebrinus*) and Pecos gambusia (*Gambusia nobilis*) (Hoagstrom and Brooks 1999b, p. 16).

Occupied habitats in the Salt Creek Wilderness include four sinkholes of varying sizes and a perennial pool within the ephemeral Salt Creek (NM) (Hoagstrom and Brooks 1999a, p. 16). Occupied habitat within the Middle Tract in Bitter Lake NWR includes Bitter Creek, which feeds into Bitter Lake and is fed by Dragonfly Spring and Lost River (springs) and seeps (Hoagstrom and Brooks 1999b, p. 22). Bitter Lake NWR also includes numerous isolated sinkholes of varying sizes, 20 of which have been occupied by Pecos pupfish (Hoagstrom and Brooks 1999b, pp. 16, 28–35; BEEC 2010, p. 13). Bitter Lake supplies water to the rest of the refuge, including waterfowl impoundments and spring runs (which also includes springs and seeps supplying additional water), and eventually flows into the Pecos River via Hunter Marsh (pictured above in *Figure 5*) (Hoagstrom and Brooks 1999b, p. 22). The Middle Tract also includes oxbows of the Pecos River (BEEC 2010, p. 15).

Under high surface water conditions, it is likely that there is some surface water connection between Salt Creek (NM), the Pecos River, and sinkholes within the North Tract Bitter Lake NWR. Water features within the Bitter Creek Drainage are likely also connected during these same surface water conditions (Hoagstrom and Brooks 1999b, pp. 21–22; BEEC 2010, p. 13). These periods of surface water connectivity in an otherwise unconnected habitat may provide an opportunity for dispersal and colonization (Hoagstrom and Brooks 1999a, p. 21). A concrete fish barrier was constructed at Bitter Lake NWR at the South Weir of the refuge, with the purpose of protecting Pecos pupfish from hybridization with the sheepshead minnow (Conservation Team 2022, pp. 3–4).

Few of the occupied sites on Bitter Lake NWR are routinely surveyed; however, Pecos pupfish are assumed to be present at all known occupied sites on the refuge. A visit to the Salt Creek Wilderness confirmed Pecos pupfish presence in Salt Creek (NM) in 2023 (Jacobsen 2023, entire). Routine monitoring at Bitter Lake NWR occurs on Bitter Creek, Sinkhole 7, Sinkhole 9, the Unit 5 spring ditch, Unit 15 spring ditch, and the Hunter Marsh Ditch; Pecos pupfish were verified extant at these sites in during surveys in 2022 (Hatt 2022, p. 5). Pecos pupfish observations have also been confirmed at additional sinkholes on Bitter Lake NWR during sampling in support of genetic research in 2021 (Whiteley 2023, p. 27). While the remainder of the potential sites at Bitter Lake NWR have not been surveyed since the 1990's, no evidence suggests that there has been a status change from prior documentation (see Appendix A for a list of sites, when they were last sampled, and current status) (Hoagstrom and Brooks 1999b, p. 16; BEEC 2010, p. 13).

Bottomless Lakes State Park

Pecos pupfish historically occurred at five sinkholes (Mirror Lake, Upper and Lower Figure 8 Lakes, Lazy Lagoon, and Lea Lake) within Bottomless Lakes State Park (Hoagstrom and Brooks 1999a, pp. 43–44; BEEC 2010, p. 16). Lea Lake provides water to the BLM Overflow Wetlands, where Pecos pupfish also occur (Hoagstrom and Brooks 1999a, p. 22; BEEC 2010, p. 17). Other fish species that may co-occur with Pecos pupfish at Bottomless Lakes State Park include plains killifish, rainwater killifish (*Lucania parva*), green sunfish (*Lepomis cyanellus*), Mexican tetra (*Astyanax mexicanus*), and western mosquitofish (*Gambusia affinis*) (Hoagstrom and Brooks 1999a, pp. 44–45). As of 2020, it appears that Pecos pupfish are no longer present in Upper Figure 8 Lake (Appendix A).

BLM Overflow Wetlands

The BLM Overflow Wetlands is a large wetland that contains multiple shallow marsh habitats and is formed by water discharged from Lea Lake and seeps (Hoagstrom and Brooks 1999b, p. 22; Kreager 2003, p. 2; BEEC 2010, p 17). Salinity varies throughout the Overflow Wetlands but increases further from Lea lake due to evaporation of surface water (Hoagstrom and Brooks 1999b, p. 22).

Pecos pupfish habitat in the Overflow Wetlands includes approximately 52.5 hectares (ha) (130 acres (ac)) of perennial wetlands (BEEC 2010, p. 18). Surveys for Pecos pupfish have been performed in the BLM Overflow Wetlands since the 1980s, and catch per unit effort has been monitored annually since 2011 at three sites (North Road, Middle Wetland, and Upper Fish Barrier) (Hoagstrom and Brooks 1999a, p. 45; Brandenburg and Farrington 2003, entire; Hatt 2022, p. 5).

There is a surface water connection between Lea Lake and the BLM Overflow Wetlands, allowing for Pecos pupfish dispersal to Lea Lake during winter months for thermal refuge (BEEC 2010, p. 18). Additionally, there is a surface water connection between the Pecos River and the BLM Overflow Wetlands, however concrete fish barriers have been constructed at the BLM Overflow Wetlands (with the purpose of protecting Pecos pupfish from hybridization with

the sheepshead minnow), impeding connectivity between the Pecos River and the BLM Overflow Wetlands (Pecos Pupfish Conservation Team (Conservation Team) 2022, p. 4).

Salt Creek (TX)

Salt Creek, Culberson and Reeves County, TX, is the only known natural population of Pecos pupfish remaining in Texas (Hoagstrom and Brooks 1999a, p. 16; BEEC 2010, p. 18). Salt Creek (TX), originates at a series of springs, which form extensive wetlands in the upper reach of Salt Creek (TX) (Hoagstrom and Brooks 1999a, p. 16; BEEC 2010, p. 18). Pecos pupfish are most abundant in Salt Creek (TX) within these extensive wetlands (Blue Earth Ecological Consultants, Inc. (BEEC) 2010, p. 18). The lower reach of Salt Creek (TX) contains a series of falls, which may be a barrier to invasion by other fish (including sheepshead minnow) (Hoagstrom and Brooks 1999a, pp. 16, 52). Although this area is not routinely monitored, a visit in 2023 confirmed the continued presence of Pecos pupfish in at least one location in Salt Creek (TX) (Montagne 2023, entire). Other fish species that may occur throughout Salt Creek (TX) include red shiner (Cyprinella lutrensis), western mosquitofish, rainwater killifish, plains killifish, inland silverside (Menidia beryllina), hybrid striped bass (Morone saxatilis X M. chrysops), hybrid pupfish (C. variegatus X C. pecosensis), and gulf killifish (Fundulus grandis) (Hoagstrom and Brooks 1999b, pp. 44–45; Delaune 2020, p. 79). Genetic work on the Pecos pupfish in Salt Creek showed the presence of alleles unique to this system though the reasons and causes for this are unknown. The authors surmise that the unique alleles could be the result of genetic drift due to isolation or a steep selection gradient (A. A. Echelle et al. 2003, p. 6).

CHAPTER 3 – STRESSORS AND SOURCES

In this chapter, we assess the influences and factors that impact Pecos pupfish, their habitats, and distribution. Factors discussed in this section are sheepshead minnow introgression (Section 3.1), water use and management (Section 3.2), climate change and drought (Section 3.3), habitat loss (Section 3.4), and golden algal blooms (Section 3.5), as well as ongoing conservation efforts (Section 3.6). The sources, stressors, causes and effects that impact Pecos pupfish habitat are often complex and intertwined (*Figure 13*). These interactions influence the current viability of the species and determine the resilience of the Pecos pupfish and its vulnerability to extinction.

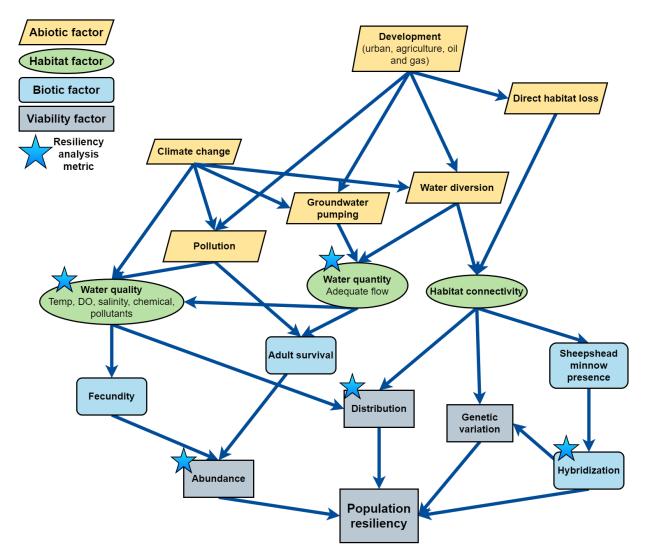


Figure 13. Influence diagram of primary relationships between factors that impact Pecos pupfish.

3.1 Sheepshead Minnow Introgression

The sheepshead minnow is a species once confined to shallow, brackish, coastal waters of the Gulf and Atlantic coasts of the continental United States and is ascribed as one of the primary causes, along with habitat degradation, of the reduction in range of the Pecos pupfish in the 1980s (Hoagstrom 2003, p. 101; 2009, entire; Conservation Team 2022, p. 5) (*Figure 11*). It was introduced into the Pecos River somewhere near Pecos, TX, in the early 1980s, and bait-bucket transfers have continued since (Conservation Team 2022, p. 5) (*Figure 14*).

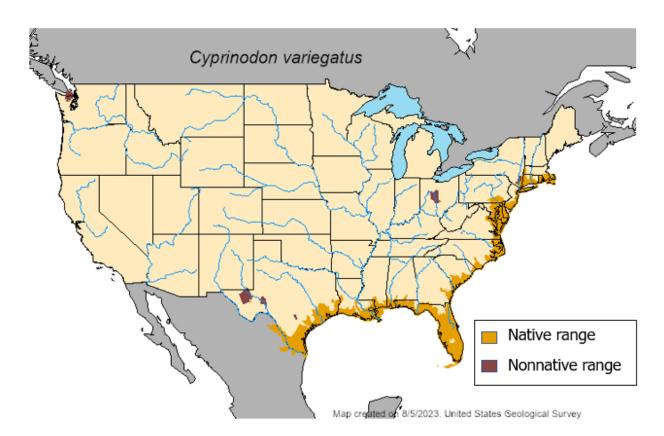


Figure 14. Map depicting the major rivers of the United States and the native and nonnative range of sheepshead minnow. Sheepshead minnow has been introduced to several streams and rivers in Texas, as shown by the dark red areas on this map, including those formerly occupied by Pecos pupfish. This map was generated by using USGS data from 1958 to 2023 but does not show all areas currently occupied by sheepshead minnow (Nico and Fuller 2023, entire).

Sheepshead minnow is a stressor to Pecos pupfish through hybridization and outcompeting for resources (Echelle and Connor 1989, pp. 725-726; A. A. Echelle et al. 2003, entire). Pecos pupfish and sheepshead minnow do not appear to have isolating mechanisms and readily interbreed (Cokendolpher 1980, entire; Kodric-Brown and Rosenfield 2004, entire) (*Figure 15*). Hybridization eventually leads to the loss of non-introgressed (genetically pure) Pecos pupfish in the area of introgression (Echelle and Connor 1989, p. 725; A. F. Echelle et al. 2003, entire). Research into growth of F1 (first generation) hybrids suggest that these hybrid fish grow faster and are larger than pure Pecos pupfish (Rosenfield et al. 2004, p. 1595). We believe Pecos pupfish hybridization with the sheepshead minnow is one of the greatest threats to this species, and is the cause of extirpation from historical sites (Echelle and Connor 1989, pp. 725–726; A. F. Echelle et al. 2003, entire; Pecos Pupfish Conservation Team (Conservation Team) 2022, p. 5).

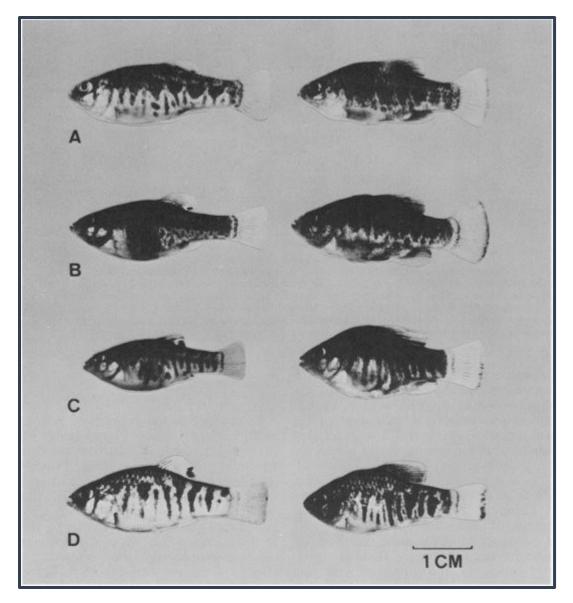


Figure 15. Illustration of Pecos pupfish x sheepshead minnow hybrids. Line A: Pecos pupfish, Lines B and C: hybrids, Line D: sheepshead minnow. The females are on the left and the males are on the right (Echelle and Connor 1989, p. 719). This figure illustrates 2 different hybrids that can be produced.

Surveys at multiple sites on the Pecos River downstream of Red Bluff Reservoir, TX indicated evidence of hybridization sometime between 1980 and 1984 (Echelle and Connor 1989, p. 717). It is presumed that this introduction occurred via a bait-bucket transfer in Red Bluff Reservoir (Childs et al. 1996, p. 2020). By the late 1980s, near Pecos, TX, Pecos pupfish were extirpated and replaced by the hybrid swarm (Connor 1987, p. 2; Echelle and Connor 1989, pp. 717–720). In 1997, hybrids were discovered on the Pecos River at Loving Crossing, Eddy County, NM (Echelle et al. 1997, p. 338; Echelle and Echelle 2007, p. 4). Further records from 2016 and 2017 indicate that sheepshead minnow likely occur as far north as Brantley Dam, Eddy County, NM (Davenport 2023a, entire).As a consequence, non-introgressed Pecos pupfish are likely

extirpated from the Pecos River below Brantley Reservoir, with the exception of the Salt Creek, TX population (*Figure 16*).

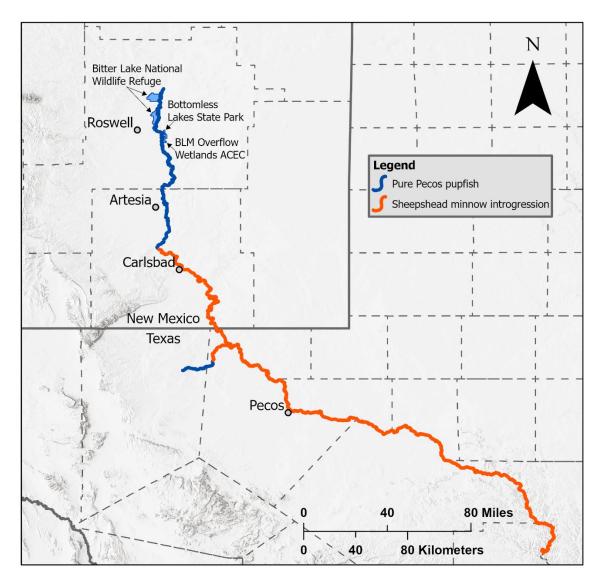


Figure 16. Sheepshead minnow introgression in the Pecos River in New Mexico and Texas. The orange line shows the progression of the sheepshead minnow to Brantley Dam. The blue lines show where Pecos pupfish are non-introgressed (Echelle et al. 2003, p. 6; Whiteley 2023, p. 2).

3.2 Water Use and Management

Adverse impacts to both water quantity and, to a lesser extent, water quality, are a threat to Pecos pupfish viability. As anthropogenic uses of water increase from urban, agricultural, and industrial development, water management will become more important to maintain adequate water for the Pecos pupfish. While we have reasonable certainty that there will be adequate aquifer levels until 2100 (Llewellyn et al. 2021, p. 100), the demand on water in the Pecos River Basin is expected to increase based on climate change projections (Sites Southwest 2008, pp. 6–

3, 6–6). Although diversions from the Pecos River are capped by existing water rights, agreements, and regulations, decreasing surface water availability can increase the demand for pumped groundwater (Dunbar et al. 2022, p. 87).

In Texas, the Delaware River is experiencing an increase in ground water pumping to support hydraulic fracturing operations and we expect the increased water usage to continue around Salt Creek (TX) (Scanlon et al. 2020, pp. 3510–3513). Both a deep and shallow aquifer (Rustler and Pecos Valley complex) may support the springs feeding Salt Creek (TX) (George et al. 2011, pp. 4, 58, 146). However, there are no specific hydrologic models detailing how the aquifers influence the flows in Salt Creek (TX).

Water use is expected to increase with a growing human population, further depleting ground water storage and negatively influencing the Pecos pupfish's future (Llewellyn et al. 2021, p. 84). Activities such as surface and groundwater withdrawals, as well as impoundments, have decreased streamflow resulting in direct habitat loss and increased habitat fragmentation (Llewellyn et al. 2021, p. 138). We are uncertain of how the aquifers will be affected and recover (rainfall and recharge), if at all, and how surface flows would be affected (Land and Newton 2008a, p. 190).

The Pecos River provides connected wetted habitat year-round and has block releases for the threatened Pecos bluntnose shiner (*Notropis simus pecosensis*). There are four Federally owned reservoirs on the Pecos River: Santa Rosa (U.S. Army Corp of Engineers; USACE); Sumner (Bureau of Reclamation; BOR); Brantley (BOR); and Avalon (BOR) (Service 2017, p. 7). The groundwater rights are owned by the NM Interstate Stream Commission (NMISC) (Service 2017, p. 11). The state and Federal agencies work together to maintain river flows that provide water for a variety of reasons, including environmental reasons. Releases from Fort Sumner Reservoir to Brantley Reservoir happened as block releases several times a year for decades, at an interval and timing contrary to the historical flow regime (Hoagstrom et al. 2008a, p. 6). When not discharging, no water was released, leading to artificially low flows (Hoagstrom et al. 2008a, p. 6). These block releases act as a buffer to drying events even though the water may be repurposed from environmental use to consumptive irrigation use (Hoagstrom et al. 2008a, p. 6).

Karst aquifer systems, like that found on Bitter Lake NWR, may affect sinkhole systems. However, to what degree karst systems influence sinkholes is poorly understood; as an effective study requires more techniques, research and data to understand the relationship and influence between them (Veni 2013, p. 57). Groundwater pumping may tap into conduits that feed springs or sinkholes (Veni 2013, p. 47). The San Andres aquifer appears to feed the region in and around Bitter Creek and Salt Creek (NM) and is supported by additional flows from the Toyah Aquifer (LaFave 1987, p. 34). Precipitation cycles and agricultural activity appear to be the two main factors causing variation in the aquifers (Land and Newton 2008a, p. 189). However, the Roswell Artesian Basin provides an example of a rechargeable artesian aquifer (Land and Newton 2008a, p. 190). Water reduction measures and high levels of rainfall in the 1970's led to a reversal in long-term hydraulic head declines in this aquifer and allowed this Roswell Artesian Basin aquifer to recharge (Land and Newton 2008a, p. 190). Because Pecos pupfish are relatively tolerant of more extreme (high temperatures, low dissolved oxygen, high salinity) water quality conditions, minor changes to water quality are generally seen as less of a concern (Propst 1999, p. 68). However, throughout the Pecos pupfish's range, water temperatures have the potential to exceed the fish's thermal tolerance (Brown and Feldmeth 1971, p. entire). Furthermore, it is surmised that extreme salinity caused declines in two historical off-channel Pecos pupfish populations (Pupfish Spring) (**Appendix A**)(Hoagstrom and Brooks 1999a, p. 13).

The entirety of the Pecos pupfish range in the Pecos River has ongoing water quality concerns and many reaches are considered impaired (Llewellyn et al. 2021, p. 29). Below Sumner Reservoir, the river improves for 160 km (100 mi) before becoming impaired again to the state line by polychlorinated biphenyls (PCB) (Llewellyn et al. 2021, p. 28). Another stressor is contamination of water by oil and gas development (Bonetti et al. 2021, entire). Pipelines present a potential route of contamination, pipelines may contain oil, gas, or brines, and leaks or ruptures may allow these materials to enter underground aquifers (Ashworth 1990, p. 31). Pipelines in the vicinity of, Bitter Lake NWR, BLM ACEC, and Bottomless Lakes State Park are protected and managed to keep these systems conserved and free from contamination.

3.3 Global Climate Change and Drought

The Southwest U.S. is thought to be extremely sensitive for increased drought and higher average temperatures caused by climate change (Sheffield and Wood 2008, p. 101). In particular, temperatures across New Mexico, including in the Pecos River Basin, have risen approximately 1.1 °C (2 °F) between 1970 and 2020 (Dunbar et al. 2022, pp. 4–5). While Pecos pupfish have persisted through historical drought conditions, observations from Bitter Lake NWR suggest that prolonged drought or higher temperatures have likely led to mortality events (Jacobsen 2023, entire). Because Pecos pupfish are able to persist in degraded water conditions that are saline, they are likely to be somewhat resilient to adverse water flow and temperature impacts (Propst 1999, pp. 67–68). However, Pecos pupfish are likely persisting at or near their thermal maximum, particularly during the hottest parts of the year (Matthews and Zimmerman 1990, p. 27). The increasing temperatures predicted by climate modeling (Figure 17) suggest that water temperatures have the potential to exceed the thermal maximum for Pecos pupfish. This is particularly critical for sites that are shallower, have limited freshwater input, or are isolated from any potential thermal refugia. Observations of the Conchos pupfish (C. eximius), a close relative of the pupfish, suggest that severe flooding and drought may have caused declines in fish numbers (Davis 1980, p. 83).

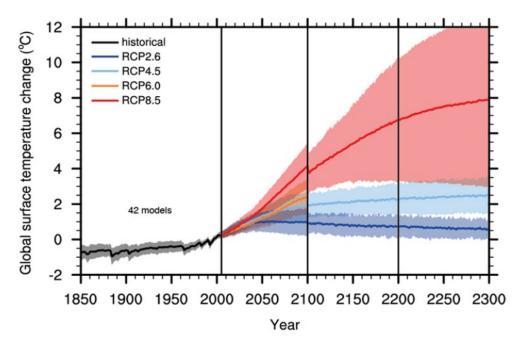


Figure 17. Projected change in global surface temperature in the Pecos River Basin. (Llewellyn et al. 2021, p. 88).

An average increase in temperature manifests itself locally as higher temperatures and higher overnight low temperatures (Hayhoe et al. 2018, p. 88)(*Figure 17*). In terms of precipitation, broadly speaking, wet areas are expected to get wetter and experience more intense precipitation events, while dry areas are expected to get drier and experience more intense drought events (Shafer et al. 2014, pp. 443–445; Kloesel et al. 2018, pp. 995–996, 1004) (*Figure 18*). Another effect of climate change is exacerbated drought due to feedback loops between high air temperatures, low humidities, and low soil moisture (Cheng et al. 2019, pp. 4437–4440). Potential effects of climate change that are likely to affect water resources include increased temperatures, evaporation, evapotranspiration, drought, earlier runoff, and reduced or increased precipitation (Llewellyn et al. 2021, p. 98). The main uncertainty of a changing climate is the resulting demands on surface and ground water aquifers that support habitat for the Pecos pupfish. Chapter 5 details the potential impacts of a changing climate and water use on Pecos pupfish populations.

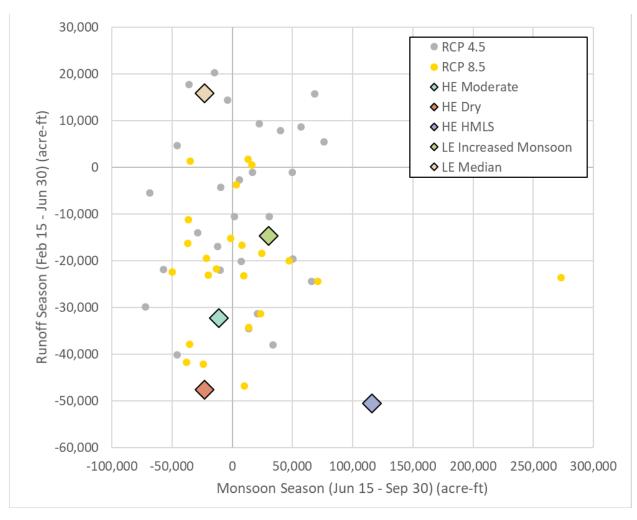


Figure 18. Predicted change in precipitation runoff in the Pecos River Basin. The change in precipitation runoff based on modeling of Pecos River Basin (Llewellyn et al. 2021, p. 103).

3.4 Direct Habitat Loss and Fragmentation

Groundwater depletion has dried up several marshes, playas, and spring ponds formerly occupied by Pecos pupfish adjacent to the Pecos River in New Mexico and Texas (Hoagstrom and Brooks 1999, p. 11). Direct habitat loss was also believed to have caused the extirpation of Comanche Springs pupfish (*C. elegans*) in Texas near Fort Stockton (Echelle et al. 2003a, p. 114). Habitat loss occurs when streams are dewatered, and surface flow is eliminated. To date, we are unaware of habitat loss within the range of the species at a scale that has caused the extirpation of Pecos pupfish at more than a single location. However, significant habitat impairment has occurred throughout the range of the Pecos pupfish. The Pecos River has been significantly altered through dam construction, channelization, and water diversions resulting in the loss of offchannel marshes, oxbows, and changes to mainstem flows (Hoagstrom and Brooks 1999b, pp. 10–12). While there is no data on to what extent the Pecos pupfish used these habitats, based on habitat descriptions of the current known occupied locations we presume that at least a portion of the available off-channel habitat may have been used by Pecos pupfish for connectivity (Hoagstrom and Brooks 1999b, p. 22). Furthermore Collyer et al. (2015, p. 191) suggest that habitat fragmentation and alternation may have rendered Pecos pupfish populations in the lower Pecos River more vulnerable to hybridization with the sheepshead minnow, as there are morphological differences between populations that are linked to aquatic habitat type and diversity. More recently, in 2020, 2022, and 2023, drought events led to the loss of portions of Bitter Creek on Bitter Lake NWR. In both 2020 and 2022, Pecos pupfish mortality was observed (Hatt 2022, p. 4). Data collected during winter surveys suggest that the Pecos pupfish is able to return to sections of the creek once sufficient water quantities are present (Jacobsen 2023, entire). Although data are lacking from the Pecos River mainstem, this scenario likely occurs there as well.

