State of California
Natural Resources Agency
Department of Fish and Wildlife

REPORT TO THE FISH AND GAME COMMISSION

FIVE-YEAR SPECIES REVIEW OF DESERT PUPFISH (*Cyprinodon macularius*)

December 2021

Desert Pupfish male and female pair. Photo by Sharon Keeney

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I. EXECUTIVE SUMMARY

The Desert Pupfish (*Cyprinodon macularius* Baird and Girard) is currently listed as endangered under the California Endangered Species Act (CESA) (Fish and G. Code § 2050 et seq.; Cal. Code Regs. tit. 14 § 670.5, subd. (a)(2)(J)). Pursuant to Fish and Game Code Section 2077, Subdivision (a), the California Department of Fish and Wildlife (Department or CDFW [formerly California Department of Fish and Game (CDFG)]) has prepared this Five-Year Species Review to evaluate whether conditions that led to the original listing of Desert Pupfish are still present or have changed. This review is based on the best scientific information currently available to the Department, regarding each of the components listed under Section 2072.3 of the Fish and Game Code and Section 670.1, subdivisions (d) and (i)(1)(A), of Title 14 of the California Code of Regulations. In addition, this document contains a review of the identification of habitat that may be essential to the continued existence of the species and the Department's recommendations for management activities and other recommendations for recovery of the species (Fish & G. Code, § 2077, subd. (a)).

Desert Pupfish are small and exceptionally hardy fish belonging to the family Cyprinodontidae. Like other pupfishes, they are adapted to extreme environmental conditions including high salinity, low dissolved oxygen, rapid temperature fluctuations, and extreme temperatures. As a result, unless other species have been introduced, pupfish are often the only fish found within their native habitats (Cox 1966). The historic range of Desert Pupfish within California encompassed portions of Imperial, Riverside, and the eastern margins of San Diego counties. Their historic distribution within the State included the Salton Sea Sink, its tributaries, and the lower Colorado River. Other populations were historically distributed and, in some cases, remain extant outside of California (USFWS 2010) but are not addressed in this report.

Currently, California's self-sustaining populations of Desert Pupfish persist in several locations within and around the Salton Sea (S. Keeney, CDFW, pers. comm. 2020; Figure 1). Among these are a total of 12 refuge populations, mostly located within the Salton Sea Sink. Three naturally occurring stream-based populations exist in Salton Sea tributary streams (S. Keeney, CDFW pers. comm. 2020). The Salton Sea itself supports small, fluctuating, and patchily distributed populations of Desert Pupfish (USFWS 2010). All remaining naturally occurring populations exist in agricultural irrigation outflow canals. These apparently functional, yet clearly suboptimal, habitats are highly susceptible to flow fluctuations and agricultural outflow control-related stream desiccation. As a result, Desert Pupfish populations relegated to the extensive network of irrigation canals in the Salton Sea Sink can, and do, expand or contract dramatically (S. Keeney, CDFW pers. comm. 2020).

Desert Pupfish continue to face multiple threats, namely: (1) restricted distribution in often suboptimal habitats; (2) predation and competition from non-native species; (3) habitat fragmentation; and (4) loss of suitable habitat, both ongoing and predicted. Increasing demands for water, particularly to provide municipal supplies and support largescale agriculture, coupled with predicted outcomes of climate change, are additional factors that may negatively affect the ability of Desert Pupfish to persist.

In completing this Five-Year Species Review for Desert Pupfish, the Department finds there is sufficient scientific information to indicate that the threats and stressors that led to the listing of Desert Pupfish as endangered under CESA are still present and, in some cases, have worsened. The Department, therefore, recommends no change to the status of Desert Pupfish on the list of California's endangered species at this time.

II. INTRODUCTION

A. Five-Year Species Review

This Five-Year Species Review addresses Desert Pupfish. Upon a specific appropriation of funds by the Legislature the Department shall, or if other funding is available, in the absence of a specific appropriation, may, review species listed as endangered or threatened under CESA every five years to determine if the conditions that led to the original listing are still present (Fish and G. Code § 2077, subd. (a)). Desert Pupfish is also listed as endangered under the Federal Endangered Species Act. Pursuant to Fish and Game Code section 2077, subdivision (b), the United States Department of the Interior, U.S. Fish and Wildlife Service (Service or USFWS) was contacted in an effort to coordinate this status review with their five-year review process (last completed in 2010). However, the Service has no plan to complete a status review at this time or in the foreseeable future (D. Duncan, USFWS, pers. comm. 2020).

Using the best scientific information available to the Department, this Five-Year Species Review includes information on the following components pursuant to section 2072.3 and section 2077, subdivision (a), of the Fish and Game Code and section 670.1, subdivision (d), of Title 14 of the California Code of Regulations: species' population trend(s), range, distribution (including a detailed distribution map), abundance, life history, factors affecting the species' ability to survive and reproduce, the degree and immediacy of threats, impact(s) of existing management efforts, availability and sources of information, identified habitat essential for the continued existence of the species, and the Department's recommendations for future management activities and other recovery measures to conserve, protect, and enhance the species.

B. Listing and Status Review History

Desert Pupfish was listed as endangered under CESA on October 2, 1980. The main identified threats to the species at the time of listing are unknown but likely included: habitat loss, restricted distribution, and threats (predation and competition) from non-native introduced species.

On March 31, 1986, the Desert Pupfish was listed as endangered and critical habitat was designated under the Federal Endangered Species Act.

On July 1, 1990, the state Five Year Status Report for Desert Pupfish was published (CDFG 1990).

On December 8, 1993, the federal Desert Pupfish Recovery Plan was published (USFWS 1993).

In 2010, the federal Five Year Status Review for Desert Pupfish was published (USFWS 2010).

On September 26, 2019, the federal Desert Pupfish Recovery Plan was amended (USFWS 2019).

This Five-Year Species Review was prepared by Jeff Weaver and Claire Ingel, in the Department's Fisheries Branch. Sharon Keeney, lead Department biologist for Desert Pupfish, and Rob Titus, Fisheries Branch, also contributed substantially to this species review.

C. Notifications and Information Received

On November 26, 2019, the Department notified persons who had expressed their interest in CESA actions in writing to the California Fish and Game Commission (Commission) and had provided contact information to the Commission (Fish and G. Code, § 2077(a)). The e-mail notification included a link to the Department's dedicated web page for five-year reviews of threatened and endangered species at https://www.wildlife.ca.gov/Conservation/CESA/Five-Year-Reviews.

III. BIOLOGY

A. Taxonomic and Physical Description

The Desert Pupfish was described by Baird and Girard (1853) from specimens collected in the San Pedro River, Arizona (USFWS 1993). The Desert Pupfish is a small (<75 mm (2.95 in) Total Length (TL)) but hardy fish that exhibits several notable physical characteristics, many of which it shares with other pupfishes. The body is thick and laterally compressed, with a protruding terminal mouth and large eyes. Males are larger than females and the species is seasonally sexually dimorphic, with males displaying iridescent bright blue body coloration, with a prominent yellow tail, during breeding season (Moyle 2002). Females are tan to olive, with 5-8 broken lateral bars, which males also possess but less conspicuously so (Moyle 2002). The caudal fin, and often pelvic fins, have a black terminal band (Moyle 2002). Prior to early systematic studies focused on this taxon (Miller 1943, Miller 1948), most cyprinodonts in the desert southwest were lumped together as *C. macularius*. Desert Pupfish are now identified as the disjunct groups present in the lower Colorado River and, in California, the Salton Sea Sink (Moyle 2002). The Owens Pupfish (*C. radiosus*) is the closest relative to Desert Pupfish in California (Echelle and Dowling 1992), although the two may have been isolated from one another (and other pupfishes of the southwestern United States) for over two million years (Moyle 2002). A broad range of studies has been performed on the Desert Pupfish including, but not limited to, those addressing taxonomy, biogeography, physiology, genetics, and behavioral ecology. Thus, the Desert Pupfish has been regarded as one of the better understood members of the family Cyprinodontidae (USFWS 1993).

B. Life History and Ecology

The Desert Pupfish's extreme tolerances for high temperatures, rapid temperature fluctuations, high salinities and low dissolved oxygen levels (see Subsection C.iii. in this report) have been described as "legendary" (Schoenherr and Feldmeth 1991). These unique physiological tolerances allowed the species to evolve and specialize in a harsh and arid desert bioregion, with exceptionally limited freshwater aquatic habitats. This aquatic habitat scarcity, along with documented "rarity hotspots," associated with several endemic pupfish taxa, including Desert Pupfish, in the inland deserts of southeastern California is highlighted in the Atlas of the Biodiversity of California (CDFG 2003). All of which underscore the Desert Pupfish's ecological uniqueness, both within their historic range and across the spectrum of freshwater fishes native to California.

