Clear Creek gambusia
(Gambusia heterochir)
5-Year Status Review:
Summary and Evaluation

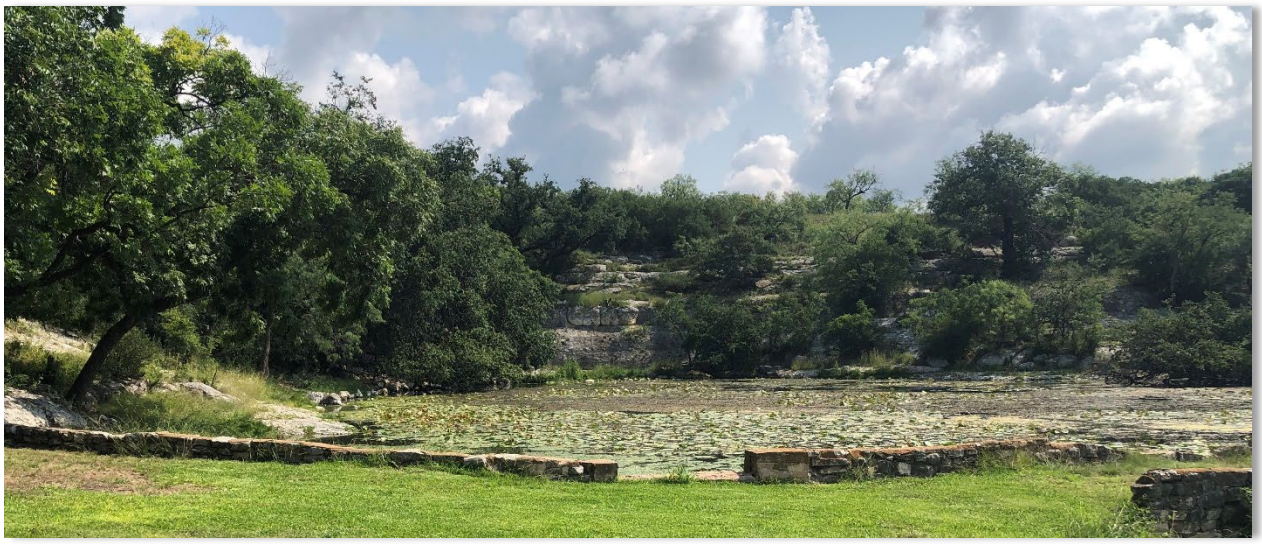

Photo by G. Pandolfi, FWS

U.S. Fish and Wildlife Service
Austin Ecological Services Field Office
Austin, Texas
August 28, 2023

5-YEAR REVIEW

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1.0 GENERAL INFORMATION

1.1 Reviewers:

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Cooperating Regional Office(s):
Not applicable

1.2 Purpose of 5-Year Reviews:

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

1.3 Methodology used to complete the review:

The U.S. Fish and Wildlife Service (Service) conducts status reviews of species on the List of Endangered and Threatened Wildlife and Plants (50 CFR 17.12) as required by section 4(c)(2)(A) of the Endangered Species Act (16 U.S.C. 1531 et seq.). The Service provides notice of status reviews via the Federal Register and requests new information on the status of the species (e.g., life history, habitat conditions, and threats). Data for this status review were solicited from interested parties through a Federal Register notice announcing this review on February 2, 2022 (87 FR 5834). This review was conducted by the Austin Ecological Services Field Office and considered both new and previously existing information from federal and state agencies, non-governmental organizations, academia, and the public. The primary sources of information used in this analysis included information from the Recovery Plan, peer-reviewed articles, agency reports, and other documents available in the Austin Ecological Services Field Office files. We also engaged in discussions with Service employees affiliated with the Fisheries and Aquatic Conservation program. Comments received were evaluated and incorporated as appropriate.

1.4 Background:

1.4.1 FR Notice citation announcing initiation of this review:

87 FR 5834; February 2, 2022

1.4.2 Listing history:

Original Listing
FR notice: 32 FR 4001
Date listed: March 11, 1967
Entity listed: Species, *Gambusia heterochir*
Classification: Endangered

1.4.3 Associated Rulemakings:

Not applicable.

1.4.4 Review History:

A five-year status review for the Clear Creek gambusia was approved in May 2010. This was the first five-year review completed for the species. The recommendation resulting from the review was that no change was warranted to the classification of the species as Endangered.

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

The species' recovery priority number was given and maintained as 2 in the five-year status review published in 2010.

1.4.6 Recovery Plan or Outline

Name of plan or outline: Clear Creek Gambusia Recovery Plan
Date issued: 1982
Dates of previous plans/amendment or outline, if applicable: Not applicable

2.0 REVIEW ANALYSIS

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

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

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

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

Not applicable; the Clear Creek gambusia is not listed as a DPS.

2.2 Updated Information and Current Species Status

2.2.1 Biology and Habitat

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

Inks Dam National Fish Hatchery (NFH) staff documented new information regarding best practices for maintaining captive populations of Clear Creek gambusia. The initial feeding regime for the captive population was flake, dry, floating, and semi-moist commercial pelleted feeds, but the fishes were not adequately responsive to this approach (Inks Dam National Fish Hatchery 2012, p. 80). The fish were switched to brine shrimp with Spirulina, which was more palatable (Inks Dam National Fish Hatchery 2012, p. 80). In 2012, Inks Dam NFH experimented with additional feed, including brine shrimp, bloodworms, krill, and plankton, all of which were eaten by Clear Creek gambusia (Inks Dam National Fish Hatchery 2012, p. 80). In 2015, staff at Inks Dam NFH discovered that, although captive Clear Creek gambusia did not respond to flake food sprinkled on the water surface, injecting flake food into the water column induced a feeding response (Inks Dam National Fish Hatchery 2015b, p. 7). Since then, adult captive Clear Creek gambusia are fed a diet of flake food and frozen brine shrimp (Inks Dam National Fish Hatchery 2015b, p. 7). Newly born young are fed a combination of finely ground flake and newly hatched, enriched brine shrimp for their first two months and then switched to the adult diet (Inks Dam National Fish Hatchery 2015b, p. 7).