Habitat fragmentation is the disruption of continuous habitat resulting in smaller disconnected areas and can be either temporary or permanent (Wiegand et al. 2005, p. 109). The natural landscape for the Pecos pupfish is comprised of isolated sinkholes with unknown sub-surface connectivity, disjunct wetlands and ephemeral streams, and a historically well-connected river system. Much of the direct habitat loss and fragmentation within the range of the Pecos pupfish is the result of de-watering of habitat as a result of anthropogenic development, and water management and use, such as demand for water for agriculture and oil and gas development (Hoagstrom et al. 2008a, p. 6). Climate change impacts in the Pecos River Basin will likely result in higher overall surface temperatures (*Figure 17*). In general, warming surface temperatures directly impact evapotranspiration rates and can lead to lowered surface water (Llewellyn et al. 2021, p. 21). However, throughout the range of the Pecos pupfish the hydrology impacting their habitat is a complicated mix of evaporation, spring flow, and groundwater recharge.

The loss of habitat connectivity and the resulting fragmentation can lead to isolation among populations, which may have caused a genetic bottleneck in some Pecos pupfish populations (Collyer et al. 2015, p. 191; Whiteley 2023, pp. 6–7). Isolated and small populations are also more susceptible to stochastic events and amplify the effects of inbreeding depression and genetic drift (Rieman and Allendorf 2001, p. 762). Fragmentation and isolation of habitats can increase the risk of local extirpation as recolonization from adjacent populations is less likely (Hoagstrom et al. 2008b, p. 13). As habitat loss and fragmentation increases, habitat diversity decreases.

3.5 Golden Algae

Fish kills resulting from toxic golden algae (*Prymnesium parvum*) blooms have the potential to affect Pecos pupfish populations. Pecos pupfish are vulnerable to golden algae blooms, which cause injury and death. Large-scale fish mortality events attributed to toxins produced by these algae have occurred in the Pecos River in New Mexico and Texas, most recently from Brantley Reservoir downstream into Texas (Rhodes and Hubbs 1992, entire; Zymonas and Propst 2007, entire; Patiño et al. 2014, entire). As golden algae is easily spread via waterfowl or wind, it is likely present throughout the current range of Pecos pupfish. Although no pure Pecos pupfish kills have been reported, fish kills of other species in nearby habitats, including within Cottonwood Lake and Devil's Inkwell Lake at Bottomless Lakes State Park, have occurred (Zymonas and Propst 2007, entire; Hatt 2021, entire). If toxic golden algae blooms occurred in

occupied Pecos pupfish habitats, particularly off-channel habitats, they would be detrimental to affected populations. A fish kill occurred in 2020 at Figure 8 Lake at Bottomless Lakes State Park, but the cause of the fish kill or the conditions that led to the fish kill were not documented (Hatt 2021, entire). Lake turnover could have been a contributing factor in the Figure 8 lake fish kill (Hatt(Hatt 2019, p. 5), 2024). Childs et al. (1996, pp. 2019–2020) speculated that the spread of hybrid Pecos pupfish x sheepshead minnow in Salt Creek (TX), upstream from its convergence with the Pecos River, occurred during a period when abundance of the native Pecos pupfish was low, possibly because of an undocumented fish kill caused by an algal bloom. The conditions needed for golden algae blooms are not well understood. We expect as water quantity decreases in the future, water quality will decrease also, potentially providing the conditions for more frequent algal blooms to occur. The positive association between organic nitrogen and golden algae suggest that reductions in nitrogen could be effective at curbing algal blooms; similarly, reductions in phosphorus may also create unfavorable conditions; therefore, the reduction in nitrogen and phosphorus may reduce algal blooms (Israël et al. 2014, p. 90).

3.6 Food Resources and Fish Communities

Food resources tend to dictate the fish community of a given area. Water quantity and water quality will influence the availability of food resources (Linam and Kleinsasser 1996, p. 8). With flooding events, organic matter increases in a system, providing different resources to various trophic levels of fish (Harrington 2021, entire). Conversely, when drying events occur a variety of food resources become scarcer, and reduced water quality influences the available food resources (Harrington 2021, entire). Intraspecific and interspecific competition is the stressor of available food resources. The Pecos pupfish will compete with other species and each other for food resources. The Pecos pupfish is omnivorous, consuming benthic animals, filamentous algae, macrophytes, sand, seeds, and the bulk of their diet consists of a diatom-detritus mixture (Davis 1981, p. 535). The Pecos pupfish is known to have dietary plasticity by consuming a range of herbivorous and carnivorous food resources (Hoagstrom et al. 2015, p. 16).

At Bitter Lake NWR, seven sites including sinkholes and Bitter Creek had the following fish: red shiner, roundnose minnow (*Dionda episcopa*), plains killifish, rainwater killifish, Pecos gambusia, western mosquitofish, and greenthroat darter (*Etheostoma lepidum*) (Brandenburg and Farrington 2003, p. 1). Predatory and/or game fish, such as catfish (*Ictalurus spp.*), bass (*Micropterus spp.*), spotted gar (*Lepisosteus oculatus*), and longnose gar (*Lepisosteus osseus*) are found in the Pecos River.

The effect of predation by other fishes, birds, and other predators on Pecos pupfish populations is unknown. However, hybrid Pecos pupfish x sheepshead minnow are a primary dietary item for the nonnative gulf killifish in the lower Pecos River (East et al. 2017, p. 62). Also, elsewhere in the southwest, predation by nonnative fishes is implicated in the decline of the desert pupfish (Schoenherr 1981, entire).

3.7 Summary of Stressors and Sources

The greatest threats to the Pecos pupfish are introgression with sheepshead minnow, loss and decline of surface and ground water, degradation of water quality, and habitat loss and fragmentation, and the effects of climate change. Introduction of sheepshead minnow into new locations occupied by Pecos pupfish could lead to rapid introgression, replacing the genetically pure population with Pecos pupfish x sheepshead minnow. Research has found Pecos pupfish populations that are already negatively impacted by habitat alteration are likely more at risk of introgression because the reduction in habitat increases competition for breeding substrates (Kodric-Brown and Rosenfield 2004, p. 121-122; Collyer et al. 2015, 191). Anthropogenic water use and management has impacts on most of the surface water and groundwater within the range of the Pecos pupfish, and continued development and climate-driven changes to water availability will continue to impact the species in the future. Stochastic events such as golden algae blooms have impacted Pecos pupfish populations and climate change may accelerate the future rate of golden algae blooms to create potentially more frequent and severe occurrences (Barkoh and Fries 2010, p. 4). Climate change impacts including higher average annual temperatures, more variable or lower average annual precipitation, and increased drought frequency, are currently impacting the Pecos pupfish and will likely continue to do so.

3.8 Conservation Agreement

In 1999, a Conservation Agreement was developed to address the threats to Pecos pupfish. Since the implementation of the Conservation Agreement, conservation efforts have included sheepshead minnow eradication, installation of fish barriers, and enforcement of state fishing rules in an effort to protect the Pecos pupfish from further introgression of sheepshead minnows or hybrids and alleviate other threats affecting this species (Conservation Team 2022, p. 3). The Conservation Agreement was amended in 2013 and in 2022 (Conservation Team 2022, pp. 1, 4).

The above-mentioned stressors, introgression, water quantity, and habitat degradation and loss, have been considered and some stressors have been reduced through the implementation of the Conservation Agreement (Conservation Team 2022, entire). There are eight signatory agencies to the agreement including TPWD, NMDGF, NM Energy, Minerals, and Natural Resources Department, NM Department of Agriculture, NM Interstate Stream Commission (NMISC), Commissioner of Public Lands and NM State Land Office, BLM, and the Service(Conservation Team 2022, pp. 8–23).. The duration of the Conservation Agreement is indefinite with formal review every 10 years (Conservation Team 2022, p. 12). The last update of this Conservation Agreement was in 2022 and a set of renewed requirements were accepted by the signatories. The agreement has been effective in establishing fish barriers that are intended to reduce the expansion of the sheepshead minnow by vested parties. This creates a stable foundation for the Pecos pupfish in that there are numerous agencies working together for the survival of the pupfish.

Since 1999, one fish barrier has been installed at Bitter Lake NWR, near the confluence with the Pecos River. In 2019 two fish barriers were replaced at the BLM Overflow Wetlands, where north and south concrete barriers were installed to prevent entrance of fish from the mainstem

Pecos River into the complex. The purpose of these concrete barriers is to halt the opportunity for the sheepshead minnow to access habitats upstream of these barriers should the species be introduced into the Pecos River upstream of Brantley Dam (*Figure 19*). The barrier on the BLM Overflow Wetlands not only protects the wetlands, but also protects the Bottomless Lakes complex from the threat of hybridization. Artificial fish barriers have been an effective method to reduce nonnative fish population expansion (Novinger and Rahel, 2003, pp. 773–774).



Figure 19. BLM Overflow Wetlands North Fish Barrier. (Photo credit: Vance Wolf, Service)

The Fort Worth Zoo and other collaborating zoos have successfully bred Pecos pupfish in captivity since 2000. Captive conservation efforts have focused on propagation techniques, animal husbandry research, and propagation for stocking. Beginning in 2012, the State of Texas began working with private landowners within the Pecos River watershed to identify opportunities for the development of Pecos pupfish production ponds. As of 2024, two have been established, and recruitment of additional landowners, and establishment of additional ponds, is ongoing. The goal of the ponds is to sustain the genetic lineage from the Salt Creek, TX, population, create stable habitats isolated from potential sheepshead minnow incursion with secure water sources, and provide a stock of fish that can be used to establish other locations.

CHAPTER 4 – CURRENT CONDITION

The results of the Pecos pupfish population condition model described below provide the basis for our analyses of the species' current status using the 3Rs. The population condition scores allow us to directly assess and compare the resiliency of each Pecos pupfish analysis unit (*Table 2*), which then support our analyses of the species' redundancy (within and among the various 9 analysis units) and representation (across its environmental settings). We emphasize that this portion of the assessment is a "snapshot in time" of the Pecos pupfishes' current condition and does not consider future trends. Chapter 5 assesses the species' potential condition under several future scenarios.

Analysis Unit	Land Ownership		
1: Upper Pecos River	BLM, State, Private		
2: Salt Creek Wilderness	FWS		
3: Bitter Creek Drainage	FWS		
4: Bitter Lake NWR Middle Tract Wetlands	FWS		
5: Bottomless Lakes State Park	State		
6: BLM Overflow Wetlands and Lea Lake	BLM, State		
7: Middle Pecos River	BLM, State, Private		
8: Salt Creek (TX)	Private		
9: Lower Pecos River	BLM, State, Private		

Table 2. Analysis units for the Pecos pupfish.

4.1 Methodology for assessing the 3Rs

We reviewed online databases, reports, scientific publications, books, hydrological spatial datasets, water quality datasets, unpublished datasets, and newspaper articles, and interviewed individual researchers in an effort to locate information that could be applied to Pecos pupfish population analysis units across its entire range.

4.1.1 Resiliency

In order to assess the resiliency of the Pecos pupfish, we developed a qualitative model that produced a condition score for each Pecos pupfish analysis unit. The model incorporates one demographic metric (occurrence) and three habitat metrics (water quantity, water quality, and habitat diversity) into the overall status for each unit (*Table 4*). We selected habitat diversity as a metric as habitats with multiple aquatic environments may better allow the fish to withstand changing or adverse conditions. We also included genetic security in the model, but we did not incorporate the score for genetic security into the overall unit score. Because empirical data relating some of these metrics directly to Pecos pupfish life history needs are sparse, for the purpose of this SSA, the selected metrics were appropriate for assessing the viability of Pecos pupfish across the species range.

Based on the available data and our understanding of Pecos pupfish ecology, we developed a basis for assigning a risk category for each metric at the population analysis unit level (*Table 3*). The risk category reflects a qualitative determination of the likelihood that the species response to the conditions described in each individual metric, over the 20-year period following the year 2023, would be extirpated from a given population analysis unit. This 20-year timeframe correlates with approximately 20 1-year generations which is near the maximum of the presumed Pecos pupfish lifespan in the wild.

Risk Category	Analysis Unit Condition	Estimated Chance of Extirpation for 20 Years	Numerical Extirpation Risk Estimate	Threats Characterization
Low Risk	High Condition	Extirpation is very unlikely	<10%	Threats to pupfish needs are minimized or limited in spatial extent within the unit.
Moderate Risk	Moderate Condition	Extirpation is unlikely	10–40%	Threats to pupfish needs are widespread throughout the unit but limited in duration or severity.
High Risk	Low Condition	Extirpation risk ranges from being about as likely as not to being very likely	>40%	Threats to pupfish are severe and pervasive throughout the unit.

Table 3. Qualitative and quantitative descriptions of the three risk categories used in the resiliency analysis.

The individual metrics, which we ranked and scored as 1 (Low), 2 (Moderate) or 3 (High) based on criteria described in *Table 4*, were then combined to produce a categorical condition score for each analysis unit. We then averaged that score across all four categories to come up with an overall unit score. For the overall unit score an average of greater than 2.6 was considered High Condition, 1.6 - 2.5 was considered Moderate Condition, and less than 1.5 was considered Low Condition. To aid in the comparison of analysis units (with each other and under various future scenarios) and assess the species' viability under the 3Rs, we categorized the final condition scores as "high" (population generally secure), "moderate" (population marginally secure) or "low" (population generally insecure). We based these categories primarily on our understanding of Pecos pupfish habitat needs, known stressors, and the principles of conservation biology. We acknowledge that there is uncertainty associated with this model and some of the supporting data, however the methodology is appropriate for assessing the status of the Pecos pupfish across its range given the available information.

4.1.2 Redundancy

To assess redundancy of the Pecos pupfish, we examined three components: (1) how many extant sites exist within each analysis unit, (2) how connected these sites are within the unit, and (3) how connected each unit is to nearby units. Importantly though, the diversity of the habitat,

and not the number of sites Pecos pupfish have been detected reflects the extent of the occupied Pecos pupfish habitat within the unit. For example, Bitter Creek (on Bitter Lake NWR) is approximately 1,546 m (5,072 ft) of variably wetted stream and is counted as a single site. Similarly, the BLM Overflow Wetlands cover over 1,000 acres (405 hectares) and is also counted as a single site. However, we assume that with the exception of the sites delineated on the upper Pecos River, which is a riverine environment, each site is representative of a discrete aquatic environment.

4.1.3 Representation

For the purposes of this SSA, we used aquatic environment as a surrogate for representation. The Pecos pupfish occupied four broad categories of aquatic environments throughout its range: riverine (Pecos River), shallow stream (Salt Creek (TX), Salt Creek (NM), Bitter Creek), sinkholes, and wetlands (BLM Overflow Wetlands, Bitter Lake NWR Middle Tract) (Hoagstrom and Brooks 1999a, entire). Although some limited genetic analysis has been done on the Pecos pupfish that indicated potential geographic structure to Pecos pupfish populations, the authors cautioned against a rigorous application of the results since the divergence was minor (Echelle and Echelle 2007, p. 7). More recent work by Whiteley (2023, entire) also showed difference between Pecos pupfish populations among the samples from Bitter Lake NWR, Bottomless Lakes SP, and the BLM Overflow Wetlands. Xu (2017, p. 22) and Collyer et al. (2015, p. 187) both noted morphological divergence in Pecos pupfish populations that corresponded to differing habitat use. While neither of these recent studies directly related morphological differences to genetic information, the results suggest that including a range of aquatic environments represents (i.e. = habitat diversity) much of the current diversity of the Pecos pupfish species.

Condition	Genetic Security	Occurrence	Water Quantity	Water Quality	Habitat Diversity
High Condition (High Resiliency)	No evidence of introgression with sheepshead minnow.	Extant or presumed extant at all sites identified in Appendix A. Fish observed at, or in the vicinity of, each of these sites at least once within the last 5 years.	Stable and sufficient water availability throughout the unit. Low flow or drying events documented, but no long-term drying events recorded.	No severe impairments to water quality documented and no recorded contamination events.	Unit has a diverse habitat assemblage within the unit (streams/river, wetlands, and sinkholes).
Moderate Condition (Moderate Resiliency)	Introgression possible in the unit, but no confirmation.	Two or fewer of the known occupied sites in Appendix A confirmed or presumed extirpated.	Occasional low flows or drying events across < 50% of the unit with rare long- term drying events documented.	Occasional water quality impairments documented, likely linked to low flows. No documented exposure to surface contaminants.	Fish restricted to just a single habitat type within the unit.
Low Condition (Low Resiliency)	Introgression only in a portion of the unit.	Pupfish extant at 50% or less of sites identified in Appendix A. Populations low enough that fish are not detected on 50% or more visits to occupied locations.	Routine low flows and drying events across the majority of the unit and regular long-term drying events.	Documented exposure to surface contaminants within much of the unit.	N/A
Likely Extirpated	Confirmed introgression throughout the unit.	N/A	N/A	N/A	N/A

Table 4. Condition criteria resiliency analysis metrics as applied to each analysis unit.

4.2 Analytical Units

Fundamental to our analysis of the Pecos pupfish was the determination of scientifically sound analytical units at a scale useful for assessing the species. As there is little information available regarding the demographic or genetic processes that define the spatial structure of Pecos pupfish populations, we relied on spatial occurrence data to define a suitable extent for our analysis units. In this report, we defined Pecos pupfish analytical units based on documented occurrences, HUC-12 sub-watershed boundaries, stream and river features, and barriers (such as Brantley Reservoir and Red Bluff Reservoir) (*Figure 20*, see Appendix B for data sources).

- Analysis Unit 1: Upper Pecos River (*Figure 21*)
- Analysis Unit 2: Salt Creek Wilderness (*Figure 22*)
- Analysis Unit 3: Bitter Creek Drainage (*Figure 23*)
- Analysis Unit 4: Bitter Lake NWR Middle Track Wetlands (*Figure 24*)
- Analysis Unit 5: Bottomless Lakes State Park (*Figure 25*)
- Analysis Unit 6: BLM Overflow Wetlands and Lea Lake (*Figure 26*)
- Analysis Unit 7: Middle Pecos River (*Figure 27*)
- Analysis Unit 8: Salt Creek (TX) (Figure 28)
- Analysis Unit 9: Lower Pecos River (*Figure 29*)

This approach is based on the assumption that the closer occurrences are (such as within the same analysis unit), the more likely similar environmental processes are influencing the sites where the fish occurs. We note that in the cases of Units 3–6 we made minor modifications to the boundaries to include occurrences that were physically connected on the landscape (Appendix B).

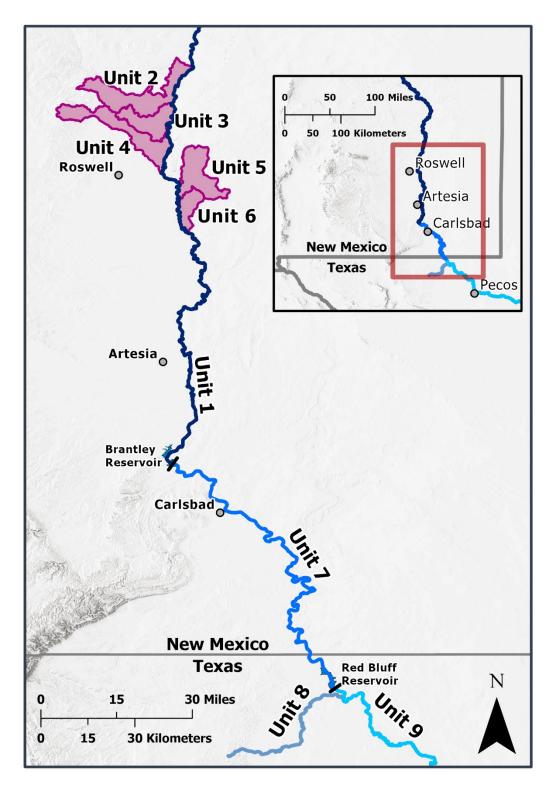


Figure 20. Map of Pecos pupfish analysis units 1-9.

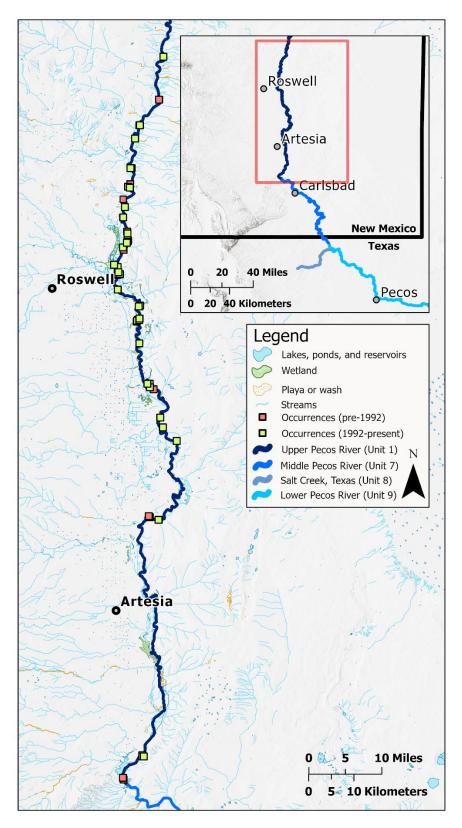


Figure 21. Unit 1 - Upper Pecos River.

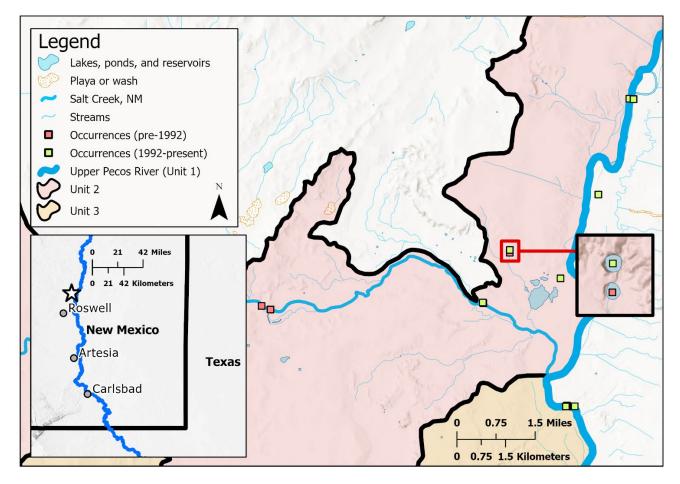


Figure 22. Unit 2 - Salt Creek Wilderness.

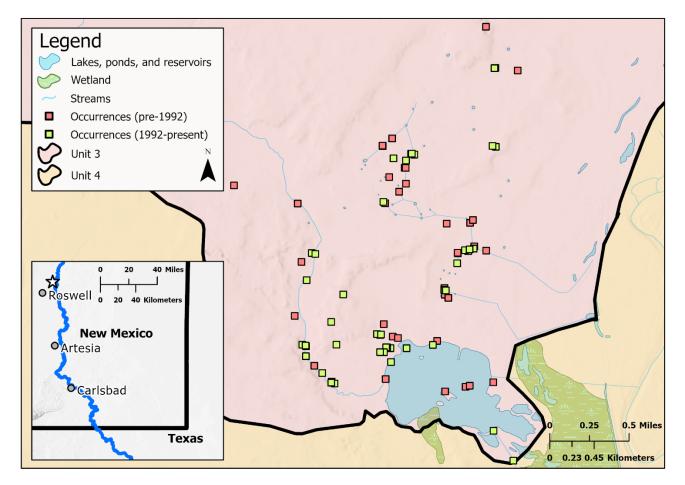


Figure 23. Unit 3 - Bitter Creek Drainage.

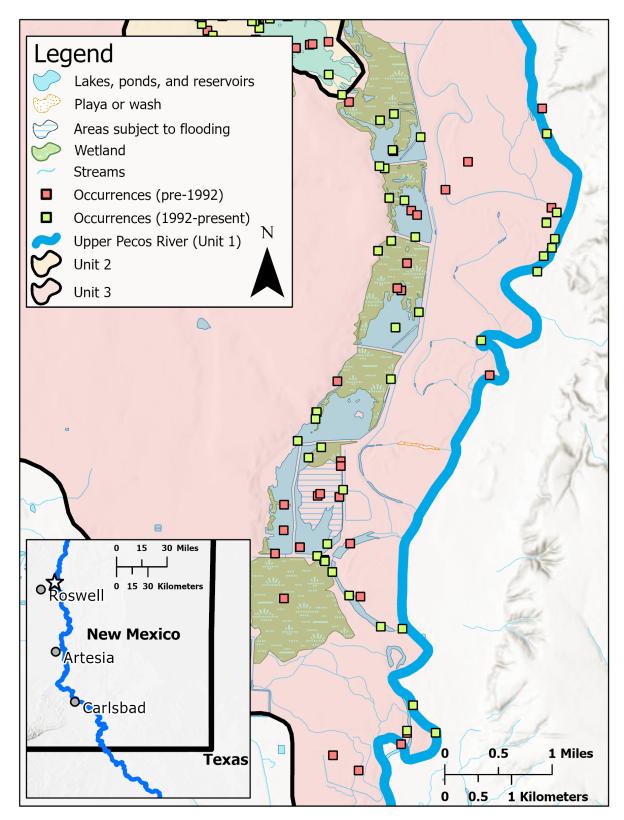


Figure 24. Unit 4 - Bitter Lake NWR Middle Tract Wetlands.

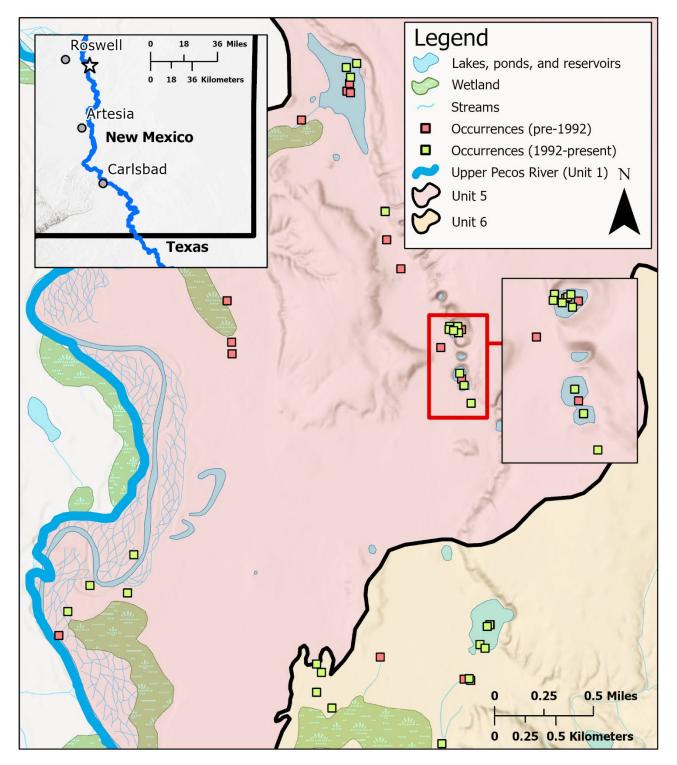


Figure 25. Unit 5 - Bottomless Lakes State Park.