Its remarkable physiological tolerances and certain life history traits, such as preference for shallow-water habitats, likely led to habitat partitioning by, and natural segregation of, Desert Pupfish from other less-tolerant fishes (Schoenherr 1992). Other life history attributes include habitat niche specialization (Schoenherr 1979), quick population rebounds when habitat

conditions are favorable, and establishment of high densities even in smaller habitats (Schoenherr 1988, Echelle et al. 2007). The lifespan of Desert Pupfish is typically 1 year but can be as long as 3 years (Moyle 2002). The species can become sexually mature as early as 6 weeks of age (15.2 mm (0.6 in) TL) but most Desert Pupfish do not breed until their second year, when they reach their maximum length of approximately 75 mm (2.95 in) TL (Barlow 1961, Moyle 2002). Females lay one to four eggs at a time and between 50-800 eggs over the breeding season, typically from late March to late September (Schoenherr 1988, CDFW 2019a). When water temperature is constant and warm, fish may breed year-round (Schoenherr 1988). Male pupfish are usually highly aggressive during the breeding season (early spring into winter when water temperature exceeds about 20°C). During this time they establish, actively patrol, and defend individual territories that are an area approximately 1-2 m^2 (11-22 ft^2) surrounding the egg deposition site (USFWS 1993, Moyle 2002). However, while guarding their territory and protecting their own fertilized eggs, subdominant males can "sneak" into the territory of a dominant male and attempt to fertilize some of the eggs (Schoenherr 1992). This alternate reproductive life history trait likely contributes to repeat spawning and is presumably a driving factor in their high fecundity and notably quick population rebounds. Interestingly, dominant male breeding territory defense is thought to have been misinterpreted by past field observers as playful; thus, the common name of pupfish was ascribed to this species complex, after a puppy's typically playful behavior (Berra 2001).

Desert Pupfish are opportunistic omnivores (Cox 1966 and 1972 in USFWS 1993). Diet varies seasonally and includes insect larvae, detritus, aquatic vegetation, snails and, at times, their own eggs and young (Schoenherr 1992, CDFW 2019a). During breeding, subdominant "sneaker" males have been observed consuming the spawn of dominant males, while invading their territories and attempting to fertilize eggs (Schoenherr 1992). However, it has also been noted that dominant males can identify eggs fertilized by other males, within their guarded territory, and consume them preferentially (Schoenherr 1992, Loiselle 1980 in USFWS 1993). Beyond this male breeding behavior-driven intraspecific competition, both sexes have also been documented to engage in this feeding behavior, known as filial cannibalism. However, the extent to which this feeding behavior occurs in Desert Pupfish, as opposed to engaging in other more conventional feeding habits, is unknown (Cox 1972 in USFWS 1993). Schoenherr (1992) also noted that physical and visual interference from large numbers of non-native cichlids can further contribute to filial cannibalism in Desert Pupfish apparently leading, in its extreme form, to recruitment failure of Desert Pupfish populations. Desert Pupfish tend to swim in loose shoals with fish of similar size. During breeding season, these shoals are almost entirely composed of females and juveniles, owing to the territorial nature of males and their limited movement during this period (Barlow 1961, Loiselle 1980 and 1982 in USFWS 1993). Juveniles often aggregate in shallower waters than do adults. Growth is rapid and varies depending upon age, water temperature, and salinity (Moyle 2002).

C. Habitat Necessary for Species Survival

Aside from locations where non-native fishes have been introduced and persist (see Threats section in this report), as noted, pupfish are often the only fish found in their native waters. Aside from the fundamental need for isolation from competitors and predators, their other known habitat requirements include:

i. Vegetation Communities

The Desert Pupfish prefers areas with rooted aquatic vegetation, such as widgeon grass (*Ruppia maritima*) and chara, or muskgrass (*Chara* spp.), along with filamentous algae to

provide food, cover, and structure for multiple pupfish life stages and their invertebrate prey (Black 1980, Environmental Science Associates (ESA) 2017). The species does not typically occupy areas with dense emergent vegetation (Schoenherr 1992, Keeney 2016 in ESA 2017).

ii. Aquatic Ecotype

Desert Pupfish historically occupied diverse habitats, in both the Lower Colorado River system and the Salton Sea Sink, including cienegas and springs, small streams, and margins of larger bodies of water. The species tends to occur in clear, shallow, slow-moving waters with silt-sand substrates and above freezing winter water temperatures. These habitat types are regarded as essential to their long-term persistence (Black 1980, USFWS 1993).

iii. Climate and Hydrology

Desert Pupfish can withstand the highest recorded thermal maximum (44.6°C (112°F)) of any fish species, allowing them to persist even in thermal hot springs where other fishes would perish (Schoenherr 1992). They can also tolerate winter seasonal temperatures as low as 4.5°C (40.1°F) (Schoenherr 1992) and salinity levels ranging from more than twice that of sea water (68 ppt) to freshwater (Moyle 2002). The Desert Pupfish has also been recorded to survive the lowest dissolved oxygen levels of any fish species, down to as low as 0.1-0.4 mg/L (Lowe et al. 1967 in Moyle 2002; Schoenherr 1988, 1990, 1992). The species can also tolerate rapid and extreme changes in these conditions (e.g., daily water temperature fluctuations of up to 26°C (78.8°F)) (Kinne 1960, Lowe and Heath 1969, Schoenherr and Feldmeth 1991). These wide tolerances apparently allow for populations to persist through periods of low flow and habitat contraction associated with drought (S. Keeney, CDFW, pers. comm. 2020; Figure 2a). In addition, the Desert Pupfish has repeatedly been documented to persist in lotic habitats (rivers, streams and creeks) after large flooding events, unlike some co-occurring non-native fish species such as Nile Tilapia (tilapia; *Oreochromis niloticus)* and Sailfin Molly (*Poecilia latipinna*) (Schoenherr 1988). These flexible adaptive attributes may be key to long-term persistence of the species, particularly in the face of shrinking freshwater habitats within the Salton Sea Sink, along with projected temperature increases in this region associated with climate change.

iv. Reproductive Habitat

Consistent holding of territories by male Desert Pupfish has been observed from mid-April through October (Barlow 1961). Territories can be maintained in different parts of their habitat, but generally do not occur in areas deeper than 1 m. These territories are generally centered around an identifiable object or outstanding feature of the benthic profile (Barlow 1961); however, it is not clear from the literature what function(s) these features may serve in the male mating territory selection process. The size of individual territories seems to vary based, in part, on fish density and relative size of males (Barlow 1961). Eggs may be laid on sandy or muddy substrate or algal mats in either freshwater or saline environments (Schoenherr 1988). Kinne (1960) found that juveniles grow faster in fresh water at lower temperatures (15°C (59°F)) and 20°C (68°F)) and faster in salt water at higher temperatures (25°C (77°F), 30°C (86°F) and 35°C (95°F)).

IV. DISTRIBUTION AND ABUNDANCE

A. Range and Distribution

The historic range of Desert Pupfish included southeastern California, southwestern Arizona, and northern Mexico, including the Salton Sea Sink and its tributaries, the Gila River watershed, as well as the lower Colorado River and its delta (Moyle 2002). The hydrological history of the Salton Sea Sink in California is a complicated one. In prehistoric times, the Salton Trough (which is a geological feature that more broadly encompasses the Salton Sea Sink) was filled, in varying stages, by Lake Cahuilla. The history of Lake Cahuilla spans the late Pleistocene and the Holocene. During the past 2,000 years, the lake formed and receded several times, when water from the Colorado River naturally rerouted and intermittently inundated the Salton Trough (Schoenherr 1992). The lake dried completely in about the year 1500, when natural rerouting of the Colorado River completely diverted its water away from historic Lake Cahuilla and into the Gulf of California, leaving Desert Pupfish isolated in springs of the Salton Sea Sink (Schoenherr 1988). Schoenherr (1992) reported that estimates indicate that Lake Cahuilla took about 55-60 years to dry completely and offers interesting evidence of progressively descending fish traps, constructed by the native Cahuilla peoples. These traps were left behind at identifiable high water line marks of the ancient receding lake. They occurred at regular intervals that corresponded to known evaporation rates for the region, clearly demonstrating the lake's recession over a discrete period of time.

More recently, the Salton Sea Sink was reportedly reflooded seven times by overflow from the Colorado River, between the years 1840 and 1905 (Schoenherr 1992). To further complicate this history, the present Salton Sea originated in 1905-1907 when, due to an engineering accident, the Colorado River flooded the Salton Sea Sink via the historic Alamo Canal and Desert Pupfish from outside the basin were reintroduced. Desert Pupfish were documented as abundant in shoreline pools of the Salton Sea in the early 1960s (Barlow 1961) but have declined overall, in both abundance and distribution since that time within the Salton Sea itself, as well as within its broader sink. They are now patchily distributed and in low numbers within the Salton Sea itself (Parmenter et al. 2004 and Keeney 2010b in USFWS 2010) and are relegated to a very limited number of populations in their natural habitats.