Inks Dam NFH also recorded new information on the species' longevity in captivity. The natural lifespan of Clear Creek gambusia in the wild averages less than one full year (Hubbs 1971, p. 19). Individuals in the captive population at Inks Dam NFH may live longer, up to five years (Inks Dam National Fish Hatchery 2015b, p. 6; 2020, p. 22).

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

Demographically, the Clear Creek gambusia population has declined severely since the last five-year status review. The risk of extinction has risen as a result. Since its discovery, Clear Creek gambusia have been found in the wild only at the headwaters of Clear Creek, in a pool created by a dam (U.S. Fish and Wildlife Service 2010b, p. 5). The source of these headwaters are a set of springs known as Wilkinson Springs and the size of the spring pool above the dam is about 0.35 acres (0.14 hectares) (U.S. Fish and Wildlife Service 2010b,

p. 5). A captive population of Clear Creek gambusia is located at Inks Dam NFH (Texas Fish and Wildlife Conservation Office 2011, p. 24). Both the wild population and the captive population are in danger of extinction within the next several years.

Wild Population

The most recent five-year status review for the Clear Creek gambusia was approved in 2010. In early 2009, the wild population in the upper pool was characterized as "in good shape" (Rio Grande Fishes Recovery Team 2009, p. 4).

The most recent (and only) population size estimate completed since the last five-year status review is from 2010 (U.S. Fish and Wildlife Service 2010a, p. 4). In May 2010, Fisheries and Aquatic Conservation staff used depletion sampling with minnow traps, collecting and releasing 2,385 individuals (Phillips 2010, p. 1). Preliminary findings were that most individuals were Clear Creek gambusia, a few were western mosquitofish (*Gambusia affinis*), and none were putative hybrids (Phillips 2010, p. 1). Based on these sampling efforts, the Service, in the Biological Opinion associated with a planned repair to the dam, estimated a population size of the upper pool of at least 5,000 individuals, and possibly much higher (U.S. Fish and Wildlife Service 2010a, p. 4).

In general, for the period from 2010 to 2015, we are confident that the population continued to persist because surveyors were able to collect Clear Creek gambusia via seining, minnow traps, and dip nets. Other sampling trips provided summary data on the number of fish captured in Wilkinson Springs pool, and the relative proportion of Clear Creek gambusia, western mosquitofish, and their hybrids. The overall trend since 2010 has been decreasing proportions of unhybridized Clear Creek gambusia.

In March 2010, the upper pool was sampled (Allan 2010a, p. 1). Using a combination of seining along the shore of Wilkinson Springs pool and setting minnow traps, surveyors collected 174 Clear Creek gambusia, 11 putative hybrids, and four western mosquitofish (Allan 2010a, p. 1). In October 2010, 254 Clear Creek gambusia were collected and taken to Inks Dam NFH (Dexter National Fish Hatchery & Technology Center 2011, p. 2). No fish were collected in 2011 (Inks Dam National Fish Hatchery 2012, p. 9). In March 2012, surveyors captured 160 Clear Creek gambusia to supplement the refuge population at Inks Dam NFH (Texas Fish and Wildlife Conservation Office 2012, p. 26).

In August 2013, 60 individuals were collected from the Wilkinson Springs pool for a fish health assessment, and the surveyors observed that the proportion of hybrids appeared to be rapidly increasing (Montagne et al. 2013, p. 1). In 2014, 265 Clear Creek gambusia individuals were captured using seines (Montagne 2015a, p. 1). In 2015, concerns about the unrepaired and leaky dam led to another effort to collect unhybridized Clear Creek gambusia from the wild in order to supplement the captive population (Napier 2015, pp. 1–3). Fish biologists with the ability to distinguish between Clear Creek gambusia, western mosquitofish, and likely hybrids in the field spent a full day evaluating fish in February 2015 (Napier 2015, pp. 1–3). Ultimately, that effort yielded 210 Clear Creek gambusia, which were brought to Inks Dam NFH (Montagne 2015b, p. 1).

The early 2015 collection effort was the last one until summer 2021, when Service personnel returned to the headwaters of Clear Creek (Wilkinson Springs) in hopes of re-assessing the status of Clear Creek gambusia in the wild and collecting individuals to supplement the refuge population (Pandolfi 2021, p. 1). Using dip nets, staff collected 80 individual fish, then conducted a visual and morphological assessment (Pandolfi 2021, pp. 1–2). All of the captured fish appeared to be western mosquitofish (Pandolfi 2021, pp. 1–2). None were added to the captive population (Conway 2023, p. 1).

Service personnel visited the ranch again in September 2022 to collect individuals for genetic analysis (Montagne 2023, p. 1). They used seines, minnow traps, and dip nets to collect 100–200 fish from the Wilkinson Springs pool (Montagne 2023, p. 1). Each fish was individually examined with the intent of classifying it as a Clear Creek gambusia, a western mosquitofish, or a hybrid (Montagne 2023, p. 1). Almost all appeared to be western mosquitofish (Montagne 2023, p. 1). A few may have been hybrids, but none looked like unhybridized Clear Creek gambusia (Montagne 2023, p. 1). A few of the collected individuals were too young to identify in the field; these were brought back to Inks Dam NFH and raised until maturity, at which time they were determined to be western mosquitofish (Conway 2023, p. 1; Montagne 2023, p. 1).