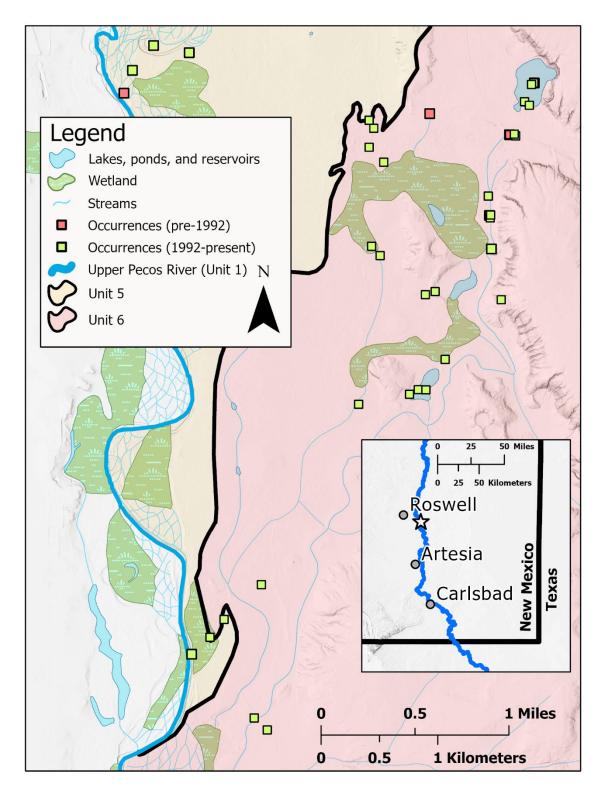


Figure 26. Unit 6 - BLM Overflow Wetland and Lea Lake.

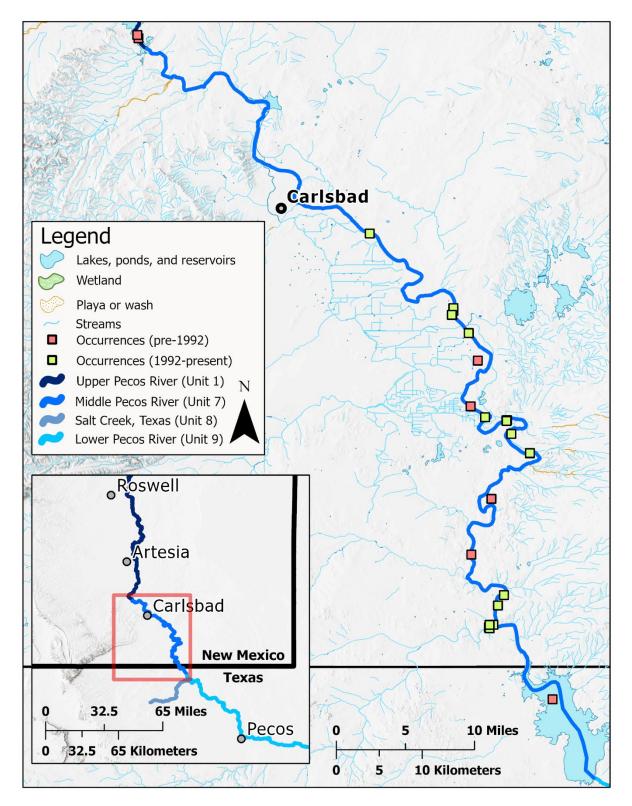


Figure 27. Unit 7 - Middle Pecos River.

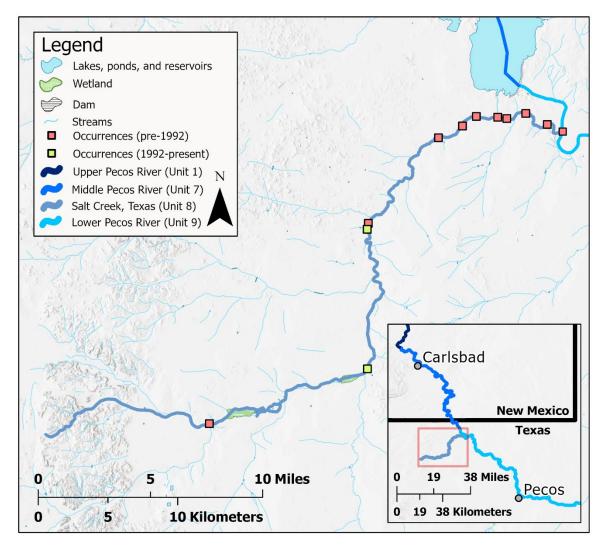


Figure 28. Unit 8 - Salt Creek, Texas.

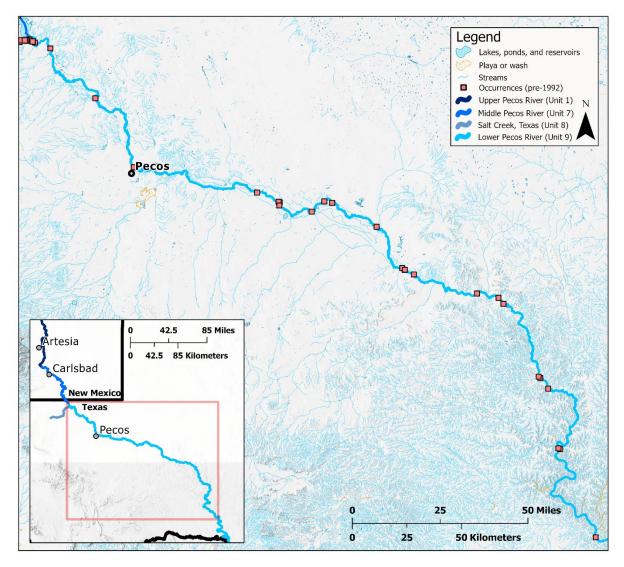


Figure 29. Unit 9 - Lower Pecos River.

Within each analysis unit, we identified discrete sites where Pecos pupfish have been documented to occur during past sampling and inventory efforts (Brooks 1992, entire; Hoagstrom and Brooks 1999b, entire; NHNM 2021, entire; GBIF 2022, entire). These sites represent the documentation of at least one Pecos pupfish at a specific location at a point in time. Many of these sites have not been routinely visited or have only been visited once. Eleven sampling locations representing nine sites have been routinely sampled (Hatt 2022, p. 5). A full list of all sites, including the last observation date, is included in Appendix A.

In some cases, such as sinkholes, these sites are analogous to subpopulations. In others, such as the Pecos River or BLM Overflow Wetlands, the documented sites only represent the accessible portion of the habitat and likely do not represent the entire population in the area.

4.3 Factors Influencing Current Condition

Based on the Pecos pupfish life history and habitat needs (Chapter 2) and stressors (Chapter 3), we identified the potential influences likely affecting the species' current condition and viability. Genetic security has historically been the most severe threat to Pecos pupfish viability and is the proximate cause for the loss of Pecos pupfish in much of the Pecos River downstream of Brantley Dam.

4.3.1 Genetic Integrity

The Pecos pupfish is vulnerable to hybridization with closely related species, specifically the nonnative and introduced sheepshead minnow (Echelle and Connor 1989, pp. 725–726; Childs et al. 1996, pp. 2019–2021). It is important to note that hybridization is considered particularly problematic in situations where nonnative species are introduced into areas outside of their natural ranges (e.g., Pecos River) (Allendorf et al. 2001, entire; Todesco et al. 2016, entire). During a period between 1980 and 1984, sheepshead minnow rapidly spread throughout approximately 430 km (267.2 mi) of the Pecos River in Texas (Echelle and Connor 1989, p. 717). Research conducted in 2007 confirmed introgression throughout the Pecos River in Eddy County, NM, and did not detect hybrids from sites sampled in Chaves County, NM (Echelle and Echelle 2007, pp. 5–6). Data from surveys conducted by the Services' New Mexico Fish and Wildlife Conservation Office (NMFWCO) indicate that sheepshead minnow are likely now found into New Mexico as far north as Brantley Dam (Davenport 2023a, entire). The introgression with sheepshead minnow has resulted in a reduction of more than half of the occupied portions of the Pecos River.

4.3.2 Occurrence

Two demographic factors, adult survival and fecundity, influence Pecos pupfish population trends and stability (Echelle and Connor 1989, p. 725; Kodric-Brown and Mazzolini 1992, 175; A. F. Echelle et al. 2003, entire). However, we have limited information on both adult Pecos pupfish lifespan and mortality in the wild, as well as little data on egg and juvenile survival (Kodric-Brown 1977, 756-758; Garrett et al. 2002, p. 366). Because of the lack of survival and recruitment data specific to the Pecos pupfish, we looked at the occurrence of Pecos pupfish at specific known sites over time as a proxy measure for survival and fecundity. Using a range-wide occurrence dataset compiled from Brooks (1992, entire) and Hoagstrom and Brooks (1999b, entire) in conjunction with monitoring reports from NMDGF (Hatt 2021, entire) and NMFWCO (Davenport 2023b, entire) we were able to assess the persistence of a site over time (Appendix A). This is possible because as a short-lived species, the continued persistence of the fish at a given site demonstrates survival and recruitment. However, given our current presence/absence data for Pecos pupfish across its range we made two broad assumptions:

1. If Pecos pupfish were observed to be present at a site (by any methodology) within the last 5 years, we considered that site to have an extant and stable population. While we recognize that this approach does not account for the

potential of a long-term slow decline, we think that persistent occupancy of a site by Pecos pupfish over 30 years likely represents a viable population.

2. We assumed that, in the absence of recent (within 5 years) data, Pecos pupfish remain extant if there have been no reported water quantity or quality issues at the site.

While agencies involved in the yearly monitoring effort have been refining sampling methods, to date the sampling methodology that has been used does not have the power to determine abundance or population trends. Use of catch per unit effort requires rigorous assumption testing and consistency in order to provide meaningful data on a fish population (Maunder and Punt 2004, pp. 155–156). Therefore, while the data on the pupfish shows numbers of fish caught each year, this data cannot be used to estimate Pecos pupfish abundance.

Data collected as part of the monitoring effort for the Pecos Pupfish Conservation Agreement have consistently detected Pecos pupfish, however there are typically significant year-to-year variations in the number of fish caught at each sampling location (Hatt 2021, p. 6). In 2021, sampling protocols were altered to collect data in a way that could be used in estimating Pecos pupfish populations. As of 2023, only two years of data have been collected and data interpretation is limited. However, this data have helped confirm that Pecos pupfish populations are likely highly variable (Hatt 2022, pp. 6–7).

4.3.3 Water Quality

Members of the Cyprinodon genus are known for their wide physiological tolerance relative to many other freshwater fishes. Pecos pupfish are able to tolerate a wide range of water quality conditions (Hoagstrom and Brooks 1999b, entire). In areas where the salinity gradient changes, Pecos pupfish dominate the areas with the highest salinities (Hoagstrom and Brooks 1999a, p. 12). However, salinities greater than 35,000 mg/L, larval and egg development are suppressed or halted in desert pupfish (Hoagstrom and Brooks 1999a, p. 21; Propst 1999, p. 67). Pecos pupfish can also tolerate low dissolved oxygen, with measurements of dissolved oxygen levels as low as 2.5 mg/L recorded during Pecos pupfish sampling (Hoagstrom and Brooks, 1999, p. 31; Propst 1999, pp. 67–68).. These tolerance limits are further supported by temperature extremes, dissolved oxygen, and salinity often resulting in fish mortality (Hoagstrom and Brooks 1999, p. 21; Propst 1999, p. 67). Though direct water quality data was not gathered, mortality events in Bitter Creek and Salt Creek (NM) were thought to be related to unsuitable water quality conditions (Jacobsen 2022, entire). In addition to natural impacts to water quality, industrial and agricultural pollutants have been shown to negatively impact Pecos pupfish (Houston et al. 2019, p. 33). Finally, golden algae blooms are an ever-present and stochastic threat to freshwater fish populations throughout the southwestern U.S. (Patiño et al. 2014, entire). This species of algae produces toxins that result in fish mortality. Because Pecos pupfish likely can persist in variable water quality conditions that are more extreme than most fish, we did not consider short-term or moderate changes to natural water quality parameters such as dissolved oxygen, temperature, or salinity as impairments to Pecos pupfish condition. Rather, for water quality to be in high condition it needs to be free of contaminants from surface sources such as industry, agriculture,

or urban development. However, long-term, or large changes to water quality variable such as salinity, temperature, and dissolved oxygen can have deleterious impacts to Pecos pupfish fecundity and survival. Thus, moderate and low condition water quality incorporates both changes to natural water quality variables and surface contamination relative to their intensity, duration, and spatial extent within the unit.

4.3.4 Water Quantity

Pecos pupfish depend on perennial water to complete all stages of their lifecycle. While Pecos pupfish persists in shallow habitats less than 1 m (3.3 ft) deep (Salt Creek, NM) they may need deeper water to provide thermal refugia and winter habitat (Kodric-Brown 1977, p. 755). Sufficient water quantity is also necessary throughout the year for breeding and adult survival (Kodric-Brown 1977, p. 754; Hoagstrom et al. 2015, p. 14). It is surmised that water quantity changes in small, ephemeral streams have led to Pecos pupfish mortality events in both Bitter Creek and Salt Creek (NM) (Hoagstrom 2009, p. 28; Davenport 2023a, entire; Jacobsen 2023, entire). Water availability for the Pecos pupfish is influenced by a variety of factors depending on the specific aquatic environment of a particular Pecos pupfish site or population. Sinkhole environments in the Pecos Basin are largely spring fed systems derived from the San Andres artesian aquifer (Land 2003, p. 230). Similarly, permanent water in the Bitter Creek and Salt Creek (NM) likely originates from spring flow from the San Andres aquifer (Land and Huff 2009, p. 1). Salt Creek (TX) likely is supported by spring flows from the Toyah Aquifer, though no direct modeling has been done on this particular stream (LaFave 1987, p. 34). These streams also hold water during precipitation events. Water in Bitter Lake NWR is managed through a series of constructed impoundments and water conveyance structures. The Bureau of Land Management (BLM) Overflow Wetlands are supported by outflows of water from Lea Lake, a sinkhole in Bottomless Lakes State Park, as well as precipitation. Finally, water in the Pecos River is managed by the Bureau of Reclamation (BOR) for water deliveries, environmental flows, and fulfilling obligations under the Pecos River Compact.

4.3.5 Habitat Diversity

Pecos pupfish are found in a diverse assemblage of habitats in the Pecos River Basin including sinkholes, springs, perennial streams, managed wetlands and marshes, and the Pecos River mainstem (Hoagstrom and Brooks 1999a, entire; Collyer et al. 2015, p. 182). Pecos pupfish prefer environments with little to no water flow, and, in areas with flows, they typically occupy pools and shallow runs and riffles (Hoagstrom and Brooks 1999a, pp. 36, 45). Within their occupied habitat, Pecos pupfish also require a diverse set of microscale habitat conditions. For reproduction, Pecos pupfish require shallow water less than 2 meters (m) (6.56 feet (ft) deep, and in areas with topographic diversity for spawning (Kodric-Brown 1977, p. 750-751). A variety of underwater features such as crevices, boulders, large rocks, scattered pebbles, and aquatic plants provide topographic diversity throughout the range of the Pecos pupfish (Kodric-Brown 1975, p. 35; 1977, pp. 750-751, 753-756, and 761-762). Rocky embankments appear to be the most desirable breeding substrate, as the most aggressive and largest males occupy these areas at Mirror Lake, Bottomless Lakes State Park, Chaves County, NM (Kodric-Brown 1975, pp. 34-

35). The percentage of males holding territory can vary year to year and is influenced by the amount of breeding and foraging habitat available (dependent on water levels), and that density of territorial males was highest in dense patches of aquatic vegetation, and lowest in flat silty areas with isolated rocks (Kodric-Brown 1975, pp. 20, 34-35). During the colder months when water temperatures drop below 10 °C (50 °F), Pecos pupfish become inactive and can be found in deeper water with dense vegetation and flocculent material (such as fine detritus or non-living organic matter) present in the substrate (Kodric-Brown 1977, p. 752; Collyer et al. 2015, p. 17).

While there is no current data on the genetic differences in Pecos pupfish across different habitat types, studies have shown that Pecos pupfish exhibit differences in morphology across different habitat types (Collyer et al. 2015, p. 189). Phenotypic plasticity, in response to differing environmental conditions, may allow the Pecos pupfish to respond to spatially and temporally variable environments (Stearns 1989, p. 436; Via et al. 1995, p. 212). Further research is needed to ascertain the scope of the phenotypic variation and how gene flow affects it. Nevertheless, maintaining a variety of different habitats in proximity (and thus with potential connection) likely preserves adaptive capacity within the Pecos pupfish population.

4.4 Current Condition

In this section, we present the results of Pecos pupfish current condition, in terms of resiliency, redundancy, and representation. Chapter 5 assesses the species' potential condition under several future scenarios. As noted previously, our evaluation of the 3Rs here only considers the current status of the populations.

4.4.1 Resiliency

Resiliency describes the ability of a population to withstand environmental or demographic stochastic disturbance and is positively related to population size and growth rate, patch size, and connectivity to other populations. Generally, healthy populations with large numbers of individuals and adequate habitat conditions are able to withstand stochastic disturbances. We analyzed the resiliency of each unit across four influence factors as described in the prior section *Table 4*. We also included genetic security in *Table 4*, although the condition for this category was not included in the overall unit score. We gave a score to each resiliency metric and summarized across each unit for an overall unit score (*Table 5* and Appendix C).

<u>Unit 1, Upper Pecos River</u>: Pecos pupfish remain extant in the upper Pecos River. While there are no surveys focused on Pecos pupfish in this analysis unit, surveys conducted in support of other fish monitoring routinely detected Pecos pupfish. It is unlikely that Pecos pupfish are uniformly distributed throughout upper Pecos River and data indicate they tend to occupy discrete patches of habitat within the channel. Based on their preference for slower, warmer, and more saline conditions, Pecos pupfish are limited to specific areas within the Pecos River channel and these areas likely shift both spatially and temporally. This is reflected in data that show varying numbers of Pecos pupfish in year-to-year sampling and seem to indicate that during periods of drying Pecos pupfish are often more represented in samples (Davenport 2023b, entire). Although it is unclear if any particular site in the Upper Pecos has been lost, the highly

variable nature of this river section and shifting populations likely means that sampling at the same site will not always detect the Pecos pupfish.

The Upper Pecos River is subject to regular severe low flows and intermittent drying (Follansbee and Dean 1915, p. 452; Hatch et al. 1985, p. 561; Hoagstrom et al., p. 6). Because this threatens the persistence of the Federally Threatened Pecos bluntnose shiner, conservation measures are in place by the Bureau of Reclamation through a Biological Opinion to minimize intermittent drying. These measures buffer the threat of river drying for Pecos bluntnose shiner and, by extension, Pecos pupfish.

The entirety of the Pecos pupfish range in the Pecos River has ongoing water quality concerns and is considered impaired due to nutrient loading, discharges from municipal wastewater treatment plant discharges, and livestock grazing (Llewellyn et al. 2021, p. 29). The current condition evaluation for the upper Pecos River population determined that occurrence, water quality, and habitat diversity are in moderate condition, and water quality is in low condition. Thus the Upper Pecos River population is determined to be in overall moderate current condition and has moderate resiliency.

<u>Unit 2, Salt Creek Wilderness</u>: No routine Pecos pupfish monitoring occurs within the Salt Creek Wilderness analysis unit. Pupfish remain extant in Salt Creek (NM) and likely at three sinkholes in the unit (Inkpot, Little Inkpot, and New Sinkhole). A visit to Salt Creek (NM) in February 2023 confirmed presence of the pupfish but also documented a mortality event of several thousand mostly juvenile pupfish from an undetermined cause (Jacobsen 2023, entire). Although Pecos pupfish remain extant at several locations in the unit, the habitat available within the unit is small so this the stream unit may be subject to mortality events. Pecos pupfish are presumed to have been extirpated from Pren's Hole, though the cause is unknown (Hatt 2019, p. 5). Pecos pupfish from Salt Creek (NM) were dispersed by a flash flood and repopulated Pren's Hole (Hoagstrom and Brooks 1999a, p. 16).

We have no recent data on water quality or quantity within this unit either from the sinkholes or Salt Creek (NM). Deeper sinkholes generally have stable conditions, both in water quantity and quality, and thus we assume that likely holds true for the sinkholes in this unit as pupfish need large populations and room for expansion (Collyer et al. 2015, p. 191). Evidence of Pecos pupfish mortality in Salt Creek (NM) in 2023 suggest some impairment of the stream (Jacobsen 2023, entire). No water quality data was taken at the time of the observation. However, Salt Creek (NM) likely experiences routine drying events throughout the year, and concurrently with those drying events, impairments to temperature, dissolved oxygen, and salinity.

This unit contains both stream and sinkhole aquatic environments. Though the permanent water in both environments is supported by water from the San Andres aquifer, the depth of the sinkholes likely provides a more stable long-term environment. Conversely, Salt Creek (NM), although ephemerally flowing, allows for Pecos pupfish dispersal throughout the unit and a potential connection to the Pecos River. This diversity of habitat helps buffer the unit against both gradual environmental changes as well as stochastic events, such as floods or golden algae, that may impact a single aquatic environment. The current condition evaluation for the Salt Creek Wilderness determined that habitat diversity is in high condition, and occurrence, water quality, and water quantity are in moderate condition. Thus, the Salt Creek Wilderness population is determined to be in overall moderate current condition and has moderate resiliency.

<u>Unit 3, Bitter Creek Drainage</u>: Routine monitoring occurs in Bitter Creek as well as two of the sinkholes in the unit. Several other sinkholes (Dragonfly Spring, Sago Spring, and Lake St. Francis) are not regularly sampled, but are routinely visited by biologists with Bitter Lake NWR. We extrapolated both the formal and informal monitoring data to the remainder of the unit, and presumed the Pecos pupfish remains extant at all documented occupied sites in the unit. There have been documented fish kills on Bitter Creek, but routine monitoring indicates that Pecos pupfish populations in the creek remain extant, though highly variable (Hatt 2021, entire).

Water quality is documented in conjunction with the Pecos pupfish monitoring and has not detected any impairment to the aquatic environments in the unit. Furthermore, all of the unit is within Bitter Lake NWR which protects the unit from direct surface contamination.

Much of the water in this unit is derived from underground springs from the San Andres aquifer. This includes all of the sinkholes as well as the springs the feed Bitter Creek such as the Dragonfly Spring and Lost River. The closest PVACD monitoring well to this unit shows a long-term stable water depth trend which likely corresponds to stable spring flows in the unit. The water in Bitter Creek is supplemented by precipitation during wet seasons or years. Because evaporation exceeds precipitation across the Pecos River Basin (Land 2003, p. 230), during drought years when precipitation is not sufficient to maintain surface flows, portions of Bitter Creek dry out.

This unit contains both stream and sinkhole aquatic environments. Though the permanent water in both environments is supported by water from the San Andres aquifer, the depth of the sinkholes likely provides a more stable long-term environment. Bitter Creek is supported by both seasonal precipitation as well as spring flows from Dragonfly Spring and the Lost River. This diversity of habitat helps buffer the unit against both gradual environmental changes as well as stochastic events, such as floods or golden algae, that may impact a single aquatic environment. The Bitter Creek Drainage population's current condition evaluation determined that occurrence, water quality and habitat diversity are in high condition, and water quality is in moderate condition. Thus, the Bitter Creek Drainage population is determined to be in overall high current condition and has high resiliency.

<u>Unit 4, Bitter Creek Middle Tract Wetlands:</u> Pecos pupfish are routinely monitored at three sites within the Middle Tract Wetlands analysis unit (Hatt 2022, p. 5). However, most of the impoundments listed as occupied (Appendix A) have not been surveyed in decades. Despite this lack of data, we presume that the Pecos pupfish remains extant at all documented occupied sites in the unit. This is due to both their connection to occupied habitat and the absence of any known mortality event. In addition, surveys after long lapses in sampling (e.g., Unit 15) have been documented as extant.

This unit is composed of artificial wetlands and ditches that are routinely managed by Bitter Lake NWR. The ditches are spring fed and retain permanent water. The wetland impoundments

vary widely in habitat extent, though likely retain permanent water in most years. Given the variable nature of the water in the impoundments the amount of habitat is presumed to vary widely in any given year, and in particularly dry years may be extremely limited.

Bitter Lake NWR staff have collected specific data on water quality impairments that would impact the Pecos pupfish. However, when water levels are low, the shallow impoundments and wetlands in the unit could be subject to adverse water quality such as increased temperature and salinity and decreased available dissolved oxygen because water becomes lentic or stagnate and soon evaporates. The entirety of the unit is within Bitter Lake NWR which likely significantly reduces the risk of surface contaminants..

Aquatic environments in this unit area are a mix of manmade channels, impoundments, and wetlands. While we do not have data on how Pecos pupfish move between these environments, the diversity of habitats likely helps buffer the Pecos pupfish from short-term environmental changes such as droughts, provides ample refugia and breeding habitat, and provides protection from stochastic events such as floods or golden algae blooms. Thus, the Bitter Lake NWR Middle Tract Wetlands population's current condition evaluation determined that occurrence, water quantity, water quality, and habitat diversity are in overall high current condition and has high resiliency.

<u>Unit 5, Bottomless Lakes State Park</u>: Pecos pupfish have been routinely monitored at three sites in this analysis unit. However, during the 2021 monitoring, Pecos pupfish were confirmed extirpated from Upper Figure 8 Lake, possibly as a result of a golden algae bloom (Hatt 2021, p. 7). Pecos pupfish remain extant throughout the remainder of the known occupied sites within the unit, including the adjacent Lower Figure 8 Lake sinkhole.

All of the Pecos pupfish sinkholes at Bottomless Lakes State Park are fed by springs from the San Andres artesian aquifer (Land 2003, p. 229). Though some historical lowering of sinkhole levels has occurred, the recent trend is an increase in surface water levels in the sinkholes (Land 2003, p. 231). Water levels in the sinkholes appear to be closely related to the overall fluctuation in water levels in the artesian aquifer (Land 2003, p. 231).

The Bottomless Lakes sinkholes are relatively protected from surface contamination as a consequence of their location and status as a New Mexico State Park. No documented water contamination either from surface sources or natural water quality parameters has been recorded in the unit. However, as mentioned above, a possible golden algae outbreak in Upper Figure 8 Lake in 2020 may have been the cause of the complete loss of Pecos pupfish from this sinkhole.