Desert Pupfish now occupy only a fraction of their historic range in California (Figure 1). Habitats currently occupied by naturally distributed populations include two streams (San Felipe and Salt creeks, which are tributary to the Salton Sea), as well as shoreline pools of, and agricultural irrigation drains that empty into, the Salton Sea (USFWS 2010). A naturally occurring population also exists in a creek near Hot Mineral Spa (USFWS 2010). In total, five natural populations exist in California and no additional wild populations have either been established or reestablished in recent history (USFWS 2010). Additionally, 12 refuge populations have been established at the following locations (S. Keeney, CDFW, pers. comm. 2020):

- Anza Borrego State Park – 1 pond
- Oasis Springs Ecological Reserve – 2 ponds (Figures 5a and 5b)
- Dos Palmas Preserve – 4 ponds
- Living Desert Zoo and Gardens – 3 ponds
- University of California Riverside Palm Desert -1 pond
- Imperial Irrigation District Grass Carp Hatchery – 2 ponds, one population (Figure 2a)

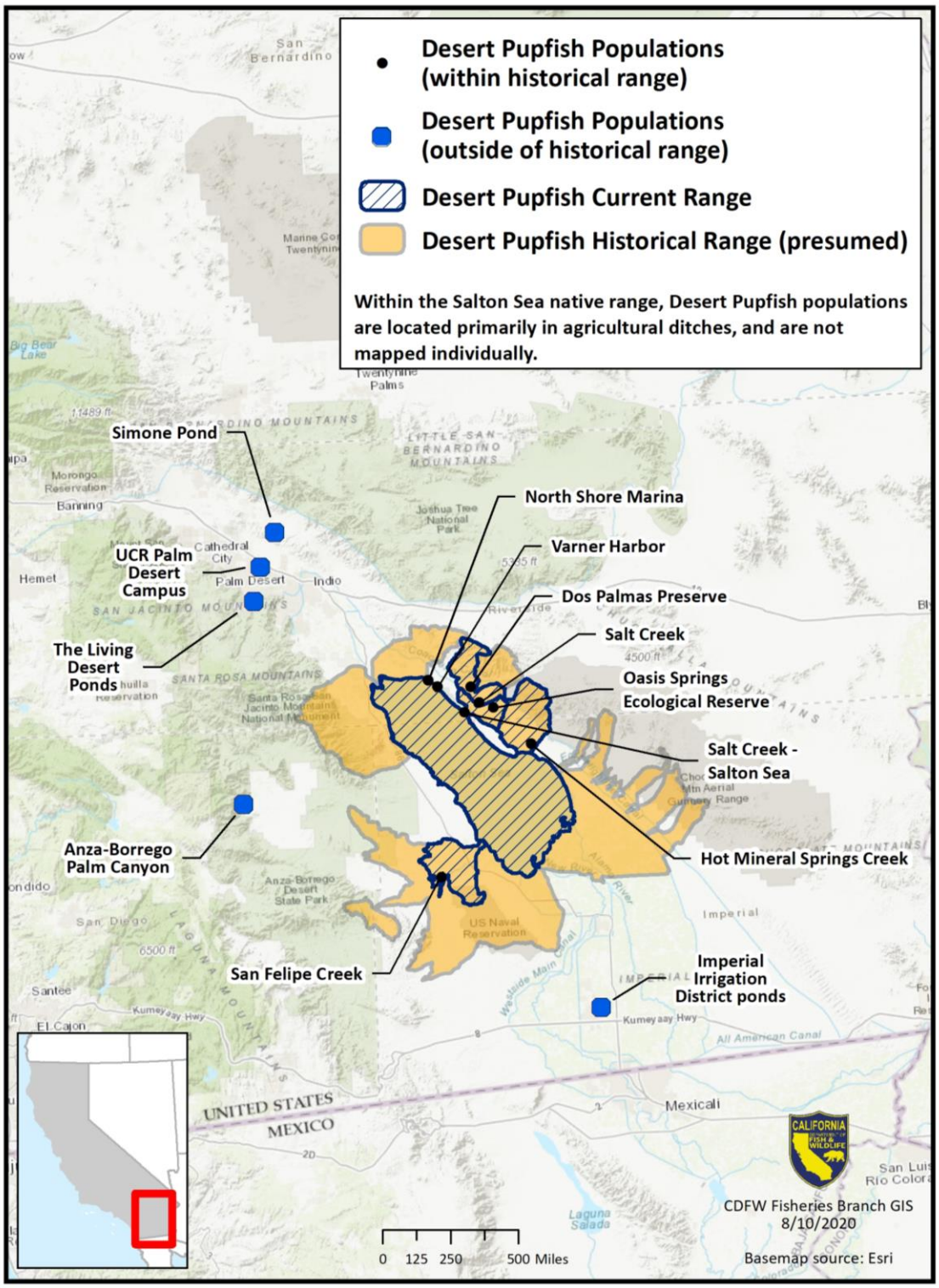

Figure 1. Presumed historic range and known current distribution of Desert Pupfish.

Figure 2a. Imperial Irrigation District refuge pond

Figure 2b. San Felipe Creek, Partially dessicated (summer 2019).

B. Population Trend and Abundance

The most recent range-wide status assessment of Desert Pupfish by the Service was summarized as “poor but stable” (USFWS 2010). Desert Pupfish numbers in the Salton Sea itself are relatively low and the fish is patchily distributed throughout (Parmenter et al. 2004 and Keeney 2010b in USFWS 2010). While populations in irrigation drains feeding into the Salton Sea can be locally abundant (Keeney 2010a in USFWS 2010), these already marginal habitats (Figure 3) remain dominated by non-native fishes (Martin and Saiki 2005) and are subject to widely varying flows. Both the Salt Creek and San Felipe Creek populations expand and contract seasonally, depending on available wetted habitat (see Figure 2a for example of partial dessication of San Felipe Creek). Lower San Felipe Creek, east of Highway 86, maintains flow provided by an irrigation drain and Desert Pupfish are seasonally abundant there (S. Keeney, CDFW, pers. comm. 2020). When conditions are suitable, Desert Pupfish have historically occurred in the lower ends of various other washes of the Salton Sea Sink; however, reduced flows have resulted in fewer of these habitats available to sustain pupfish populations. Shoreline pools of the Salton Sea that were historically large, perennial, and typically utilized by Desert Pupfish have also been substantially reduced in size in recent decades, further contributing to the notably restricted distribution of Desert Pupfish (S. Keeney, CDFW, pers. comm. 2020).

V. THREATS AND SURVIVAL FACTORS

A. Factors Affecting Ability to Survive and Reproduce

Title 14 C.C.R. section 670.1(i)(1)(A) requires the Commission to consider the following factors when determining whether a species should be listed as threatened or endangered in California: present or threatened modification or destruction of its habitat; overexploitation; predation; competition; disease; and other natural occurrences or human-related activities.

i. <u>Modification or destruction of habitat</u>

Desert Pupfish habitats have declined in quality or been lost altogether since the mid-19th century due to human landscape alteration and natural resource consumption, including such activities as urban development, water use, agriculture, and introduction of non-native

species. Suitable habitat for California populations has also been intermittently reduced or lost due to periodic contraction of the Salton Sea (e.g., during the severe drought from 2012-2016) and due to water diversion via the All-American Canal, which led to nearly complete dewatering of the lower portions of the Colorado River and its delta (USFWS 2010).

Agriculture, Water Impoundments and Diversions. Agriculture is a principal land use in Imperial and Riverside counties, particularly within the Imperial and Coachella valleys. According to the 2018 Imperial County Office of the Agricultural Commissioner's (ICAC) "Agricultural Crop & Livestock Report 2018," over 500,000 acres of harvested land for crop production were reported in Imperial County and the associated gross production value, including livestock, was over $2 billion (ICAC 2018). Agriculture is also listed as the largest industry in the Imperial Valley, which encompasses the Salton Sea Sink. Agricultural impacts within the Salton Sea Sink include water quality impairment via pollutant inputs (i.e., pesticides, herbicides, fertilizers). Accumulation of environmental contaminants (particularly mercury) in Salton Sea Sink waters were noted as threats by the USFWS at the time of federal listing (USFWS 2010). Since that time, selenium levels have become a greater concern, along with heavy metals and organochlorides accumulating in waterways emptying into the Salton Sea (USFWS 2010). Agricultural drainage outputs in the basin are also highly regulated and can vary widely in terms of seasonal flows, depending on the season, crop type, available water rights, and other factors (S. Keeney, CDFW, pers. comm. 2020).

While impacts associated with agricultural runoff may negatively affect the long-term habitat viability and persistence of Desert Pupfish populations, diminished agricultural runoff and reduced drainage flows may be of equal, if not greater, concern (S. Keeney, CDFW, pers. comm. 2020). This is a somewhat counterintuitive situation: on the one hand, agricultural runoff contributes known pollutants to Salton Sink waterways and to the Salton Sea itself, degrading water quality (and, thus, potentially habitat suitability for pupfish) while, on the other, a reduction in those flows can have the twofold impact of increasing pollutant concentrations while also reducing surface flows and available habitat quantity. One of the principal contributors to reduced agricultural water supplies in the Salton Sea Sink in recent decades is the 2003 Quantification Settlement and Agreement (QSA) and its associated water transfer agreements. This multi-faceted and complex contractual arrangement was entered upon by the San Diego County Water Authority (SDCWA), Coachella Valley Water District (CVMD), Imperial Irrigation District (IID), and the Metropolitan Water District of Southern California. The agreement reallocated irrigation water from the Colorado River, previously delivered to farms in Coachella and Imperial valleys, for municipal water use in southern coastal California. To avoid ongoing water overdrafts to support the increasing demands of municipal supply in San Diego and surrounding areas, the agreement provides for the eventual transfer of up to 30 million acre-feet of water (or up to 200,000 acre-feet per year), for up to 75 years. This constitutes the largest farm-to-city transfer of water in U.S. history (Water Education Foundation 2021).