Captive Population

The refuge population at Inks Dam NFH was founded in 2011 with 242 Clear Creek gambusia collected in October 2010 (Dexter National Fish Hatchery & Technology Center 2011, p. 2; Texas Fish and Wildlife Conservation Office 2011, p. 24; Inks Dam National Fish Hatchery 2012, p. 9). In 2012, an additional 160 wild Clear Creek gambusia were added to the refuge population (U.S. Fish and Wildlife Service 2023, p. 3). At the end of Fiscal Year (FY)

2012, the total population was 215 fish. These fish were never used in captive-breeding.

We do not have fiscal year-end totals for the captive population for FY2013 or FY2014. In 2014, 265 more Clear Creek gambusia were collected and brought to Inks Dam NFH (Inks Dam National Fish Hatchery 2015b, p. 4). The first captive-bred fish were produced in February 2015 from the stock collected in 2014 (Inks Dam National Fish Hatchery 2015b, p. 4). The numbers of wild-caught fish in the refuge population, the yearly offspring produced, and the total size of the refuge population beginning with the first year of propagation in 2015 are shown in Table 1. In 2015, the refuge population was again supplemented with 210 wild Clear Creek gambusia (Inks Dam National Fish Hatchery 2015b, p. 4). At the close of FY2015, the total captive population was 474 fish, including 171 wild and 303 captive-bred individuals (Inks Dam National Fish Hatchery 2015b, p. 4).

At the end of FY2016, the total population at Inks Dam NFH was 813 individuals (Inks Dam National Fish Hatchery 2017, p. 4). Of these, 68 were wild, and 597 were born in FY2016. At the end of FY2017, the population had risen to 1,237 individuals, 43 of which were wild and 591 of which were born that year (Inks Dam National Fish Hatchery 2017, p. 3). At the end of FY2018, the total population had risen again to 1,900 individuals, ten of which were wild and 825 of which were born that year (Inks Dam National Fish Hatchery 2018, p. 20). Peak fecundity occurred in FY18.

At the end of FY2019, the total captive population was 2,445, three of which were wild and 718 of which were born that year (Inks Dam National Fish Hatchery 2019, p. 20). At the end of the FY2020 breeding season, the total captive population was 2,564, one of which was wild and 409 of which were born that year (Inks Dam National Fish Hatchery 2020, p. 22). Peak total population occurred in FY2020.

As of December 2021, the total captive population was 2,166, one of which was wild and 111 of which were born that year (Conway 2023, p. 1). As of December 2022, the total population was 775 (Conway 2023, p. 1). The last wild fish died in 2022, and only eight offspring were produced (Conway 2023, p. 1). As of July 2023, the population continues to be in decline due to a lack of recruitment of new individuals (Conway 2023, p. 1). The 2023 breeding season has so far produced just three offspring (Conway 2023, p. 1). Given the decline in fecundity, 2023 is likely to be the last year that the species is able to be bred in captivity.

Table 1. The numbers of wild-caught Clear Creek gambusia in the refuge population, the yearly offspring produced, and the total size of the refuge population beginning with the first year of propagation in 2015.

Year	Number of Wild-Caught Individuals in Captivity	Number of Offspring	Total Population Size
2015	171	303	474
2016	68	597	813
2017	43	591	1,237
2018	10	825	1,900
2019	3	718	2,445
2020	1	409	2,564
2021	1	111	2,166
2022	0	8	775

The reason for the decline in fecundity has not been established, but a goal of the in-progress Science Support Partnership project is to determine if inbreeding depression is occurring within the captive population (U.S. Fish and Wildlife Service 2023, p. 4). Based on a maximum lifespan in captivity of five years, the captive population will be eliminated by 2030 if no new wild Clear Creek gambusia can be introduced to the captive population, or if genetic testing of the captive population does not yield insights into how to rescue the population (U.S. Fish and Wildlife Service 2023, p. 4).

Summary

Although data are currently lacking to make a definitive statement about the abundance of Clear Creek gambusia in the wild, the evidence that is available points to a population in decline, in which abundance is very low. It is possible that the species may have been extirpated from the wild. The refuge population at Inks Dam NFH is also in decline and on track to die out within the next several years. The potential to change that trend is uncertain.

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

The genetic health of both the wild and captive populations of Clear Creek gambusia has declined severely since the last five-year status review. The risk of extinction has risen as a result.

Wild Population

In October 2010, 254 wild Clear Creek gambusia were collected and taken to Inks Dam NFH (Dexter National Fish Hatchery & Technology Center 2011, p. 2). Staff at what is now the Southwestern Native Aquatic Resources and Recovery Center (SNARRC) in Dexter, New Mexico analyzed the genetics of all 254 fish in order to determine whether any were hybrids with western mosquitofish (Dexter National Fish Hatchery & Technology Center 2011, p. 2). Of the 254 individuals, 11 (4%) were found to be hybrids, and one died after being fin clipped, leaving 242 as the unhybridized Clear Creek gambusia population (Dexter National Fish Hatchery & Technology Center 2011, pp. 4–7).