Pecos pupfish in the Bottomless Lakes State Park are found exclusively in sinkhole habitat. Although sinkholes may exhibit more stable water quantity and quality, events such as the 2020 mortality event in Upper Figure 8 Lake illustrate the susceptibility of these habitats to stochastic events. The Bottomless Lakes State Park population's current condition evaluation determined that occurrence, water quantity, and water quality are in high condition, and habitat diversity is in moderate condition. Thus, the Bottomless Lakes State Park population is in overall high current condition and high resiliency. <u>Unit 6, BLM Overflow Wetlands and Lea Lake</u>: Pecos pupfish in this unit are surveyed in limited accessible areas of the BLM Overflow Wetlands (Hatt 2022, p. 2). Pecos pupfish are presumed extant throughout the suitable habitat within the wetland because installed fish barriers protect the unit from sheepshead minnow introgression (Hoagstrom et al. 2015, p. 16).

Lea Lake typically exhibits stable water quantity throughout the year (Hoagstrom and Brooks 1999a, p. 16). Water from Lea Lake flows through an outflow channel into the BLM Overflow Wetlands. In addition to flows from Lea Lake, wetland water is supplied by several springs throughout the complex as well as surface flows during precipitation events. As a result of the different sources of water to the wetland, the extent of aquatic habitat varies both seasonally and annually. However, owing to the constant source of water from Lea Lake, as well as the springs in the complex, permanent water remains in many locations. There are no documented water contamination issues in this unit.

This unit contains a large wetland complex and the largest sinkhole in Bottomless Lakes State Park. This sinkhole was not included in Unit 5 as it is not hydrologically connected to the other sinkholes in Unit 5 and is hydrologically connected to the BLM Overflow Wetlands. The habitat diversity represented by these aquatic environments provides a buffer from stochastic events. The BLM Overflow Wetlands and Lea Lake population's current condition evaluation determined that occurrence, water quantity, and habitat diversity are in high condition, and water quality is in moderate condition. The BLM Overflow Wetlands and Lea Lake population is in overall high current condition and has high resilience.

<u>Unit 7, Middle Pecos River</u>: It is likely that Pecos pupfish are extirpated from the Pecos River between Brantley Dam and Red Bluff Reservoir. Sheepshead minnow are regularly caught between Brantley Dam and Red Bluff Reservoir, which indicates that they are present throughout this segment of the Pecos River system (Davenport 2023a, entire).

The Middle Pecos River has regular issues with severe low flows and intermittency, water quality impairments, and stochastic events (Zymonas and Propst 2007, p. 45). The Middle Pecos River population's current condition evaluation determined that water quantity and habitat diversity are in moderate condition, and water quality is in low condition. Due to the presence of sheepshead minnow the Middle Pecos River population is considered extirpated.

<u>Unit 8, Salt Creek (TX)</u>: Pecos pupfish in Salt Creek (TX) are currently present in only a single reach of the stream. The fish at this location are not routinely monitored, however a TPWD biologist visited the site in 2023 and confirmed that fish are present (Montagne 2023, p. 2). Pecos pupfish from the lower reach of Salt Creek (TX), near the confluence with the Pecos River were confirmed introgressed with sheepshead minnow from the Pecos River. An unidentified physical barrier in the lower reaches of Salt Creek (TX) appears to have limited the spread of introgressed fish further upstream (A. F. Echelle et al. 2003, pp. 4–6). The Salt Creek (TX) population's current condition evaluation determined that occurrence, water quantity, and habitat diversity are in moderate condition, and water quality is in low condition. Thus, the Salt Creek (TX) population is in overall moderate current condition and has moderate resilience.

<u>Unit 9, Lower Pecos River</u>: Pecos pupfish have been extirpated from the lower Pecos River due to introgression with the sheepshead minnow.

The flow of the lower Pecos River north of Independence Creek is subject to frequent and ongoing intermittency issues, regularly experiencing no flow events, especially during the irrigation season and during periods of drought. South of Independence Creek the character of the river changes to one with steeper bank and canyon and permanent water flow. The water in this unit has very high salinity and increasing ongoing impacts from contaminants (Hoagstrom 2009, pp. 35-36). Hazardous material spills or leaks associated with oil and gas production are an ongoing problem in this unit and may be increasing in both number and frequency (Scanlon et al. 2020, p. 3511). The Lower Pecos River population's current condition evaluation determined that water quantity and habitat diversity are in moderate condition, and water quality is in low condition. Due to the presence of sheepshead minnow the Lower Pecos River population is considered extirpated..

Unit	Genetic Security	Occurrence	Water Quantity	Water Quality	Habitat Diversity	Overall Condition
Unit 1: Upper Pecos River (above Brantley Reservoir)	High	Moderate	Moderate	Low	Moderate	Moderate
Unit 2: Salt Creek Wilderness	High	Moderate	Moderate	Moderate	High	Moderate
Unit 3: Bitter Creek Drainage	High	High	High	Moderate	High	High
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High	High	High	High	High	High
Unit 5: Bottomless Lakes State Park	High	High	High	High	Moderate	High
Unit 6: BLM Overflow Wetlands and Lea Lake	High	High	High	Moderate	High	High
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated
Unit 8: Salt Creek (TX)	Low	Moderate	Moderate	Low	Moderate	Moderate
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated

 Table 5. Current Condition and resilience of nine analysis units.

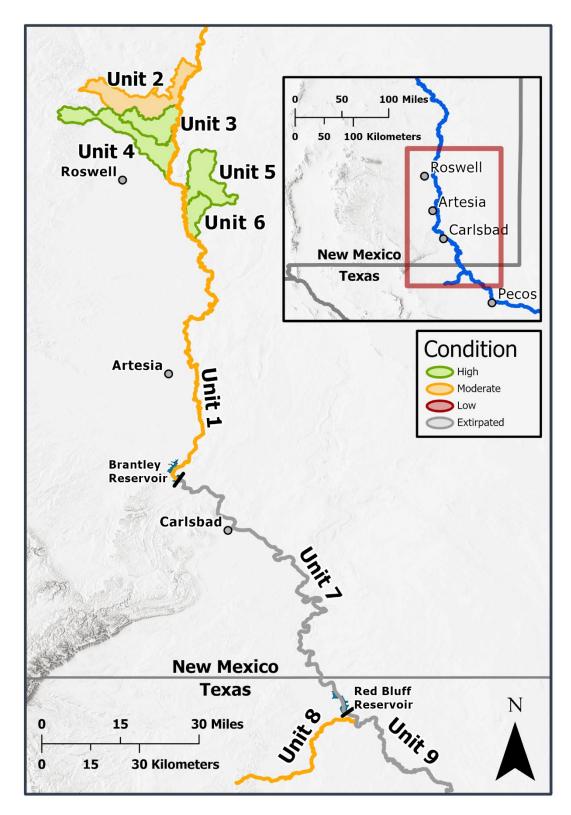


Figure 30. Map of the current condition of nine analysis units.

4.4.2 Redundancy

Redundancy describes the ability of a species to withstand catastrophic events by maintaining multiple, resilient populations distributed (and connected) within the species' varied habitats and across the species' range. We assessed Pecos pupfish redundancy at two scales, within the individual analysis units and across the range of the species. Within the analysis unit we looked at connection both internal to the unit and across adjacent units to characterize the overall redundancy of a unit (*Table 4*). The overall redundancy of the unit could not be higher than the lowest internal or external redundancy score. Important to the discussion of redundancy in Pecos pupfish populations is the consideration of sheepshead minnow introgression. While connectivity enhances redundancy within and among analysis units, this same connectivity increases the threat of sheepshead minnow introgression. A well-connected Pecos pupfish population is one that allows for dispersal and recolonization but is also one that is at increased risk of introgression. Redundancy throughout the species range, coupled with healthy populations may help lower the risk of introgression. A healthy, robust Pecos pupfish population may be more resistant to introgression and, thus, less likely to contribute to spread of hybrid fish (Kodric-Brown and Rosenfield 2004, p. 122).

The Upper Pecos analysis unit (Unit 1) is well connected throughout its length and the pattern of flow within the river likely creates a variety of microhabitat sites that are suitable for the Pecos pupfish. The Upper Pecos is moderately connected to adjacent off-channel units, though only at times of high flow.

The BLM Overflow Wetlands analysis unit (Unit 6) is the only other unit that has high internal redundancy. Similar to the Pecos River, the Overflow Wetlands provide a large area with many microhabitats. This unit was historically connected to the Pecos River during high flows, but fish barriers installed to protect the unit from sheepshead minnow introgression have limited this connection.

The Salt Creek Wilderness (Unit 2) and the Middle Tract Wetlands (Unit 4) units are the only two units that are currently connected to the Pecos River during periods of high flow. In the Salt Creek Wilderness unit, the connection to the unit is only in Salt Creek (NM) proper and the Pecos pupfish may only be found in one permanent pool in Salt Creek (NM). Within the Salt Creek Wilderness unit, Pecos pupfish are distributed among several sinkholes and Salt Creek, (NM), and there is no known, above-ground connection between these areas. The Middle Tract unit (Unit 4) is connected to the Upper Pecos at the southern end of the unit during periods of high flow. While pupfish are likely widely distributed within the Middle Tract unit, connection among the different occupied sites is managed through a series of diversions and manmade impoundments. Water flow through the unit is generally north to south, and when the gates between the impoundments are open flow is likely to great to allow Pecos pupfish to move up the current.

Bitter Creek Drainage (Unit 3) and Bottomless Lakes State Park (Unit 5) do not have any known connection to adjacent analysis units. Internally, many of the occupied sites within the Bitter Creek Drainage are isolated sinkholes. There is connection along Bitter Creek and to the springs

that feed the creek, however, there is no known connection between the creek and adjacent sinkholes. It is also surmised that there may be some underground connection between the springs in the Dragonfly Spring sinkhole area (Land and Huff 2009, p. 20). It is currently unknown how extensive this connection is (if at all) or if a Pecos pupfish would be able to move between sinkholes underground. The Bottomless Lakes State Park sinkholes are fully isolated from each other and from adjacent analysis units.

Salt Creek (TX) (Unit 8) is directly connected to the lower Pecos River. There is a presumed natural barrier within Salt Creek (TX) upstream from the confluence that moderates this connectivity. The barrier has allowed non-introgressed Pecos pupfish to remain extant in the upper reaches of Salt Creek (TX) despite the presence of an introgressed population downstream. It is currently unknown how many extant sites are found in the upper areas of Salt Creek (TX) but the connection between them likely varies seasonally with the amount of water in the creek.

Since the introduction of sheepshead minnow into the Pecos River in the early 1980s, the introgressed population has continued to expand north. This represents a reduction of over half of the presumed riverine range of the Pecos pupfish by 1997 (Echelle et al. 1997, pp. 336–337). Since then, the introgressed population has expanded as far north as Brantley Dam (*Figure 16* and *Figure 27*).

Analysis Unit	In-Unit Distribution	External Connection
1: Upper Pecos River	Contiguous, connected	Limited, natural barrier
2: Salt Creek Wilderness	Discrete, disconnected	Limited, natural barrier
3: Bitter Creek Drainage	Discrete, disconnected	None
4: Bitter Lake NWR Middle Tract Wetlands	Discrete, connected	Limited, natural and manmade barrier
5: Bottomless Lakes State Park	Discrete, disconnected	None
6: BLM Overflow Wetlands and Lea Lake	Contiguous, connected	Limited, manmade barrier
7: Middle Pecos River	Contiguous, connected	Contiguous
8: Salt Creek (TX)	Discrete, connected	Limited, natural barrier
9: Lower Pecos River	Contiguous, connected	Contiguous

Table 6. Analysis unit redundancy.

4.4.3 Representation

Representation describes the ability of a species to adapt to changing environmental conditions over time and is characterized by the breadth of genetic and environmental diversity within and among populations. As discussed in Chapter 2, Pecos pupfish are known from a variety of different environmental settings and show specific morphological variation related to these environmental settings. Populations have been documented in sinkholes, streams, marshes, managed wetlands, and rivers with varying physical characteristics (i.e., size, gradient, elevation, temperature, etc.).

Genetic studies of Pecos pupfish have largely been conducted as part of efforts to understand the dynamics of hybridization with the sheepshead minnow and the spread of introgressed populations. Nevertheless, data collected as part of these investigations have revealed important

genetic relationships in across the range of the Pecos pupfish. The population of Pecos pupfish in the upper reaches of Salt Creek (TX) shows a specific allele, Gpi-A¹⁵⁷, that is found only at this location (Echelle et al. 2003, p. 6). Neither the mechanism for the unique allelic frequency nor the importance to the population is known at this time (Echelle et al. 2003, p. 6). Recent work in the northern portion of the Pecos pupfish range has further examined the genetic relationships of these populations. Pecos pupfish in the BLM Overflow Wetland and Lower Figure 8 Lake (Bottomless Lakes State Park) were both highly genetically differentiated from each other and from other populations at Bottomless Lakes State Park and Bitter Lake NWR (Whiteley 2023, p. 18). Additionally, both of these sites had low within-sample genetic variation and high inbreeding coefficients (Whiteley 2023, pp. 7–8). Analysis showed distinct clustering of Pecos pupfish at two sites at Bottomless Lakes State Park (Mirror Lake and Lazy Lagoon) and all of the sampled sites at Bitter Lake NWR (Whiteley 2023, p. 18). On Bitter Lake NWR two distinct clusters were observed that may indicated gene flow (Whiteley 2023, p. 8). The sampled sites in the Middle Tract Wetlands clustered with each other and Bitter Creek, while the four sample sinkholes all clustered with each other (Whiteley 2023, p. 8). While that data analyzed by Whitely (2023, entire) did not attempt to infer a relationship between environmental factors, a result that might reflect either developmental plasticity or local genetic adaptation, research does suggest that Pecos pupfish morphology differs depending on the environmental setting (Collyer et al. 2015, p. 189; Xu 2017, pp. 22, 26–27).

Pecos pupfish have been extirpated from the Pecos River in southern New Mexico and Texas and have likely been extirpated from several off-channel locations in the same region. In New Mexico, Pecos pupfish are found in nearly all the localities from Brooks (1992, entire) and Hoagstrom and Brooks (1999, entire). However, several sinkhole locations have not been visited since the late 1990's, and it is unknown if fish persist in these locations. Currently, the Pecos pupfish is found within all of the historically occupied environmental settings. However, the only remaining riverine analysis unit has moderate resiliency which reflects a potential increase in the loss of representation of riverine Pecos pupfish, and the only remaining extant analysis unit in Texas also has moderate resiliency which presents a risk to representation of Pecos pupfish in Texas. Pecos pupfish have likely experienced some reduction in representation as a result of the large range reduction following extirpations from the Pecos River and off-channel locations in Texas and southern New Mexico.

4.4.4 Assessment of Current Viability

The Pecos pupfish is currently distributed across seven of nine analysis units covering the historical range. Within those seven analysis units, four were assessed to be in high resiliency condition and three in moderate condition. Across the range of the species, we identified 66 distinct locations (sites) where Pecos pupfish have been recorded since 1992. As of 2023, eight (12.1 percent) of these sites are confirmed or presumed extirpated and four are in unknown status. The remaining 54 sites (81.2 percent) are extant or presumed extant. Twenty-one sites (31.8 percent) have been confirmed as extant within the last 5 years. This does not take into account losses that may have occurred before the first comprehensive range-wide surveys occurred. There has been a large decline in the extent of the occupied range because of the

extirpation of Pecos pupfish from their historical range in the Pecos River below Brantley Dam (southern New Mexico and Texas). Pecos pupfish were historically found in riverine, stream, wetland, and sinkhole habitats and currently continue to be recorded from all these habitats. Because of the reduction in the range caused by the extirpation of Pecos pupfish from a large section of the Pecos River, the species has experienced a reduction in both redundancy and representation. However, we do not have the data on the historical size of the Pecos pupfish population in the Pecos River or the genetic relationship between this population and others to adequately assess the relative importance of this population to the species.

Although there is uncertainty surround the demography of differing Pecos pupfish populations and their genetic relationships, data suggests that the Pecos pupfish still occurs in multiple populations representing the historical range of habitat variation for the species. Though declines in range extent and likely population size has occurred, monitoring data suggest that the Pecos pupfish continues to have multiple, long-term persistent populations throughout its range.

CHAPTER 5 – FUTURE SCENARIOS

5.1. Approach

Using the same methods described for Current Condition, we assessed viability of the Pecos pupfish under three future scenarios at 2050 and 2100, consistent with the available information. All of the scenarios were focused on different climate projections for the Pecos River Basin. We also assessed the risk of sheepshead minnow introgression into other parts of the species' range.

Although development such as urbanization, agriculture, and oil and gas extraction may have local effects on some pupfish sites, we do not expect substantial effects from these sources at the species or analysis unit level. The exception to this is the potential for oil and gas development in the vicinity of Salt Creek (TX) to cause significant variation in stream flow. Oil and gas development in this area is expected to increase as energy demands are needed with increased human development (Llewellyn et al. 2021, pp. 81, 163, and 171). While we do not have ongoing monitoring on Salt Creek (TX), stream gauges on the Black River in New Mexico have shown a direct correlation between oil and gas activities and reductions in stream flow.

Population growth in Chaves County, NM, which contains the majority of current occupied Pecos pupfish sites, averaged a 1.3 percent annual growth rate between 1960 and 2010 (Consensus Planning, Inc. 2016, p. 10) but a 1.9 percent annual decline between 2010 and 2020. The PVACD regulates groundwater use within the aquifer and supplies water to about 110,000 acres of crops/year (Llewellyn et al. 2021, p. 47). The amount of water withdrawn causes seasonal variability in aquifer levels, but yearly fluctuations in groundwater levels typically remain similar (PVACD 2023, entire). The long-term average water level has remained constant. Water availability in the Pecos River is influenced by a variety of factors including human development, primarily agriculture. However, this river is currently managed for multiple uses, including endangered species conservation, and future human water use from the river is not expected to substantially increase in the future. Given these factors, we think the most important abiotic factors affecting Pecos pupfish viability will result from potential changes in water availability resulting from changing climatic conditions. The most important biotic factor is the potential for hybridization and genetic introgression by sheepshead minnow.

As discussed in *Section 3.3 Global Climate Change and Drought*, the southwest is predicted to continue to become hotter and dryer (Garfin et al. 2013, pp. 5–6). Llewellyn et al. (2021, entire) developed a report specific to the Pecos Basin in New Mexico that we used to assess potential future conditions that could impact the Pecos pupfish. This report looked at three "storylines" (or scenarios) to assess future water availability across the Pecos River Basin in New Mexico. For this SSA, we selected three of those scenarios to represent the variability of potential future conditions that could impact the Pecos pupfish and its habitat.

Scenario 1: Hot and Dry (RCP 8.5) – Steep increase in annual average temperature coupled with steep decreases in annual precipitation.

Scenario 2: Hot and Wet (RCP 8.5) – Steep increase in annual average temperature coupled with an increase in annual average precipitation.

Scenario 3: Warm and Dry (RCP 4.5) – Modest increase in annual average temperature and modest decrease in annual average precipitation.

For each of the scenarios we assumed that the Pecos Pupfish Conservation Agreement will remain active, and the signatory agencies will continue monitoring the biological condition of the species and working to prevent spread of sheepshead minnow.

5.1.1 Assumptions and Limitations

As with any analysis, we made many assumptions that have consequences for our projections and interpretation of Pecos pupfish viability. First, we only used occurrence data starting in 1992 as the basis for our analysis. This was the first published range-wide survey of the Pecos pupfish and therefore provided the most comprehensive data set on Pecos pupfish occurrence. Sites that were only recorded prior to 1992 were excluded from our analysis but were included in the overall picture of historical distribution.

We were unable to locate information on thresholds or water body sizes that equate to an increase in extirpation risk specifically for Pecos pupfish. It is logical to assume populations that occupy smaller and shallower habitats are less resilient, but there are no clear thresholds at which the size raises extinction risk that we found in the literature. We also did not find any specific thresholds for water quality impacts to Pecos pupfish populations that equate to a specific extirpation risk. Pupfish, including the Pecos pupfish, are known for their tolerance for water quality conditions that inhibit the fecundity and survival of other fish. We assumed that populations experiencing long-term high temperatures or elevated salinity are less resilient, but there are no clear thresholds at which this long-term exposure raises extirpation risk. Thus, our categorization methodology may over- or under-estimate resiliency of populations depending on where the real biological thresholds are.

A critical assumption is that the primary stressors we identified, sheepshead minnow presence, and water quality and quantity alteration that leads to habitat loss and fragmentation, which are exacerbated due to climate change are the primary threats to the species long-term viability. We are confident that stressors such as vegetation changes are unlikely to be long-term threats. Although land use practices and development have impacted the species historically, given the current distribution of Pecos pupfish populations, we anticipate that these activities would not have a large future impact.

In order to characterize sheepshead minnow introgression into the future, we separately assessed this stressor. This stressor is a low probability, high consequence event where if the event occurs a population could be extirpated or highly degraded. We did not think it was appropriate to project the condition of the species under the assumption the stressor does or does not occur, as it would provide two dramatically different future outcomes. Such an exercise would provide limited insights, as we assume that if sheepshead minnow gain access to sites occupied by Pecos pupfish, the Pecos pupfish would be replaced by the sheepshead minnow. Therefore, the main question concerns the likelihood that a given population will experience the sheepshead minnow invasion.

Another assumption in this SSA regards the role of conservation in future viability of the Pecos pupfish. With the current conservation agreement in place, the Conservation Team has been proactive in supporting the species. We incorporated these efforts into several aspects of our analysis, such as our evaluation of the probability of current Pecos pupfish populations being invaded by nonnatives taking into consideration conservation measures to prevent such invasion. However, there were other aspects of the conservation efforts that we did not incorporate in our future projections. For example, securing water rights to maintain groundwater can improve the resiliency of existing populations. We also were unable to incorporate future water conservation measures into our analysis. While past water conservation, particularly in the Pecos Valley Artesian Conservancy District, has had beneficial impacts to groundwater supply within the range of the Pecos pupfish, we were uncertain of the direct link between these measures and Pecos pupfish habitat and the plausibility of future water conservation efforts.

5.1.2 Surface Temperature

Average annual surface temperatures as well as the incidence of extreme heat events are projected to increase across the entirety of the southwest including the Pecos Basin (Vose et al. 2017, entire). Within the Pecos Basin average surface temperatures could increase by as much as 13.32 °F (-10.4 °C) (*Table 7*) to an average surface temperature in excess of 70 °F (21.1 °C) (*Figure 31*).

Table 7. Projected temperature and precipitation change under each of the three scenarios.(Llewellyn et al. 2021, p. 101)

Scenario	Annual change in temperature	Difference in temperature, 2010 – 2100	Annual change in precipitation	Difference in annual precipitation, 2010 – 2100
1	+0.148 °F	+13.32 °F	-0.093 in	-8.37 in
	(+0.082 °C)	(+7.40 °C)	(-0.236 cm)	(-21.26 cm)
2	+0.137 °F	+12.33 °F	+0.022 in	+1.98 in
	(+0.076 °C)	(+6.85 °C)	(+0.056 cm)	(+5.03 cm)
3	+0.028 °F	+2.25 °F	-0.007 in	-0.63 in
	(+0.016 °C)	(+1.25 °C)	(-0.018 cm)	(-1.60 cm)

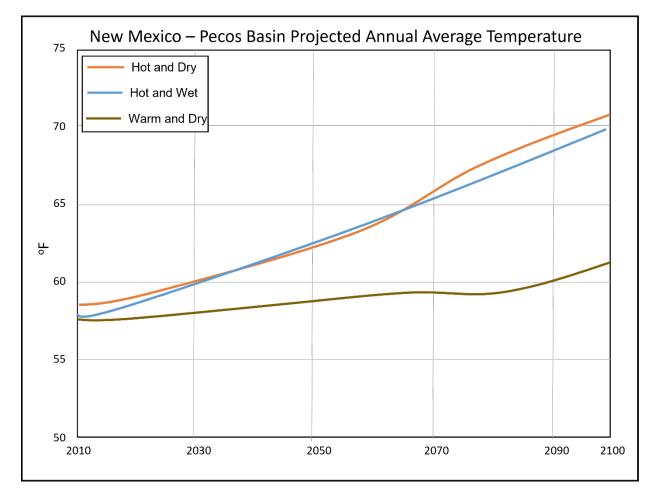


Figure 31. Average surface temperature increases in the Pecos Basin. Figure adapted and simplified from (Llewellyn et al. 2021, p. 101) to show only the three scenarios selected for this SSA.

As temperatures increase across the region, we also anticipate a corresponding increase in evapotranspiration rates (*Figure 32*). Both temperature and evapotranspiration rates can have negative effects on Pecos pupfish and their habitat. Increasing temperatures increase the risk of golden algae blooms as well as increasing the chances that shallow habitat could exceed the

thermal tolerance of Pecos pupfish. Greater evapotranspiration leads to lowering of water levels with the potential for corresponding increases in salinity and water temperatures and lowered dissolved oxygen. Lowered water levels also may lead to a reduction in the overall habitat available to Pecos pupfish along with the potential of the complete loss of water in shallow aquatic environments.

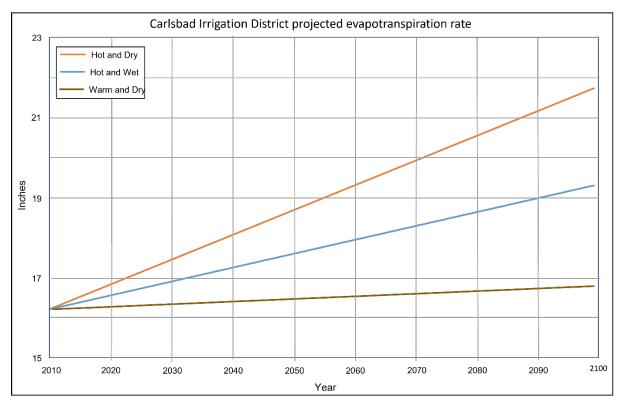


Figure 32. Projected evapotranspiration rates. Figure adapted and simplified from (Llewellyn et al. 2021, p. 108) to show only the three scenarios selected for this SSA.

5.1.3 Precipitation and Aquifer Levels

Precipitation changes related to climate change are more variable and less certain than those changes projected for temperature. In the southwest, the occurrence of seasonal monsoons complicates the picture for overall projected changes to precipitation in the Pecos River Basin. Though generally models predict a drying trend across the Pecos Basin, under certain RCP 8.5 conditions, monsoon moisture increases leading to an increase in average annual precipitation (*Table 7*). Under both RCP 4.5 and RCP 8.5 scenarios, snowpack in the headwater of the Pecos River decreases with a corresponding earlier snowmelt runoff (Llewellyn et al. 2021, p. 191). Though precipitation changes could potentially reduce flows into the San Andres aquifer from the Sacramento Mountains, the effect of lower snowpack and runoff will likely be most impactful to the Pecos River (*Figure 33*).