Once the QSA and associated agreements are fully implemented (scheduled for 2021), agricultural inflow that sustains the Salton Sea's current levels is expected to substantially decrease, causing the already shrinking sea to lose more of its current surface acreage. The ongoing and predicted extent of lake level reduction, primarily owing to reduced agricultural inflows combined with climate change impacts, may lead to permanent isolation of Desert Pupfish populations in Salton Sea tributaries and irrigation inflow channels. These waterways have, in the past, been intermittently connected to the Salton Sea (S. Keeney, CDFW, pers. comm. 2020), providing some level of interbreeding between usually isolated populations. During past wetter periods, Desert Pupfish were able to utilize the shallow

near-shore environment of the lake to migrate and interbreed, leading to increased genetic diversity (USFWS 1993). In short, permanent isolation associated with shrinking aquatic habitats across the Salton Sea Sink remains a principle threat to the persistence of Desert Pupfish.

Decreases in agricultural outflows, associated with the QSA, will also likely impact Desert Pupfish, and perhaps other fishes and aquatic organisms in these habitats, via reduced surface flows and associated increased water quality impairment in agricultural drains they occupy (Figure 3). For example, selenium levels are projected to increase within Salton Sea irrigation drains, which could lead to reduced egg viability, increased mortality of larval Desert Pupfish, and increased vulnerability to pathogens (S. Keeney, CDFW, pers. comm. 2020). In addition, increased salinity associated with lake level reduction will presumably exclude Desert Pupfish from utilizing the majority of the Salton Sea itself, likely leading to further reduction in gene flow between populations (USFWS 2002). As mentioned in Moyle (2002), if remaining natural populations of Desert Pupfish remain largely reliant upon agricultural irrigation outflows, which are often unreliable in both quantity and quality, their status is not likely to improve.

Figure 3. A typical Salton Sea irrigation drain, representing much of the Desert Pupfish's remaining available habitat (aside from a few natural streams and mostly artificial refuges), all of which require ongoing human intervention, management, and maintenance. Populations of Desert Pupfish in irrigation outflow drains are particularly vulnerable to abrupt habitat dessication, due to agricultural irrigation reductions or other outflow modifications, as well as ongoing threats from agricultural effluents and other pollutants and resulting poor water quality.

Groundwater Pumping. Groundwater, or aquifer, pumping is largely associated with large-scale agricultural irrigation and municipal water demands. Increased groundwater pumping often occurs when a region's surface water budget is insufficient to meet demands. Freshwater demand continues to increase in the Salton Sea Sink region, as among many other parts of the State, taxing already limited supplies in the notably arid environment of

much of inland southern California. Excessive groundwater pumping can lead to overextraction of aquifers and cause a range of environmental impacts, including documented loss or degradation of aquatic habitats supported by aquifer-fed spring systems (Pinter and Keller 1991). Given their already tenuous situation, further reduction or loss of habitat could pose a substantial threat to Desert Pupfish. This threat is underscored by the extremity of the desert environment in which they occur, amplifying the severity of loss of any remaining suitable aquatic habitats.

ii. Overexploitation

Overexploitation, as a result of commercial, recreational, scientific, or educational activities, was not considered a threat at the time of listing, and there is no information to suggest that it has since become a threat (CDFG 1990, S. Keeney, CDFW, pers. comm. 2019).

iii. Predation and Competition

Black (1980) noted that the abundance of non-native fish species in Desert Pupfish habitats should be considered a principal threat and justification for listing as an endangered species. The State of California's 1990 Five-Year Status Report (CDFG 1990) also indicated that the presence of non-native species was a principal factor affecting the Desert Pupfish's abundance, distribution, and ability to persist. The 2019 USFWS Recovery Plan amendment cited the introduction, and largely unchecked spread, of non-native species as one of the two main threats to Desert Pupfish (USFWS 2019). The amendment also notes that continued non-native species proliferation and associated increasing impacts to Desert Pupfish are expected in the future (USFWS 2019). More specifically, threats to Desert Pupfish associated with non-native species include: (1) competition and displacement by three species of tilapia (Nile Tilapia, Redbelly Tilapia (*Tilapia zillii*) and Mozambique Tilapia (*Oreochromis mossambicus*)(Schoenherr 1985), as well as Sailfin Molly, Porthole Livebearer (*Poeciliopsis gracilis*), and Mosquitofish (*Gambusia affinis*); (2) predation from Largemouth Bass (*Micropterus salmoides*), Mosquitofish, tilapia, and potentially American Bullfrog (*Lithobates catesbeianus*); (3) habitat alteration and/or reduction (mainly from tamarisk (genus Tamarix) invasion); (4) interference with Desert Pupfish reproduction by Sailfin Molly and other non-native fishes; (5) potential disease transmission; and (6) habitat displacement (by tilapia in particular) (S. Keeney, CDFW, pers. comm. 2019). In addition, Shortfin Molly (*Poecilia mexicana*), Red Shiner (*Cyprinella lutrensis*), and the Mozambique Mouthbrooder (*Oreochromis mossambicus*) have also been reported as established in the Salton Sea (Black 1980), potentially further threatening native aquatic species, including Desert Pupfish

Other invasive fauna in the Salton Sea Sink include: Yellow Bullhead (*Ameiurus natalis*), Bluegill (*Lepomis macrochirus*), Carp (*Cyprinus carpio*), Goldfish (*Carassius auratus*), spiny softshell turtle (*Apalone spinifera*), Rio Grande leopard frog (*Lithobates berlandieri*), red swamp crayfish (*Procambarus clarkii*), and multiple unknown/unidentified snail species. Occasionally, other exotic fishes are found in the Salton Sea Sink, generally presumed to originate from "aquarium dumps," including Oriental Weatherfish (*Misgurnus anguillicaudatus*), Swordtail (*Xiphophorus hellerii*), and Jumping Guabine (*Anablepsoides hartii*) (S. Keeney, CDFW, pers. comm. 2020). However, the extent to which these lesser-detected species may impact Desert Pupfish remains unknown.

iv. Disease

Disease was not known to be a threat to Desert Pupfish at the time of listing, and there is no information to suggest that it has since become a threat (CDFG 1990). However, the USFWS (2010) noted that Desert Pupfish are susceptible to parasites, are known to exhibit infestations of anchor worm (*Lernea* spp.) and that Desert Pupfish populations may be at risk for pathogen outbreaks. This threat would likely be exacerbated by high fish densities in already suboptimal habitats (e.g., agricultural drainage ditches), where existing stressors, such as poor water quality, may enhance disease transmission.

v. Other natural occurrences or human-related activities

Other factors that may negatively affect the ability of Desert Pupfish to persist primarily include genetic threats and climate change.

Genetics. Recent genetic studies indicate that the three naturally occurring populations of Desert Pupfish in the Salton Sea Sink (San Felipe Creek, Salt Creek, and the Salton Sea and its associated irrigation drains) should be treated as two separate management units: (1) the San Felipe Creek/San Sebastian Marsh system; and (2) the other remaining populations, as a collective, within the Salton Sea Sink (Echelle et al. 2007). Loftis et al. (2009) found that the remnant populations of wild Desert Pupfish in the Salton Sea Sink retain a high degree of genetic variation; therefore, preserving their population interconnectivity, whether naturally or artificially via human intervention, will be required to maintain this high level of genetic diversity into the future. Already largely disjunct Desert Pupfish populations have been naturally and artificially further isolated by changes in hydrology and human water use in the Salton Sea, Salt Creek, and San Felipe Creek; as such, some local populations are now smaller, restricted to limited and suboptimal habitats, and are subject to genetic bottlenecks or extirpation. These conditions, if not corrected, will likely lead to both reduced genetic diversity and evolutionary adaptability of the species. It is imperative, therefore, that a genetics management plan and scientifically rigorous associated approach toward management of Desert Pupfish is implemented (S. Keeney, CDFW, pers. comm. 2020).

Climate Change. The inland deserts region of California, which encompasses the Salton Sea Sink, is the hottest and driest part of the State and the region's climate is becoming more extreme, with daily average high temperatures projected to increase by up to 4-8°C (8-14ºF) by the end of this century (Hopkins 2018). Rainfall rates are currently low (generally between 8-13 cm (3-5") per year) and are highly variable from year to year. This variability is projected to increase in coming decades, with extreme drought and extreme wet events both predicted to become more common (Hopkins 2018). Given the area's already heavy dependence on water from outside sources, coupled with competing demands for remaining in-basin water supplies to meet agricultural, municipal, recreational, and ecological needs, future climate warming and increased variability and extremity of weather patterns will undoubtedly exacerbate existing challenges. Desert Pupfish, with their already very limited distribution in generally marginal and shallow-water habitats, are highly vulnerable to climate change impacts (Moyle et al. 2012). Consequently, ongoing establishment of refuge populations in climate change-resilient habitats will be imperative to ensure their persistence into the future.

B. Degree and Immediacy of Threats

The Desert Pupfish continues to face multiple threats, including the restricted and disjunct areas the species currently occupies in often suboptimal habitats. Other primary threats include: (1) predation and competition from non-native species; (2) genetic impacts from founder effects and bottlenecks, coupled with ongoing isolation of small populations with limited or no gene flow; and (3) predicted climate change impacts within the already arid historic range of Desert Pupfish. Future water use, along with associated management and conservation measures in the Salton Sea Sink will be critical factors influencing the ability of the species to persist (USFWS 2010). The degree and immediacy of these threats, in aggregate, is not clearly understood; however, they are likely synergistic and may pose compounding risks to the species. Rapidly increasing human development and demand for water, particularly in the desert region where Desert Pupfish exist, along with potential for more extreme weather patterns associated with climate change (especially prolonged drought), may dramatically increase the rate and severity of habitat loss and other future threats to this species.