Although hybrid levels were low in 2010, they have increased in the years since. In 2013, 60 wild Clear Creek gambusia were collected for fish health analysis (Montagne et al. 2013, p. 1). Based on the morphology of the collected specimens, the surveyors reported a concern that the hybridization rate was increasing (Montagne et al. 2013, p. 1). An analysis by the Texas Parks and Wildlife Department evaluated hybridization among fish in the upper pool (Lutz-Carillo 2014, entire). Fifty-one fish were collected from the upper pool in 2013, and genetic testing revealed that 76.5% were pure Clear Creek gambusia, 19.6% were pure western mosquitofish, and 3.9% were hybrids (Lutz-Carillo 2014, p. 1). Below the upper pool, all fish tested were either western mosquitofish (93.3%) or hybrids (6.7%) (Lutz-Carillo 2014, p. 1). Given the known status of the dam as leaky (Allan 2009, p. 2), the increase in the proportion of hybrids is not surprising. Collection trips in 2014 and 2015 continued to locate Clear Creek gambusia in the upper pool at Wilkinson Springs, but the surveyors on these trips noted that the rate of hybridization appeared to be increasing (Montagne 2015b, p. 1). The surveyors also observed that even within the upper pool, Clear Creek gambusia were only found, as of 2014–2015, in the uppermost portion near the spring openings (Montagne 2015b, p. 1). Sampling occurring near the dam yielded almost exclusively western mosquitofish and hybrids (Montagne 2015b, p. 1).

Captive Population

The genetics of the current captive population appear to be free of introgression from western mosquitofish. In 2016, all of the FY2014 and FY2015 wild-caught fish were fin-clipped for genetic analysis, which was performed by Texas Parks and Wildlife Department (Inks Dam National Fish Hatchery 2016, p. 18). Of the 154 fin clips, 13 were found to be western mosquitofish or hybrids of Clear Creek gambusia and western mosquitofish, and three samples were unresolved (Inks Dam National Fish Hatchery 2016, p. 18). Those individuals, including the unresolved ones, were euthanized and thus removed from the captive population (Inks Dam National Fish Hatchery 2016, p. 18). In 2017, genetic testing was conducted on 96 captive-bred fish from FY15 and FY16 (48 from each year) by SNARRC (Inks Dam National Fish Hatchery 2017, p. 3). No hybrids were found (Inks Dam National Fish Hatchery 2017, p. 3). Later, 200 captive-bred fish from the same year classes (100 from each year) were tested and again, no fish were found to be hybrids (Inks Dam National Fish Hatchery 2017, p. 3). Some of the samples were undeterminable due to issues with the genetic testing process (Inks Dam National Fish Hatchery 2017, p. 3). The fish belonging to those samples were euthanized and thus removed from the captive population (Inks Dam National Fish Hatchery 2017, p. 3).

In July 2022, a Science Support Partnership grant was awarded to the Texas Fish and Wildlife Conservation Office and the U.S. Geological Survey Upper Midwest Environmental Science Center. Project objectives are to determine the status of the wild population of Clear Creek gambusia and provide management recommendations for the species, both in the wild and for the refuge population currently held at Inks Dam NFH (U.S. Fish and Wildlife Service 2023, p. 3). Field collections of wild Clear Creek gambusia, hybrids, and local western mosquitofish to support this work are ongoing. This study was motivated in part from a need to better understand the declining fecundity in the captive population, which is not currently established, but could be a consequence of genetic drift or inbreeding depression (U.S. Fish and Wildlife Service 2023, p. 4).

Summary

Although data are currently lacking to make a definitive statement about the genetic health of Clear Creek gambusia in the wild, the evidence that is available points to a decline in abundance that puts the species at elevated risk for genetic drift and inbreeding depression, potentially compromising its overall genetic health. The refuge population at Inks Dam NFH is also facing fecundity declines that are likely related to a decline in genetic health. The potential to

change that trend is uncertain, and as a result the species is in danger of extinction due to declines in genetic health.

2.2.1.4 Taxonomic classification or changes in nomenclature:

There have not been any questions or concerns regarding the taxonomic classification or nomenclature of Clear Creek gambusia since the previous five-year review was approved in 2010.

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

When the dam on Clear Creek below Wilkinson Springs is in disrepair, Clear Creek gambusia and western mosquitofish may cross that partial barrier (U.S. Fish and Wildlife Service 2010b, pp. 10–11, 17–18). The last time the dam was in significant disrepair, in the 1970s, Clear Creek gambusia were observed both above and below the dam, and many hybrids between the two fishes could be found (U.S. Fish and Wildlife Service 2010b, pp. 21–23). During the current period of dam disrepair (2007–present), observations of Clear Creek gambusia below the dam have not been reported (Rio Grande Fishes Recovery Team 2007, p. 2). In addition, whereas in the past Clear Creek gambusia could be found, albeit at varying densities, throughout the upper pool, in 2014–2015 surveyors observed that they were absent or nearly absent from the portion of the upper pool near the dam (Montagne 2015b, p. 1; 2015a, p. 1). That is, within their limited range of the upper pool, they were only found along the periphery, near spring outlets (Montagne 2015b, p. 1; 2015a, p. 1). As of 2021–2022, the abundance of Clear Creek gambusia has decreased further (Pandolfi 2021, p. 2; Montagne 2023, p. 1), complicating our ability to specifically describe the occupied portion of the pool. However, it is likely that any remaining Clear Creek gambusia in the upper pool are confined almost exclusively to a small area around spring outlets where conditions are most suitable for their persistence. These conditions represent a probable decline in the spatial distribution of the species since the last five-year status review, which is an increased threat to the continued persistence of the species in the wild.

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

As detailed in the 2010 five-year status review, Clear Creek gambusia are adapted to the stenothermal (i.e., constant temperature) waters associated with the portion of Clear Creek adjacent to Wilkinson Springs (U.S. Fish and

Wildlife Service 2010b, p. 10). Historically, prior to dams being installed along the creek, Clear Creek gambusia would have inhabited not only the waters immediately adjacent to the springs, but also the upper portion of Clear Creek where water quality was heavily influenced by spring flow (U.S. Fish and Wildlife Service 2010b, p. 10). Since the last five-year status review was conducted, the dam closest to Wilkinson Springs has severely deteriorated, impairing its ability to function as a barrier that maintains stable spring habitat conditions in the Wilkinson Springs pool (Montagne et al. 2013, p. 8; Montagne 2015b, p. 1; Napier 2015, pp. 4–6; U.S. Fish and Wildlife Service 2015, p. 1; Pandolfi 2021, pp. 1, 4–8). As a direct result of the dam's degradation and the loss of the barrier between the two pools, there has been a decline in the total area of habitat best suited for Clear Creek gambusia, and in the area of habitat where Clear Creek gambusia are able to successfully outcompete and maintain high abundance relative to western mosquitofish.