Figure 33. Project Pecos River flow changes at the Acme Gauge. Figure adapted and simplified from Llewellyn et al. 2021, p. 121) to show only the three scenarios selected for this SSA.

The levels of the San Andres aquifer likely directly impacts the water sources for most nonriverine Pecos pupfish habitats, except for those in Salt Creek (TX) (Land 2003, p. 228). Although we do not know the exact relationship between aquifer levels and the springs that provide flows to sinkholes, wetlands, and streams that provide Pecos pupfish habitat, we can infer that changes to the aquifer level will likely produce a corresponding change in spring flows. Levels in the San Andres aquifer are influenced by the amount of historical water in the aquifer and current inputs (Land and Huff 2009, p. 20) as well as pumping by users in the PVACD. As illustrated in *Figure 34* there is a significant variation in the potential changes to the San Andres aquifer.

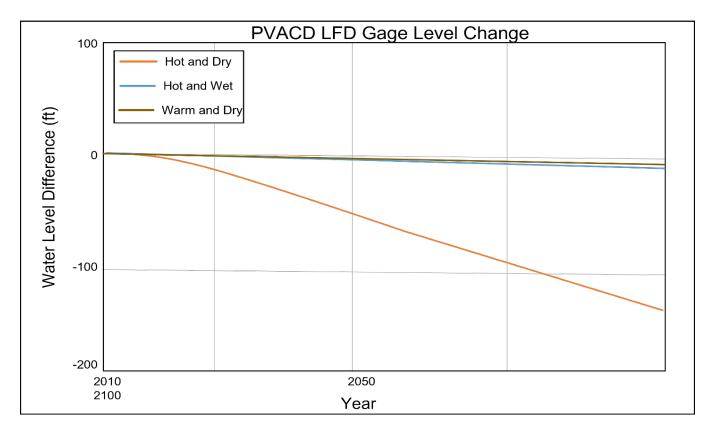


Figure 34. Aquifer drawdown as measured at the PVACD LFD Gage. Figure adapted and simplified from Llewellyn et al. 2021, p. 128) to show only the three scenarios selected for this SSA.

5.2 SSA Future Scenarios

5.2.1 Scenario 1 – Hot and Dry (RCP 8.5)

In this scenario, future annual air temperature increases slightly, and annual precipitation decreases throughout the Pecos River Basin (*Table 7*). Though temperatures increase in all seasons, summer and autumn temperatures are predicted to increase more than winter and spring temperatures. By 2100 (and likely much sooner), conditions in the Pecos River Basin would be much drier than the historical average. Precipitation would be greatly decreased in all seasons,

though decreases would be most extreme during the monsoon season. Runoff inflow into the Pecos River Basin will decrease across every season and the inflow that will occur is anticipated to be the result of very few large storm events (Llewellyn et al. 2021, p. 105).

5.2.2 Scenario 2 – Hot and Wet (RCP 8.5)

In this scenario, both temperature and annual precipitation increase throughout the Pecos River Basin (*Table 7*). In this scenario increased temperatures retain more moisture in the atmosphere leading to increased monsoons. This scenario is more seasonally variable, with sharply increased inflow during the monsoon season and a steep decrease of inflow during the spring runoff. In this scenario, spring and summer temperatures increase more rapidly than fall and winter temperatures. While precipitation decreases during winter and spring, precipitation increases during the summer and autumn monsoon season, leading to an overall increase in precipitation within the Pecos River Basin. As a result of decreased winter precipitation, spring runoff is anticipated to decrease. However, a large increase in monsoon flows make up for the spring runoff decrease (Llewellyn et al. 2021, pp. 105–106).

5.2.3 Scenario 3 – Warm and Dry (RCP 4.5)

This scenario anticipates the smallest changes to temperature and precipitation of the three scenarios (*Table 7*). By 2100, this scenario predicts slightly higher average temperatures and a slightly dryer climate. Importantly, summer and fall temperatures are anticipated to increase almost twice as much as winter and spring temperatures (Llewellyn et al. 2021, p. 101).

5.3 Future Condition Projections

Using the projections for temperature, precipitation, and San Andres aquifer under the three scenarios outlined above, we then predicted the potential range of outcomes these scenarios could have on the Pecos pupfish. Future conditions were analyzed for each resiliency metric and summarized for each unit (**Appendix C**).

5.3.1 Scenario 1 – Hot and Dry (RCP 8.5)

This scenario forecasts extreme drying and higher temperatures across the Pecos River Basin (*Table 7*). A decrease in precipitation across the basin along with increased air temperatures and overall drying trends is projected to lead to decreases in stream flow, spring output, and potentially a lowering of the aquifer that supports wetland and sinkhole habitats for the pupfish. Although the Pecos River is managed for flows that support endangered species such as the Pecos bluntnose shiner, decreasing precipitation will impede the ability of the upstream storage to deliver reliable water to both downstream users and retain adequate flow in the Pecos River and lead to an increase in drying days (*Figure 33*). Higher temperatures, particularly during the summer, will lead to an increase in water needs and increase groundwater pumping by agriculture in the PVACD. Higher temperatures also increase evaporative loss from water bodies and could lead to decreases in habitats available for the pupfish.

This scenario will have some negative effects on all Pecos pupfish analysis units. The most severe impacts are anticipated to be to small streams. Salt Creek (TX), Salt Creek (NM), and Bitter Creek are all projected to dry and cease flowing during the hottest parts of the year leading to local fish kills, or in the case of Salt Creek (TX), possibly the loss of all habitats in the analysis unit. All of these creeks currently experience intermittent drying events, and lower precipitation and increased temperatures in the future will exacerbate this existing condition that stresses these habitats. Wetland areas such as the BLM Overflow Wetlands and the managed wetlands on Bitter Lake NWR are also anticipated to be significantly impacted in this scenario. At the 2050 timestep, given the climate projections, habitat conditions are projected to be similar to current conditions with minimal changes to most aquatic environments anticipated over that time period with the exception of small streams which are already experiencing impacts from warming and drying temperatures. By 2100, significant degradation to Pecos pupfish habitat and a decline in distribution are expected. Shallow streams will likely no longer support permanent water leading to the loss of fish in Bitter Creek, Salt Creek (NM), and Salt Creek (TX), which would mean the extirpation of Pecos pupfish from Texas. Habitat extent in wetland habitats in the BLM Overflow Wetlands and Middle Tract on Bitter Lake NWR will be greatly reduced, and pupfish would be expected to persist only in deeper channels or near springs.

Historically the San Andres aquifer has been resilient and rebounded after extended drought (Land and Newton 2008b, pp. 189-190). However, the conditions under this scenario, RCP 8.5, at 2100 will be much hotter and drier than the historical average and is expected to lead to unprecedented conditions in aquifer levels and surface water quality and quantity. Across the range of the Pecos pupfish, we anticipate substantial increases in salinity as a result of increased evapotranspiration. Although Pecos pupfish can tolerate higher salinity levels than most fish, significant salinity impairment could lead to a reduction in suitable conditions for breeding. Increasing surface temperatures will lead to an increase in water temperatures and likely lowered dissolved oxygen saturation. This will be particularly pronounced in shallow habitat such as streams and wetlands. A substantial reduction in the aquifer level would reduce the outflow of springs leading to a loss of fish in habitats that rely on steady, perennial spring flow and a reduction (or elimination) of available habitat in shallower sinkholes. Additionally, the increasing temperature and evaporation could cause shallower habitats to exceed the thermal and saline tolerances of the Pecos pupfish. Consequently, we anticipate a reduction in both the number, extent, and population sizes of extant sites in sinkhole units. Finally, we anticipate greatly reduced flows in the Pecos River under this scenario. While reduced flows in the Pecos River has the potential to benefit the pupfish on a seasonal basis, long-term drying events will lead to the disconnection of occupied sites and increased impairment of water quality. Given these assumed future changes in the environment, we projected the future conditions under Scenario 1 for the Pecos pupfish and its habitats in each analysis unit).

At 2050, two units are projected to remain in high condition, four units are in moderate condition, and one unit is in low condition. At 2100, one unit is in high condition, four units are in moderate condition, one unit is in low condition, and one unit is extirpated (*Table 8* and *Table 9* and *Figure 35*). Although habitat conditions are expected to generally decline across the range, the Bottomless Lakes unit is anticipated to remain in high condition because the sinkhole habitats there are expected to be less affected by potential aquifer declines. While only two units (Unit 3 and Unit 8) experience declines from current condition at 2050 (*Table 8*), by 2100 all units except Unit 5 experience declines from current condition (*Table 8*).

Table 8. Scenario 1 - Hot and Dry. Time Step 2050.

Unit	Current Condition	Occurrence	Water Quantity	Water Quality	Habitat Diversity	S1 2050 Condition
Unit 1: Upper Pecos River (above Brantley Reservoir)	Moderate	Moderate	Moderate	Low	Moderate	Moderate
Unit 2: Salt Creek Wilderness	Moderate	Low	Moderate	Moderate	High	Moderate
Unit 3: Bitter Creek Drainage	High	High	Moderate	Moderate	High	Moderate
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High	High	High	High	High	High
Unit 5: Bottomless Lakes State Park	High	High	High	High	Moderate	High
Unit 6: BLM Overflow Wetlands and Lea Lake	High	High	High	Moderate	High	High
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated
Unit 8: Salt Creek (TX)	Moderate	Low	Low	Low	Moderate	Low
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated

Table 9. Scenario 1 - Hot and Dry. Time Step 2100.

Unit	Current Condition	Occurrence	Water Quantity	Water Quality	Habitat Diversity	S1 2100 Condition
Unit 1: Upper Pecos River (above Brantley Reservoir)	Moderate	Moderate	Moderate	Low	Moderate	Moderate
Unit 2: Salt Creek Wilderness	Moderate	Low	Low	Low	Moderate	Low
Unit 3: Bitter Creek Drainage	High	Moderate	Moderate	Moderate	Moderate	Moderate
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High	Low	Low	Moderate	High	Moderate
Unit 5: Bottomless Lakes State Park	High	High	High	High	Moderate	High
Unit 6: BLM Overflow Wetlands and Lea Lake	High	Moderate	Moderate	Moderate	High	Moderate
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Low	Low	Moderate	Extirpated
Unit 8: Salt Creek (TX)	Moderate	Extirpated	Low	Low	Moderate	Extirpated
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated

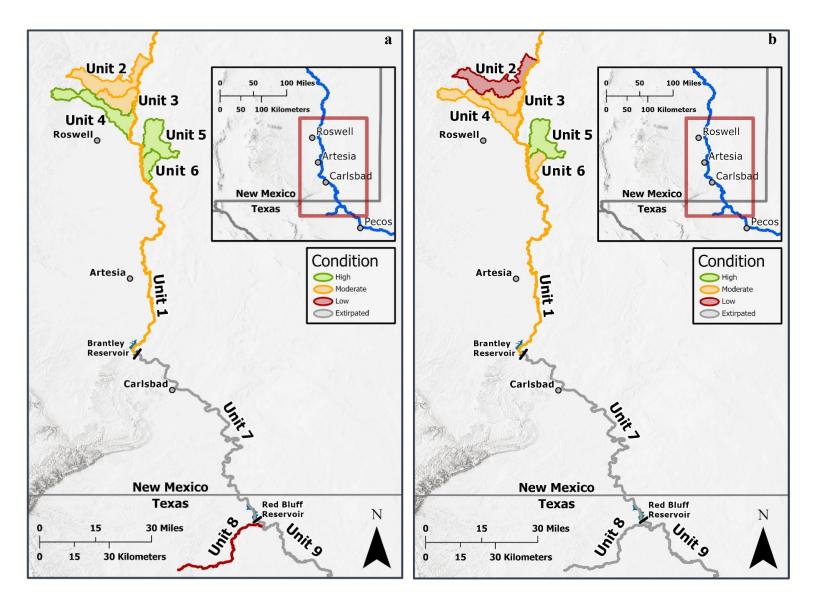


Figure 35. Analysis Unit Overview of Scenario 1 (Hot and Dry) Future Condition at Time Steps 2050 (a) and 2100 (b).

5.3.2 Scenario 2 - Hot and Wet (RCP 8.5)

This scenario forecasts a significantly higher average annual surface temperature across the Pecos River Basin. Unlike Scenario 1, higher summer temperatures result in more moisture in the atmosphere, consequently leading to an increase in precipitation during the summer monsoon season (June – September). Overall higher surface temperatures will lead to similar outcomes as described under Scenario 1, such as water quality impairment, and reduction in habitat extent. However, the predicted increased monsoons may buffer some systems from the most severe impacts of increasing average temperatures.

As with Scenario 1, the small streams are most likely to experience the most severe adverse impacts from increasing annual temperatures. These aquatic environments will likely experience more drying events and subsequent impairments to salinity, water temperatures, and dissolved oxygen. At the 2050 timestep, conditions appear similar to current conditions throughout much of the Pecos pupfish range. However, small streams will likely stop flowing during the hottest parts of the year leading to local fish kills, or in the case of Salt Creek (TX) possibly the loss of all habitats in the analysis unit. By 2100 rising annual temperatures may eliminate year-round stream flow in all but the wettest years. Consequently, we anticipate the loss of the Pecos pupfish population at Salt Creek (TX) and a reduction in occupied sites in Bitter Creek and the Salt Creek Wilderness. Increased temperatures will have an impact on shallower wetlands in the BLM Overflow Wetlands and Bitter Lake NWR Middle Tract Wetlands. Prolonged extreme air temperatures can adversely impact water quality and could result in decreased fitness, hinder breeding, or lead to fish kills. Sinkholes are the most stable environment for the Pecos pupfish, and this is unlikely to change in this scenario. The San Andres aquifer responds quickly to precipitation inputs, and an increase in monsoon season precipitation will likely prevent significant declines in sinkhole water levels.

Given these assumed future changes in the environment, we projected the future conditions under Scenario 2 for the Pecos pupfish and its habitats in each analysis unit (*Table 10, Table 11*, and *Figure 36*). At 2050, three units, Bitter Lake NWR Middle Tract Wetlands, Bottomless Lakes State Park, and BLM Overflow Wetlands and Lea Lake, are projected to remain in high condition, three units, Upper Pacos River, Salt Creek Wilderness, and Bitter Creek Drainage, are in moderate condition, and one unit, Salt Creek (TX), is in low condition, and two units, Middle Pecos River and Lower Pecos River, remain extirpated. Under this scenario only two units, Bitter Creek Drainage and Salt Creek (TX), experience a decrease from current condition (*Table 10*). At 2100, two units, Bottomless Lakes State Park and BLM Overflow Wetlands and Lea Lake, are in high condition, three units, Upper Pecos River, Bitter Creek Drainage, and Bitter Lake NWR Middle Tract Wetlands, are in moderate condition, one unit, Salt Creek Wilderness, is in low condition, and three units, Middle Pecos River, Salt Creek (TX), and Lower Pecos River, are extirpated with all units except Upper Pecos River, Bottomless Lakes State Park, and BLM Overflow Wetlands and Lea Lake experience a decrease from current condition (*Table 11*).

Table 10. Scenario 2 - Hot and Wet. Time Step 2050.

Unit	Current Condition	Occurrence	Water Quantity	Water Quality	Habitat Diversity	S2 2050 Condition
Unit 1: Upper Pecos River (above Brantley Reservoir)	Moderate	Moderate	Moderate	Low	Moderate	Moderate
Unit 2: Salt Creek Wilderness	Moderate	Low	Moderate	Moderate	High	Moderate
Unit 3: Bitter Creek Drainage	High	High	Moderate	Moderate	High	Moderate
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High	High	High	High	High	High
Unit 5: Bottomless Lakes State Park	High	High	High	High	Moderate	High
Unit 6: BLM Overflow Wetlands and Lea Lake	High	High	High	Moderate	High	High
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated
Unit 8: Salt Creek (TX)	Moderate	Low	Low	Low	Moderate	Low
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated

Table 11. Scenario 2 - Hot and Wet. Time Step 2100.

Unit	Current Condition	Occurrence	Water Quantity	Water Quality	Habitat Diversity	S2 2100 Condition
Unit 1: Upper Pecos River (above Brantley Reservoir)	Moderate	Moderate	Moderate	Low	Moderate	Moderate
Unit 2: Salt Creek Wilderness	Moderate	Low	Moderate	Low	Moderate	Low
Unit 3: Bitter Creek Drainage	High	Moderate	Moderate	High	Moderate	Moderate
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High	Moderate	Moderate	Moderate	High	Moderate
Unit 5: Bottomless Lakes State Park	High	High	High	High	Moderate	High
Unit 6: BLM Overflow Wetlands and Lea Lake	High	High	High	Moderate	High	High
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Low	Low	Moderate	Extirpated
Unit 8: Salt Creek (TX)	Moderate	Extirpated	Low	Low	Moderate	Extirpated
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated

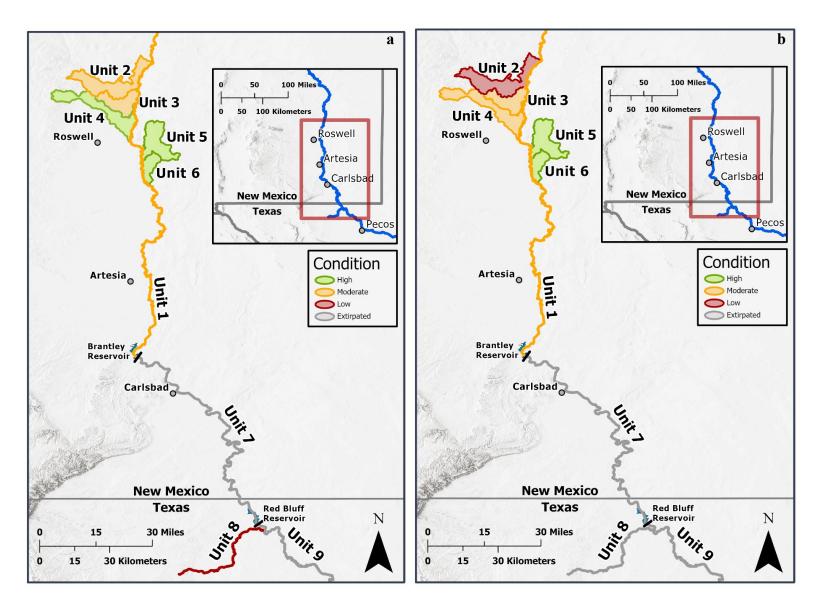


Figure 36. Analysis Unit Overview of Scenario 2 (Hot and Wet) Future Condition. Time Step 2050 (a) and Time Step 2100 (b).

5.3.3 Scenario 3 – Warm and Dry (RCP 4.5)

This scenario forecasts a minimal increase in yearly average temperatures and a minimal decrease in precipitation across the basin. Even minimal decreases in precipitation could have consequences for shallow streams in several units (Salt Creek Wilderness, Bitter Creek Drainage, and Salt Creek (TX)). Like the prior scenarios, the 2050 time step appears fairly similar to current condition. By 2100, small streams are likely experiencing increased water stress, and in dry years likely most of the stream environments will be dry. However, under this scenario, we anticipate minimal impacts to groundwater resources and thus minimal impacts to sinkhole, spring fed, and river habitats.

At 2050, four units are projected to be in high condition and three units in moderate condition and two units are extirpated. At 2100, three units are projected to be in high condition, three units in moderate condition, and one unit in low condition and two units extirpated (*Table 12*, *Table 13*, and *Figure 37*). In Scenario 3, no units experience decreases from current condition at 2050 (*Table 12*), however, at 2100 two units (Unit 3 and Unit 8) experience decreases from current condition (*Table 13*).

Table 12. Scenario 3 – Warm and Dry. Time Step 2050

Unit	Current Condition	Occurrence	Water Quantity	Water Quality	Habitat Diversity	S3 2050 Condition
Unit 1: Upper Pecos River (above Brantley Reservoir)	Moderate	Moderate	Moderate	Low	Moderate	Moderate
Unit 2: Salt Creek Wilderness	Moderate	Moderate	Moderate	Moderate	High	Moderate
Unit 3: Bitter Creek Drainage	High	High	High	Moderate	High	High
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High	High	High	High	High	High
Unit 5: Bottomless Lakes State Park	High	High	High	High	Moderate	High
Unit 6: BLM Overflow Wetlands and Lea Lake	High	High	High	Moderate	High	High
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Low	Moderate	Low	Moderate	Extirpated
Unit 8: Salt Creek (TX)	Moderate	Moderate	Moderate	Low	Moderate	Moderate
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated

Table 13. Scenario 3 - Warm and Dry. Time Step 2100.

Unit	Current Condition	Occurrence	Water Quantity	Water Quality	Habitat Diversity	S3 2100 Condition
Unit 1: Upper Pecos River (above Brantley Reservoir)	Moderate	Moderate	Moderate	Low	Moderate	Moderate
Unit 2: Salt Creek Wilderness	Moderate	Moderate	Low	Low	High	Moderate
Unit 3: Bitter Creek Drainage	High	Moderate	Moderate	Moderate	High	Moderate
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High	High	High	High	High	High
Unit 5: Bottomless Lakes State Park	High	High	High	High	Moderate	High
Unit 6: BLM Overflow Wetlands and Lea Lake	High	High	High	Moderate	High	High
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated
Unit 8: Salt Creek (TX)	Moderate	Moderate	Low	Low	Moderate	Low
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate	Low	Moderate	Extirpated

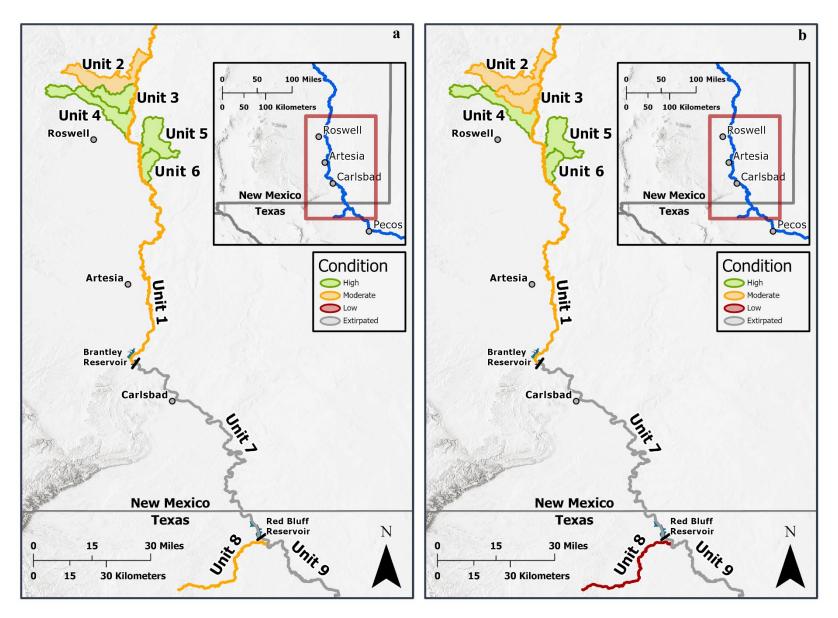


Figure 37. Analysis Unit Overview of Scenario 3 (Warm and Dry) Future Condition. Time Step 2050 (a) and Time Step 2100 (b).

5.3.4 Sheepshead Minnow

Along with the three scenarios described above we also considered the risk of sheepshead minnow introgression into the different analysis units (Table 14). Because sheepshead minnow are often used as bait fish, we think the most likely path for the sheepshead minnow to move into units existing with non-introgressed Pecos pupfish populations is through a bait bucket transfer into the Pecos River upstream of Brantley Reservoir. Based on data collected from the lower Pecos River, this scenario would be highly likely to result in the introgression of the entire population of Pecos pupfish in the Pecos River (Analysis Unit 1, Upper Pecos River) (Whiteley 2023, p. 2). Bait bucket transfers are highly unlikely to occur in any of the other analysis units, as these units are generally either well controlled or do not contain game fish species. As such, the most likely route for sheepshead minnow introgression into other analysis units would be natural movement of sheepshead minnow from the Upper Pecos River Analysis Unit if they gained access there. The analysis units most at risk of sheepshead minnow introgression from the upper Pecos River are the Salt Creek Wilderness and Middle Tract Wetlands, which are both connected hydrologically to the Pecos River during flooding events allowing for potential movement of sheepshead minnow into these off-channel habitats. In the case of the Salt Creek Wilderness, only Salt Creek (NM) itself is vulnerable to sheepshead minnow invasion, as the isolated sink holes in that analysis unit are not likely to be inundated during Pecos River flooding events. Because of the managed nature of the Middle Tract Wetlands by the Bitter Lake NWR staff and the existence of numerous water control structures that can reduce opportunities for fish movement, the vulnerability within the unit decreases with distance from the Pecos River. Managed water flows, manmade barriers, and direct human intervention would likely be employed to manage the spread of sheepshead minnow throughout the unit if the species were to gain access to the Upper Pecos River. The lower portion of Salt Creek (TX) is already introgressed with sheepshead minnow, however, despite that there is no clear barrier preventing upstream movement, upstream portions of the stream have maintained non-introgressed pupfish. Because we cannot identify a barrier, we assume the risk of introgression remains high. The remaining three units adjacent to the Pecos River (Salt Creek Wilderness, Bitter Creek Drainage, and Bottomless Lakes State Park) have either manmade or natural barriers that would prevent or minimize the chance of the spread of sheepshead minnow from the Pecos River into these units resulting in low introgression risk.

Table 14. Risk of sheepshead minnow introgression within each analysis unit out to year 2100 assuming expansion upstream of Brantley Reservoir. If sheepshead minnow invade a site with Pecos pupfish, we assume the pupfish will be lost from that site due to introgression with sheepshead minnow.

Unit	Genetics
Unit 1: Upper Pecos River (above Brantley Reservoir)	Extirpated: Based on prior introductions, without barriers sheepshead minnow are assumed to eventually spread through all the accessible portions of the habitat and hybridize with extant Pecos pupfish populations.
Unit 2: Salt Creek Wilderness	Low risk of introgression into Salt Creek (NM) during extremely high flow events on the Pecos River. The sinkholes are expected to be well protected from introgression due to their isolation.
Unit 3: Bitter Creek Drainage	Low risk of introgression due to isolated sinkholes are well protected from sheepshead minnow introgression.
Unit 4: Bitter Lake NWR Middle Tract Wetlands	High risk of introgression due to isolated sinkholes are well protected from sheepshead minnow introgression. High flow events such as the one that occurred in 2022 have the potential to introduce sheepshead minnow to the managed wetlands. There are fish barriers to prevent passage during normal flow, but these are overtopped in high flow events.
Unit 5: Bottomless Lakes State Park	Low risk of introgression due isolated sinkholes and wetlands are well protected from sheepshead minnow introgression.
Unit 6: BLM Overflow Wetlands and Lea Lake	Moderate risk of introgression due to maintenance of constructed fish barriers are expected to be effective and continue to prevent sheepshead minnow movement into the Overflow Wetlands under most conditions. However, because sheepshead minnow would be closer to the population, there is an increased risk to this area should there be a severe flood that overtopped or washed out the fish barriers.
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated: If not already extirpated (2023), sheepshead minnow are expected to spread through the entirety of the Middle Pecos River.
Unit 8: Salt Creek (TX)	High risk of introgression because downstream section of the unit has already been invaded (closest to Pecos River). We expect upstream portions to eventually experience introgression as well.
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated: Confirmed introgression already occurred and sheepshead minnow have replaced Pecos pupfish.