VI. MANAGEMENT AND RECOVERY

A. Impact of Existing Management Efforts

Management and recovery of Desert Pupfish has, to date, been largely funded and supported by several local, state, and federal partners. These organizations include: the Center for Natural Lands Management (CNLM), the Living Desert Zoo and Gardens, Coachella Valley Mountains Conservancy, Coachella Valley Conservation Commission (CVCC), Southern Low Desert Resource Conservation District (RCD), Southwest Resource Management Association (SRMA), IID, SDCWA, CVMD, other Coachella Valley Multiple Species Habitat Conservation Plan (CVMSHCP) partners, the Department, California Natural Resources Agency, California State Parks (State Parks), University of California, Department of Water Resources (DWR), the Bureau of Land Management (BLM), Bureau of Reclamation, and the Service. Conservation measures are being planned and implemented to maintain connectivity among irrigation drains, mitigate the effects of impaired water quality and selenium increases in these drainage systems, and to minimize the impacts of potential increases in Salton Sea salinity levels. These measures are intended to offset surface water discharge reductions resulting from the 2003 QSA. Fortunately, much of the Desert Pupfish's remaining habitat is located within the boundaries of the area defined under the CVMSHCP. This multi-species plan incorporates conservation of all pupfish habitat, monitoring, and planning in its implementation. In addition, partners have secured recovery grant funds to stabilize and create new Desert Pupfish refuges (S. Keeney, CDFW, pers. comm. 2020).

i. Desert Pupfish Population Monitoring

Ongoing population monitoring is a key management element for evaluating Desert Pupfish status and trends. Monitoring surveys, using minnow traps and catch per unit effort, are consistently conducted in selected habitats (Table 1). Surveys are generally conducted from late March through late October or early November, during which Desert Pupfish are most readily observable. The frequency of surveys ranges from quarterly to every 5-10 years (S. Keeney, CDFW, pers. comm. 2020).

ii. Creating and Maintaining Desert Pupfish Refuges

Over the last 30 years, several Desert Pupfish refuge ponds have been created, both within the historic range of Desert Pupfish and outside of the Salton Sea Sink. Refuge populations established since the last CDFW Desert Pupfish 5-year review (CDFW 1990) include populations at the UCR Palm Desert campus, the Imperial Irrigation District, and at the Living Desert Zoo and Gardens and Dos Palmas Ecological Reserve (S. Keeney, CDFW, pers. comm. 2020; Figure 1).

Some Desert Pupfish refuge populations have been extirpated in recent years. Actions are being taken at many of these sites to secure and restore habitat in order to reintroduce Desert Pupfish populations. Refuge sites at the Anza-Borrego Visitors Center and Palm Spring were lost after the natural springs that fed pupfish ponds dried. At the Coachella Valley Preserve (Simone/McCallum Pond), predation and competition from crayfish and other non-native species led to the extirpation of the Desert Pupfish population (S. Keeney, CDFW, pers. comm. 2020).

Table 1. Desert Pupfish monitoring activities and population trends (Keeney 2006). See Figure 1 for site locations.

Site	**Frequency**	**General Trend**	**Comments**
San Felipe Creek	1-2x per year	Extant; may be increasing with improved flow	Department staff perform weekly/biweekly site visits with photo documentation; notably reduced flows from 2016-2019 with drying of previously wetted habitats observed
Salt Creek	3-4x per year	Unknown	Stream system experienced severe desiccation during summer 2020
Hot Mineral Spa Creek	1-2x per year	Extant and abundant; likely relatively stable	Perennial flow
Coachella Valley Water District Irrigation Drains (north Salton Sea)	Irregular, every 1-6 years	Extant in most drains; abundant in some drains; probably stable	Department staff survey all CVWD drains that historically contained desert pupfish; tilapia numbers appear to be significantly lower than during the 1990s and 2000s
Imperial Irrigation District Irrigation drains	Intermittent; every 5 years or longer	Extant in some drains, presence and abundance unknown in others; possibly decreasing, in aggregate, due to habitat loss	Numerous drains were previously inaccessible due to private land access denial; recent access revealed habitat desiccation and reduction in connectivity of large shoreline pools
A4 Pond (USFWS Refuge Unit 1) and associated drain	Annual (1x per year)	Extant and abundant; likely stable	Not an official refuge

Site	Frequency	General Trend	Comments
Salton Sea (Varner Harbor)	1-2x per year	Extant and abundant; likely stable (near-term)	Reduced water quantity and increased salinity; however, apparently fewer non-natives
Salton Sea (North Shore Marina)	1-2x per year	Extant and abundant; likely stable	
Anza-Borrego Palm Canyon Pond	1-2x per year	Extant and abundant; likely stable	Long-established emergent vegetation removal needed to maintain required open water habitats
Dos Palmas Barn Pond Refuge	3x per year	Extant; appears to be decreasing although still abundant	BLM and Department staff plan pond restoration; non-native species include crayfish, Sailfin Molly, mosquitofish, snails
Dos Palmas S-1 pond Refuge	3x per year	Extant and abundant; stable to increasing	
Dos Palmas S-2 Pond Refuge	3x per year	Extant and abundant; stable to increasing	
Dos Palmas S-3 Pond Refuge	3x per year	Extant and abundant; likely stable to increasing	Non-native species present; Department and BLM staff perform ongoing suppression and removal of non-natives until eradicated
UCR Palm Desert Pond Refuge	1-2x per year	Extant; stable	
Imperial Irrigation District Ponds Refuge	1-2x per year	Extant and abundant; probably increasing	Two small ponds adjacent to each other; managed as one population
Oasis Springs Ecological Reserve Ponds (2 refuge ponds)	4x per year	Extant; likely stable to decreasing	New well in lower pond - pupfish reintroduced in 2020; non-natives in upper pond - pupfish recruitment is limited; Department staff continue non-native species suppression and removal until eradicated
The Living Desert Sonoran Pond	2x per year	Extant	Goldfish, mosquitofish and cattails removed during 2020 restoration; pond stocked with pupfish rescued from drying conditions in Salt Creek
The Living Desert S. Palm Garden Pond Refuge	1-2x per year	Decreasing or extirpated	Causes include mosquitofish presence and excessive shading

Site	Frequency	General Trend	Comments
The Living Desert N. Palm Garden Pond Refuge	1-2x per year	Decreasing or extirpated	Causes include mosquitofish presence and excessive shading
The Living Desert Sharon Pond Refuge	1-2x per year	Extant, abundant, and increasing; new pond (est. 2020)	Received pupfish from depopulated Borrego Springs High School Pond in May and June, 2020
The Living Desert Chase Pond Refuge	1-2x per year	Extant, abundant, and increasing; new pond (est. 2020)	Sonoran Pond pupfish relocated to this pond prior to 2020 restoration
Anza-Borrego Visitor Center Pond Refuge	N/A	Extirpated September 2017	Repairs needed; potential pupfish reintroduction site
Anza-Borrego Palm Spring Pond Refuge	N/A	Extirpated in 2011	Natural spring source dried during summer 2011 for the first time; no current plans to reintroduce pupfish
Salton Sea State Recreation Area Pond Refuge	N/A	Extirpated in 2011	Likely causes of extirpation include predation by raccoons and damage to water delivery system; potential site for pupfish reintroduction
Coachella Valley Preserve Simone Pond Refuge	N/A	Extirpated 2009; last two remaining pupfish were relocated to another refuge	Extirpation likely caused by non-native species (crayfish, various snail species, mosquitofish, platy, Asian clam); CNLM currently restoring pond; pupfish may be reintroduced in 2021
Coachella Valley Preserve Visitor Center Pond Refuge	N/A	Extirpated 2004-2005	Likely causes of extirpation include competition with mosquitofish and dense emergent vegetation encroachment

iii. Coachella Valley Multiple Species Habitat Conservation Plan

The Salt Creek Desert Pupfish population, CVWD irrigation drains and shoreline pools, and several refuge populations are all located within the boundaries of the CVMSHCP (Figure 4). The CVMSHCP was finalized in 2008, when the Department issued the Natural Community Conservation Plan Permit on September 9, and the Service issued its HCP Permit on October 1. The general approach for the CVMSHCP Reserve System is to maintain and preserve habitats and ecological processes in a size and configuration that will provide for the conservation of covered species and natural communities (CVMSHCP$_a$ 2016). Conservation measures specific to Desert Pupfish include: (1) ensuring conservation of occupied habitat; (2) ensuring maintenance of refuge populations in the Thousand Palms and Dos Palmas Conservation areas; 3) protecting essential ecological processes; and (4)

implementing biological monitoring and adaptive management to ensure long-term persistence (CVMSHCP$_b$ 2016).

Agricultural Drain Management and 25-Acre Replacement Habitat. The CVMSHCP requires that the agricultural drain populations be conserved through a Management Program that ensures maintenance of agricultural drains in a manner that maintains viable habitat. The program's associated Management Plan is being drafted but has not yet been implemented (S. Bigley, CVWD, pers. comm. 2020). In addition to implementing the Management Plan, CVWD will establish at least 25 acres of Desert Pupfish replacement habitat to mitigate for disturbance of drain and flood control channel habitat resulting from maintenance activities. The 25-acre replacement habitat will be located on the north shore of the Salton Sea (Figure 4). CVMSHCP will take over management of the site after suitable habitat has been established. In addition to sustaining the 25 acres in-perpetuity for Desert Pupfish, CVWD will develop a study to determine the impacts of drain maintenance on pupfish occupying these environments, with the intended goal of adjusting maintenance practices to minimize potential take of Desert Pupfish (CVMSHCP$_c$ 2016).