Because Clear Creek exists as a series of dammed pools, as the uppermost dam deteriorates, the uppermost pool is at risk of taking on the characteristics of the second pool (U.S. Fish and Wildlife Service 2010b, pp. 8–11). That is, the biotic and abiotic qualities associated with the spring inflows to the upper flow differ from those associated with the impoundments further down the watercourse (Hubbs 1959, p. 242; 2001, p. 231; U.S. Fish and Wildlife Service 2010b, pp. 8–11). When the water from the second pool and the upper pool are no longer separated by the barrier of the dam, these waters mix, diluting the influence of streamflow (U.S. Fish and Wildlife Service 2010b, pp. 8–11). This leads to conditions more similar to the second impoundment or to fluctuating conditions, both of which are likely to favor western mosquitofish over Clear Creek gambusia (Hubbs 1971, pp. 36–44; Johnson and Hubbs 1989, p. 308; U.S. Fish and Wildlife Service 2010b, pp. 8–11, 17–18, 21–23). Because the transition from spring-influenced water to non-spring-influenced water occurs over a narrow area spatially, it is likely that, under the current conditions, Clear Creek gambusia will be unable to maintain their population in the small amount of suitable habitat over the long term (Hubbs 1971, pp. 36–44; Johnson and Hubbs 1989, p. 308; U.S. Fish and Wildlife Service 2010b, pp. 21–23). Repairing the dam would address this issue (U.S. Fish and Wildlife Service 2009, pp. 1–3; 2010d, pp. 1–3), but at present there are no known plans to repair the dam.

Water quality data was collected from the Wilkinson Springs pool for portions of FY2011 and FY2012 (Inks Dam National Fish Hatchery 2012, p. 30). Across the two years, temperature ranged from 20.61–20.86° Celsius (C) (69.1–69.55° F) with an average of 20.68° C (69.22° F), dissolved oxygen ranged from 5.8–8.71 milligrams/Liter (mg/L), and pH ranged from 6.48–6.91 (Inks Dam National Fish Hatchery 2012, p. 30). This effort was repeated in FY2015; the

average temperature measured was 20.77° C (69.39° F) and the average dissolved oxygen was 5.83 mg/L (Inks Dam National Fish Hatchery 2015b, p. 8).

In addition, during the period from 2011 to 2012, which includes the drought-of-record for the area, staff from Inks Dam NFH observed a decline in water levels in the upper pool (Inks Dam National Fish Hatchery 2012, p. 81). The cause of this is not known with certainty, but was likely due to a combination of evaporation from the pool and the loss of inflow from some of the springs (Inks Dam National Fish Hatchery 2012, p. 81). Water levels did not drop so much that the fishes in that pool were in danger (Inks Dam National Fish Hatchery 2012, p. 81).

The decreased abundance of Clear Creek gambusia and increased abundance of western mosquitofish in Wilkinson Springs pool indicates a probable change in habitat conditions, while the increased abundance of western mosquitofish on its own represents a change in ecosystem conditions. Overall, habitat and ecosystem conditions for Clear Creek gambusia have declined since the last five-year status review, increasing the risk of extinction for the species in the wild.

2.2.1.7 Other:

Not applicable.

2.2.1.8 Conservation Measures:

A two-pronged approach to secure the future of Clear Creek gambusia as a species was conducted from 2008 to 2010, immediately prior to and during the development of the previous five-status year review. Grant funds were obtained to initiate a refuge population of the species at Inks Dam NFH and to design and implement major repairs to the failing dam on Clear Creek below Wilkinson Springs (U.S. Fish and Wildlife Service 2010b, p. 28).

As described in the species' previous five-year status review, in 2008 funding was provided to initiate a refuge population of Clear Creek gambusia (U.S. Fish and Wildlife Service 2010b, p. 28). As of early 2010, Inks Dam NFH had created facilities and begun a draft management plan for the species (U.S. Fish and Wildlife Service 2010b, p. 28). A Biological Opinion covering the dam repair that included terms and conditions requiring the establishment of a captive stock at Inks Dam NFH prior to beginning the dam repair was developed during 2009 and 2010 and signed in summer 2010 (U.S. Fish and Wildlife Service 2010c, pp. 1–5; 2010a, p. 1). Clear Creek gambusia were

collected from the wild in 2010 and 2012 and kept at Inks Dam NFH starting in early 2011 (Texas Fish and Wildlife Conservation Office 2011, p. 24). Although in 2008 there were steps taken to treat the captive population as a long-term backup population—the development of a captive propagation and genetic management plan for the captive population at Inks Dam NFH was a desired outcome of the Preventing Extinction Initiative funding—for the first few years the species was held, no propagation was done (U.S. Fish and Wildlife Service 2008, p. 2; Inks Dam National Fish Hatchery 2015a, entire). It appears that this was due to the fact that by the time Inks Dam NFH began bringing the species on station in 2011, it was thought that the dam repair would be completed within the following year (Inks Dam National Fish Hatchery 2012, p. 28). As a result, the purpose of the captive population as a backup while the dam repair occurred was more salient, reducing the relative importance of finalizing the management plans and implementing propagation (Inks Dam National Fish Hatchery 2012, pp. 13, 28). In 2013, concerns about the viability of the wild population were raised among members of the Rio Grande Fishes Recovery Team due to the fact that work on the dam repair had not been initiated (Montagne et al. 2013, p. 1). The Team recommended that the staff at Inks Dam NFH begin breeding the captive fish (Montagne et al. 2013, entire). By June 2015, it was clear that the dam repair was unlikely to be initiated before the expiration of all funding in July 2015 (U.S. Fish and Wildlife Service 2015, pp. 1–2). At that time, the Service predicted that without additional action, Clear Creek gambusia would be extinct in the wild by 2020 as a result of consequences associated with the deterioration and poor condition of the dam, and drafted a contingency plan for management going forward (U.S. Fish and Wildlife Service 2015, entire). Inks Dam NFH began breeding the species and offspring were produced each year beginning in 2015 (Inks Dam National Fish Hatchery 2015a, p. 5).