5.3 Assessment of Future Viability

We considered what the Pecos pupfish needs to maintain viability and characterized the status of the species in terms of its resiliency, redundancy, and representation. For the purpose of this assessment, we define viability as the ability of the species to sustain populations in natural ecosystems within a biologically meaningful timeframe: in this case, out to 2100. We chose 2100 because we have information to reasonably project the potential significant effects of stressors within the range of the Pecos pupfish within this timeframe. Based on the Pecos pupfish life history and habitat needs, and in consultation with the species' experts, we identified the potential stressors (negative influences), and the contributing sources of those stressors, that are likely to affect the species' future viability. We then evaluated how these stressors would interact with current stressors, and how, and to what extent they would affect the species in the future. Based on the available information, we believe the two largest influences on the future viability of the Pecos pupfish are the potential of introgression with sheepshead minnow and climate change-driven impacts to water quantity, water quality, and loss of habitat diversity. While water pollution and human development (particularly agricultural and oil and gas development) activities have likely influenced the species current condition and may affect some areas (Salt Creek, TX) in the future, we found that the changing climate and the related effects to water availability to sustain habitats has, and will continue to have, the greatest influence on the status of the Pecos pupfish. Sheepshead minnow introduction, while much less predictable, does have the potential to threaten Pecos pupfish populations above Brantley Dam should an introduction occur.

5.3.1 Analysis Unit Summary of Future Condition

Our assessment of best available information indicates that currently two of the nine known Pecos pupfish populations have been extirpated, and three others are in moderate condition (*Table 15*). The majority of known occupied Pecos pupfish sites are within the five units around Bitter Lake NWR and Bottomless Lakes State Park in New Mexico (Analysis Units 2-6). Within these units, four were found to be in high condition and one in moderate condition, indicating that multiple areas across the species core range have high resiliency. The small Salt Creek analysis unit in Texas is currently in moderate condition. This unit is disconnected from the remainder of the range in New Mexico, providing some redundancy in maintaining a relatively large geographic range. The two large units of the Lower and Middle Pecos River have been previously extirpated due to the introgression of the sheepshead minnow. Loss of these parts of the range represent a significant reduction in the overall range and redundancy for the species and loss of a large segment of the riverine habitats historically available to the species. The riverine habitats continue to be represented by the Upper Pecos River Unit.

Analysis	Current	2050	2050	2050	2100	2100	2100
Condition	Current	Scenario 1	Scenario 2	Scenario 3	Scenario 1	Scenario 2	Scenario 3
High	4	3	3	4	1	2	3
Moderate	3	3	3	3	4	3	3
Low	0	1	1	0	1	1	1
Extirpated	2	2	2	2	3	3	2

Table 15. Summary of the current and future projected conditions of Pecos pupfish. Data is summarized across the 9 analysis units under three future scenarios at time steps 2050 and 2100.

Under all three plausible future scenarios described in Section 5.1 species condition would be reduced by 2100 (Table 15). In the hottest and driest scenario (Scenario 1), shallow streams are likely to be lost leading to the extirpation of Pecos pupfish in Salt Creek (TX) and a reduction in redundancy and representation in the Salt Creek Wilderness and Bitter Creek Drainage units. Deeper sinkholes and wetlands are more stable and are expected to maintain suitable conditions for the Pecos pupfish under all scenarios. However, units such as Bitter Creek Drainage, Middle Tract, and BLM Overflow Wetlands are more vulnerable to losses in redundancy in Scenario 1 due to susceptibility to habitat losses from future drying climatic conditions. The Pecos River may also be unable to sustain year-round flows under conditions predicted in Scenario 1. The increased stream flows from projected increase in monsoons in Scenario 2 help maintain sinkhole habitats throughout the range of the Pecos pupfish, and to a lesser extent, likely may buffer wetland habitats from the most severe impacts of increased temperatures. However, small streams are likely still at elevated risk of being lost or experiencing long-term drying or mortality events. Finally, in the mildest future climate scenario (Scenario 3), further effects to most habitat (wetlands, sinkholes, and riverine) are anticipated to be minimal. However, like the other two scenarios, shallow streams likely will experience drying and mortality events.

Under all three scenarios, we anticipate some reductions to resilience, redundance, and representation. Although some additional changes to Pecos pupfish status are projected to occur by 2050, we anticipate that measurable changes to viability will be more apparent by 2100. This is largely due to the resilience of the aquifer and the adaptability of the Pecos pupfish to withstand variable habitat conditions. Under all scenarios, at least one analysis unit remains in high condition. Under both Scenarios 1 and 2 Pecos pupfish are projected to be extirpated from Salt Creek (TX) eliminating the only population outside of New Mexico that has been described as genetically different from the core populations in New Mexico. Pecos pupfish experience most losses of known occupied sites under Scenario 1, though losses would be likely to occur under scenarios 2 and 3 as well. Bottomless Lakes State Park remains the only analysis unit that would be in high condition under all 3 scenarios.

Concurrent with the effects of climate change is the risk of expansion of sheepshead minnow and subsequent hybridization with Pecos pupfish. Salt Creek (TX) is already at high risk of loss due to sheepshead minnow introgression. The Upper Pecos River is currently highly vulnerable to sheepshead minnow introduction via a bait bucket transfer. Should this introduction occur, non-

introgressed Pecos pupfish would likely be extirpated from this unit, and, as a consequence there would be no remaining Pecos pupfish in the Pecos River. This would also increase the potential for sheepshead minnow invasion into portions of the Salt Creek Wilderness, the Middle Tract Wetlands, and possibly the Overflow Wetlands units.

The Pecos Pupfish Conservation Agreement will continue to provide guidance for agencies and partners working toward Pecos pupfish conservation through several means. First, the monitoring outlined in the Agreement will provide a long-term data set on the persistence of Pecos pupfish and, as methods have been refined, population trends within four analysis units (Bitter Creek Drainage and Bitter Lake NWR Middle Tract Wetlands, BLM Overflow Wetlands and Lea Lake, and Bottomless Lakes State Park). This monitoring will allow partners to detect potential sheepshead minnow introgression and allow for the detection of long-term declines or extirpations of Pecos pupfish. Secondly, the agreement will help provide for ongoing maintenance (or potentially additional) barriers to fish passage that may protect some of the analysis units from sheepshead minnow introgression should a bait bucket transfer into the Upper Pecos River occur. Finally, the agreement can reduce the opportunity for further invasions by a collaborative effort of state and Federal entities to enforce existing baitfish regulations.

5.3.2 Future Condition Summary of 3Rs

Based on our future projections of environmental changes and Pecos pupfish response we anticipate the following conditions under the three scenarios at 2100.

Under the hottest and driest scenario (Scenario 1 – Hot and Dry [RCP 8.5]) we anticipate that the Pecos pupfish will experience *reduced resiliency* across its range. Many of the analysis units are projected to have reduced water quantity and quality conditions that would significantly impacting the Pecos pupfish population. Where Pecos pupfish remain extant, these water conditions may reduce the survival and fecundity of individual populations because of a reduction in available habitat or an increase in salinity and water temperature. These extant populations are also at increased risk from stochastic and catastrophic events such as golden algae blooms. We anticipate that known occupied sites would be lost in several units and that Pecos pupfish will be completely extirpated from Salt Creek (TX). Losses of extant sites would *decrease redundancy* both within units and across the species range, leading to an increased risk of extirpation from stochastic events or declining water conditions. This is particularly acute in units such as Salt Creek (NM) where redundancy is already low. Finally, the loss of Salt Creek (TX) would *decrease representation* by eliminating a genetically unique population of Pecos pupfish. Further losses of representation may occur as Pecos pupfish populations in all stream habitats will be extirpated and populations of Pecos pupfish in wetland habitat will likely be reduced.

At 2100 in Scenario 2 (Hot and Wet [RCP 8.5]) Pecos pupfish will also experience *reduced resiliency* across its range though not to the extent as seen in Scenario 1. This is largely due to the increased monsoon precipitation that helps maintain spring flows that support sinkhole habitats and may offset the increased evapotranspiration occurring wetland habitats. Increased temperatures across the Pecos Basin would likely result in impairments to water quality in most

aquatic environments that could result in losses of individual known occupied sites. Consequently, we anticipate a *slight decrease in redundancy* primarily due to the loss of shallow stream environments (Salt Creek [TX], Salt Creek [NM], and Bitter Creek). The loss of stream environments would likely result in the extirpation of the genetically unique Pecos pupfish population in Salt Creek (TX) and a subsequent *decrease in representation* across the species range.

Finally in the scenario with the most modest projected climate changes (Scenario 3 – Warm and Dry [RCP4.5]) we project a *slight decrease in resiliency* across the species range. Although, surface temperatures are projected to increase and precipitation is projected to decrease, we anticipate that these changes will not be substantial enough to cause the loss of known occupied sites. Therefore, we projected *no change to either redundancy or representation* of the Pecos pupfish under this scenario.

Literature Cited

- Allendorf, Fred W., Robb F. Leary, Paul Spruell, and John K. Wenburg. 2001. "The Problems with Hybrids: Setting Conservation Guidelines." *Trends in Ecology & Evolution* 16 (11): 613–622. https://doi.org/10.1016/S0169-5347(01)02290-X.
- Ashworth, John B. 1990. "Evaluation of Ground-Water Resources in Parts of Loving, Pecos, Reeves, Ward, and Winkler Counties, Texas."
- Barkoh, Aaron, and Loraine T. Fries. 2010. "Aspects of The Origins, Ecology, And Control Of Golden Alga Prymnesium Parvum: Introduction To The Featured Collection." Journal of the American Water Resources Association 46 (1): 1–5. https://doi.org/10.1111/j.1752-1688.2009.00394.x.
- Bennett, Wayne A., and Thomas L. Beitinger. 1997. "Temperature Tolerance of the Sheepshead Minnow, *Cyprinodon variegatus*." *Copeia* 1997 (1): 77. https://doi.org/10.2307/1447842.
- Blann, Kristen, Eloise Kendy, Dale Turner, Dannielle Galloway, Ryan Gronewold, Aubrey Harris, John Hickey, et al. 2022. "Identifying Environmental Flow Requirements for the Pecos River: Background Literature Review and Summary." Background Literature Review and Summary. Accessed from https://www.hec.usace.army.mil/sustainablerivers/publications/docs/Pecos%20-%20Identifying%20environmental%20flow%20requirements.pdf.
- Blue Earth Ecological Consultants, Inc. (BEEC). 2010. "Monitoring Plan for Pecos Pupfish (*Cyprinodon pecosensis*)." New Mexico Department of Game and Fish.
- Boghici, Radu, and Norman G Van Broekhoven. 2001. "Hydrogeology of the Rustler Aquifer, Trans-Pecos Texas." 356. Aquifers of West Texas, Texas Water Development Board Report.
- Bonetti, Pietro, Christian Leuz, and Giovanna Michelon. 2021. "Large-Sample Evidence on the Impact of Unconventional Oil and Gas Development on Surface Waters." *Science* 373 (6557): 896–902. https://doi.org/10.1126/science.aaz2185.
- Brandenburg, W. Howard, and Michael Farrington. 2003. "Population Monitoring of Pecos Pupfish in the Bureau of Land Management's Overflow Wetlands Wildlife Habitat Area, New Mexico." American Southwest Ichthyological Research Foundation.

- Brooks, James. 1992. "Appendix Extant Pecos Pupfish Locality Descriptions. Rio Grande Fishes Recovery Team."
- Brown, James H., and C. Robert Feldmeth. 1971. "Evolution in Constant and Fluctuating Environments: Thermal Tolerances of Desert Pupfish (*Cyprinodon pecosensis*)." *Evolution* 25: 390–398.
- Caldwell, John. 2014. "Pecos Pupfish Monitoring Report 2014." Monitoring. Santa Fe, NM: New Mexico Department of Game and Fish.
- Cantu De Leija, Antonio. 2021. "Effects of Wetland Management and Associated Abiotic Factors on Rare Plant Communities of Spring-Fed Arid Wetlands." Master of Science, Louisiana State University and Agricultural and Mechanical College. https://doi.org/10.31390/gradschool_theses.5407.
- Cheng, Linyin, Martin Hoerling, Zhiyong Liu, and Jon Eischeid. 2019. "Physical Understanding of Human-Induced Changes in U.S. Hot Droughts Using Equilibrium Climate Simulations." *Journal of Climate* 32 (14): 4431–4443. https://doi.org/10.1175/JCLI-D-18-0611.1.
- Childs, Michael R., Anthony A. Echelle, and Thomas E. Dowling. 1996. "Development of the Hybrid Swarm between Pecos Pupfish (Cyprinodontidae: *Cyprinodon pecosensis*) and Sheepshead Minnow (*C. veriegatus*): A Perspective from Allozymes and MtDNA." *Evolution* 50 (5): 2014–2022. https://doi.org/10.1111/j.1558-5646.1996.tb03588.x.
- Cokendolpher, James C. 1980. "Hybridization Experiments with the Genus Cyprinodon (Teleostei: Cyprinodontidae)." *Copeia* 1980 (1): 173. https://doi.org/10.2307/1444160.
- Collyer, Michael L., Megan E. Hall, Melissa D. Smith, and Christopher W. Hoagstrom. 2015. "Habitat-Morphotype Associations of Pecos Pupfish (*Cyprinodon Pecosensis*) in Isolated Habitat Complexes." *Copeia* 103 (1): 181–199. https://doi.org/10.1643/OT-14-084.
- Connor, Patrick James. 1987. "A Genetic Analysis of a Hybrid Swarm between Two Pupfishes, *Cyprinodon pecosensis* and *C. variegatus* (Cyprinodontidae): Geographical Pattern." Stillwater, Oklahoma: Oklahoma State University.
- Consensus Planning, Inc. 2016. "Chaves County Comprehensive Plan."
- Davenport, Stephen. 2023a. Call with S. DavenportPhone call.
- . 2023b. "Pecos Pupfish Data and Quick Narrative," March 10, 2023.
- Davis, Jack R. 1980. "Rediscovery, Distribution, and Populational Status of (*Cyprinodon eximius*) (Cyprinodontidae) in Devil's River, Texas." *The Southwestern Naturalist* 25 (1): 81–88. https://doi.org/10.2307/3671213.
- ———. 1981. "Diet of the Pecos River Pupfish, *Cyprinodon pecosensis* (Cyprinodontidae)." *The Southwestern Naturalist* 25 (4): 535. https://doi.org/10.2307/3670854.
- Delaune, Kelbi. 2020. "Aquatic Biodiversity in the Pecos River: Investigating Threats, Resources, and New Monitoring Methods." Dissertation, Lubbock, Texas: Texas Tech University.
- Delaune, Kelbi, Matthew A. Barnes, and Allison Pease. 2017. "EDNA Detection of Species of Greatest Conservation Need in the Lower Pecos River System." Lubbock, Texas: Texas Tech University.
- Doege, Robyn. 2023. "Pecos Pupfish Longevity in Captivity," October 13, 2023.
- Dunbar, N.W., D.S. Gutzler, K.S. Pearthree, F.M. Phillips, P.W. Bauer, C.D. Allen, D. DuBois, et al. 2022. *Climate Change in New Mexico Over the Next 50 Years: Impacts on Water Resources*. Bulletin 164. New Mexico Bureau of Geology and Mineral Resources. https://doi.org/10.58799/B-164.

- East, Jessica L., Christopher Wilcut, and Allison A. Pease. 2017. "Aquatic Food-web Structure along a Salinized Dryland River." *Freshwater Biology* 62: 681–694.
- Echelle, Alice F., and Anthony A. Echelle. 2007. "Genetic Status of Pecos Pupfish Populations in New Mexico." New Mexico Department of Game and Fish, Santa Fe, New Mexico U. S. Bureau of Land Management, Las Cruces, New Mexico.
- Echelle, Alice F., Anthony A. Echelle, and William L. Fisher. 2003. "Genetic Status of *Cyprinodon pecosensis* in Texas." Final Report. Austin, Texas: U.S. Fish and Wildlife Service.
- Echelle, Anthony A., Evan W. Carson, Alice F. Echelle, R. A. Van Den Bussche, Thomas E. Dowling, and Axel Meyer. 2005. "Historical Biogeography of the New-World Pupfish Genus Cyprinodon (Teleostei: Cyprinodontidae)." *Copeia* 2005 (2): 320–339.
- Echelle, Anthony A., and Patrick J. Connor. 1989. "Rapid, Geographically Extensive Genetic Introgression After Secondary Contact Between Two Pupfish Species (Cyprinodon, Cyprinodontidae)." *Evolution* 43 (4): 717–727. https://doi.org/10.1111/j.1558-5646.1989.tb05171.x.
- Echelle, Anthony A., and Alice F. Echelle. 1978. "The Pecos River Pupfish, *Cyprinodon pecosensis* n. Sp. (Cyprinodontidae), with Comments on Its Evolutionary Origin." *Copeia* 1978 (4): 569. https://doi.org/10.2307/1443683.
- Echelle, Anthony A., Alice F. Echelle, Salvador Contreras-Balderas, and Ma. de Lourdes Lozana Vilano. 2003. "Pupfishes of the Northern Chihuahuan Desert: Status and Conservation. Aquatic Fauna of the Northern Chihuahuan Desert." *Museum of Texas Tech University, Special Publications* 46: 113–126.
- Echelle, Anthony A., Alice F. Echelle, and Loren G. Hill. 1972. "Interspecific Interactions and Limiting Factors of Abundance and Distribution in the Red River Pupfish, *Cyprinodon rubrofluviatilis*." *American Midland Naturalist* 88 (1): 109. https://doi.org/10.2307/2424492.
- Echelle, Anthony A., Christopher W. Hoagstrom, Alice F. Echelle, and James E. Brooks. 1997.
 "Expanded Occurrence of Genetically Introgressed Pupfish (Cyprinodontidae: Cyprinodon pecosensis X variegatus in New Mexico." The Southwestern Naturalist 42 (3): 336–339.
- Fagan, William F. 2002. "Connectivity, Fragmentation, and Extinction Risk in Dendritic Metapopulations." *Ecology* 83 (12): 3243–3249. https://doi.org/10.2307/3072074.
- Farrington, Michael A, and W Howard Brandenburg. 2003. "Life History of Pecos Pupfish (*Cyprinodon pecosensis*) in Bitter Lake National Wildlife Refuge, New Mexico." New Mexico Department of Game and Fish Conservation Services Division.
- Farrington, Michael A, W. Howard Brandenburg, and Steven P. Platania. 2010. "Population Monitoring of Pecos Pupfish, *Cyprinodon pecosensis*, in the Bureau of Land Management's Overflow Wetlands Wildlife Habitat Area, New Mexico 2007-2008."
 Final Report. Pecos Las Cruces District, Las Cruces, NM: U.S. Bureau of Land Management.
- Follansbee, Robert, and H J Dean. 1915. "WATER RESOURCES OF THE RIO GRANDE BASIN 1888-1913."

- Follansbee, Robert, H J Dean, William W. Follett, and Glenn A. Gray. 1915. *Water Resources of the Rio Grande Basin, 1888-1913*. Washington: Government Printing Office. Accessed from https://www.loc.gov/item/gs15000446/.
- Garfin, Gregg, Angela Jardine, Robert Merideth, Mary Black, and Sarah LeRoy, eds. 2013. Assessment of Climate Change in the Southwest United States: A Report Prepared for the National Climate Assessment. Washington, DC: Island Press/Center for Resource Economics. https://doi.org/10.5822/978-1-61091-484-0.
- Garrett, Gary P. 1982. "Variation in the Reproductive Traits of the Pecos Pupfish, *Cyprinodon pecosensis.*" *The American Midland Naturalist* 108 (2): 355–363. https://doi.org/10.2307/2425496.
- Garrett, Gary P., Clark Hubbs, and Robert J. Edwards. 2002. "Threatened Fishes of the World: Cyprinodon Pecosensis Echelle & Echelle, 1978 (Cyprinodontidae)." *Environmental Biology of Fishes* 65 (3): 366–366. https://doi.org/10.1023/A:1020521230163.
- George, Peter G., Robert E. Mace, and Rima Petrossian. 2011. "Aquifers of Texas." 380. Texas Water Development Board.
- Global Biodiversity Information Facility (GBIF). 2022. "Occurrence Download." GBIF.org. Accessed from https://doi.org/10.15468/dl.n3p937.
- Harrington, E R. 2021. "Sinkholes, Bottomless Lakes, and the Pecos River."
- Hatch, Michael D., William H. Baltosser, and C. Gregory Schmitt. 1985. "Life History and Ecology of the Bluntnose Shiner (*Notropis simus pecosensis*) in the Pecos River of New Mexico." *The Southwestern Naturalist* 30 (4): 555–562. https://doi.org/10.2307/3671049.
- Hatt, Joanna. 2019. "Pecos Pupfish Monitoring Report 2019." Fisheries Management Division, Santa Fe, New Mexico: New Mexico Department of Game and Fish.
- ———. 2021. "Pecos Pupfish Monitoring Report 2021." Fisheries Management Division, Santa Fe, New Mexico: New Mexico Department of Game and Fish.
 - —. 2022. "Pecos Pupfish Monitoring Report 2022." Monitoring. Santa Fe, NM: New Mexico Department of Game and Fish.
- Havenor, Kay C. 1968. Structure, Stratigraphy, and Hydrogeology of the Northern Roswell Artesian Basin, Chaves County, New Mexico. Circular 93. Socorro, NM: New Mexico Bureau of Geology and Mineral Resources. https://doi.org/10.58799/C-93.
- Hayhoe, Katharine, Donald J. Wuebbles, David R. Easterling, David W. Fahey, Sarah Doherty, James P. Kossin, William V. Sweet, Russell S. Vose, and Michael F. Wehner. 2018.
 "Chapter 2 : Our Changing Climate. Impacts, Risks, and Adaptation in the United States: The Fourth National Climate Assessment, Volume II." U.S. Global Change Research Program. https://doi.org/10.7930/NCA4.2018.CH2.
- Hoagstrom, Christopher W. 2003. "Historical and Recent Fish Fauna of Lower Pecos River." In Aquatic Fauna of the Northern Chihuahuan Desert, 46:91–110. Sul Ross University, Alpine, Texas: Museum of Texas Tech University.
- Hoagstrom, Christopher W. 2009. "Causes and Impacts of Salinization in the Lower Pecos River" 19 (1).
- Hoagstrom, Christopher W., and James E. Brooks. 1999a. "Distribution, Status, and Conservation of the Pecos Pupfish, *Cyprinodon pecosensis*." Technical Report No. 2. Santa Fe, NM: New Mexico Department of Game and Fish.
 - ———. 1999b. "Distribution, Status, and Conservation of the Pecos Pupfish, *Cyprinodon pecosensis*." 2. Santa Fe, NM: New Mexico Department of Game and Fish.

- Hoagstrom, Christopher W., James E. Brooks, and Stephen R. Davenport. 2008a.
 "Spatiotemporal Population Trends of *Notropis simus pecosensis* in Relation to Habitat Conditions and the Annual Flow Regime of the Pecos River, 1992–2005." *Copeia* 2008 (1): 5–15. https://doi.org/10.1643/CE-07-002.
- 2008b. "Spatiotemporal Population Trends of *Notropis simus pecosensis* in Relation to Habitat Conditions and the Annual Flow Regime of the Pecos River, 1992–2005." *Copeia* 2008 (1): 5–15. https://doi.org/10.1643/CE-07-002.
- Hoagstrom, Christopher W, and Megan J Osborne. 2021. "Biogeography of Cyprinodon Across the Great Plains-Chihuahuan Desert Region and Adjacent Areas." In *Desert Fishes Council Special Publications*.
- Hoagstrom, C.W., C.A Caldwell, D.L. Peterson, and J. Dick. 2015. "Wetland Habitat Associations of Imperiled Pecos Pupfish *Cyprinodon pecosensis* in a Brackish Ciénega," 36.
- Houston, N, J Thomas, P Ging, A Teeple, D Pedraza, and D Wallace. 2019. "Pecos River Basin Salinity Assessment, Santa Rosa Lake, New Mexico, to the Confluence of the Pecos River and the Rio Grande, Texas, 2015." U.S. Geological Survey Scientific Investigations Report 2019–5071. Reston, Virginia: U.S. Geological Survey. Accessed from https://pubs.usgs.gov/sir/2019/5071/sir20195071.pdf.
- Israël, Natascha M.D., Matthew M. VanLandeghem, Shawn Denny, John Ingle, and Reynaldo Patiño. 2014. "Golden Alga Presence and Abundance Are Inversely Related to Salinity in a High-Salinity River Ecosystem, Pecos River, USA." *Harmful Algae* 39 (October): 81– 91. https://doi.org/10.1016/j.hal.2014.06.012.
- ITIS. 2023. "(*Cyprinodon pecosensis*) Echelle and Eschelle, 1978." Integrated Taxonomic Information System. Accessed from https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=165 639&print version=PRT&source=to print#null.
- Jacobsen, Carl. 2023. "Pupfish on Salt Creek," 2023.
- Kennedy, Stephen E. 1977. "Life History of the Leon Springs Pupfish, *Cyprinodon bovinus*." *Copeia* 1977 (1): 93. https://doi.org/10.2307/1443509.
- Kloesel, Kevin, Bill Bartush, Jay Banner, David Brown, Jay Lemery, Xiaomao Lin, Cindy Loeffler, et al. 2018. "Chapter 23 : Southern Great Plains. Impacts, Risks, and Adaptation in the United States: The Fourth National Climate Assessment, Volume II." U.S. Global Change Research Program. https://doi.org/10.7930/NCA4.2018.CH23.
- Kodric-Brown, Astrid. 1975. "Breeding Territories in Two Freshwater Fishes of the Genus *Cyprinodon* (Pisces, Cyprinodontidae) in the Southwestern United States." Dissertation, Los Angeles, California: University of Southern California.
- ------. 1977. "Reproductive Success and the Evolution of Breeding Territories in Pupfish (Cyprinodon)." *Evolution* 31 (4): 750–766. https://doi.org/10.2307/2407437.
- ———. 1983. "Determinants of Male Reproductive Success in Pupfish (*Cyprinodon pecosensis*)." Animal Behaviour 31 (1): 128–137. https://doi.org/10.1016/S0003-3472(83)80180-8.
- ——. 1986. "Satellites and Sneakers: Opportunistic Male Breeding Tactics in Pupfish (*Cyprinodon pecosensis*)." *Behavioral Ecology and Sociobiology* 19 (6): 425–432.
- Kodric-Brown, Astrid, and Patricia Mazzolini. 1992. "The Breeding System of Pupfish, *Cyprinodon pecosensis*: Effects of Density and Interspecific Interactions with the

Killifish, Fundulus Zebrinus." *Environmental Biology of Fishes* 35 (2): 169–176. https://doi.org/10.1007/BF00002191.