Dos Palmas Conservation Area. In 1980, the California Desert Conservation Area (CDCA) Plan designated 2,503 non-contiguous acres as the Salt Creek Desert Pupfish/Rail Habitat Area of Critical Environmental Concern (ACEC) to protect washes, seeps, and springs, which provide habitat for Desert Pupfish and other species. In 1998, the CDCA Plan was amended to expand the ACEC to 14,419 acres and was named the Dos Palmas ACEC. Ownership includes federal, state, private (both conservation and non-conservation based), and SDCWA mitigation lands. The Dos Palmas Conservation Area includes the Dos Palmas ACEC, managed by BLM, the Oasis Springs Ecological Reserve, managed by the Department, and a portion of the Salton Sea State Recreation Area, managed by State Parks. This area comprises part of the CVMSHCP Conservation Boundary (CVCC 2011; Figure 4).

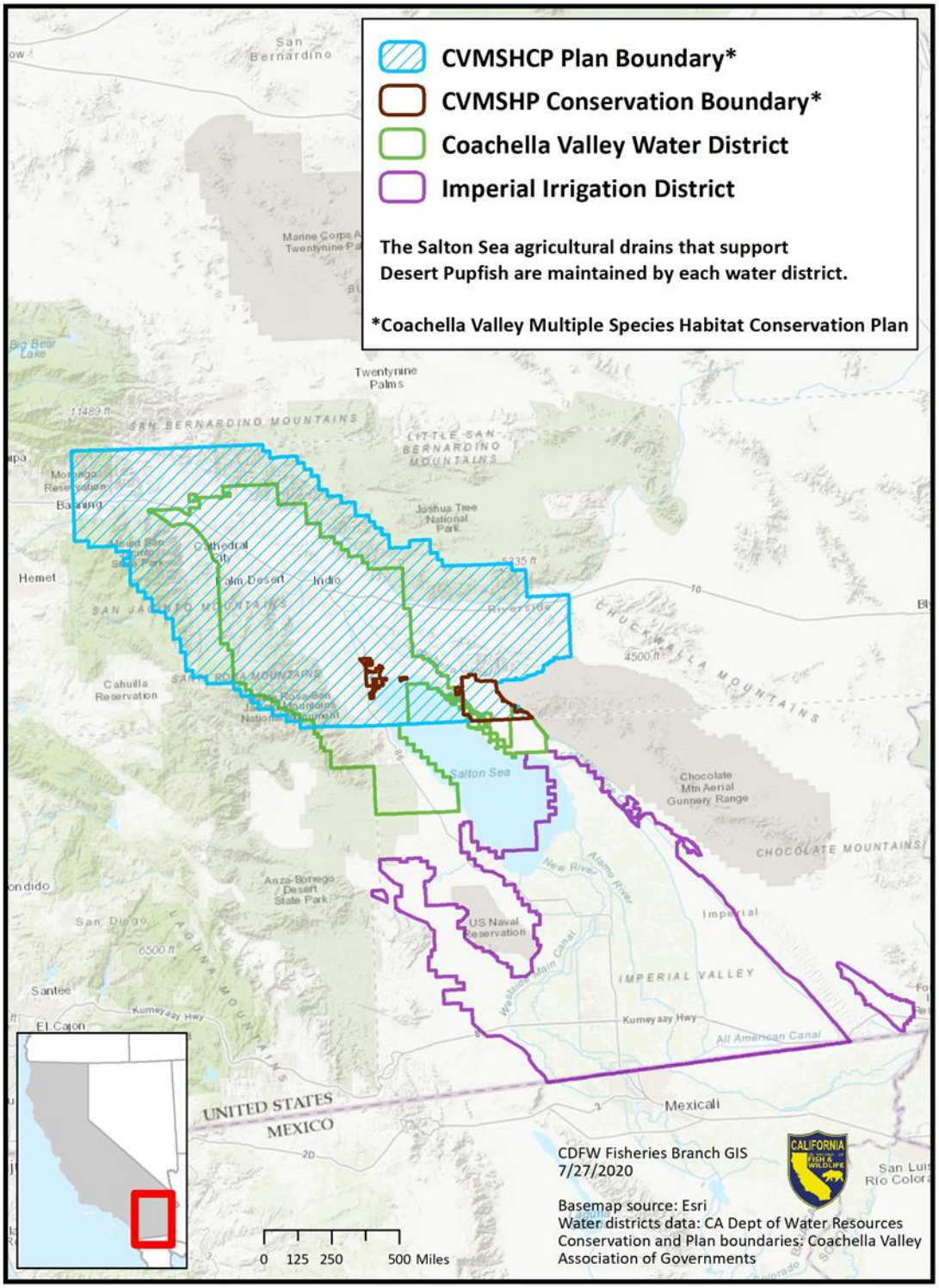

Figure 4. Area boundaries of the Coachella Valley Multiple Species Habitat Conservation Plan (CVMSHCP), CVMSHCP Desert Pupfish Conservation, Coachella Valley Water District, and Imperial Irrigation District.

The Oasis Springs Ecological Reserve includes two oases, each containing a pond watered by an artesian well (Figure 1, Figures 5a and 5b). The Department installed a new well for the upper pond in the late 1990s; however, the lower pond's supply well was not replaced. The Desert Pupfish population in the lower pond was subsequently lost, likely due to reduced groundwater aquifer levels and resultant degradation of water quality (Keeney 2019). The Coachella Valley Mountain Conservancy awarded a Proposition 1 Grant to the Department for the construction of a new well in September, 2016 and well construction was completed in September, 2018. Desert Pupfish were re-established in the lower pond in 2020 (S. Keeney, CDFW, pers. comm. 2020).

In Upper Salt Creek (see Figure 1 for general location), the SDCWA manages instream flow to maintain a surface outflow, which provides Desert Pupfish habitat. These flows are part of the SDCWA's required mitigation for the Coachella Canal Lining Project (SDCWA 2019). Historically, the average volume of outflow water required to create surface flow in the steambed has been approximately 623 acre-feet per year (SDCWA 2019). However, in recent years, as much as 4,850 acre-feet per year have been required (S. Keeney, CDFW, pers. comm. 2020). This large disparity likely speaks to reduced aquifer groundwater recharge in the basin, coupled with increasing temperatures and associated landscape-level changes, creating the need for more water to achieve past flow levels and provide required habitat. Given the importance of this artificially sustained stream habitat, CVWD and Department staff regularly monitor water quality and flows in upper Salt Creek to inform ongoing and adaptive management of water releases. A monitoring network of wells, piezometers, and gages has also been installed to monitor groundwater recharge and surface flows in the Core Marsh and Main Drain Hydrological areas (SDCWA 2019).

Figure 5a. Oasis Springs Ecological Reserve, Lower Oasis

Figure 5b. Oasis Springs Ecological Reserve, Lower Pond

Vegetation control and removal are ongoing tasks required to maintain suitable Desert Pupfish habitat, both within the Dos Palmas ACEC, and elsewhere within their range. The Department, BLM, CNLM, the Southern Low Desert RCD, private property owners, and SDCWA have partnered to take a systematic approach to tamarisk control in the northern and western portions of the Salt Creek watershed. These efforts are intended to reduce habitat encroachment and water loss through transpiration. BLM is removing tamarisk in the Salt Creek watershed and the Department has completed limited control activities in the Oasis Springs Ecological Reserve. Department staff also assisted BLM in applying for, and

obtaining, a grant from the Wildlife Conservation Board for tamarisk control. The SDCWA has provided funds to BLM to control tamarisk and restore native vegetation, as part of its required mitigation for the Coachella Canal lining (CVCC 2011). Part of this funding source has been allocated to support ongoing tamarisk removal and replanting with suitable vegetation (CVCC 2018). In 2020, the Living Desert received a Proposition 1 Grant from the Coachella Valley Mountains Conservancy to remove and control 70 acres of tamarisk in the lower reaches of Salt Creek (Danoff-Burg, pers. comm. 2020). Treatment and removal activities occurred during the winter months of 2020 and out-planting of native vegetation is planned for spring 2021 (S. Keeney, CDFW, pers. comm. 2020).

iv. San Felipe Creek Ecological Reserve

The San Felipe Creek Ecological Reserve encompasses approximately 1,900 acres of marsh, creosote bush scrub, mesquite woodland, mesquite dune, alkali sink scrub, and wash community (CDFW 2019b). The property was designated as an ecological reserve by the Commission in 1994; a checkerboard of Department and BLM land ownership within the reserve includes most of the Desert Pupfish's federally-designated critical habitat in San Felipe Creek.