With respect to the dam repair, in 2010 the Service entered into a Private Lands Agreement with the landowner to repair the dam (U.S. Fish and Wildlife Service 2010a, pp. 1–2). Funding for the project was obligated through the Partners for Fish and Wildlife Program and the Endangered Species Program's Preventing Extinction Initiative (U.S. Fish and Wildlife Service 2010a, pp. 1–2) and the landowner also agreed to contribute funding (Allan 2010b, p. 2). In 2010, the Service anticipated that the dam repair would be completed between 2011 and 2015 (Allan 2010b, p. 2; Texas Fish and Wildlife Conservation Office 2010, p. 10; 2011, p. 24; U.S. Fish and Wildlife Service 2015, p. 1). Service personnel were in contact with the landowner and local land manager throughout this time and received assurances that the landowner intended to complete the work (Napier 2015, pp. 5–6). However, the landowner did not arrange for the dam repair to be completed, nor did the landowner grant

permission to the Service to initiate work on the dam in 2011 (Texas Fish and Wildlife Conservation Office 2011, p. 24), 2012 (Inks Dam National Fish Hatchery 2012, p. 28), 2013 (Texas Fish and Wildlife Conservation Office 2013, p. 20), 2014, or 2015 (Napier 2015, p. 6; U.S. Fish and Wildlife Service 2015, p. 1).

The Science Support Partnership grant (described in Section 2.2.1.3) is the first step in a renewed effort to determine whether the refuge population currently at Inks Dam NFH can be rescued with wild Clear Creek gambusia or a new breeding methodology (U.S. Fish and Wildlife Service 2023, p. 3). If so, the Service's goal is to implement a breeding program based on genetic analyses (U.S. Fish and Wildlife Service 2023, p. 4). Restoration of the wild Clear Creek gambusia habitat, in the form of repairing the dam, is necessary if the species is to continue to exist in the wild.

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

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

The five-year status review published in 2010 identified the species' limited range and the fact that there is only one population of wild Clear Creek gambusia as an important exacerbating factor to the threats associated with habitat loss and degradation (U.S. Fish and Wildlife Service 2010b, p. 16). The review also analyzed threats to habitat from increasing groundwater withdrawals, reduced springflows, water quality degradation, and existing (previous) habitat alteration ((U.S. Fish and Wildlife Service 2010b, pp. 11–18). We reviewed these potential threats as of 2023.

Of the identified threats to Clear Creek gambusia habitat, the only one with a continuing significant impact is the existing (previous) habitat alteration. However, the impacts of this habitat alteration are severe. The damming of Clear Creek reduced the habitat where Clear Creek gambusia successfully outcompete western mosquitofish to a 0.35 acre (0.14 hectare) pool at the headwaters of Clear Creek (U.S. Fish and Wildlife Service 2010b, pp. 5, 17). Today, the dam separating this upper pool from the next impoundment has severely deteriorated, with the result being degradation of the spring pool habitat (U.S. Fish and Wildlife Service 2010b, p. 17; Texas Fish and Wildlife Conservation Office 2013, pp. 19–20; Montagne 2015a, p. 1; Napier 2015, pp. 4–6; Pandolfi 2021, entire). Vegetation management and the manipulation of the edges and bottom of the second impoundment, which was performed in the 1980s, transformed marginal Clear Creek gambusia habitat into unsuitable

habitat (U.S. Fish and Wildlife Service 2010b, p. 17). The current condition of the only site in the wild that can sustain Clear Creek gambusia is a habitat that is severely curtailed and at risk of being lost completely in the near term (Napier 2015, pp. 5–6; 2015; U.S. Fish and Wildlife Service 2015, pp. 1–2).

We reviewed the 2022 Menard County Underground Water District Management Plan and the 2021 Region F Water Plan. Most of the water use in Menard County comes from surface water supplies (Freese and Nichols, Inc. 2020, p. 1-23). The entire county pumps less than 450 acre-feet/year (555,066 cubic meters/year) from the Edwards-Trinity Plateau Aquifer on average, which is the aquifer that supplies Wilkinson Springs (Brune 1975, p. 55; Freese and Nichols, Inc. 2020, p. 3-31). The modeled available groundwater through 2070 is about 2,200 acre-feet/year (2,713,656 cubic meters/year), about five times the current annual use (Freese and Nichols, Inc. 2020, pp. 3-23–3-24). In addition, the Menard County Underground Water District Management Plan has implemented rules to regulate groundwater withdrawals and protect groundwater resources (Menard County Underground Water District 2017, pp. 4–5). Consequently, a reduction in spring flow due to groundwater withdrawals appears unlikely. That said, a significant increase in withdrawals, perhaps as a result of future drought, could present a threat to spring flows. Withdrawals should be monitored to better understand the relationship between seasonal weather conditions and groundwater withdrawals.