- Kodric-Brown, Astrid, and Jonathan A. Rosenfield. 2004. "Populations of Pecos Pupfish (*Cyprinodon pecosensis*) Differ in Their Susceptibility to Hybridization with Sheepshead Minnow (*C. variegatus*)." *Behavioral Ecology and Sociobiology* 56 (2): 116–123.
- Kreager, T R. 2003. "Overflow Wetlands Area of Critical Environmental Concern Activity Plan." Roswell, New Mexico: Roswell Field Office, Bureau of Land Management.
- LaFave, John I. 1987. "Groundwater Flow Delineation in the Toyah Basin of Trans-Pecos, Texas." Accessed from http://hdl.handle.net/2152/46536.
- Land, Lewis. 2003. "Evaporite Karst and Regional Ground-Water Circulation in the Lower Pecos Valley of Southeastern New Mexico." *Oklahoma Geological Survey Circular* 109: 227–232.
- Land, Lewis, and G. Huff. 2009. "Multi-Tracer Investigation of Groundwater Residence Time in a Karstic Aquifer: Bitter Lakes National Wildlife Refuge, New Mexico, USA." *Hydrogeology Journal* 18 (December): 455–472. https://doi.org/10.1007/s10040-009-0522-3.
- Land, Lewis, and Brad T. Newton. 2008a. "Seasonal and Long-Term Variations in Hydraulic Head in a Karstic Aquifer: Roswell Artesian Basin, New Mexico1." JAWRA Journal of the American Water Resources Association 44 (1): 175–191. https://doi.org/10.1111/j.1752-1688.2007.00146.x.
 - 2008b. "Seasonal and Long-Term Variations in Hydraulic Head in a Karstic Aquifer: Roswell Artesian Basin, New Mexico1." *JAWRA Journal of the American Water Resources Association* 44 (1): 175–191. https://doi.org/10.1111/j.1752-1688.2007.00146.x.
- Letcher, Benjamin H., Keith H. Nislow, Jason A. Coombs, Matthew J. O'Donnell, and Todd L. Dubreuil. 2007. "Population Response to Habitat Fragmentation in a Stream-Dwelling Brook Trout Population." *PLOS ONE* 2 (11): e1139. https://doi.org/10.1371/journal.pone.0001139.
- Linam, Gordon W., and Leroy J. Kleinsasser. 1996. "Relationship between Fishes and Water Quality in the Pecos River, Texas." River Studies 9. Texas Parks and Wildlife Department.
- Llewellyn, Dagmar, Lucas Barrett, Emma Kelly, Deena Larsen, Ashlee Rudolph, Dan Davis, Alec Norman, and Hannah Riseley-White. 2021. "Pecos River Basin Study - New Mexico." Bureau of Reclamation.
- Matthews, William J., and Earl G. Zimmerman. 1990. "Potential Effects of Global Warming on Native Fishes of the Southern Great Plains and the Southwest." *Fisheries* 15 (6): 26–32. https://doi.org/10.1577/1548-8446(1990)015<0026:PEOGWO>2.0.CO;2.
- Maunder, Mark N., and André E. Punt. 2004. "Standardizing Catch and Effort Data: A Review of Recent Approaches." *Fisheries Research*, Models in Fisheries Research: GLMs, GAMS and GLMMs, 70 (2): 141–159. https://doi.org/10.1016/j.fishres.2004.08.002.
- Montagne, Michael. 2023. "2023 Trip Report.Doc."
- Natural Heritage New Mexico (NHNM). 2021. "Pecos Pupfish NMBiotics Database." Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM.
- Nico, Leo, and Pam Fuller. 2023. "Sheepshead Minnow (*Cyprinodon variegatus*) Species Profile." USGS Nonindigenous Aquatic Species. 2023. Accessed from https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=662.

- Novinger, Douglas C., and Frank J. Rahel. 2003. "Isolation Management with Artificial Barriers as a Conservation Strategy for Cutthroat Trout in Headwater Streams." *Conservation Biology* 17 (3): 772–781. https://doi.org/10.1046/j.1523-1739.2003.00472.x.
- Page, Lawrence, Héctor Espinosa-Pérez, Lloyd Finley, Carter Gilbert, Robert Lea, Nicholas Mandrak, Richard Mayden, and Joseph Nelson. 2013. "Scientific Name, Occurrence, and Accepted Common Name." In Common and Scientific Names of Fishes from the United States, Canada, and Mexico, 7th ed., 144. The American Fisheries Society.
- Patiño, Reynaldo, Dan Dawson, and Matthew M. VanLandeghem. 2014. "Retrospective Analysis of Associations between Water Quality and Toxic Blooms of Golden Alga (*Prymnesium parvum*) in Texas Reservoirs: Implications for Understanding Dispersal Mechanisms and Impacts of Climate Change." *Harmful Algae* 33 (March): 1–11. https://doi.org/10.1016/j.hal.2013.12.006.
- Pecos Pupfish Conservation Team (Conservation Team). 2022. "Pecos Pupfish Conservation Agreement 2022."
- Pecos Valley Artesian Conservancy District (PVACD). 2023. "Roswell Artesian Basin September 2023 Water Graph." 2023. Accessed from https://www.pvacd.com/images/31758-2023-9%20SEPTEMBER%20WATER%20GRAPH.pdf.
- Peterson, Douglas P., Bruce E. Rieman, Dona L. Horan, and Michael K. Young. 2014. "Patch Size but Not Short-Term Isolation Influences Occurrence of Westslope Cutthroat Trout above Human-Made Barriers." *Ecology of Freshwater Fish* 23 (4): 556–571. https://doi.org/10.1111/eff.12108.
- Propst, David. 1999. "Threatened and Endangered Fishes of New Mexico." Technical Report 1. Santa Fe, NM: New Mexico Department of Game and Fish.
- Redford, Kent H., George Amato, Jonathan Baillie, Pablo Beldomenico, Elizabeth L. Bennett, Nancy Clum, Robert Cook, et al. 2011. "What Does It Mean to Successfully Conserve a (Vertebrate) Species?" *BioScience* 61 (1): 39–48. https://doi.org/10.1525/bio.2011.61.1.9.
- Rhodes, Kevin, and Clark Hubbs. 1992. "Recovery of Pecos River Fishes from a Red Tide Fish Kill." *The Southwestern Naturalist* 37 (2): 178. https://doi.org/10.2307/3671666.
- Rieman, B. E., and F. W. Allendorf. 2001. "Effective Population Size and Genetic Conservation Criteria for Bull Trout." North American Journal of Fisheries Management 21 (4): 756– 764. https://doi.org/10.1577/1548-8675(2001)021<0756:EPSAGC>2.0.CO;2.
- Rosenfield, Jonathan A., Stacy Nolasco, Steven Lindauer, Claudette Sandoval, and Astrid Kodric-Brown. 2004. "The Role of Hybrid Vigor in the Replacement of Pecos Pupfish by Its Hybrids with Sheepshead Minnow: Genetic Homogenization of Endemic Pupfish." *Conservation Biology* 18 (6): 1589–1598. https://doi.org/10.1111/j.1523-1739.2004.00356.x.
- Sabo, John L., and David M. Post. 2008. "Quantifying Periodic, Stochastic, and Catastrophic Environmental Variation." *Ecological Monographs* 78 (1): 19–40. https://doi.org/10.1890/06-1340.1.
- Scanlon, Bridget R., Svetlana Ikonnikova, Qian Yang, and Robert C. Reedy. 2020. "Will Water Issues Constrain Oil and Gas Production in the United States?" *Environmental Science & Technology* 54 (6): 3510–3519. https://doi.org/10.1021/acs.est.9b06390.
- Schoenherr, Allan A. 1981. "The Role of Competition in the Displacement of Native Fishes by Introduced Species." In *Fishes in North American Deserts*, 173–203. Wiley-Interscience.

- Schoenherr, Allan A., and C. Robert Feldmeth. 1992. "Thermal Tolerances for Relict Populations of Desert Pupfish, (*Cyprinodon maculariuss*)." Proceedings of the Desert Fishes Council XXII and XXII: 49–51.
- Shafer, M., D. Ojima, J. M. Antle, D. Kluck, R. A. McPherson, S. Petersen, B. Scanlon, and K. Sherman. 2014. "Great Plains. Climate Change Impacts in the United States: The Third National Climate Assessment." U.S. Global Change Research Program. https://doi.org/10.7930/J0D798BC.
- Shaffer, Mark L, and Bruce A Stein. 2000. "Safeguarding Our Precious Heritage." In Precious Heritage: The Status of Biodiversity in the United States, edited by Bruce Stein, Lynn S Kutner, and Jonathan S Adams, 0. Oxford University Press. https://doi.org/10.1093/oso/9780195125191.003.0017.
- Sheffield, Justin, and Eric F. Wood. 2008. "Projected Changes in Drought Occurrence under Future Global Warming from Multi-Model, Multi-Scenario, IPCC AR4 Simulations." *Climate Dynamics* 31 (1): 79–105. https://doi.org/10.1007/s00382-007-0340-z.
- Sites Southwest. 2008. "Eddy County Comprehensive Plan." Final Plan. Carlsbad, NM: Eddy County. Accessed from https://www.nrc.gov/docs/ML1034/ML103430034.pdf.
- Smith, David R., Nathan L. Allan, Conor P. McGowan, Jennifer A. Szymanski, Susan R. Oetker, and Heather M. Bell. 2018. "Development of a Species Status Assessment Process for Decisions under the U.S. Endangered Species Act." *Journal of Fish and Wildlife Management* 9 (1): 302–320. https://doi.org/10.3996/052017-JFWM-041.
- Stearns, Stephen C. 1989. "The Evolutionary Significance of Phenotypic Plasticity." *BioScience* 39 (7): 436–445. https://doi.org/10.2307/1311135.
- Tear, Timothy H., Peter Kareiva, Paul L. Angermeier, Patrick Comer, Brian Czech, Randy Kautz, Laura Landon, et al. 2005. "How Much Is Enough? The Recurrent Problem of Setting Measurable Objectives in Conservation." *BioScience* 55 (10): 835–849. https://doi.org/10.1641/0006-3568(2005)055[0835:HMIETR]2.0.CO;2.
- Todesco, Marco, Mariana A. Pascual, Gregory L. Owens, Katherine L. Ostevik, Brook T. Moyers, Sariel Hübner, Sylvia M. Heredia, et al. 2016. "Hybridization and Extinction." *Evolutionary Applications* 9 (7): 892–908. https://doi.org/10.1111/eva.12367.
- U.S. Fish and Wildlife Service (Service). 2017. "20171205_Carlsbad Project Water Operations_BiOp_Final (1).Pdf."
- Veni, George. 2013. "Impact Of Climate Change On Human And Ecological Use Of Karst Groundwater Resources: A Case Study From The Southwestern USA." In , 51–60.
- Via, Sara, Richard Gomulkiewicz, Gerdien De Jong, Samuel M. Scheiner, Carl D. Schlichting, and Peter H. Van Tienderen. 1995. "Adaptive Phenotypic Plasticity: Consensus and Controversy." *Trends in Ecology & Evolution* 10 (5): 212–217. https://doi.org/10.1016/S0169-5347(00)89061-8.
- Vose, R.S., D.R. Easterling, K.E. Kunkel, A.N. LeGrande, and M.F. Wehner. 2017. "Climate Science Special Report." In *Climate Science Special Report: Fourth National Climate Assessment*, 1:1–470. U.S. Global Change Research Program, Washington, DC. Accessed from https://science2017.globalchange.gov/chapter/6/.
- Whiteley, Andrew. 2023. "Population Genomics of Pecos Pupfish (*Cyprinodon pecosensis*)." Final Report. New Mexico Department of Game and Fish.
- Wiegand, Thorsten, Eloy Revilla, and Kirk A. Moloney. 2005. "Effects of Habitat Loss and Fragmentation on Population Dynamics." *Conservation Biology* 19 (1): 108–121.

- Xu, Qianna. 2017. "Body Shape Diversification of Pecos Pupfish (*Cyprinodon pecosensis*) on Varying Habitats as Evaluated by Geometric Morphometrics." M.S>, Bowling Green, Kentucky: Western Kentucky University.
- Zymonas, Nikolas D, and David L Propst. 2007. "Ecology of Blue Sucker and Gray Redhorse in the Lower Pecos River, New Mexico 2000-2006." Santa Fe, NM: Conservation Services Division New Mexico Department of Game and Fish.

Location	Analysis Unit	Last Sampled	Current Status
Lake Arthur Falls	1: Upper Pecos River	1995	Presumed Extant
Rio Felix confluence	1: Upper Pecos River	1994	Presumed Extant
Dexter Bridge	1: Upper Pecos River	1996	Presumed Extant
Rio Hondo Confluence	1: Upper Pecos River	1996	Presumed Extant
US 380 Bridge	1: Upper Pecos River	1996	Presumed Extant
Pecos @ Bitter Lake NWR	1: Upper Pecos River	1997	Presumed Extant
US 70 Bridge	1: Upper Pecos River	1997	Presumed Extant
Gasline Crossing	1: Upper Pecos River	1995	Presumed Extant
Salee Ranch	1: Upper Pecos River	1997	Presumed Extant
Sinkhole W15 (Inkpot)	2: Salt Creek Wilderness	1998	Presumed Extant
Salt Creek (NM)	2: Salt Creek Wilderness	2023	Extant
Little Inkpot (W16)	2: Salt Creek Wilderness	1998	Presumed Extant
New Sinkhole	2: Salt Creek Wilderness	2022	Extant
Pren's Hole (W17)	2: Salt Creek Wilderness	2013	Presumed Extirpated
Sinkhole 1	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 7	3: Bitter Creek Drainage	2021	Extant
Sinkhole 9	3: Bitter Creek Drainage	2023	Extant
Sinkhole 10	3: Bitter Creek Drainage	2015	Presumed Extant
Sinkhole 11	3: Bitter Creek Drainage	2022	Extant
Sinkhole 16	3: Bitter Creek Drainage	1998	Presumed Extant
Sinkhole 20	3: Bitter Creek Drainage	2021	Extant
Sinkhole 21	3: Bitter Creek Drainage	2021	Presumed Extirpated
Sinkhole 24	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 25	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 26	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 27	3: Bitter Creek Drainage	1995	Presumed Extant
Sinkhole 28	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 29	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 31	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 32	3: Bitter Creek Drainage	1997	Presumed Extant
Sinkhole 37 (Lake St. Francis)	3: Bitter Creek Drainage	2022	Extant
Sinkhole 38	3: Bitter Creek Drainage	Unknown	Unknown
Sinkhole 39	3: Bitter Creek Drainage	Unknown	Unknown
Dragonfly Spring	3: Bitter Creek Drainage	2022	Extant
Sago Spring	3: Bitter Creek Drainage	2022	Extant
Bitter Creek	3: Bitter Creek Drainage	2023	Extant
Bitter Lake	3: Bitter Creek Drainage	2023	Extant
	0-		

Appendix A. List of Pecos Pupfish Sites

Location	Analysis Unit	Last Sampled	Current Status
Sinkhole 2	3: Bitter Creek Drainage	1996	Presumed Extant
Sinkhole 3	3: Bitter Creek Drainage	1996	Presumed Extant
Hunter Oxbow	4: BLNWR Middle Tract Wetlands	1993	Presumed Extant
Hunter Marsh	4: BLNWR Middle Tract Wetlands	2023	Extant
Unit 5 Oxbow	4: BLNWR Middle Tract Wetlands	2006	Presumed Extant
Unit 3 Oxbow	4: BLNWR Middle Tract Wetlands	2006	Presumed Extant
Unit 3 Spring	4: BLNWR Middle Tract Wetlands	2023	Extant
Unit 3 Lake	4: BLNWR Middle Tract Wetlands	2006	Presumed Extant
Unit 5 Spring	4: BLNWR Middle Tract Wetlands	2021	Extant
Unit 6 Lake	4: BLNWR Middle Tract Wetlands	2006	Presumed Extant
Unit 7 Lake	4: BLNWR Middle Tract Wetlands	2001	Presumed Extant
Unit 15 Spring	4: BLNWR Middle Tract Wetlands	2023	Extant
Unit 16 Lake	4: BLNWR Middle Tract Wetlands	2001	Presumed Extant
Unit 17 Lake	4: BLNWR Middle Tract Wetlands	1987	Presumed Extant
Mirror Lake	5: Bottomless Lakes State Park	2023	Extant
Figure 8 Lake	5: Bottomless Lakes State Park	2023	Extant
Lazy Lagoon	5: Bottomless Lakes State Park	2023	Extant
BLM Overflow Wetlands	6: BLM Overflow Wetlands and Lea Lake	2023	Extant
Lea Lake	6: BLM Overflow Wetlands and Lea Lake	2022	Extant
Lea Lake outflow	6: BLM Overflow Wetlands and Lea Lake	1995	Presumed Extant
Pierce Canyon Crossing	7: Middle Pecos River	1996	Extirpated
Malaga Crossing	7: Middle Pecos River	1995	Extirpated
Salt Creek (TX)	8: Salt Creek (TX)	2023	Extant
Pecos River below Red Bluff Dam	9: Lower Pecos River	1984	Extirpated
Gravel Pit Pond	N/A	1998	Presumed Extirpated
Borrow Pit	N/A	Unknown	Unknown
Laguna Grande Spring 1 (Pupfish Spring)	N/A	1995	Presumed Extirpated
Laguna Grande Spring 2 (Surprise Spring)	N/A	1995	Presumed Extirpated

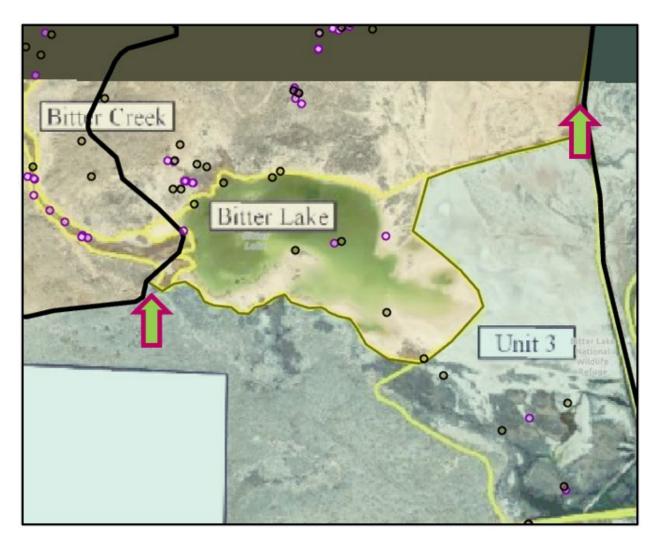
Unit	HUC12s in unit (merge together)	Contains	Methods	Source
2: Salt Creek Wilderness	130600031807	Pren's Hole	We removed everything east of the Pecos River.	 "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas. Pecos River, from "USA National Hydrography Dataset – High Resolution" in ArcGIS Living Atlas.
2: Salt Creek Wilderness	130600050905	Salt Creek (NM), Inkpot, Little Inkpot	We made no changes to this HUC12.	• "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas.
3: Bitter Creek Drainage	130600070402	Bitter Lake, Lake St. Francis, Sinkholes 1-4, 8, 16, 19, 20, 21	We split the HUC12 based on management units identified in Cantu de Leija (2021; see below). We kept the northern part of this HUC12, which includes Bitter Lake, in Unit 3. We placed the southern portion of this HUC12 in Unit 4. Additional information on what we did can be found in NOTE A below.	 "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas. (Cantu De Leija 2021, p. 4, Figure 1.1)
3: Bitter Creek Drainage	130600070404	Sinkhole 5 and 7	We removed everything east of the Pecos River. We split this HUC12 into two using soils data. We kept the northern portion of this HUC12 in Unit 3 and placed the southern portion of this HUC 12 in Unit 4. Additional information on what we did can be found in NOTE B below.	 "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas. Pecos River, from "USA National Hydrography Dataset – High Resolution" in ArcGIS Living Atlas. "USA Soils Map Units" in ArcGIS Living Atlas.
3: Bitter Creek Drainage	130600070401	Bitter Creek, Lost River, Dragonfly Spring	We made no changes to this HUC12.	• "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas.

Appendix B. Delineation of Analysis Units

Unit	HUC12s in unit (merge together)	Contains	Methods	Source
4: BLNWR Middle Tract Wetlands	130600070402	Middle tract wetlands (Units 3, 5, 5, 6, 15,16)	Following the steps above in Unit 3 (HUC12: 130600070402), we included the southern portion of this HUC12 in Unit 4. Additional information on what we did can be found in NOTE A below.	 "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas. (Cantu De Leija 2021, p. 4, Figure 1.1)
4: BLNWR Middle Tract Wetlands	130600070404	Hunter Oxbow and Hunter Marsh, and other oxbows of the Pecos River	We removed everything east of the Pecos River. Following the steps above in Unit 3 (HUC12: 130600070404), we included the southern portion of this HUC12 in Unit 4. Additional information on what we did can be found in NOTE B below.	 "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas. Pecos River, from "USA National Hydrography Dataset – High Resolution" in ArcGIS Living Atlas. "USA Soils Map Units" in ArcGIS Living Atlas.
5: Bottomless Lakes State Park	130600070502	Lazy Lagoon	We made no changes to this HUC12.	• "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas.
5: Bottomless Lakes State Park	130600070504	Mirror Lake and Figure Eight Lake	We removed everything west of the Pecos River. We split this HUC12 into two using wetlands data. We removed palustrine wetlands from this HUC12 and included them in Unit 6. Additional information on what we did can be found in NOTE C below.	 "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas. Pecos River, from "USA National Hydrography Dataset – High Resolution" in ArcGIS Living Atlas. "USA Wetlands" in ArcGIS Living Atlas.
6: BLM Overflow Wetlands and Lea Lake	130600070507	Overflow Wetlands and Lea Lake	We removed everything west of the Pecos River.	 "Watershed Boundary Dataset: HUC12s" in Living Atlas. Pecos River, from "USA National Hydrography Dataset – High Resolution" in ArcGIS Living Atlas.
6: BLM Overflow Wetlands and Lea Lake	130600070504	Small portion of Overflow Wetlands	Following the steps above in Unit 5 (HUC12: X), we included the palustrine wetlands in this HUC12 in Unit 6. Additional information on what we did can be found in NOTE C below.	 "Watershed Boundary Dataset: HUC12s" in ArcGIS Living Atlas. "USA Wetlands" in ArcGIS Living Atlas.

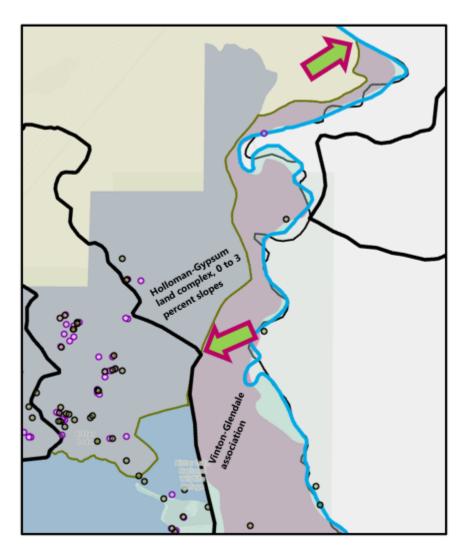
NOTE A: Unit 3 and Unit 4, boundaries based on management units

- 1. We georeferenced Figure 1.1 from Cantu de Leija (2021).
- 2. We created a new line feature of the boundary between "Bitter Lake" and Unit 3".
- 3. We used the new line feature to split this HUC12 (130600070402) into two. We kept the northern portion of this HUC12 in Unit 2, and we place the southern portion of this HUC12 in Unit 4.



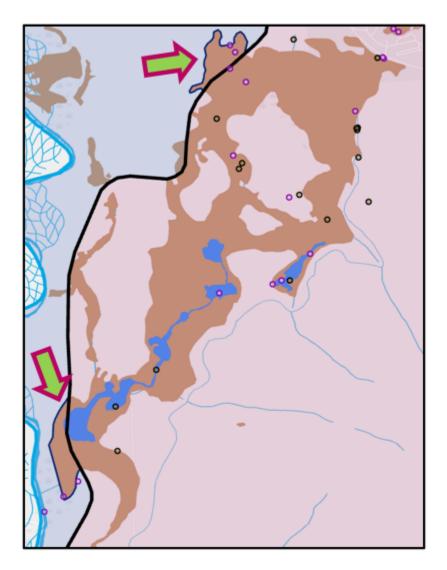
NOTE B: Unit 3 and Unit 4, boundaries based on soil types

- 1. We downloaded "USA Soils Map Units" feature layer in the ArcGIS Living Atlas.
- 2. We zoomed to Bitter Lake NWR Refuge and selected the two Entisol soils found in the northern portion of Bitter Lake NWR. We selected for the following attributes:
 - a) Mapunit Name = Vinton-Glendale association
 - b) Mapunit Name = Holloman-Gypsum land complex, 0 to 3 percent slopes.
- 3. We exported these selected features.
- 4. We created a new line feature of the boundary between the two soil layers that intersect the Pecos River and the existing HUC boundary (130600070404).
- 5. We used the new line feature to split this HUC12 (130600070404) into two. We kept the northern portion of this HUC12 in Unit 2, and we place the southern portion of this HUC12 in Unit 4.



NOTE C: Unit 5 and Unit 6, boundaries based on wetlands data

- 1. We downloaded "USA Wetlands" in the ArcGIS Living Atlas.
- 2. We zoomed into the Overflow Wetlands area. We clipped the HUC12 (130600070504) by the two palustrine wetlands that have locality data. We removed these palustrine wetlands from Unit 5 and included them in Unit 6.



Appendix C. Condition Category Tables

The tables in Appendix C provide more detail on the rationale and analysis underlying our resiliency condition score for each unit.