By 1992, planned construction of a barrier to exclude tilapia from Desert Pupfish habitat in San Felipe Creek was suspended indefinitely. The barrier was deemed unnecessary because: (1) tilapia populations in the Salton Sea were declining; (2) portions of the stream system became intermittently dry and prevented upstream migration of tilapia; and (3) periodic flash floods often wash tilapia downstream out of the creek (CDFG 1996). In 1993 and 1994, Department and BLM personnel participated in a prescribed burn and pothole blasting project at Carrizo Marsh, near San Felipe Creek, to create pool habitat and remove tamarisk (CDFG 1996). In the 2000s, BLM and the Department partnered in tamarisk control within strategically planned areas of San Felipe Creek, including Harper's Well Wash and Tarantula Wash (S. Keeney, CDFW, pers. comm. 2020). The Department is currently partnering with BLM, USGS, SRMA, State Parks, and the Service to seek grant funding to implement San Felipe Creek management actions, including the installation of monitoring wells and further invasive vegetation control (S. Keeney, CDFW, pers. comm. 2020).

v. Salton Sea Species Conservation Habitat Project

The Salton Sea Species Conservation Habitat (SCH) Project is located along the southern shore of the Salton Sea, at the mouth of the New River, in Imperial County. The project is sponsored by the California Natural Resources Agency and implemented through the Department and DWR. The SCH Project created 3,770 acres of replacement ponds on the lakeshore's exposed playa to mitigate for near-term losses of shallow, saline habitats. These mitigation measures were intended to specifically support piscivorous bird species and, more generally, to create sustainable aquatic habitats and natural communities. Additional goals of the project are to provide suitable water quality for fish species, minimize the risk of the bioaccumulation of selenium, and minimize the risk of disease and toxicity to wildlife and plants (ESA 2017). Habitat conditions in the SCH ponds are expected to be suitable for Desert Pupfish. Desired conditions for Desert Pupfish in these ponds include: (1) maintaining connectivity for populations in adjacent irrigation drains and the Salton Sea until the ponds are filled; and, (2) ensuring that self-sustaining Desert Pupfish populations are established (minimum of 2,000 adults per pond) and persist for the life of the project (ESA 2017).

B. Recommendations for Management Activities and Other Recommendations for Recovery of the Species

The Department recommends the following actions to ensure the long-term persistence of Desert Pupfish:

1. Continue maintenance of existing habitats and population monitoring:
    - Monitor all existing Desert Pupfish populations (Table 1) in order to identify threats and inform adaptive management strategies. Finalize, distribute, and implement the Desert Pupfish Refuge Management Plan with fellow agencies and conservation partners.
    - Continue providing supplemental flows to Salt Creek, in accordance with the Coachella Canal Lining Project mitigation requirement.

2. Protect and expand populations and their habitats:
    - Develop a comprehensive groundwater basin model for the San Felipe Creek watershed to identify the water source(s), define variability of the natural discharge of San Felipe Creek springs, and evaluate susceptibility of these springs against climate change and groundwater development models.
    - Develop and implement comprehensive non-native vegetation control for San Felipe Creek, Salt Creek, the creek near Hot Mineral Spa, and Salton Sea irrigation drains. Water-consumptive tamarisk and other non-native plants should be removed and replaced with native plants.
    - Maintain open water habitat in refuge ponds containing cattails and bulrush. Ideally, ponds should contain approximately 75% open water mixed with 25% rooted aquatic vegetation (S. Keeney, CDFW, pers. comm. 2020).
    - Develop and implement an aquatic invasive species control plan for all Desert Pupfish habitats, both existing and planned.
    - Identify backup water sources for all refuges to mitigate for potential water system failures.
    - Coordinate with county planners to identify planned or approved expansion of development and groundwater pumping in areas that will directly or indirectly affect Desert Pupfish populations, especially in locations outside of the CVMSHCP plan area.
    - Coordinate with conservation partners to acquire and conserve habitat within and adjacent to tributaries accessible to Desert Pupfish and protect existing habitat from degradation (e.g., excluding off-highway vehicle access).

3. Develop and implement a genetic management plan to guide managed gene flow between all populations:
    - Monitor local populations for abundance and genetic diversity, in adherence with a genetics management plan. The USFWS (2010) indicated that the findings of several studies and associated papers (Echelle et al. 2007, Koike et al. 2008, Loftis et al. 2009) could provide a framework for such a genetic management and monitoring plan. The 2019 amendment to the Desert Pupfish Recovery Plan (USFWS 2019) also identified multiple genetic studies on Desert Pupfish that have occurred in the past decade and should be incorporated into any planning efforts. Of particular concern is the potential for further gene flow restriction of Salton Sea tributary populations, as migratory corridors may become too saline for Desert Pupfish passage. Increasing salinity levels of the shrinking Salton Sea are also of concern,

potentially precluding future use of the nearshore sea environment by pupfish for migration and population dispersal. Monitoring and translocation planning should be adaptive and incorporate such environmental changes into the iterative planning, implementation, and evaluation process.

- Integrate, where warranted and feasible, the findings and recommendations of Echelle et al. (2007), including managing the Salton Sink populations as two separate genetic management units. Further recommendations include translocating adult Desert Pupfish between sites, within each management unit, at predetermined intervals and in adherence with a genetics management plan. Implementation of these measures should increase genetic diversity and maintain the overall genetic health of Desert Pupfish across all populations.

4. Expand existing distribution:
    - Establish new refuge populations near San Felipe Creek.
    - Continue to implement Desert Pupfish rescues to reduce mortality from habitat desiccation. Potentially utilizing rescued fish for population expansion purposes, as guided by a genetics management plan.
    - Create new refuge populations in suitable locations (e.g., The Living Desert and Dos Palmas Preserve). Refuges should include large (>1 acre) ponds, with stable water sources, that are fishless prior to pupfish introduction.

VII. RECOMMENDATION TO THE COMMISSION

Pursuant to Fish and Game Code section 2077, the Department has prepared this Five-Year Species Review based upon the best scientific information available to the Department to determine if conditions that led to the original listing are still present.

In completing this Five-Year Species Review for Desert Pupfish, the Department finds there is sufficient scientific information to indicate that the conditions that led to the listing of Desert Pupfish as endangered are still present and, in some cases, have worsened. The Department, therefore, recommends no change to the status of Desert Pupfish on the list of California's endangered species at this time.

VIII. SOURCES

A. Literature Cited

BAIRD, S.F. AND C. GIRARD. 1853. Descriptions of new species of fishes collected by Mr. John H. Clark, on the U.S. and Mexican Boundary Survey, under Lt. Cal. Jas. D. Graham. Proceedings of the Academy of Natural Sciences of Philadelphia 6:387-390.

BARLOW, G.W. 1961. Social behavior of the Desert Pupfish, *Cyprinodon macularius*, in the field and in the aquarium. The American Midland Naturalist, The University of Notre Dame 65(2):339-359.

BERRA, T. M. (2001). Freshwater Fish Distribution. Academic Press, San Diego, California, USA.

BLACK, G.F. 1980. Status of the Desert Pupfish *Cyprinodon macularius* (Baird and Girard), in California. State of California. The Resources Agency. Department of Fish and Game. Inland Fisheries, Region 5. Special Publication 80-1.

CALIFORNIA DEPARTMENT OF FISH AND GAME. 1990. Five-year status report for the Desert Pupfish. Internal administrative report.

CALIFORNIA DEPARTMENT OF FISH AND GAME. 1996. The status of rare, threatened, and endangered animals and plants of California.

CALIFORNIA DEPARTMENT OF FISH AND GAME. 2003. Atlas of the Biodiversity of California. 103 pp.

CALIFORNIA DEPARTMENT OF FISH AND WILDLIFE. 2019a. Desert Pupfish (*Cyprinodon macularius*) [Internet]. Available from: https://www.wildlife.ca.gov/Regions/6/Desert-Fishes/Desert-Pupfish

CALIFORNIA DEPARTMENT OF FISH AND WILDLIFE. 2019b. San Felipe Creek Ecological Reserve [Internet]. Available from: https://wildlife.ca.gov/Lands/Places-to-Visit/San-Felipe-Creek-ER

COACHELLA VALLEY CONSERVATION COMMISSION. 2011. Dos Palmas Conservation Area Reserve Management Unit 4. Available from: http://www.cvmshcp.org/pdf%20files/Dos_Palmas_RMUP_Rev_Mar_2011_RMOC_Final.pdf

COACHELLA VALLEY CONSERVATION COMMISSION. 2018. Coachella Valley Multiple Species Habitat Conservation Plan/Natural Community Conservation Plan, 2018 Annual Report. Available from: http://www.cvmshcp.org/Annual%20Reports/Annual%20Report%202018.pdf

COACHELLA VALLEY MULTIPLE SPECIES HABITAT CONSERVATION PLAN ($_a$). 2016. Establishment of the MSHCP Reserve System, Final Major Amendment to the CVMSHCP, 4-1. 222 pp. Available from: http://www.cvmshcp.org/Plan%20Documents/11.%20CVAG%20MSHCP%20Plan%20Section%204.0.pdf

COACHELLA VALLEY MULTIPLE SPECIES HABITAT CONSERVATION PLAN ($_b$). 2016. Species Accounts and Conservation Measures, Final Major Amendment to the CVMSHCP, 9-77. 267 pp. Available from: http://www.cvmshcp.org/Plan%20Documents/16.%20CVAG%20MSHCP%20Plan%20Section%209.0.pdf

COACHELLA VALLEY MULTIPLE SPECIES HABITAT CONSERVATION PLAN ($_c$). 2016. MSHCP Reserve System Management and Monitoring Program, Final Major Amendment to the CVMSHCP. 124 pp. Available from: https://cvmshcp.org/Plan%20Documents/15.%20CVAG%20MSHCP%20Plan%20Section%208.0.pdf

COX, T.J. 1966. A behavioral and ecological study of the desert pupfish (*Cvprinodon macularius*) in Quitobaquito Springs, Organ Pipe Cactus National Monument, Arizona. Dissertation, University of Arizona, Tucson.