Similarly, based on the available information about flows in Clear Creek, there is no indication that spring flows are likely to cease under future drought, although they could be significantly curtailed. Spring flows continued throughout the drought of the 1950s and the drought of 2011 (Brune 1975, p. 55; U.S. Geological Survey 2011, p. 1; 2012, p. 1; 2013, p. 1; 2023). Some of the smaller spring outlets at Wilkinson Springs were observed to have reduced or stopped flows during the 2011 drought, causing a small drop in the pool level (Inks Dam National Fish Hatchery 2012, p. 81). Thus, future drought could lead to reduced springflow and pool levels over a period of many months to several years.

With respect to water quality, we reviewed potential sources of water contamination. We reviewed the Texas Commission on Environmental Quality's (TCEQ) Leaking Petroleum Storage Tank and Industrial and Hazardous Waste Corrective Action datasets and found no sites closer than the city of Menard, TX, which is over 15 kilometers (km) (over 10 miles) from Wilkinson Springs (TCEQ 2019b; 2019a). There are few oil and gas wells or pipelines in the area and none in the uplands above Wilkinson Springs, so the

risk of water contamination from any spills or leaks appears to be minimal (IHS 2022a; 2022b).

Overall, while threats associated with degraded water quality and quantity are currently low, the threats to Clear Creek gambusia from the alteration and curtailment of its habitat are severe and ongoing, and directly threaten the continued existence of the species in the wild.

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

This factor is not relevant to the Clear Creek gambusia.

2.2.2.3 Disease or predation:

Although disease or parasites could impact Clear Creek gambusia, and predation has been observed, these factors do not represent a threat at the scale of the population (species) level.

2.2.2.4 Inadequacy of existing regulatory mechanisms:

At the time of listing, the primary threat to Clear Creek gambusia was the extensive habitat modification of Clear Creek that forced it into closer sympatric competition with western mosquitofish than what occurred historically (Hubbs 1957, pp. 8–9; 1971, p. 1). Since listing, the species' continued existence in the wild relies upon the continued maintenance of the dam impounding Wilkinson Springs at the headwaters of Clear Creek (U.S. Fish and Wildlife Service and Rio Grande Fishes Recovery Team 1982, p. 5; U.S. Fish and Wildlife Service 2010b, pp. 11, 17–18, 21–24). The dam is currently in a severe state of disrepair and no longer functions as a barrier to separate Clear Creek gambusia from western mosquitofish and maintain the spring-influenced conditions under which the Clear Creek gambusia evolved (Napier 2015, entire; Inks Dam National Fish Hatchery 2020, p. 20; Pandolfi 2021, entire). No regulatory mechanisms exist to repair the dam. When the dam is functioning as a barrier, western mosquitofish cannot access the upper pool and compete or hybridize with Clear Creek gambusia (Edwards and Hubbs 1985, pp. 22–29; U.S. Fish and Wildlife Service 2010b, p. 22). The current risk of near-term extinction for the Clear Creek gambusia species is attributable to the absence of a functional dam.

2.2.2.5 Other natural or manmade factors affecting its continued existence:

Competition and hybridization with western mosquitofish, which is correlated with the integrity of the dam, is a major threat to Clear Creek gambusia. Hubbs (1971, entire) completed extensive studies of Clear Creek gambusia and western mosquitofish, both in situ and in the lab, that demonstrated these phenomena.

Clear Creek gambusia evolved to be best adapted to the stenothermal environment found near the outlets of the Wilkinson Springs (U.S. Fish and Wildlife Service and Rio Grande Fishes Recovery Team 1982, pp. 2–3). The species also evolved a preference for lower-than-typical aquatic pH and for Clear Creek amphipods (*Hyalella texana*) as a food source (U.S. Fish and Wildlife Service and Rio Grande Fishes Recovery Team 1982, pp. 2–3). In contrast, western mosquitofish are well adapted to eurythermal (i.e., changing temperature) environments, prefer a higher aquatic pH, and prefer insects to amphipods (U.S. Fish and Wildlife Service and Rio Grande Fishes Recovery Team 1982, pp. 2–3). When the dam is intact, the entire upper pool takes on the aquatic characteristics of the springs flowing into it (U.S. Fish and Wildlife Service and Rio Grande Fishes Recovery Team 1982, pp. 2–3). Under these circumstances, Clear Creek gambusia outcompete and eventually eliminate western mosquitofish because Clear Creek gambusia grow faster and produce more offspring in the spring-influenced aquatic conditions (Hubbs 1971, pp. 41–42).

Hybrids between the two species occur but are at an apparent competitive disadvantage in the presence of pure Clear Creek gambusia or pure western mosquitofish (Hubbs 1971, pp. 39–42). This inference is based on the fact that when the dam is slightly leaky, there is a stable hybrid swarm, but each original species remains and is present in higher numbers than the hybrids (Hubbs 1971, pp. 39–42). Thus, the threat from hybridization alone is not the same as the case of the Pecos pupfish (*Cyprinodon pecosensis*), which hybridizes with sheepshead minnow (*Cyprinodon variegatus*) if sheepshead minnow is introduced to the same water body (Echelle and Connor 1989, pp. 725–726). In that case, the hybrid swarm becomes the dominant entity, and all Pecos pupfish and most sheepshead minnow are eliminated (Echelle and Connor 1989, pp. 725–726).

When the dam impounding Wilkinson Springs is leaky, western mosquitofish migrate into the upper pool in winter and compete directly with Clear Creek gambusia for food (Hubbs 1971, pp. 26–28). As long as the number of western mosquitofish that do this remains small, Clear Creek gambusia will outcompete them by the end of the following breeding season thanks to their superior

adaptation to the environmental conditions in the spring pool (Hubbs 1971, pp. 38–43).