CURRENT CONDITION

Unit	Genetics	Occurrence	Water Quantity	Water Quality	Habitat Diversity	OVERALL CONDITION
Unit 1: Upper Pecos River (above Brantley Reservoir)	High: No known introgression, but direct connection to Lower Pecos River introgressed population (connectivity mitigated through dams).	Moderate: Detected throughout unit except at Willow Creek and 6 Mile Draw (extant 2021)	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	MODERATE
Unit 2: Salt Creek Wilderness	High: Isolated sinkholes and creek with no documented introgression and no connection to introgressed populations.	High: Extant in unit. Extirpated from Pren's Hole	Moderate: Salt Creek likely experiencing drying event. Sinkholes have perennial water.	Moderate: Documented fish kill (Jan 2023). May be related to water quality.	High: Only sinkhole and ephemeral creek habitats.	MODERATE
Unit 3: Bitter Creek Drainage	High: Isolated sinkholes with no documented introgression and no connection to introgressed populations.	High: All occupied sites not regularly monitored. Extant in monitored sites. No negative reports.	High: Documented low flows in stream habitat Bitter Creek. Sinkholes perennial.	Moderate: Occasional low flow leading to high water temps. Known high salinity in some sinkholes (e.g., Sinkhole 21).	High: Stream and sinkhole habitat.	нідн
Unit 4: BLNWR Middle Tract Wetlands	High: No introgression and no direct connection to introgressed populations.	High: Confirmed extant and no negative reports.	High: Portions of the unit include ephemeral streams and managed wetlands. These may have low flows.	High: No documented water quality or contamination issues.	High: Unit contains a diverse suite of habitats including managed wetlands, streams, seeps, and oxbows.	HIGH
<i>Unit 5:</i> Bottomless Lakes State Park	High: No introgression and no direct connection to introgressed populations.	High: Extant populations throughout most of unit. Extirpated from Upper Figure 8 Lake.	High: Perennial sinkholes	Moderate: Water quality issues in Upper and Lower Figure 8 Lake.	Moderate: Sinkhole habitat only	MODERATE
Unit 6: BLM Overflow Wetlands & Lea Lake	High: No introgression and no direct connection to introgressed populations.	High: Extant at all surveyed sites. No negative surveys.	High: Perennial wetlands	High: No documented water quality or contamination issues.	High: Wetland and sinkhole habitat	HIGH
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Moderate: Documented low flows on the Pecos River	Low: Pecos River exposed to contaminants from oil & gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED
Unit 8: Salt Creek, TX	Low: Introgression in lower section of the unit (closest to Pecos River).	Moderate: Extant in headwaters but extirpated from confluence area (introgression).	Moderate: Low flows documented in Salt Creek	Low: Surrounded by significant oil & gas development with potential for contamination.	Moderate: Stream habitat only	MODERATE
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED

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Scenario 1 – Hot and Dry: 2050

Future Condition - 2050

Unit	Genetics	Occurrence	Water Quantity	Water Quality	Habitat Diversity	OVERALL CONDITION
Unit 1: Upper Pecos River (above Brantley Reservoir)	High: No known introgression, but direct connection to Lower Pecos River introgressed population (connectivity mitigated through dams).	Moderate: Expected loss of some sites due to drying.	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	MODERATE
Unit 2: Salt Creek Wilderness	High: Isolated sinkholes and creek with no documented introgression and no connection to introgressed populations.	Moderate: Loss of Pren's Hole and likely loss of Salt Creek. Sinkholes remain extant.	Moderate: Salt Creek experiences regular drying events.	Moderate: High temps and low DO anticipated in Salt Creek.	Moderate: Sinkhole habitat only	MODERATE
Unit 3: Bitter Creek Drainage	High: Isolated sinkholes with no documented introgression and no connection to introgressed populations.	High: Some drying of Bitter Creek, but fish remain extant in deeper portions. Sinkholes extant.	Moderate: Routine drying of Bitter Creek during dry years (Lost River inlet remains).	Moderate: Occasional low flow leading to high water temps. Known high salinity in some sinkholes (e.g., Sinkhole 21).	High: Stream and sinkhole habitat.	MODERATE
Unit 4: BLNWR Middle Tract Wetlands	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations.	Moderate: Some managed wetland units may experience occasional drying.	Moderate: Occasional low flow leading to high water temps and other impairments.	High: Unit contains a diverse suite of habitats including managed wetlands, streams, seeps, and oxbows.	MODERATE
<i>Unit 5:</i> Bottomless Lakes State Park	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial sinkholes	Moderate: Water quality issues in Upper and Lower Figure 8 Lake.	Moderate: Sinkhole habitat only	MODERATE
Unit 6: BLM Overflow Wetlands & Lea Lake	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial wetlands	High: No documented water quality or contamination issues.	High: Wetland and sinkhole habitat	HIGH
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated	Extirpated	Moderate: Documented low flows on the Pecos River	Low: Pecos River exposed to contaminants from oil & gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED
Unit 8: Salt Creek, TX	Low: Introgression in lower section of the unit (closest to Pecos River).	Low: Potential for loss of population.	Low: Routine drying events anticipated in Salt Creek.	Low: Surrounded by significant oil & gas development with potential for contamination.	Moderate: Stream habitat only	LOW
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED

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Scenario 1 – Hot and Dry: 2100

Future Condition - 2100

Unit	Genetics	Occurrence	Water Quantity	Water Quality	Habitat Diversity	OVERALL CONDITION
Unit 1: Upper Pecos River (above Brantley Reservoir)	Extirpated: Based on prior introductions, without barriers sheepshead minnow are able to eventually spread through all the accessible portions of the habitat and hybridize with extant Pecos pupfish populations.	Moderate: Expected loss of some sites due to drying.	Low: River experiences regular long-term drying events.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities. Low water levels lead to increasing salinity, temperature, and DO impairment.	Moderate: River habitat only	Low
Unit 2: Salt Creek Wilderness	High: Low risk of introgression into Salt Creek during extremely high flow events on the Pecos River. However, given the likely scenario where the Salt Creek population is no longer extant, the sinkholes are likely well protected from introgression.	Low: Expected loss of Salt Creek population and at least one additional sinkhole (along with Pren's Hole).	Moderate: Salt Creek dries up except for wettest years. Sinkholes remain intact, though possibly lower.	Moderate: Documented water quality issues in Salt Creek.	Moderate: Only sinkhole habitat.	MODERATE
Unit 3: Bitter Creek Drainage	High: Isolated sinkholes are well protected from sheepshead minnow introgression.	Moderate: Expected losses of fish in Bitter Creek and several shallower sinkholes and springs (e.g., Dragonfly Spring, Sinkhole 37, 28, & 31).	High: Likely loss of most of Bitter Creek. Lowered water levels or loss of some sinkholes, but not the majority of the unit.	Moderate: Low water levels lead to increasing salinity, temperature, and DO impairment.	Moderate: Only sinkhole habitat (spring areas that feed into Bitter Creek likely remain and are treated as default sinkholes).	MODERATE
Unit 4: BLNWR Middle Tract Wetlands	Low: High flow events such as the one that occurred in 2022 have the potential to introduce sheepshead minnow to the managed wetlands. There are fish barriers to prevent passage during normal flow, but these are overtopped in high flow events.	Low: Impacts to spring flows and marsh habitats could result in the loss of some extant pops in shallower managed wetlands.	Low: Low flows expected in marshes and possible drying of oxbows.	Moderate: Low water levels lead to increasing salinity, temperature, and DO impairment.	High: Unit contains a diverse suite of habitats including managed wetlands, streams, seeps, and oxbows.	MODERATE
<i>Unit 5:</i> Bottomless Lakes State Park	High: No introgression and no direct connection to introgressed populations.	High: Fish remain extant through all known occupied sites in the unit (except Upper Figure 8 Lake).	High: Perennial sinkholes	Moderate: Water quality issues in Upper and Lower Figure 8 Lake.	Moderate: Sinkhole habitat only	HIGH
Unit 6: BLM Overflow Wetlands & Lea Lake	Moderate: Maintenance of fish barriers are likely to prove effective and continuing to prevent sheepshead minnow movement into the Overflow Wetlands under most conditions. However, because sheepshead minnow are closer to the population there is an increased risk to this area should there be a severe flood and it becomes easier	High: Higher temps and lower rainfall likely lead to a reduction in wetland extant. However, Pecos pupfish remain extant in the complex.	Moderate: Routine drying events in portions of the marsh.	Moderate: Reported (need citation) high salinity and potential for adverse water condition.	High: Wetland and sinkhole habitat	MODERATE

	for a bait-bucket introduction to occur.					
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Extirpated: If not already extirpated (2023), sheepshead minnow are expected to spread through the entirety of the Middle Pecos reach.	Extirpated	Low: Expected routine drying.	Low: Pecos River exposed to contaminants from oil & gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED
Unit 8: Salt Creek, TX	Low: Introgression in lower section of the unit (closest to Pecos River).	Extirpated: Routine long- term drying likely leads to the loss of this population.	Low: Routine drying expected.	Low: Surrounded by significant oil & gas development with potential for contamination.	Extirpated: Likely no permanent water remains in unit.	EXTIRPATED
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED

Scenario 2 – Hot and Wet: 2050

Future Condition - 2050

Unit	Genetics	Occurrence	Water Quantity	Water Quality	Habitat Diversity	OVERALL CONDITION
Unit 1: Upper Pecos River (above Brantley Reservoir)	High: No known introgression, but direct connection to Lower Pecos River introgressed population (connectivity mitigated through dams).	Moderate: Expected loss of some sites due to drying.	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	MODERATE
Unit 2: Salt Creek Wilderness, NM	High: Isolated sinkholes and creek with no documented introgression and no connection to introgressed populations.	Moderate: Loss of Pren's Hole and likely loss of Salt Creek. Sinkholes remain extant.	Moderate: Salt Creek experiences regular drying events.	Moderate: High temps and low DO anticipated in Salt Creek.	High: Stream and sinkhole habitat.	MODERATE
Unit 3: Bitter Creek Drainage	High: Isolated sinkholes with no documented introgression and no connection to introgressed populations.	High: Some drying of Bitter Creek, but fish remain extant in deeper portions. Sinkholes extant.	Moderate: Routine drying of Bitter Creek during dry years (Lost River inlet remains).	0 0	High: Stream and sinkhole habitat.	MODERATE
Unit 4: BLNWR Middle Tract Wetlands	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations.	Moderate: Some managed wetland units may experience occasional drying.	Moderate: Occasional low flow leading to high water temps and other impairments.	High: Unit contains a diverse suite of habitats including managed wetlands,	MODERATE

					streams, seeps, and oxbows.	
<i>Unit 5:</i> Bottomless Lakes State Park	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial sinkholes	Moderate: Water quality issues in Upper and Lower Figure 8 Lake.	Moderate: Sinkhole habitat only	MODERATE
Unit 6: BLM Overflow Wetlands & Lea Lake	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial wetlands	High: No documented water quality or contamination issues.	High: Wetland and sinkhole habitat	нісн
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Low: Likely introgression up to Pecos near Loving.	Extirpated	Moderate: Documented low flows on the Pecos River	Low: Pecos River exposed to contaminants from oil & gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED
Unit 8: Salt Creek, TX	Low: Introgression in lower section of the unit (closest to Pecos River).	Low: Potential for loss of population.	Low: Routine drying events anticipated in Salt Creek.	Low: Surrounded by significant oil & gas development with potential for contamination.	Moderate: Stream habitat only	MODERATE
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED

Scenario 2 – Hot and Wet: 2100

Future Condition – 2100

Unit	Genetics	Occurrence	Water Quantity	Water Quality	Habitat Diversity	OVERALL CONDITION
Unit 1: Upper Pecos River (above Brantley Reservoir)	High: No known introgression, but direct connection to Lower Pecos River introgressed population (connectivity mitigated through dams).	Moderate: Expected loss of some sites due to drying.	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	MODERATE
Unit 2: Salt Creek Wilderness, NM	High: Isolated sinkholes and creek with no documented introgression and no connection to introgressed populations.	Moderate: Loss of Pren's Hole and likely loss of Salt Creek due to higher average surface temperatures. Sinkholes remain extant.	Moderate: Routine low flows	Low: Documented water quality issues in Salt Creek.	High: Sinkhole habitat remains. Monsoon rainfall keeps Salt Creek extant in most years.	MODERATE

Unit 3: Bitter Creek Drainage	High: Isolated sinkholes with no documented introgression and no connection to introgressed populations.	Moderate: Higher average annual surface temperatures lead to the loss of fish in Bitter Creek.	High: Likely loss of most of Bitter Creek. Monsoon flows keep sinkhole levels stable.	Moderate: Occasional low flow leading to high water temps and other impairments.	High: Sinkhole habitat perennial. Higher monsoon rainfall keeps Bitter Creek from complete drying.	MODERATE
Unit 4: BLNWR Middle Tract Wetlands	High: No introgression and no direct connection to introgressed populations.	Moderate: Higher average annual temperatures cause losses of fish in shallower wetlands.	Moderate: Low flows possible in dry years, but increased monsoon activity offsets higher temps.	Moderate: Occasional low flow leading to high water temps and other impairments.	High: Unit contains a diverse suite of habitats including managed wetlands, streams, seeps, and oxbows.	MODERATE
<i>Unit 5:</i> Bottomless Lakes State Park	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial sinkholes	High: No documented water quality or contamination issues.	Moderate: Sinkhole habitat only	HIGH
Unit 6: BLM Overflow Wetlands & Lea Lake	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial wetlands	Moderate: Shallower portions of wetland experience high temps during hottest times of year.	High: Wetland and sinkhole habitat	HIGH
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Low: Likely introgression up to Pecos near Loving.	Extirpated	Moderate: Documented low flows on the Pecos River	Low: Pecos River exposed to contaminants from oil & gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED
Unit 8: Salt Creek, TX	Low: Introgression in lower section of the unit (closest to Pecos River).	Low: Potential for loss of population.	Low: Routine drying events anticipated in Salt Creek.	Low: Surrounded by significant oil & gas development with potential for contamination.	Moderate: Stream habitat only	LOW
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED

Scenario 3 – Warm and Dry: 2050

Future Condition - 2050

Condition Condition Condition Condition Condition Condition Condition Condition	Unit	Genetics	Occurrence	Water Quantity	Water Quality	Habitat Diversity	OVERALL CONDITION
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Unit 1: Upper Pecos River (above Brantley Reservoir)	High: No known introgression, but direct connection to Lower Pecos River introgressed population (connectivity mitigated through dams).	Moderate: Detected throughout unit except at Willow Creek and 6 Mile Draw (extant 2021)	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	MODERATE
Unit 2: Salt Creek Wilderness, NM	High: Isolated sinkholes and creek with no documented introgression and no connection to introgressed populations.	High: Extant in unit. Extirpated from Pren's Hole	Moderate: Salt Creek likely experiencing drying event. Sinkholes have perennial water.	Moderate: Documented fish kill (Jan 2023). May be related to water quality.	High: Only sinkhole and ephemeral creek habitats.	MODERATE
	High: Isolated sinkholes with no documented introgression and no connection to introgressed populations.	High: All occupied sites not regularly monitored. Extant in monitored sites. No negative reports.	High: Documented low flows in stream habitat Bitter Creek. Sinkholes perennial.		High: Stream and sinkhole habitat.	HIGH
Unit 4: BLNWR Middle Tract Wetlands	High: No introgression and no direct connection to introgressed populations.	High: Confirmed extant and no negative reports.	High: Portions of the unit include ephemeral streams and managed wetlands. These may have low flows.		High: Unit contains a diverse suite of habitats including managed wetlands, streams, seeps, and oxbows.	нісн
<i>Unit 5:</i> Bottomless Lakes State Park	High: No introgression and no direct connection to introgressed populations.	High: Extant populations throughout most of unit. Extirpated from Upper Figure 8 Lake.	High: Perennial sinkholes	Moderate: Water quality issues in Upper and Lower Figure 8 Lake.	Moderate: Sinkhole habitat only	MODERATE
Unit 6: BLM Overflow Wetlands & Lea Lake	High: No introgression and no direct connection to introgressed populations.	High: Extant at all surveyed sites. No negative surveys.	High: Perennial wetlands	High: No documented water quality or contamination issues.	High: Wetland and sinkhole habitat	HIGH
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Low: Likely introgression up to Pecos near Loving.	Extirpated	Moderate: Documented low flows on the Pecos River	Low: Pecos River exposed to contaminants from oil & gas and agricultural activities.		EXTIRPATED
Unit 8: Salt Creek, TX	Low: Introgression in lower section of the unit (closest to Pecos River).	Moderate: Extant in headwaters but extirpated from confluence area (introgression).	Moderate: Low flows documented in Salt Creek	Low: Surrounded by significant oil & gas development with potential for contamination.	Moderate: Stream habitat only	MODERATE
Unit 9: Lower Pecos River (south of Red Bluff Reservoir)	Extirpated	Extirpated	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED

Scenario 3 – Warm and Dry: 2100

Future Condition - 2100

Unit	Genetics	Occurrence	Water Quantity	Water Quality	Habitat Diversity	OVERALL CONDITION
Unit 1: Upper Pecos River	High: No known introgression, but direct connection to Lower Pecos River introgressed population (connectivity mitigated through dams).	Moderate: Expected loss of some sites due to drying.	Moderate: River sections dry out on occasion.	Low: Upper Pecos River experiences occasional contamination from oil and gas and agricultural activities.	Moderate: River habitat only	MODERATE
Unit 2: Salt Creek Wilderness, NM	High: Isolated sinkholes and creek with no documented introgression and no connection to introgressed populations.	Moderate: Loss of Pren's Hole and likely loss of Salt Creek. Sinkholes remain extant.	Moderate: Salt Creek experiences regular drying events.	Moderate: High temps and low DO anticipated in Salt Creek.	High: Stream and sinkhole habitat.	MODERATE
Unit 3: Bitter Creek Drainage	High: Isolated sinkholes with no documented introgression and no connection to introgressed populations.	High: Some drying of Bitter Creek, but fish remain extant in deeper portions. Sinkholes extant.	Moderate: Routine drying of Bitter Creek during dry years (Lost River inlet remains).	Moderate: Occasional low flow leading to high water temps. Known high salinity in some sinkholes (e.g., Sinkhole 21).	High: Stream and sinkhole habitat.	MODERATE
Unit 4: BLNWR Middle Tract Wetlands	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations.	Moderate: Some managed wetland units may experience occasional drying.	Moderate: Occasional low flow leading to high water temps and other impairments.	High: Unit contains a diverse suite of habitats including managed wetlands, streams, seeps, and oxbows.	MODERATE
<i>Unit 5:</i> Bottomless Lakes State Park	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial sinkholes	Moderate: Water quality issues in Upper and Lower Figure 8 Lake.	Moderate: Sinkhole habitat only	MODERATE
Unit 6: BLM Overflow Wetlands & Lea Lake	High: No introgression and no direct connection to introgressed populations.	High: No expected losses of extant populations	High: Perennial wetlands	High: No documented water quality or contamination issues.	High: Wetland and sinkhole habitat	HIGH
Unit 7: Middle Pecos River (Brantley to Red Bluff)	Low: Likely introgression up to Pecos near Loving.	Extirpated	Moderate: Documented low flows on the Pecos River	Low: Pecos River exposed to contaminants from oil & gas and agricultural activities.	Moderate: River habitat only	EXTIRPATED
Unit 8: Salt Creek, TX	Low: Introgression in lower section of the unit (closest to Pecos River).	Low: Potential for loss of population.	Low: Routine drying events anticipated in Salt Creek.	Low: Surrounded by significant oil & gas development with potential for contamination.	Moderate: Stream habitat only	MODERATE

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Unit 9: Lower Pecos River (south of Red Bluff Reservoir)ExtirpatedExtirpatedModerate: River sections dry out on occasion.experiences occasional ontamination from oil and gas and agricultural activities.Moderate: River habitat onlyEXTIRPATED		Extirpated	Extirpated		contamination from oil and gas and agricultural		EXTIRPATED
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Appendix D. Pecos Pupfish Conservation Agreement Conservation Measures

VII. Conservation measures in order to address the threats to Pecos pupfish described in Section V, the Signatories hereby agree to the following:

A. The TPWD will:

(1). enforce bait use regulations within the Pecos River basin as Pecos Pupfish Conservation Agreement 2022 8 described in 31 Texas Administrative Code §57.972(e);

(2). in collaboration with the Service's Texas Fish and Wildlife Conservation Office, monitor population trends and genetic status of Pecos Pupfish populations in Texas;

(3). in cooperation with willing landowners, implement actions needed to ensure persistence of existing populations of Pecos Pupfish and seek to establish additional secure populations of the species within the Pecos River basin;

(4). in cooperation with willing landowners, local communities, nongovernmental organizations, and other agencies, restore and preserve natural riparian and aquatic habitat conditions in the Pecos River basin to support healthy aquatic ecosystems and the long-term persistence of Pecos Pupfish;

(5). support continued maintenance of the existing refuge population of Pecos Pupfish located at the Fort Worth Zoo and seek to identify, evaluate, and establish additional potential refuges that would guard against catastrophic, stochastic events; and

(6). in biennial rotation with NMDGF, coordinate the Conservation Team, including maintaining a file of literature relevant to Pecos Pupfish, updating the Conservation Team contact information, and preparing an annual summary of activities accomplished under this Agreement.

B. The NMDGF will:

(1). enforce bait programs as defined in 19.31.10.14 NMAC for the Pecos River and prohibit the use of live bait on Bitter Lake NWR and Bottomless Lakes State Park;

(2). restrict the importation of nonnative fish species to occupied Pecos Pupfish habitat in accordance with 19.35.7.14 NMAC;

(3). lead development of the Pecos Pupfish Conservation Strategy as described in Section VI with assistance from the other Signatories;

(4). lead population monitoring and genetic assessments of Pecos Pupfish at all sites in New Mexico as described in the 2010 monitoring plan and determine actions needed to assure security of the populations;

(5). with cooperation of other agencies, establish and maintain refuge populations of Pecos Pupfish to guard against stochastic events, such as fish kills resulting from golden alga blooms; and

(6). in biennial rotation with TPWD, coordinate the Conservation Team, including maintaining a file of literature relevant to Pecos Pupfish, updating the Conservation Team contact information, and preparing an annual summary of activities accomplished under this Agreement.

C. The NM Energy, Minerals and Natural Resources Department will:

(1). recognize and maintain the management and protection of Pecos Pupfish in the Bottomless Lakes State Park Management Plan;

(2). in cooperation with the NMDGF and the Service, conduct aquatic faunal surveys according to the collaboratively designed monitoring plan;

(3). in cooperation with the NMDGF and the Service, investigate removal and control of undesirable aquatic species and conservation of nontarget species;

(4). in cooperation NMGFD and the Service, establish, when necessary, and maintain Pecos Pupfish in Mirror, Figure Eight, Pasture, Lost, and Lea lakes and Lazy Lagoon; and

(5). prohibit sport fishing in south Figure Eight, Lost, and Lea lakes, ponds 1, 2, and 3 in the Lea Lake wetland area, and Lazy Lagoon to promote Pecos Pupfish conservation.

D. The NM Department of Agriculture will:

(1). identify and act as intermediaries with private landowners as needed to establish Pecos Pupfish refuge populations; and

(2). review and advise on legislative and political initiatives that will enhance the conservation of Pecos Pupfish.

E. The NMISC will:

(1). upon notification by Signatories of proposed activities to conserve Pecos Pupfish under this Agreement, including, but not limited to, water acquisition and leasing, and habitat restoration, in cooperation with signatory agencies, evaluate the hydrologic impacts of such proposed activities to New Mexico waters; and

(2). assist in the identification and acquisition of water rights that may be utilized to offset all water depletions resulting from implementation of Pecos Pupfish Conservation Agreement 2022 10 proposed activities.

(3). the Signatories acknowledge that NMISC's participation in this Agreement is expressly made contingent upon sufficient appropriation and authorization for appropriation being granted by the Legislature of New Mexico and the State of New Mexico for performance of this Agreement. Absence of appropriation or allotment of funds shall relieve the NMISC from any obligations under this Agreement. No liability shall accrue to NMISC should funds not be appropriated or allotted.

F. The NM State Land Office will:

(1). work with other agencies to evaluate potential refugia for Pecos Pupfish on state trust lands and evaluate the efficacy of additional introductions;

(2). facilitate access to New Mexico state trust lands for research and monitoring of Pecos Pupfish populations, and for habitat restoration or enhancement projects benefiting Pecos Pupfish; and

(3). work with other agencies to evaluate, prioritize, and, as capabilities and appropriations are available, implement riparian restoration that benefits Pecos Pupfish.

G. The BLM will:

(1). work with the NMDGF to identify management actions that will protect and enhance Pecos Pupfish populations in the Overflow Wetlands ACEC;

(2). continue implementation of management and protection measures for Pecos Pupfish in accordance with the Overflow Wetlands ACEC Plan;

(3). in cooperation with the NMDGF and the Service, monitor populations of Pecos Pupfish at the Overflow Wetlands ACEC as described in the collaboratively designed monitoring plan;

(4). in cooperation with the NMDGF and the Service, work with private landowners and public lessees to identify off-channel sites such as perennial springs for potential introduction of Pecos Pupfish; and

(5). continue implementation of the Overflow Wetlands ACEC Activity Plan and management prescriptions found in the Roswell Approved Resource Management Plan (page 65). Pecos Pupfish Conservation Agreement 2022 11

H. The Service will:

(1). provide funding as available to support genetic, habitat, and other needed research on Pecos Pupfish;

(2). assist with Pecos Pupfish monitoring as described in the collaboratively designed 2010 monitoring plan (BEEC 2010, entire) including at points of ingress of sheepshead minnow on Bitter Lake NWR and in Texas;

(3). incorporate the management and protection of Pecos Pupfish in the Bitter Lake NWR Comprehensive Conservation Plan and Habitat Management Plan; and

(4). assess the feasibility of using, with modifications, if necessary, constructed wetlands at the Dexter National Fish Hatchery and Technical Center or other appropriate site, as a refugium population site for Pecos Pupfish.

I. All Signatories will:

(1). provide representation to the Conservation Team established by this Agreement;

(2). assist with the development of the Conservation Strategy within two years of execution of this Agreement;

(3). participate in all necessary meetings to discuss the species status and progress toward achieving the conservation objectives for Pecos Pupfish and addressing threats outlined in the Agreement, in the 2009 proposal to list, and in the Conservation Strategy (described in Section VI);

(4). provide in-kind contributions of personnel, field equipment, supplies, etc., to assist in investigations and re-establishment efforts, subject to individual agency capabilities and appropriations; and

(5). annually provide information and review the written report of the Conservation Team documenting the status of accomplishments under this Agreement and the proposed Conservation Strategy (see Section VI). This assessment will determine the effectiveness of the Agreement and whether revisions are warranted and will be provided to the Conservation Team each year.