COX, T.J. 1972. The food habits of desert pupfish (*Cvprinodon macularius*) in the Quitobaquito Springs, Organ Pipe Cactus National Monument, Arizona. Journal of the Arizona-Nevada Academy of Science 7: 25—27.

ECHELLE, A.A., AND T.E. DOWLING. 1992. Mitochondrial DNA variation and evolution of the Death Valley Pupfishes (Cyprinodon, Cyprinodontidae). Evolution 46(1):193-206.

ECHELLE, A.A., D. LOFTIS, H. KOIKE, AND R.A. VAN DEN BUSSCHE. 2007. Pupfish Genetics: Genetic Structure of Wild and Refuge Stocks of Desert Pupfish. Final Report to U.S. Fish and Wildlife Service, Coop. Agreement No. 201814J826, Oklahoma State University, Stillwater. 69 pp.

ESA ASSOCIATES. 2017. Final Salton Sea Species Conservation Habitat Project, Desert Pupfish Adaptive Management and Monitoring Plan. Prepared for California Department of Water Resources. 54 pp.

HOPKINS, F. 2018. Inland Deserts Summary Report. California's Fourth Climate Change Assessment. University of California, Riverside. Publication number: SUM-CCCA4-2018-008.

IMPERIAL COUNTY, OFFICE OF THE AGRICULTURAL COMMISSIONER. 2018. Imperial County Agricultural Crop & Livestock Report, 2018. Available from: https://agcom.imperialcounty.org/wp-content/uploads/2020/02/2018_Imperial_County_Crop_and_Livestock_Report.pdf

KEENEY, S. 2006. Status of the desert pupfish (*Cyprinodon macularius*) in California. Abstracts, Desert Fishes Council, 38th Annual Meeting, 15-18 November 2006, Death Valley, California. Pg. 35.

KEENEY, S. 2010a. Status of pupfish populations. Unpublished Report, California Department of Fish and Game. 3 pp.

KEENEY, S. 2010b. Desert pupfish 5-year review. California Department of Fish and Game. E-mail 2 August 2010 to Doug Duncan, U.S. Fish and Wildlife Service. 1 pg.

KEENEY, S. 2016. California Department of Fish and Wildlife, Inland Deserts Region 6. Bermuda Dunes, California. Email and phone communications regarding status of pupfish and habitat in Salton Sea area, desert pupfish genetics, protection and capture methods, decontamination protocols with Ramona Swenson, Environmental Science Associates, Sept-Oct, 2016.

KEENEY, S. 2019. New or Restored Well for the Endangered Desert Pupfish (*Cyprinodon macularius*) at Oasis Springs Ecological Reserve. Final Proposition 1 Grant Report to the Coachella Valley Mountains Conservancy. 5 pp.

KINNE, O. 1960. Growth, food intake, and food conversion in a euryplastic fish exposed to different temperatures and salinities. Physiological Zoology, 33(4):288-317.

KOIKE, H., A.A. ECHELLE, D. LOFTIS, AND R.A. VAN DEN BUSSCHE. 2008. Microsatellite DNA analysis of success in conserving genetic diversity after 33 years of refuge management for the desert pupfish complex. Animal Conservation 11(2008):321-329.

LOFTIS, D.G., A.A. ECHELLE, H. LOILE, R.A. VAN DEN BUSSCHE, AND C.O. MCKINLEY. 2009. Genetic structure of wild populations of the endangered Desert Pupfish complex. Conservation Genetics 10:453-463.

LOISELLE, P.V. 1980. Spawn recognition by male *Cvprinodon macularius californiensis*. Proceedings of the Desert Fishes Council XI (1979): 46. (abstract).

LOISELLE, P.V. 1982. Male spawning—partner preference in an arena—breeding teleost *Cyprinodon macularius californiensis* Girard (Atherinomorpha: Cyprinodontidae). The American Naturalist 120: 721—732.

LOWE, C.H., D.S. HINDS, AND E.A. HALPERN. 1967. Experimental catastrophic selection and tolerances to low oxygen concentrations in native Arizona freshwater fishes. Ecology 48: 1013—1017.

LOWE C.H., AND W.G. HEATH. 1969. Behavioral and physiological responses to temperature in the desert pupfish, *Cvprinodon macularius*. Physiological Zoology 42: 53-59.

MARTIN, B.A. AND M.K SAIKI. 2005. Relation of Desert Pupfish abundance to selected environmental variables in natural and manmade habitats in the Salton Sea Basin. Environmental Biology of Fishes. 73:97-107.

MILLER, R.R. 1943. The status of *Cyprinodon macularius* and *Cyprinodon nevadensis*, two desert fishes of western North America. Occasional Papers of the Museum of Zoology, University of Michigan 473:1-25.

MILLER, R.R. 1948. The cyprinodont fishes of the Death Valley System of eastern California and southwestern Nevada. University of Michigan Museum of Zoology Miscellaneous Publications #68. 155 pp.

MOYLE, P.B. 2002. Inland Fishes of California. Berkeley: University of California Press.

MOYLE, P.B., J.D. KIERNAN, P.K. CRAIN, AND R.M. QUINONES. 2012. Projected effects of future climates on freshwater fishes of California. California Energy Commission Publication number: CEC-500-2012-028.

PARMENTER, S.C., M.T. BOGAN, R. BLOOM, S. KEENEY, AND E. KONNO. 2004. 2002 California Area Report. Hendrickson, D.A., and L.T. Findley, eds., Proceedings of the Desert Fishes Council, Desert Fishes Council, Bishop, California 34:34-35.

PINTER, N., AND E.A. KELLER. 1991. Hydrology of Fish Slough. Pages III-11 to III-13 in Ferren, Jr., and Davis editors. Biotic inventory and ecosystem characterization for Fish Slough Inyo and Mono Counties, California. University of California, Santa Barbara. Prepared for the Resources Agency, Department of Fish and Game, Award No. FG-83890.

SCHOENHERR, A.A. 1979. Niche separation within a population of freshwater fishes in an irrigation drain near the Salton Sea, California. Bulletin of the Southern California Academy of Science 78:46-55.

SCHOENHERR, A.A. 1985. Replacement of *Cvnrinodon macularius* by *Tilapia zilli* in an irrigation drain near the Salton Sea. Proceedings of the Desert Fishes Council XIII (1981):65-66 (abstract).

SCHOENHERR, A.A. 1988. A review of the life history and status of the Desert Pupfish, *Cyprinodon macularius*. Bulletin of the Southern California Academy of Sciences. 87(3):104-134.

SCHOENHERR, A.A. 1990. A comparison of two populations of the endangered desert pupfish (*Cyprinodon macularius*). First Annual Report. California Department of Fish and Game.

SCHOENHERR, A.A. AND C.R. FELDMETH. 1991. Thermal tolerances for relict populations of desert pupfish, *Cyprinodon macularius*. Proceedings of the Desert Fishes Council XXIII (1991):49-54 (abstract).

SCHOENHERR, A.A. 1992. A Natural History of California. Berkeley: University of California Press.

SAN DIEGO COUNTY WATER AUTHORITY. 2019. Dos Palmas Oasis 2018 Annual Water Supply Monitoring Summary Report. 68 pp.

U.S. FISH AND WILDLIFE SERVICE. 1993. Desert Pupfish Recovery Plan. Phoenix, Arizona. 67 pp.

U.S. FISH AND WILDLIFE SERVICE. 2002. Biological Opinion on the Bureau of Reclamation's Voluntary Fish and Wildlife Conservation Measures and Associated Conservation Agreements with the California Water Agencies. Carlsbad Fish and Wildlife Office. Carlsbad, California. 80 pp.

U.S. FISH AND WILDLIFE SERVICE. 2010. Desert Pupfish 5-Year Review: Summary and Evaluation. Arizona Ecological Services Office. Phoenix, Arizona.

U.S. FISH AND WILDLIFE SERVICE. 2019. Recovery Plan for Desert Pupfish (*Cyprinodon macularius*) Amendment 1. U.S. Fish and Wildlife Service Southwest Office. Albuquerque, New Mexico. 20 pp.

WATER EDUCATION FOUNDATION. 2021. Quantification Settlement Agreement. [Internet]. Available from: https://www.watereducation.org/aquapedia/quantification-settlement-agreement

B. Personal Communication

E-mail message from Doug Duncan, USFWS Arizona Ecological Services Office, regarding the status and plans for a federal 5-year status review to update the existing 2010 review (no plans in place). March 10, 2019.

Multiple email messages with Sharon Keeney (CDFW, Bermuda Dunes Field Office) 2019 - 2021.

Telephone conversation and email message from Steve Bigley, Director of Environmental Services, Coachella Valley Water District, regarding the Garfield Street 25-acre Desert Pupfish mitigation area. February 18, 2020.

Telephone conversation with Dr. James Danoff-Burg (Director of Conservation, The Living Desert Zoo and Gardens), February 14, 2020.

IX. LIST OF FIGURES

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