However, when the dam is severely leaky, as it has been since at least 2013 (Montagne et al. 2013, p. 1), two problems arise. First, a higher number of western mosquitofish are able to move into the upper pool (Hubbs 1971, pp. 39–43). When higher numbers of western mosquitofish are present, their likelihood of interbreeding with Clear Creek gambusia and producing hybrids goes up, further reducing the absolute proportion of pure Clear Creek gambusia individuals in the upper pool (Hubbs 1971, pp. 38–43). The western mosquitofish also compete directly for food resources in the winter when resources are less scarce, increasing mortality among the Clear Creek gambusia population (Hubbs 1971, pp. 26–28). The second problem with a severely leaky dam is that the environment of the upper pool is less likely to be consistently maintained in the conditions created by the spring outflows. If water temperatures become eurythermal in the upper pool and aquatic pH values rise, it could inhibit the amphipod that is the Clear Creek gambusia's preferred food source, inhibit the amphipod's preferred food and shelter plant, and increase the reproductive fitness of western mosquitofish, ultimately reducing the ability of Clear Creek gambusia to successfully compete with western mosquitofish (Hubbs 1971, pp. 5, 41–43). In addition, we know that Clear Creek gambusia compete poorly with western mosquitofish below the dam because Clear Creek gambusia are virtually absent from that habitat (Edwards and Hubbs 1985, pp. 41–42). Because of this, we infer that if the upper pool becomes more like the second pool in terms of temperature, pH, and vegetation and invertebrate community structure, that western mosquitofish will outcompete Clear Creek gambusia there as it does below the dam. As a result, Clear Creek gambusia would be eliminated from the upper pool.

Because the dam is currently severely compromised, we assume that both hybridization and competition are ongoing. These factors are the force behind the observed decline in pure Clear Creek gambusia abundance since 2013 and are likely to lead to the extinction of the species in the wild in the near future.

2.3 Synthesis

At the time the last five-year status review was developed (2009–2010), the one known wild population of Clear Creek gambusia was stable and the captive population was beginning to be developed. The dam that maintains Clear Creek gambusia habitat and the Wilkinson Springs pool was leaking, but not to a degree that western mosquitofish could invade the pool in large numbers. In addition, the owner of the property that contains the wild population of Clear Creek gambusia had signed an agreement with the Service and funding was in place to repair the dam, thus ensuring the long-term viability of the species.

Funding

was available to repair the dam from July 2010 to July 2015. During this time, no repairs were completed. In 2015, the funding that was obtained through Service grants and the Partners for Fish and Wildlife program agreement expired. Since then, the dam has deteriorated further and is currently in very poor condition. As a result, the wild Clear Creek gambusia population has declined and persists, at best, in very low abundance. No Clear Creek gambusia were observed on visits to the site in 2021 or 2022.

A captive population of Clear Creek gambusia was developed at Inks Dam NFH in order to hold a portion of the wild population safely while then-anticipated repairs to the dam were underway. The intention was not originally to propagate the species, but the hatchery did so following reports of increased hybridization levels in 2013 following continued deterioration of the dam deterioration and concerns about when repairs would be completed. Propagation went well initially, with the number of offspring produced and total population size rising. However, population fecundity peaked in 2018 and total population peaked in 2020, following by precipitous declines. During 2022, the last full breeding season, eight offspring were produced, down from a peak of 825 offspring. Even accounting for increased individual longevity in the captive environment, the population at Inks Dam NFH is likely to die out by 2030.

Taken together, the future of the Clear Creek gambusia, both in the wild at Wilkinson Springs pool and in refuge at the Inks Dam NFH, is that of continued decline and likely extirpation from the wild and captivity. Therefore, we recommend that the classification of the species remain Endangered.

3.0 RESULTS

3.1 Recommended Classification:

No change is needed

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

No change recommended.

Brief Rationale:

Taxonomically, it is one of several species in its genus. The threats to the species remain very high. However, if the dam is repaired and subsequently maintained in good condition, the habitat required by the species should continue to exist for the foreseeable future. In the past, when the dam is in good repair, the Clear Creek population in the Wilkinson Springs pool was stable.

3.3 Listing and Reclassification Priority Number, if reclassification is recommended (see 48 FR 43098):

Not applicable.

4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

1) A critical step to take to prevent extinction of the Clear Creek gambusia is to repair the dam just below Wilkinson Springs on upper Clear Creek. If this cannot be accomplished, the species will likely become extinct, rendering all other actions moot. [Recovery Action 1.411] Because the species exists only on one parcel of private property, and there is no legal mechanism to allow the dam to be repaired, securing the cooperation of the landowner will be a necessary step in completing the dam repair action.
2) The current captive population of Clear Creek gambusia is in decline, likely as a consequence of genetic drift or inbreeding depression. Genetic analyses of the captive population are in progress to determine if and how to rescue the captive population. Any recommendations that result from this project should be implemented if possible given available resources. [Recovery Action 1.32]
3) Other future actions that may be pursued if the dam is repaired and resources are available include monitoring of the wild population, supplementing the captive population, captive propagation, monitoring water quality and quantity at Wilkinson Springs pool, restoring Clear Creek, and revising the species' recovery plan. [Recovery Actions 1.31, 1.32, and 1.42]

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U.S. FISH AND WILDLIFE SERVICE

5-YEAR REVIEW of Clear Creek gambusia (*Gambusia heterochir*)

Current Classification: Endangered

Recommendation resulting from the 5-Year Review:

No change needed.

Appropriate Listing/Reclassification Priority Number, if applicable:

FIELD OFFICE APPROVAL:

Karen Myers, Lead Field Supervisor, Fish and Wildlife Service, Austin Ecological Services Field Office

Approve KAREN MYERS Digitally signed by KAREN MYERS Date: 2023.08.28 11:35:21 -05'00' _____